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

ToxR 011221

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Molinate: Subchronic Neurotoxicity Study

TO: Paul Parsons
PM Team Reviewer (62)
Reregistration Branch/SRRD (7508C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 9/9/94
Toxicology Branch II, Section II,
Health Effects Division (7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 9/20/94
Section II Head, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 9/20/94
Chief, Toxicology Branch II/HFAS/HED (7509C)

Registrant: ZENECA Inc. Ag Products
Chemical: Molinate; S-ethyl hexahydro-1H-azepine-1-carbothioate
Synonym: Ordram
Caswell No.: 444
MRID No.: 432707-01
Submission: S468596
DP Barcode: D204763
Action Requested: None specified.

Comment: The Registrant has submitted a subchronic neurotoxicity study in rats, which is a data requirement for Molinate. This study has been reviewed, and the DER is appended.

MOLINATE: Subchronic Neurotoxicity Study in Rats. Study # PR0949; Report # CTL/P/4289, dated May 10, 1994.

EXECUTIVE SUMMARY: Under the conditions of the study, administration of Molinate to Alpk:APfSD rats of both sexes [12/sex/group] at dose levels of 50 [♂ 4.0/♀ 4.5 mg/kg/day], 150 [♂ 11.7/♀ 13.9 mg/kg/day], and 450 ppm [♂ 35.5/♀ 41.0 mg/kg/day] for at least 90 days resulted in a dose-related decrease in body weight [♂♂ 86%/♀♀ 84% of control at the high dose; ♀♀ 93% of control at the mid dose; ♀♀ 94% of control at the low dose], body-weight gain

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[♂♂ 78%/♀♀ 65% of control at the high dose; ♀♀ 84% of control at the mid dose; ♀♀ 88% of control at the low dose], food consumption, and food utilization. At the high-dose level, (1) females displayed an increase in landing foot splay at week 5; (2) both sexes displayed an increase in the time to tail flick at week 14; (3) both sexes displayed a decrease in forelimb grip strength during week 9; (4) females displayed a decrease in hindlimb grip strength during week 5 and males displayed a decrease during week 14. Additionally, a decrease in the time to tail flick was observed at week 5 in males at all dose levels, but there was no dose response. No apparent effect was demonstrated on overall motor activity in males, but when compared to pretest values, high-dose females displayed increased motor activity following treatment. There was a dose-related decrease in both brain and erythrocyte cholinesterase activities in both sexes, and a dose-related decrease in NTE activity at all dose levels in both sexes compared to the control values. Absolute brain weight was decreased significantly in both sexes at the high-dose level. Microscopically, nerve fiber degeneration in the sciatic nerve and the sural nerve was increased in the high-dose males compared to the control incidence. **Several FOB parameters were altered during the study, and motor activity in the high-dose females appears to have been increased relative to pre-treatment values. Males displayed nerve fiber degeneration in the sciatic and sural nerves at the high-dose level. There was a dose-related decrease in brain and erythrocyte cholinesterase activity, and decreased NTE activity was observed in both sexes at all dose levels. Although no NOEL was attained in this study for the decreased brain cholinesterase activity observed in females, the decrease at the low dose in both sexes is considered minimal [93% of control].** This study is classified Core Minimum, and it satisfies the guideline requirement [82-7-SS] for a subchronic neurotoxicity study in the rat.

cc: Debbie McCall, CCB

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 9/19/94
Tox. Branch II, Section II (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 9/20/94
Tox. Branch II, Head Section II (7509C)
Tertiary Reviewer: William F. Sette, Ph.D. William F Sette 9/19/94
Science Support and Special Review Section (7509C)

DATA EVALUATION REPORT

STUDY TYPE: subchronic neurotoxicity - rats

Guideline: 82-7-SS TOX. CHEM. NO.: 444

Shaughnessey No.: 041402

MRID NO.: 432707-01

TEST MATERIAL: Molinate

SYNONYMS: S-ethyl hexahydro-1H-azepine-1-carbothioate; Ordram

STUDY NUMBER: PRO949; Report # CTL/P/4289; CTL Ref. Y06367/025

SPONSOR: Zeneca Agrochemicals

TESTING FACILITY: Zeneca Central Toxicology Laboratory/UK

TITLE OF REPORT: Molinate: Subchronic Neurotoxicity Study in Rats

AUTHOR: JM Horner

REPORT ISSUED: May 10, 1994

QUALITY ASSURANCE: Both a quality assurance statement and a GLP compliance statement were provided.

EXECUTIVE SUMMARY: Under the conditions of the study, administration of Molinate to Alpk:APfSD rats of both sexes [12/sex/group] at dose levels of 50 [♂ 4.0/♀ 4.5 mg/kg/day], 150 [♂ 11.7/♀ 13.9 mg/kg/day], and 450 ppm [♂ 35.5/♀ 41.0 mg/kg/day] for at least 90 days resulted in a dose-related decrease in body weight [♂♂ 86%/♀♀ 84% of control at the high dose; ♀♀ 93% of control at the mid dose; ♀♀ 94% of control at the low dose], body-weight gain [♂♂ 78%/♀♀ 65% of control at the high dose; ♀♀ 84% of control at the mid dose; ♀♀ 88% of control at the low dose], food consumption, and food utilization. At the high-dose level, (1) females displayed an increase in landing foot splay at week 5; (2) both sexes displayed an increase in the time to tail flick at week 14; (3) both sexes displayed a decrease in forelimb grip strength during week 9; (4) females displayed a decrease in hindlimb grip strength during week 5 and males displayed a decrease during week 14. Additionally, a decrease in the time to tail flick was observed at week 5 in males at all dose levels, but there was no dose response. No apparent effect was demonstrated on overall motor activity in males, but when compared to pretest values, high-dose females displayed increased motor activity following treatment. There was

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a dose-related decrease in both brain and erythrocyte cholinesterase activities in both sexes, and a dose-related decrease in NTE activity at all dose levels in both sexes compared to the control values. Absolute brain weight was decreased significantly in both sexes at the high-dose level. Microscopically, nerve fiber degeneration in the sciatic nerve and the sural nerve was increased in the high-dose males compared to the control incidence. Several FOB parameters were altered during the study, and motor activity in the high-dose females appears to have been increased relative to pre-treatment values. Males displayed nerve fiber degeneration in the sciatic and sural nerves at the high-dose level. There was a dose-related decrease in brain and erythrocyte cholinesterase activity, and decreased NTE activity was observed in both sexes at all dose levels. Although no NOEL was attained in this study for the decreased brain cholinesterase activity observed in females, the decrease at the low dose in both sexes is considered minimal [9?% of control]. This study is classified Core Minimum, and it satisfies the guideline requirement [82-7-SS] for a subchronic neurotoxicity study in the rat.

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A. MATERIALS:

1. Test Compound: Molinate; Description: amber liquid; Batch #: CTL reference # Y06367/025; Purity: 96.8%; Source: ICI Agrochemicals/UK. **NOTE:** Appendix A consists of a Certificate of Analysis [dated 12/17/91] and a Certificate of Re-Analysis [dated 8/31/93]. On the former certificate under Supplementary information is the statement: For Study No: A1'91/0169 and at the top of the page, Y06367/025/001 is hand-written and dated 12/23/91. The latter certificate has nothing listed under Supplementary information, but Y06367/025 is hand-written at the top and dated 9/6/93. Additionally, TB II notes that the Data reference, the Analytical reference, and Methods of analysis differ between the two certificates. Both certificates show 96.8% as the purity.

2. Test Animals: Species: rat; Strain: Alpk:APfSD; Age: ≈28 days on arrival; Weight: Week 1: males 180-183 g/females 151-153 g; Source: Specific Pathogen Free (SPF) colony maintained at the Barriered Animal Breeding Unit at Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK.

3. Statistics: body weights - analysis of covariance on initial [week 1] body weight per sex; weekly food consumption, food utilization, motor activity measurements, time to tail-flick, landing foot splay, fore- and hind-limb grip strength, cholinesterase activity, NTE activity, and GFAP levels - analysis of variance per sex; brain weight, brain length, and brain width - analysis of variance and covariance on final body weight and method of kill per sex. Analysis of variance and covariance were carried out using the GLM procedure in SAS (1989). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. 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 were tested statistically using a two-sided Student's t-test, based on the error mean square in the analysis.

B. STUDY DESIGN

1. Methodology: Fifty two males and 52 females were allocated to the various groups [randomization method designed to ensure only healthy rats (excluding those failing pre-test tail-flick test) were used; those at weight extremes were excluded also] of the study. The groups were arranged on two racks in 6 single-sex replicates (randomized blocks; Appendix E, copy attached). Each replicate consisted of 4 cages, one for each treatment group. The sequence of the groups in each replicate was determined using computer-generated sequences of numbers 1 to 4, and 4 individual animal numbers were assigned to each cage sequentially. During the acclimatization period [≈ 2 weeks], the rats were assigned to cages using the following procedure. Body weights were recorded with the heaviest listed

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first, and shuffle cards were numbered 1 through 4, corresponding to the group numbers on the replicate. Rats were taken in weight order, and a card was chosen at random and the heaviest rat chosen, ear-punched with the lowest available experimental number for that cage shown on the rack plan and allocated to the group indicated by the number on the card. The next heaviest rat was chosen and allocated to the group shown on the next card, etc. This procedure continued until all cages in each replicate contained one rat, and it was then repeated until each cage contained 4 rats.

Molinate was administered via the diet at dose levels of 0, 50, 150, and 450 ppm (control groups received basal diet only). The rats were housed 4 per cage per sex during the study and were fed CT1 diet supplied by Special Diets Services Limited, Stepfield, Witham, Essex, UK ad libitum. Water was available ad libitum also.

Dose preparation: The test diets were prepared [frequency not reported; only one date is referenced in the results] in 30 kg batches from a premix prepared by grinding Molinate with 500 grams of milled diet. The premix was added to 29.5 kg of additional diet and mixed. The amounts of Molinate [based on a purity of 96.8% w/w] added to 30 kg of diet to attain the required dietary concentrations are listed in Table 1. The diets were stored in a deep freeze and were thawed before use. Samples of each dietary level were taken at intervals during the study and analyzed to verify the concentration attained. The stability [at -20° and at room temperature] of Molinate in the diet was determined over a 5-week period. Homogeneity was determined by analyzing samples from the low- and high-dose levels.

Table 1. Diet Preparation

Concentration of Molinate [ppm]	Molinate [grams] in 30 kg of diet
0	0
50	1.55
150	4.65
450	13.95

RESULTS

The diets were found to be homogeneously mixed, being within 2% of the overall mean value. The stability of Molinate in the diet was determined to be acceptable for a period of 9/14 days at room temperature for the 50/450 ppm dose levels and for a period of 5 weeks [period of use] at -20°C for both dose levels. The overall mean dietary concentrations attained were within 8% of the nominal concentrations.

2. Clinical Observations: The rats were observed [cageside checks] daily for changes in clinical condition and behavior,

and each rat was removed from its cage weekly and physically examined for changes in general health status. Individual body weights were recorded in replicate order weekly (one week prior to study initiation, immediately prior to study start, and on the same day of each week thereafter). Food consumption (per cage) was recorded continuously throughout the study and calculated on a weekly basis, and the food utilization value per cage was calculated as the body weight gained by the rats in the cage per 100 grams of food eaten.

RESULTS

Survival and Clinical Observations: One low-dose male was euthanized *in extremis* during week 7 [broken nasal cavity], but the death was not considered treatment related. All other rats survived to study termination. No clinical signs were observed that could be attributed to treatment.

Body Weight and Food Consumption: The high-dose rats of both sexes displayed significant reductions in body weight compared to their respective controls throughout the study (♀♀ 85-89% of control value and ♂♂ 84-92% of control value). Slight, but statistically significant, decreases in body weight were observed during weeks 2 and 3 in males at the mid-dose level [96% of control], and females at the mid- and low-dose levels displayed statistically significant decreases in body weight [dose-related] throughout the study [89-94% (mid dose)/93-96% (low dose) of control]. A dose-related decrease in overall body-weight gain was displayed by both sexes compared to the respective controls [calculated by TB II; statistics not performed; Table 2]. Throughout the study, high-dose rats of both sexes displayed statistically significant decreases [weeks 4 and 8 for females not significant] in food consumption compared to the control values, and females at the low- [week 3] and mid-dose [weeks 2 and 3] also displayed statistically significant decreases compared to the controls [Table 2]. Decreased food utilization was observed during the 1-4 week interval and overall by both sexes at the high dose and by the mid-dose females. During the 5-8 week interval, the mid-dose females displayed a significant increase in food utilization.

Table 2. Body Weight, Body-Weight Gain, and Food Consumption [grams (% of control)]

Parameter/Time Frame/Sex/Dose	0 ppm	50 ppm	450 ppm	450 ppm
Body Weight (g)				
MALES				
week 1	183.2	182.9	182.0	180.2
week 2	232.4	229.3	222.4**(96)	207.5**(89)
week 3	279.6	273.4	267.6**(96)	246.1**(88)
week 4	311.8	306.8	301.2*(97)	272.6**(87)
week 5	341.7	333.2	329.2(96)	296.8**(87)
week 6	366.5	357.7	352.1	317.7**(87)
week 7	386.8	376.3	372.7	334.8**(87)
week 8	408.1	392.8	389.6(95)	346.4**(85)
week 9	426.0	412.9	407.9	362.9**(85)
week 10	439.6	426.7	421.3	375.2**(85)
week 11	449.7	442.2	433.0	387.8**(86)
week 12	461.7	449.6	444.6	398.5**(86)
week 13	474.8	461.7	456.9	404.3**(85)
week 14	483.8	471.7	463.4	414.5**(86)
FEMALES				
week 1	152.3	151.3	151.9	153.0
week 2	175.9	168.5**(96)	165.1**(94)	162.2**(92)
week 3	200.7	185.6**(92)	178.8**(89)	174.4**(87)
week 4	211.8	197.9**(93)	191.1**(90)	182.3**(86)
week 5	221.0	208.9**(95)	202.2**(91)	192.2**(87)
week 6	233.3	222.8**(95)	216.8**(93)	201.2**(86)
week 7	241.6	228.9**(95)	225.1**(93)	207.3**(86)
week 8	248.5	235.1**(95)	232.0**(93)	212.4**(85)
week 9	251.7	238.7**(95)	237.2**(94)	217.3**(86)
week 10	259.6	245.3**(94)	240.6**(93)	219.0**(84)
week 11	269.0	249.1**(93)	246.7**(92)	224.9**(84)
week 12	271.5	257.1**(95)	253.3**(93)	229.5**(85)
week 13	274.5	262.3**(96)	255.8**(93)	232.1**(85)
week 14	276.5	261.1**(96)	256.8**(93)	233.4**(84)
Body-Weight Gain (g)				
MALES				
weeks 1-2	49.2	46.4(94)	40.4(82)	27.3(55)
weeks 2-3	47.2	44.1	45.2	38.6(82)
weeks 3-4	32.2	33.4	33.6	26.5(82)
weeks 1-3	96.4	90.5	85.6(89)	65.9(68)
weeks 1-4	128.6	123.9	119.2(93)	92.4(72)
weeks 1-9	242.8	230.0	225.9(93)	182.7(75)
weeks 1-14	300.6	288.8(96)	281.4(94)	234.2(78)
FEMALES				
week 1-2	23.6	17.2(73)	13.2(56)	9.2(39)
weeks 2-3	24.8	17.1(69)	13.7(55)	12.2(49)
weeks 3-4	11.1	12.3	12.3	7.9(71)
weeks 1-3	48.4	34.3(71)	26.9(56)	21.4(44)
weeks 1-4	59.5	46.6(78)	39.2(66)	29.3(49)
weeks 1-9	99.4	87.4(88)	85.3(86)	64.3(65)
weeks 1-14	124.2	109.8(88)	104.9(84)	80.4(65)
Food Consumption (g/rat/day)				
MALES				
week 1	25.2	25.3	23.9(95)	22.1**(88)
week 2	27.7	27.0	26.1(94)	24.6**(89)
week 3	28.0	27.9	27.2	24.4**(87)
week 4	28.5	27.7	27.6	25.1**(88)
week 7	28.3	27.1	20.7(95)	24.6*(87)
week 12	28.1	28.0	27.2	24.3**(86)
week 13	28.2	28.3	27.5	25.2**(89)
FEMALES				
week 1	20.8	18.9*(91)	19.3(93)	19.0*(91)
week 2	21.2	19.8(93)	19.0**(90)	18.6**(88)
week 3	21.6	20.3*(94)	19.5**(90)	18.1**(84)
week 4	20.3	19.8	20.5	18.7(92)
week 7	20.5	20.2	20.3	18.5*(90)
week 12	20.4	19.9	19.4	17.7**(87)
week 13	20.2	19.3	19.6	17.8*(88)

Table 2. Body Weight, Body-Weight Gain, and Food Consumption [grams (% of control)]				
Parameter/Time Frame/Sex/Dose	0 ppm	50 ppm	150 ppm	450 ppm
Food Utilization (g growth/100 g food)				
MALES				
weeks 1-4	20.7♦	19.9	20.1	17.3**(83)
weeks 5-8	10.7	10.1	10.3	9.5(89)
weeks 9-13	5.9	5.9	5.8	5.9
weeks 1-13	11.8	11.4	11.5	10.4**(88)
FEMALES				
weeks 1-4	11.7	10.5	9.1*(78)	7.5**(64)
weeks 5-8	5.3	5.3	6.3*(118)	4.9
weeks 9-13	3.5	3.3	2.8(82)	2.6(74)
weeks 1-13	6.6	6.1	5.8*(88)	4.8**(73)

♦ numbers have bee rounded off; * $p<0.05$; ** $p<0.01$; ♦ (% of control); ∴ statistics not performed

The mean dose of Molinate consumed by each group is listed in Table 3 below.

Dietary Level (ppm)	Table 3. Mean Calculated Molinate Consumed (mg/kg/day)	
	MALES	FEMALES
50	4.0	4.5
150	11.7	13.9
450	35.5	41.0

3. Behavioral Investigations - **Subchronic Neurotoxicity**

The behavioral investigations incorporated a functional observational battery (FOB) and a test for locomotor activity (LA). Both tests were conducted prior to study initiation and during the 5th, 9th, and 14th weeks. Testing was performed "blind" by one observer. All animals (12/group/sex) were observed for the following parameters (see below).

Functional Observational Battery (FOB) - Detailed clinical observations [removal of rat from cage and a physical examination for changes in general health status; see below] and quantitative assessments of landing foot splay, sensory perception [tail-flick test], and muscle weakness [fore- and hind-limb grip strength] were performed at each time point. The clinical observations included the following list of measures: (1) assessment of autonomic function [lacrimation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis; (2) description, incidence, and severity of any convulsions, tremors, abnormal motor function, abnormal behavior, etc.; (3) reactivity to stimuli; (4) changes in level of arousal; (5) sensorimotor responses; and (6) alterations in respiration.

Motor Activity (MA) - Motor activity was tested by placing each rat into an automated activity recording device (not further described), and each session was divided into 10 scans of 5 minute duration. No statement was made as to whether the

initial time in the cage was included in the assessment. The groups were "counter balanced across test times and across devices", and at each repeated trial the rats were returned to the same activity monitor at approximately the same time of day.

RESULTS

Functional Observational Battery (FOB): Clinical Observations: There were no clinical signs observed following dosing that could be attributed to treatment. Landing Foot Splay: There was a statistically significant increase in landing foot splay observed at the week 5 test period in females, but no apparent difference at any other time point or in males compared to the controls [Table 4]. Time to Tail Flick: There was a statistically significant [at all dose levels] decrease in the time to tail flick in males at the week 5 test period compared to the control, but there was no dose response. During the week 9 test period, females at all dose levels displayed decreased values compared to the control, but only the mid dose attained statistical significance and there was no dose response [Table 5]. At week 14, the high-dose rats of both sexes displayed an increase compared to their respective controls, and the value for males attained statistical significance. TB II notes that the value for the high-dose males at week 14 is increased relative to its pretest value, whereas all other groups [both sexes] display decreased values relative to their pretest values. Both sexes at the high-dose level displayed lower values initially and at week 5 compared to the controls. Fore- and hind-limb grip strength: There was a dose-related decrease in forelimb grip strength during the week 9 testing period, and the high-dose level displayed statistical significance [both sexes]. No other significant differences were observed in forelimb grip strength. Hindlimb grip strength was significantly decreased in the high-dose females during the week 5 testing period and in the high-dose males during the week 14 testing period. A similar decrease was evident in the high-dose females during the pre-test interval [Table 6].

Table 4. Landing Foot Splay (mm)

Dose [ppm]/interval	Pretest	week 5	week 9	week 14
MALES				
0	45.5	67.3	62.5	66.9
50	50.6	69.5	65.8	63.5
150	49.2	77.4	64.0	62.4
450	49.2	71.7	61.8	64.6
FEMALES				
0	43.5	56.4	59.4	57.8
50	44.6	60.2	63.8	52.8
150	44.0	58.8	58.8	56.7
450	44.0	66.0*(117)	60.1	58.8

* p<0.05; ♦ [% of control]

Table 5. TIME TO TAIL FLICK [seconds]

Dose [ppm]/interval	pretest	week 5	week 9	week 14
MALES				
0	5.1	7.6	4.4	4.0
50	6.3(124)♦	5.7**(75)	5.2(118)	4.4(110)
150	5.2(102)	6.2*(82)	3.6(82)	4.4(110)
450	4.6(90)	5.9*(78)	4.7(107)	6.6*(165)
FEMALES				
0	7.0	6.1	4.2	4.3
50	6.4	4.6*(75)	3.6(86)	4.0
150	6.8	5.0(82)	3.3*(79)	3.6(86)
450	6.0	5.7	3.6(86)	5.2(121)

* p<0.05; ** p<0.01; ♦ (% of control value)

Table 6. Neuromuscular Observations

Parameter/Sex/Dose	0 ppm	50 ppm	150 ppm	450 ppm
MALES				
forelimb grip strength (g)				
pretest	421	398	400	413(98)
5 weeks	1165	1231	1223	1140(98)
9 weeks	1569	1570	1517	1417*(90)
14 weeks	1513	1561	1492	1460(96)
hindlimb grip strength (g)				
pretest	275	223(81)	235(85)	260
5 weeks	719	690	744	681
9 weeks	738	773	758	727
14 weeks	985	995	1010	863*(88)
FEMALES				
forelimb grip strength (g)				
pretest	450	406	433	440(98)
5 weeks	1021	1058	979	988(97)
9 weeks	1342	1308	1265	1098**(82)
14 weeks	1217	1263	1171	1135(93)
hindlimb grip strength (g)				
pretest	283	254	292	246(87)
5 weeks	642	669	577	548*(85)
9 weeks	613	646	606	588
14 weeks	675	721	579	733

* p<0.05; ♦ (% of control value)

Locomotor Activity (LA): Overall motor activity values were lower than the control values for all treatment groups [both sexes] pretest [Table 7a]. At 5, 9, and 14 weeks, males at the low dose displayed an increase [147%, 117% and 104% of control, respectively] and the mid- and high-dose males continued to displayed decreases compared to the control values. Females at the high dose level during the week 14 testing period displayed a statistically significant decrease [86% of control value] in overall motor activity, and statistical significance was attained for two of the five-minute individual sessions. During the week 9 testing session, an increase in overall motor activity was observed in the high-dose females, which may be treatment-related, based on the relationship of the values among the groups throughout the study; i.e., at all other time points, including the pretest, the high-dose females displayed lower activity levels and at 9 weeks they achieved comparable [slightly greater] activity

levels to the controls. When the data are viewed relative to pretest values, the females display a dose-related increased activity level compared to their pretest activity levels, and the high-dose females overall display the greatest sustained increase relative to pretest values [Table 7b].

Group/ Week/ Dose (ppm)	Table 7a. Overall Motor Activity Counts (total counts of 10 Five-Minute Subsessions)			
	0	50	150	450
MALES				
-1	213.1	159.5(75)♦	200.2(94)	177.8(83)
5	353.3	518.0(147)	313.8(89)	252.8(72)
9	251.8	293.4(117)	217.3(86)	205.9(82)
14	299.7	312.8(104)	267.6(89)	231.1(77)
FEMALE				
-1	308.7	214.4(69)	228.8(74)	170.8(55)
5	656.0	570.8(87)	674.5(103)	563.3(86)
9	597.3	592.3(99)	595.3(100)	605.2(101)
14	639.0	614.4(96)	647.3(101)	552.0*(86)

♦ (% of control value) * $p<0.05$

Group/ Week/ Dose (ppm)	Table 7b. Overall Motor Activity Counts (relative to pretest values)			
	0	50	150	450
MALES				
5	166	325	157	142
9	118	184	109	116
14	141	196	134	130
FEMALE				
5	213	266	295	330
9	193	276	260	354
14	207	287	283	323

4. Gross Necropsy/Histopathology/Neuropathologic Evaluation

At scheduled sacrifice, any rat sacrificed in extremis and up to 6 designated [not further defined] rats/sex/dose were exsanguinated under terminal anesthesia with halothane vapor and subjected to a full post mortem examination. The brain, vertebral column including spinal cord, gasserian ganglia, dorsal root ganglia including spinal roots, gastrocnemius muscle, sciatic nerve, sural nerve, and tibial nerve were removed and immerse fixed [except brain] in 10% neutral buffered formol saline. The brain was submitted for enzymatic and protein determination and was weighed, the length and width recorded. The left half was kept on ice and the right half was weighed and frozen in acetone and cardice and stored at -70°C. Also at termination, 6 additional rats/sex/group were anesthetized with intraperitoneal sodium pentobarbitone and sacrificed by perfusion fixation with modified Karnovsky's fixative. The tissues listed above were removed and the brain

weight, length, and width were recorded. Only tissues from these latter rats [perfused] were processed for histology. The brain and gastrocnemius muscle were embedded in paraffin wax, and sections were cut and stained with hematoxylin and eosin. Additionally, transverse sections of the vertebral column containing samples from the lumbar and cervical regions of the spinal cord, with dorsal root ganglia and spinal roots attached, were decalcified, embedded in paraffin wax, and sections were cut and stained as noted above. The remaining tissues were embedded in ARALDITE and semi-thin sections were cut and stained with toluidine blue. Samples of the spinal cord and peripheral nerve were embedded in ARALDITE also and processed as above. Initially, the brain of 1 rat/sex of the high-dose group was examined in the transverse plane at 12 levels. On the basis of this examination, the brain of the remaining 5 rats/sex from the high-dose group and 6 rats/sex from the control group were examined in the transverse plane at the following 6 levels: 6, 7, 8, 9, 10, 12. Spinal cord from the cervical region [C3-C6] and from the lumbar region [L1-L4] was examined in the transverse plane. Spinal roots and dorsal root ganglia from the C3-C6 and L1-L4 levels and the gasserian ganglia from the trigeminal nerve were examined, as were transverse and longitudinal sections of the sciatic nerve and transverse sections of sural and tibial nerves. Samples of gastrocnemius muscle were examined in the transverse plane.

Neuropathological examination of all tissues was performed on control and high-dose rats initially, with the sciatic nerve and spinal cord of the low- and mid-dose rats being examined [light microscopy] also.

RESULTS

Brain parameters - Decreased absolute brain weight was displayed in both sexes at the high-dose level compared to the controls [Table 8]. The high-dose males also displayed a statistically significant decrease in relative [adjusted for final body weight] brain weight. No apparent effect was observed on either brain length or brain width at any dose level, although the decrease in width observed at the mid-dose level in both sexes and in the low-dose males attained statistical significance.

Macroscopic findings -No treatment-related findings were observed in either sex.

Microscopic findings - Minimal neuronal cell necrosis was observed in the granular neurons in the ventrolateral region of the dentate gyrus of the brain in two high-dose males and in one high-dose female, and slight neuronal cell necrosis was observed in one high-dose female. One control male and 2 control females displayed minimal neuronal cell necrosis also.

Minimal nerve fiber degeneration was observed in the sciatic nerve of 4 of the 6 high-dose males examined compared to one in the concurrent control of both sexes. One high-dose male also displayed minimal nerve fiber degeneration in the sural nerve. No other changes were observed [Table 9], although for one high-dose female brain, gasserian ganglia and spinal cord were listed as missing, as was one low-dose female sciatic nerve.

Table 8. Brain Parameters

Parameter/sex/Dose	0 ppm	50 ppm	150 ppm	450 ppm
BRAIN WEIGHT				
MALES				
absolute	2.08	2.02	2.02	1.97**(95)♦
relative to body weight	0.43	0.43	0.44	0.48(112)
adjusted for body weight	2.07	2.02	2.02	1.99*(96)
FEMALES				
absolute	1.93	1.91	1.89	1.86**(96)
relative to body weight	0.70	0.74	0.74	0.81(116)
adjusted for body weight♦	1.92	1.91	1.89	1.87(97)
BRAIN LENGTH				
MALES				
absolute	28.08	27.73	27.75	28.25
relative to body weight	5.84	5.92	6.03	6.89(118)
adjusted for body weight	27.93	27.57	27.74	28.59
FEMALES				
absolute	26.55	26.42	26.33	27.33
relative to body weight	9.71	10.15(105)	10.32(106)	11.84(122)
adjusted for body weight	27.00	26.51	26.32	26.85
BRAIN WIDTH				
MALES				
absolute	15.17	14.27**(94)	14.30*(96)	14.83
relative to body weight	3.16	3.05	3.15	3.61(114)
adjusted for body weight	14.99	14.22(95)	14.45	15.13
FEMALES				
absolute	15.00	14.33	13.92**(93)	14.42
relative to body weight	5.47	5.52	5.45	6.26(114)
adjusted for body weight	15.04	14.37	13.93*(93)	14.31
TERMINAL BODY WEIGHT				
MALES	481.7	469.3	462.3(96)	412.4(86)
FEMALES	275.1	261.3(95)	255.7(93)	231.1(84)

♦adjusted for final body weight; ♦ (% of control); * $p<0.05$; ** $p<0.01$

Table 9. Microscopic Observations.

Lesion/ Sex/ Dose [ppm]	MALES				FEMALES			
	0	50	150	450	0	50	150	450
BRAIN - Neuronal cell necrosis N=	6	0	0	6	6	0	0	5
dentate gyrus [total]	1	0	0	2	2	0	0	2
minimal	1	0	0	2	2	0	0	1
slight	0	0	0	0	0	0	0	1
SCIATIC NERVE N=	6	6	6	6	6	6	6	6
Nerve fiber degeneration	1	0	0	4	1	0	0	0
SURAL NERVE N=	6	0	0	6	6	0	0	6
Nerve fiber degeneration	0	0	0	1	0	0	0	0

5. Enzyme and Protein Measurements

Cholinesterase Activity: At scheduled sacrifice, blood obtained during exsanguination of 6 rats/sex/group [5 for low-dose males] was assessed for plasma and erythrocyte cholinesterase activities. Additionally, the left half of the brain of these rats was submitted for cholinesterase activity assessment [Ellman, 1961].

Neuropathy Target Esterase Activity: The right half of the brain from 3 rats/sex/group [2 for low-dose males] listed above were analyzed for neuropathy target esterase [NTE] activity [Johnson, 1977 and 1982].

Glial Fibrillary Acidic Protein Measurements: The right half of the brain from the 3 remaining rats/sex/group listed above were analyzed [O'Callaghan, 1991] for glial fibrillary acidic protein [GFAP], and the total protein content of the brain was measured also [Pierce BCA Kit; analyzed at Unilever].

RESULTS

CHOLINESTERASE ACTIVITY: There was a dose-related decrease in both brain and erythrocyte cholinesterase activities in both sexes, and the decrease in brain cholinesterase in females was statistically significant at all dose levels [Table 10]. Plasma cholinesterase activity was comparable among the groups for both sexes.

NEUROPATHY TARGET ESTERASE ACTIVITY: Both sexes displayed a dose-related reduction [25-61% lower than control] in NTE activity, which was statistically significant at all dose levels [Table 10].

GLIAL FIBRILLARY ACIDIC PROTEIN MEASUREMENTS: No adverse effect on GFAP levels was observed in either sex. The lowest levels were observed at the high-dose level in both sexes [Table 10].

Table 10. Cholinesterase Activities/NTE Activity/Glial Fibrillary Acidic Protein Levels				
Parameter/Time Frame/Sex/Dose	0 ppm	50 ppm	150 ppm	450 ppm
Brain cholinesterase (μmoles/min/g)				
MALES	12.44	11.51(93)•	10.46**(84)	7.22**(58)
FEMALES	12.0[illegible]	11.19*(93)	9.25**(77)	6.35**(53)
Erythrocyte cholinesterase (U/L)				
MALES	2692	2582	2388(89)	1967**(73)
FEMALES	2648	2397(91)	2060**(78)	1810**(68)
Plasma cholinesterase (U/L)				
MALES	595	589	600	647(109)
FEMALES	1614	1571	1496(93)	1676(104)
NTE activity				
MALES	860	691**(80)	541**(63)	352**(41)
FEMALES	865	646*(75)	505**(59)	335**(39)
GFAP (μg/mg protein)				
MALES	2.82	2.94	2.42(86)	2.04(72)
FEMALES	2.60	2.04(78)	2.17(83)	1.97(75)
GFAP (ng/g tissue)				
MALES	60686	59568	52253(86)	49783(82)
FEMALES	56354	45107(80)	51118(91)	43854(78)

• [% of control]; * $p<0.05$; ** $p<0.01$

Positive Control Data

Positive control data for the Motor Activity, Functional Observational Battery, and Neuropathology tests were not provided or referenced as evidence of the capability of the methods utilized at the testing facility to detect major neurotoxic endpoints. Information from the testing facility that performed this study demonstrating the ability of the testing procedures utilized in this study to detect major neurotoxic endpoints were submitted to the Agency previously [personal communication with B. Sette].

DISCUSSION

The administration of Molinate to rats via the diet at dose levels up to 450 ppm for at least 90 days resulted in dose-related decreases [both sexes] in body weight and body-weight gains, as well as decreased food consumption and food utilization. There was no effect on survival of either sex, and clinical signs were comparable among the groups. Females at the high-dose level displayed a significant increase in landing foot splay at 5 weeks only. Decreased time to tail flick was observed at all dose levels in males at week 5, but there was no dose response. Both sexes at the high-dose level displayed an increase in time to tail flick at week 14, although only the increase in the males attained statistical significance. There was a dose-related decrease in forelimb grip strength in both sexes at weeks 9 and 14, but statistical

significance was attained only at the high-dose level in both sexes at week 9. A statistically significant decrease in hindlimb grip strength was observed at week 5 in the high-dose females compared to the control, but a similar decrease had been displayed prior to treatment. Males at the high-dose level displayed a significant decrease in hindlimb grip strength at week 14. There was no clear effect of treatment on motor activity. Because of the variability in the motor activity data, interpretation is complicated. Based on the relative change from pretest values, it appears that females at the high-dose level displayed an increase in activity level following treatment, although prior to treatment and at the 5- and 14-week test periods, decreased motor activity was observed in this group compared to the control. There was a dose-related decrease in brain cholinesterase activity in both sexes, and the decreases were statistically significant in the low-dose females and in both sexes at the mid- and high-dose levels compared to the control values. Erythrocyte cholinesterase activity was decreased in both sexes [dose-related], with the decreases attaining statistical significance in the mid-dose females and the high-dose rats of both sexes. There was a dose-related decrease in NTE activity at all dose levels in both sexes compared to the control values, and statistical significance was attained at all dose levels. No apparent effect was observed in GFAP levels in either sex. There was a significant decrease in absolute brain weight in both sexes at the high-dose level, but brain length and width were unaffected. There were no macroscopic lesions that could be attributed to treatment. Microscopically, nerve fiber degeneration in the sciatic nerve and the sural nerve was increased in the high-dose males compared to the control incidence.

CONCLUSION

Under the conditions of the study, administration of Molinate to Alpk:APfSD rats of both sexes [12/sex/group] at dose levels of 50 [♂ 4.0/♀ 4.5 mg/kg/day], 150 [♂ 11.7/♀ 13.9 mg/kg/day], and 450 ppm [♂ 35.5/♀ 41.0 mg/kg/day] for at least 90 days resulted in a dose-related decrease in body weight [♂♂ 86%/♀♀ 84% of control at the high dose; ♀♀ 93% of control at the mid dose; ♀♀ 94% of control at the low dose], body-weight gain [♂♂ 78%/♀♀ 65% of control at the high dose; ♀♀ 84% of control at the mid dose; ♀♀ 88% of control at the low dose], food consumption, and food utilization. At the high-dose level, (1) females displayed an increase in landing foot splay at week 5; (2) both sexes displayed an increase in the time to tail flick at week 14; (3) both sexes displayed a decrease in forelimb grip strength during week 9; (4) females displayed a decrease in hindlimb grip strength during week 5 and males displayed a decrease during week 14. Additionally, a decrease in the time to tail flick was observed at week 5 in males at all dose

levels, but there was no dose response. No apparent effect was demonstrated on overall motor activity in males, but when compared to pretest values, high-dose females displayed increased motor activity following treatment. There was a dose-related decrease in both brain and erythrocyte cholinesterase activities in both sexes, and a dose-related decrease in NTE activity at all dose levels in both sexes compared to the control values. Absolute brain weight was decreased significantly in both sexes at the high-dose level. Microscopically, nerve fiber degeneration in the sciatic nerve and the sural nerve was increased in the high-dose males compared to the control incidence. Several FOB parameters were altered during the study, and motor activity in the high-dose females appears to have been increased relative to pre-treatment values. Males displayed nerve fiber degeneration in the sciatic and sural nerves at the high-dose level. There was a dose-related decrease in brain and erythrocyte cholinesterase activity, and decreased NTE activity was observed in both sexes at all dose levels. Although no NOEL was attained in this study for the decreased brain cholinesterase activity observed in females, the decrease at the low dose in both sexes is considered minimal [93% of control]. This study is classified Core Minimum, and it satisfies the guideline requirement [82-7-SS] for a subchronic neurotoxicity study in the rat.

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