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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Fonofos (Dyfonate®). Review of Supplemental Data Submitted on Acute and Subchronic Mammalian Neurotoxicity Studies

PC Code: 041701
Tox. Chem. No. 454B
Project No. D197441
Submission No. S454725

TO: Judith Loranger, CRM Team # 73
Special Review and
Reregistration Division (7508W)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 5/16/94
Section I, Toxicology Branch I
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THRU: Roger L. Gardner, Section Head Roger Gardner 5/26/94 5/27/94
Section I, Toxicology Branch I
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Background and Request:

The Toxicology Branch (TB-I) had previously reviewed and commented on the acute and subchronic mammalian neurotoxicity studies conducted with Fonofos (Dyfonate®) and has graded them Core Supplementary, upgradable pending submission of additional data and responses to specific questions (81-8, MRID 42777801; 82-7, MRID 42792601). Zeneca Ag Products has submitted the additional data and has responded to the specific questions in order to upgrade the studies for regulatory purposes. The MRID numbers for the supplemental information are 430311-01 for the responses and 430133-01 through -05 for the positive control data. TB-I has been asked to respond to the comments and to upgrade the two studies to acceptable if possible.

Toxicology Branch Response:

TB-I has reviewed the supplemental information and upgrades both studies to Core Guideline. Both studies are acceptable and thus fulfill the regulatory requirements for acute and subchronic mammalian neurotoxicity studies for Fonofos (Dyfonate®; 81-8, 82-

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7). The following paragraphs summarize the results of the studies.

Fonofos (Dyfonate®, 94.6%) was tested in an acute mammalian neurotoxicity study in Alpk:APfSD rats at the following dose levels: 0, 2, 4 or 7 mg/kg. Ten animals/sex were tested at each dose level. The doses were administered once in corn oil by gavage at 1 ml/100 g body weight. The following parameters were observed and measured: clinical signs of toxicity, body weights, food consumption, functional observational battery, motor activity, brain measurements and neuropathology.

At 7 mg/kg, one female displayed reduced foot withdrawal reflex, shaking, signs of urinary incontinence, tip toe gait and upward curvature of the spine 6 hours after dosing. Recovery in this animal was observed by 24 hours.

The NOEL is 4 mg/kg and the LOEL is 7 mg/kg based on clinical signs of toxicity. Appropriate positive control data were provided which indicated that the test system is capable of detecting neurotoxicological effects. In addition, results from a preliminary range-finding study were submitted which indicated that the dose levels selected were appropriate.

This study is graded Core Guideline and is acceptable as having satisfied the regulatory requirements for an acute mammalian neurotoxicity study (81-8) for fonofos.

Fonofos (Dyfonate®) was tested in a subchronic mammalian neurotoxicity feeding study in Alpk:APfSD rats at the following dose levels for 90 days: 0, 15, 50 or 125/150 ppm (0, 0.75, 2.5, or 6.25/7.5 mg/kg/day). The highest dose level was changed from 125 ppm to 150 ppm at week 5. Twelve rats/sex were tested at each dose level. The following parameters were observed and measured: clinical signs of toxicity, body weights, food consumption, functional observational battery, motor activity, brain measurements, cholinesterase activity and neuropathology. Six animals/sex in each group were designated for terminal neuropathology, although only the high dose group and controls were ultimately examined.

At 15 ppm, statistically significant decreases in erythrocyte cholinesterase activity (both sexes) and in plasma cholinesterase activity (females) were observed. At 50 ppm and above, statistically significant decreases in cholinesterase activity were observed in both sexes for all 3 parameters. At 125/150 ppm, treatment related clinical signs were observed in females. These included upward curvature of the spine, tiptoe gait, signs of urinary incontinence, pinched in sides, reduced splay reflex, splayed gait, eye bulging and shaking. In addition to these, decreases in the motor activity observations were noted for females. There were no microscopic indications of neurotoxicity.

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The NOEL is 50 ppm and the LEL is 125/150 ppm based on clinical signs of toxicity and on decreases in motor activity. The NOEL for cholinesterase inhibition is 15 ppm and the LEL is 50 ppm based on decreases in cholinesterase activity in all 3 parameters at 50 ppm.

This study is graded Core Guideline and is acceptable as having satisfied the regulatory requirements for a subchronic mammalian neurotoxicity study (82-7) for fonofos.

Detailed Review:

Acute Mammalian Neurotoxicity Study in Rats (Guideline 81-7)

1. Comment # 1: TB-I had asked for positive control data.

   The positive control data were sent with this submission and are acceptable. Detailed reviews of these data are in the Appendix of this memorandum.

2. Comment # 2: TB-I had stated that "there is concern over the interpretation of the clinical findings in the one female rat at 7 mg/kg...the Registrant has interpreted these findings to be systemic when the symptoms mimic neurological dysfunction."

   The Registrant replied that the adverse clinical signs were observed 6 hours after dosing. These clinical changes occurred at a "peri-lethal" dose (a dose of 7.5 mg/kg which had previously caused lethality in the preliminary dosing study). They stated that "some of these clinical changes (e.g. shaking, tiptoe gait) are not inconsistent with a neurotoxic response; however, in the absence of neuropathological change, these clinical findings are considered to reflect systemic toxicity rather than neurotoxicity."

   TB-I agrees with the Registrant, especially since there was no evidence of an effect in males or in any of the other parameters in females. However, TB-I also believes that in some cases when conducting the functional battery, it is sometimes difficult to tell the difference between pharmacologic, systemic and neurotoxic effects.

3. Comment # 3: TB-I had expressed concern over the fact that at a dose close to the LD_{50} (7.5 mg/kg), signs were only seen in one animal (female). Therefore, TB-I had requested a submission of the results from the preliminary study used to select dose levels.

   The Registrant submitted the preliminary study. In this study, males were tested at levels of 7.5 - 18.75 mg/kg and

females were tested at levels of 5.0 - 7.5 mg/kg (4 animals/sex/group). There were 2 deaths during the study; one male at 18.75 mg/kg and one female at 7.5 mg/kg. These animals had been originally dosed at 12.5 mg/kg (males) and 5.0 mg/kg (females). Two days later, these animals were redosed with 18.75 mg/kg (males) and 7.5 mg/kg (females). Both animals had severe clinical signs, including reduced activity, reduced foot withdrawal reflex, gasping, pallor, piloerection, pinna reflex absent, salivation, shaking, subdued behavior and signs of urinary incontinence (male); and reduced activity, irregular breathing, fasciculations, reduced foot withdrawal reflex, hunched posture, lachrymation, reduced righting reflex, shaking, subdued behavior, signs of urinary incontinence and reduced visual response (female). The other animals in these groups also displayed similar clinical signs. It was noted that 1 male and 1 female were not affected by treatment at the time of termination, and 1 male had only shown peri-nasal staining 4-6 hours after dosing.

In the second part of the study, which was conducted in order to confirm that females were the more sensitive sex, 3 males and 3 females were dosed at 7.5 mg/kg. Minimal clinical signs were observed for the females which included peri-nasal staining and signs of diarrhea and urinary incontinence. Minimal clinical signs were also observed for the males, which included diarrhea and reduced splay reflex for 1 male.

In the third part of the study, 4 females were tested at 6.5 mg/kg. Two animals displayed ptosis, signs of diarrhea, shaking, sides pinched in, signs of urinary incontinence, upward curvature of the spine, lachrymation, rolling gait, peri-nasal staining and decreased activity. The other two animals displayed tiptoe gait, upward curvature of the spine, signs of diarrhea and signs of urinary incontinence. Four males were dosed with 18 mg/kg. Two animals displayed pinna reflex absent, diarrhea, rolling gait, salivation, signs of urinary incontinence, reduced activity, reduced foot withdrawal reflex, shaking, upward curvature of the spine, lachrymation and peri-nasal staining. The other two animals displayed diarrhea and lachrymation.

The preliminary study indicates that females are the more sensitive sex, that other females displayed clinical signs at doses close to the dose given in the main study (6.5 mg/kg versus 7 mg/kg in the main study) and that there is some biological variation, which was also apparent in the main study.

4. Comment # 4: TB-I had requested any historical control data available for sciatic nerve fiber degeneration in the Alpk:APfSD strain of rat. The Registrant submitted available data on 5 acute and 5 subchronic neurotoxicity studies. The data are summarized in the following table.

Historical Control Incidence of Nerve Fiber Degeneration in Sciatic Nerve of Alderley Park Rats[a]

Acute Oral Studies: N = 5 Rats/Sex/Group

Month/Year	Males	Females
May 1992	2	0
June 1992	0	0
July 1992	2	1
December 1992	1	1
February 1993	1	0

Historical Control Incidence of Nerve Fiber Degeneration in Sciatic Nerve of Alderley Park Rats[a]

Subchronic Oral Studies: N = 6 Rats/Sex/Group

Month/Year	Males	Females
April 1992	0	0
May 1992	0	0
July 1992	0	1
April 1993	4	0
February 1993	0	1

[a]The data refer to the Alpk:APfSD (Wistar-derived) strain of rat. Nerve fiber degeneration is defined as foci of either Wallerian type degeneration/axonal swellings and/or areas of demyelination. The grading of nerve fiber degeneration seen was minimal for all animals. A grading criteria of minimal represents one to several small foci of Wallerian type degeneration originating from 1-2 nerve fibers.

Subchronic Mammalian Neurotoxicity Study (Guideline 82-7)

1. Comment # 1: TB-I had requested that positive control data be provided.

The positive control data were sent with this submission and are acceptable. Detailed reviews of these data are in the Appendix of this memorandum.

2. Comment # 2: TB-I had requested that the Registrant define "splay reflex". The Registrant submitted the following definition.

"The animal is lifted by gripping at the base of the tail. Normal animals extend and splay hind legs. Reduced splay reflex is scored as follows:

Slight - animal does not fully splay legs to side of body, legs may not be fully extended.

Moderate - animal tends to hold hind legs in front of body and is unable to fully extend them.

Extreme - animal tends to adduct legs on to abdomen and/or is unable to extend limbs.

N - absent (i.e. response is normal).

TB-I asknowledges and thanks the Registrant for the definition.

3. Comment # 3: TP-I had the following comment on the study:

Treatment related clinical signs were observed in high dose females...In addition to these, there were several possible effects in the functional observational battery and in the motor activity observations. These included: statistically significant increases in the mean time to tail flick at week 14 in high dose males, in mean landing foot splay at week 9 in high dose females and in motor activity at various times in high dose females; a statistically significant decrease in mean forelimb grip strength at week 14 in high dose females; non-statistically significant increases in mean landing foot splay in high dose females at week 14; and non-statistically significant decreases in forelimb grip strength in high dose males at week 14 and in mean hindlimb grip strength in both sexes at week 14 (it is especially noted in the latter that while the control and lower dose groups all showed increases in this measure between weeks 9 and 13, high dose males and females showed decreases).

The Registrant replied to each of these observations. Their comments are as follows:

a. "Tail flick

A statistically significant increase in tail flick response time was seen in males dosed with 125/150 ppm in week 14, when compared to the concurrent control group (2.9 v 4.9). Comparison of the latter value with control mean values at

the other time points shows that a value of 4.9 secs is below both of these control mean values (6.0 at week 5 and 7.0 at week 9). Since a corresponding change was not seen in female rats which are more sensitive to the effects of fonofos than male rats, the isolated finding at week 14 in males is considered to be incidental to treatment."

TB-I accepts the Registrant's explanation of the increase in tail flick response in males at week 14.

b. "Motor activity

This was generally lower at 125/150 ppm in females throughout the study with statistically significant differences seen at weeks 5 and 14 when compared with concurrent control values. There was a tendency toward lower pre-experimental values but this was not sufficient to explain subsequent differences. Thus, an effect of treatment with fonofos on motor activity was seen in females only at the highest dose level, which was broadly consistent across all time points. However, as the effect was confined to females at a dose level which produced toxicity, the decreases in motor activity cannot be ascribed with any confidence as evidence for a neurotoxic effect."

TB-I agrees with the Registrant on this effect, especially since there were no microscopic indications of neurotoxicity. At dose levels that are close to systemically toxic levels, it is difficult to tell the difference between neurotoxicity and systemic or pharmacologic effects. As noted in the previous section of this memorandum, there were effects (and even death) at 7.5 mg/kg in females in the acute study. In this study, both sexes were originally dosed with 125 ppm (6.25 mg/kg/day) for 5 weeks and then dosed with 150 ppm (7.5 mg/kg/day). The effects were observed at 9 and 14 weeks after the dose was raised to 7.5 mg/kg/day, which is slightly above the dose in which effects were observed in the acute study (7 mg/kg). What is surprising in this study is that none of the females died.

c. "Landing foot splay

A statistically significant increase in landing foot splay was seen in females at 125/150 ppm in week 9, when compared to the concurrent control group. This difference from the control was not consistent across the time points and at week 14 was much reduced in magnitude and not statistically significant. Thus the apparent difference from control at week 9 is not permanent, shows evidence of recovery by week

14, and if treatment related, is considered not to be of toxicological significance."

TB-I accepts the Registrant's discussion of the increase in landing foot splay in females at 9 weeks.

d. "Grip strength

A statistically significant reduction in forelimb grip strength was seen in females at 125/150 ppm in week 14, when compared with the concurrent control group. Differences in forelimb grip strength of similar magnitude were not observed at the earlier time points and there was no corresponding reduction of similar magnitude in hindlimb grip strength at any time point. Since hindlimb grip strength is known to be more sensitive to neuroactive agents than forelimb grip strength, this isolated finding at week 14 in females in considered to be incidental to treatment.

Small differences in both forelimb and hindlimb grip strength were observed in males dosed with 125/150 ppm at week 14. None of these differences [were] statistically significant and in the absence of similar changes at earlier time points and of any clinical or neuropathological changes at this dose level in males, these differences are considered to be incidental and unrelated to treatment."

TB-I accepts the Registrant's discussion on forelimb and hindlimb grip strength and notes that in the positive control study, hindlimb grip strength was a more sensitive indicator than forelimb grip strength. In this study, high dose males and females displayed decreases in mean hindlimb grip strength at week 14, but these were not statistically significant. It is also noted that at 9 weeks, all groups show comparable means, which indicates that the effect is probably not consistent.

4. Comment # 4: TB-I had stated that "neuropathological findings included a slight but statistically significant increase in mean brain weight in high dose females and 1 high dose female with a 'collapsed brain, consistent with hydrocephalus', both of which the authors stated were either within the historical control range or were common in this particular strain of rat." The Registrant submitted the following reply and historical control data.

"A statistically significant increase in brain weight was noted in females at a dose of 125/150 ppm (control 1.93g v treated 2.03g). There were no corresponding changes in brain length or width in this group. The report states

that this difference is considered to be within the normal range for animals of this strain and age, and therefore, not treatment related. Historical control data for females from four other related studies are shown in Table 1."

Historical Control Data of Brain Weights of Female Alderley Park Rats

Month/Year	Mean	SD	N	Min	Max	Relative Brain Weight	Mean Terminal Bodyweight
April 1992	1.96	0.06	12	1.884	2.100	0.711	276
May 1992	1.95	0.11	12	1.644	2.088	0.721	272
July 1992	1.93	0.08	12	1.843	2.108	0.707	274
Feb 1993	1.95	0.10	12	1.785	2.130	0.698	280
April 1993	1.99	0.06	12	1.893	2.110	0.736	271

"It is clear that the controls in the fonofos study had the lowest mean brain weight, whilst those from the study conducted in April 1993 had a mean brain weight much closer to the mean value of the rats in top dose of the fonofos study (2.03g). The mean brain weight at 125/150 ppm fonofos is therefore marginally above the historical control mean value on the basis of four other similar studies. Also, the difference between the control and the top dose mean brain weight values is exaggerated by one top dose animal (F92) which showed hydrocephalus macroscopically and had the highest brain weight of 2.13g for all animals which were killed by cardiac puncture.

It should be noted that although not included in the guidelines pertaining at the time these studies were conducted, Zeneca concurred with EPA's comment that measurement of brain, rbc and plasma cholinesterase activities would be helpful (science review of protocols, by Pamela Hurley). To this end, brains from rats not predesignated for neuropathological assessment were used to determine acetylcholinesterase activity. Female 92, in which "collapsed brain" consistent with hydrocephalus was recorded macroscopically, was pre-designated for enzyme measurement and not for pathological evaluation. This animal exhibited no obvious clinical signs of an adverse nature which were considered to be treatment related, and its brain CHE activity was not noticeably different from its counterparts in the same group."

TB-I accepts the Registrant's explanation of the observed increase in mean brain weight in high dose females.

5. Comment # 5: TB-I stated that "microscopic findings included minimal nerve fibre degeneration in high dose males. Considering the fact that HED does not have access to the historical control data on these animals, that these lesions did appear in high dose group males and not in the controls and that there were some indications of possible effects in the high dose animals in motor activity and in the functional observational battery, HED has considered these lesions in defining the NOEL."

The Registrant submitted the following reply: "The 'minimal fibre degeneration' refers to between one and several small foci of Wallerian type degeneration in a longitudinal section of the sciatic nerve, usually originating from a single nerve fibre. The transverse section of the sciatic nerve showed no such foci. In addition there were no degenerative changes noted in the dorsal column of the spinal cord in either of the fonofos studies. The historical control indicence of this minimal grade lesion is presented in table 2 [presented previously in this memorandum]. These data clearly show that the incidence of 2 in the high dose group males in the fonofos study was within the control range of 0-4."

TB-I accepts the Registrant's explanation of these observations and the submitted historical control data.

6. Comment # 6: Zeneca concurred with a comment from TB-I that additional microscopic examinations at the lower dose levels would not add any significant information.

Appendix: Review of Positive Control Data for Neurotoxicity Studies Conducted in Zeneca Laboratories

Reviewed By: Pamela Hurley, Toxicologist Pamela M. Hurley 5/13/94
Section I, Tox. Branch (7509C)
Secondary Reviewer: Roger L. Gardner, Section Head 5/26/94
Section I, Tox. Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity - Positive Control Study for Assessment of Sensory Perception in the Rat

ACCESSION NO./MRID NO.: 430133-02

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Morphine sulphate

STUDY NUMBER: XR2287

REPORT NUMBER: CTL/P/3689

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, Delaware

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Assessment of Sensory Perception in the Rat

AUTHOR(S): S. L. Allen

REPORT ISSUED: 6/26/92

CONCLUSION: Morphine sulphate (99%) was tested as a positive control in the tail flick test in male and female Alpk:APfSD rats. The rats received a single dose by gavage either 0, 50, 75 or 100 mg/kg of the test material in deionized water at a volume of 1 ml/100g bodyweight. The tail flick response test for pain perception was conducted one hour after dosing.

At 100 mg/kg, an increase in the tail flick response time (219 - 234% of control time) was observed in both sexes. No clear treatment-related effects were observed at the lower dose levels.

The NOEL for tail flick time response is 75 mg/kg and the LEL is 100 mg/kg.

This study is acceptable as a positive control study for the laboratory in which it was conducted, for chemicals that induce an analgesic or soporific effect. As a general comment, the animals were examined in the tail flick test at one hour after dosing and not at any time afterwards (it is assumed that this is because the analgesic effect of morphine does not last for a lo

time). For some other chemicals that have been examined with this test, a response was observed during the first few hours after dosing but then had disappeared by day 8 (the next observation time in the protocol). It was stated by the testing laboratory that the positive response for these chemicals was due to systemic toxicity (not neurotoxicity) because the animals had been tested at levels that were close to the LD_{50}. Since this particular positive control study was terminated after one hour, the test data cannot be compared with any other test data in which the animals were observed beyond one hour (i.e. up to 15 days for an acute neurotoxicity study). Therefore, when using this particular positive control study alone, it is difficult to tell the difference between pharmacological effects, systemic toxicity (i.e. malaise) and neurotoxicity for other chemicals which are being compared to this one.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Morphine sulphate

Description: White solid
Batch #(s), Other #(s): CTL Ref. No. YO5725/005
Purity: 99% w/w
Source: Sigma Chemical Company
Vehicle (if applicable): deionized water

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats
Age: Between 5 and 8 weeks
Weight(s): 130-184g (males); 107-164g (females)
Source(s): Barriered Animal Breeding Unit at ICI Pharmaceuticals, Alderley Park, Macclesfied, Cheshire, UK

3. Procedure:

a. Dosage Preparation: The test material was weighed out and added to an appropriate amount of deionized water.

Frequency of preparation: Only one time.

Storage conditions: The test material was stored at ambient temperature in the dark.

Stability Analyses: The Supplier had stated that the test material was stable for at least one year under the conditions of the storage used.

Homogeneity Analyses: Not applicable.

Concentration Analyses: Acute study - not conducted.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of studies published in the literature and also, of results from studies previously conducted in this laboratory with this particular strain of rat.

c. Animal Assignment and Dose Levels: The rats were dosed on day 1 of the study, by gavage at 1 ml/100g bodyweight.

Test Group	Dose Administered mg/kg	Main Study male	female
Contr.	0	10	10
1	50	10	10
2	75	10	10
3	100	10	10

d. Measurement of Tail Flick Response: The tail flick time of each animal was measured the day before dosing. Any animal with a response time of greater than 9.5 seconds was replaced. Tail flick time of each animal was again measured 1 hour following dosing. The report stated that "the test involved the application of a thermal stimulus to the tail and measurement of the latency to withdraw the tail. A cut-off time of 20 seconds was used."

No other measurements were conducted.

e. Statistical Analyses: Time to tail flick was analyzed by analysis of variance. Differences from the control values were statistically tested by comparing each treatment group least square mean with the control group least square mean using a two-sided Student's t-test, based on the error mean square in the analysis.

B. RESULTS:

Measurement of Tail Flick Response

The test chemical prolonged the tail flick response time in both sexes at the highest dose tested (100 mg/kg). There were no clear treatment-related effects at the lower dose levels. The following table, taken directly from the report summarizes the results.

Intergroup Comparison of Tail Flick Times (seconds)

Dose Level of Morphine Sulfate (mg/kg)			
0	50	75	100
	Males		
5.47 ± 2.42	7.31 ± 4.99	5.65 ± 2.28	12.83 ± 5.42**
	Females		
4.58 ± 2.10	6.53 ± 3.31	5.28 ± 1.21	10.06 ± 4.67**

**Statistically significant ($p < 0.01$)

Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability and achieved concentration of the test substance in the vehicle used were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that the tail flick response test is valid in this test system, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity of the study. The study shows that sensory perception in the rat can be measured using the tail flick test. The report stated that "the characteristic analgesic effect of morphine sulphate was demonstrated in this study through an increase in response times at 100 mg/kg."

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Section I, Tox. Branch (7509C)
Secondary Reviewer: Roger L. Gardner, Section Head Roger Gardner 5/26/94
Section I, Tox. Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity - Positive Control Study for Assessment of Muscular Weakness in the Rat

ACCESSION NO./MRID NO.: 430133-01

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Chlordiazepoxide

STUDY NUMBER: XR2286

REPORT NUMBER: CTL/P/3688

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, Delaware

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Assessment of Muscular Weakness in the Rat

AUTHOR(S): S. L. Allen

REPORT ISSUED: 6/26/92

CONCLUSION: Chlordiazepoxide hydrochloride (98%) was tested as a positive control in hindlimb and forelimb grip tests in male and female Alpk:APfSD rats. The rats received a single dose by gavage either 0, 5, 10 or 20 mg/kg of the test material in corn oil at a volume of 1 ml/100g bodyweight. The grip strength tests for muscle weakness was conducted one hour after dosing in 3 replicate trials.

The test chemical reduced hindlimb grip strength in both sexes at all dose levels (74 - 83% of controls). In males, there was a clear dose response. Forelimb grip strength was less affected than hindlimb grip strength. Significant reductions in forelimb grip strength were only observed in males at 20 mg/kg only (71 - 75% of controls). A comparison of the replicate trials indicated that the data were reproducible.

No NOEL was established for reduction in hindlimb grip strength and the NOEL for reduction in forelimb grip strength was 10 mg/kg. The LEL for reduction in forelimb grip strength was 20 mg/kg.

This study is acceptable as a positive control study for the laboratory in which it was conducted. As a general comment, the animals were examined in the fore- and hindlimb grip strength tests at one hour after dosing and not at any time afterwards. For some other chemicals that have been examined with this test, a response was observed during the first few hours after dosing but then had disappeared by day 8 (the next observation time in the protocol). It was stated by the testing laboratory that the positive response for these chemicals was due to systemic toxicity (not neurotoxicity) because the animals had been tested at levels that were close to the LD_{50}. Since this particular positive control study was terminated after one hour, the test data cannot be compared with any other test data in which the animals were observed beyond one hour (i.e. up to 15 days for an acute neurotoxicity study). Therefore, when using this particular positive control study alone, it is difficult to tell the difference between pharmacological effects, systemic toxicity (i.e. malaise) and neurotoxicity for other chemicals which are being compared to this one.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Chlordiazepoxide hydrochloride

Description: Solid
Batch #(s), Other #(s): CTL Ref. No. YO5672/003
Purity: > 98% w/w
Source: Sigma Chemical Company
Vehicle (if applicable): Corn oil

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats
Age: Between 5 and 8 weeks
Weight(s): 216-264g (males); 152-164g (females)
Source(s): Barriered Animal Breeding Unit at ICI Pharmaceuticals, Alderley Park, Macclesfied, Cheshire, UK

3. Procedure:

a. Dosage Preparation: The test material was weighed out and added to an appropriate amount of corn oil.

Frequency of preparation: Only one time.

Storage conditions: The test material was stored at ambient temperature in the dark.

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Stability Analyses: The Supplier had stated that the test material was stable for at least one year under the conditions of the storage used.

Homogeneity Analyses: Not applicable.

Concentration Analyses: Acute study - not conducted.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of studies published in the literature and also, of results from studies previously conducted in this laboratory with this particular strain of rat.

c. Animal Assignment and Dose Levels: The rats were dosed on day 1 of the study, by gavage at 1 ml/100g bodyweight.

Test Group	Dose Administered mg/kg	Main Study male	female
Contr.	0	10	10
1	5	10	10
2	10	10	10
3	20	10	10

d. Measurement of Forelimb and Hindlimb Grip Strength: One hour following dosing, each rat was tested for muscle relaxation by measuring fore- and hindlimb grip strength. The report stated that the following was used as a procedure: "the apparatus consisted of two strain gauges, one with a triangular ring attached and the second with a T-bar attached, with a perspex channel between. A measurement of grip strength was made by placing tha animal into the channel with its forepaws inside the triangular grasping ring of the forelimb meter. The animal was grasped by the tail and steadily pulled from away from the ring. When the grip was broken the animal was continued to be pulled along the channel so that its hindlimbs grasped the T-bar. The trial was completed when the grip of the hindlimbs was broken." Replicate trials were conducted.

No other measurements were conducted.

e. Statistical Analyses: Forelimb and hindlimb grip strength were analyzed by analysis of variance. Differences from the control values were statistically tested by comparing each treatment group least square mean with the control group least square mean using a two-sided Student's t-test, based on the error mean square in the analysis.

B. RESULTS:

Measurement of Forelimb and Hindlimb Grip Strength

The test chemical reduced hindlimb grip strength in both sexes at all dose levels. In males, there was a clear dose response. Forelimb grip strength was less affected than hindlimb grip strength. Significant reductions in forelimb grip strength were only observed in males at 20 mg/kg only. A comparison of the replicate trials indicated that the data were reproducible. The following tables, taken directly from the report summarize the results.

Intergroup Comparison of Grip Strength Data - Post Dosing Males

Grip Strength	Dose Level of Chlordiazepoxide (mg/kg) 0	5	10	20
Forelimb, trial 1	825	827	822	582**
Forelimb, trial 2	811	743	766	633**
Forelimb, trial 3	814	724	725	628**
Mean Forelimb	817	765	771	614**
Hindlimb, trial 1	652	632	577	501**
Hindlimb, trial 2	720	606*	557**	536**
Hindlimb, trial 3	699	597*	554**	531**
Mean Hindlimb	690	612*	562**	523**

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

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Intergroup Comparison of Grip Strength Data - Post Dosing Females

Grip Strength	Dose Level of Chlordiazepoxide (mg/kg) 0	5	10	20
Forelimb, trial 1	720	765	817	705
Forelimb, trial 2	731	735	757	758
Forelimb, trial 3	702	710	711	779
Mean Forelimb	717	736	761	747
Hindlimb, trial 1	641	587	524**	517**
Hindlimb, trial 2	612	5(*	476**	525
Hindlimb, trial 3	585	543	483**	490*
Mean Hindlimb	612	544*	494**	511**

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability and achieved concentration of the test substance in the vehicle used were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that the hindlimb and forelimb grip strength tests are valid tests for assessment of muscular weakness, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity of the study. The study shows that muscular weakness in the rat can be measured using the hindlimb and forelimb grip tests. The report stated that "the validity of grip strength measurement for the assessment of muscular weakness in the rat has been demonstrated using the known muscle relaxant chlordiazepoxide hydrochloride."

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Section I, Tox. Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Neurotoxicity - Positive Control Study for Assessment of Motor Activity in the Rat

ACCESSION NO./MRID NO.: 430133-03

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Amphetamine Sulphate or Chlorpromazine Hydrochloride

STUDY NUMBER: XR2285

REPORT NUMBER: CTL/P/3687

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, Delaware

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Measurement of Motor Activity in the Rat

AUTHOR(S): S. A. Horner

REPORT ISSUED: 8/7/92

CONCLUSION: Amphetamine sulphate (99%) or chlorpromazine hydrochloride (99%) were tested as positive controls in a motor activity test in male and female Alpk:APfSD rats. The rats received a single dose of either chemical by gavage at either 0, 0.1, 1.0 or 10.0 mg/kg of the test material in deionized water at a volume of 1 ml/100g bodyweight. The motor activity tests were conducted one hour after dosing.

Amphetamine sulphate induced a dose-dependent increase in motor activity in both sexes at both 10 and 1 mg/kg. At 10 mg/kg, activity for both sexes remained at 3-6 fold above controls throughout the study. At 1 mg/kg, activity scores were approximately twice the control values. Males showed in increase in activity during the first 35 minutes, whereas females showed an increase primarily during minutes 26-50. No effects were observed at 0.1 mg/kg. **The NOEL for amphetamine sulphate is 0.1 mg/kg and the LEL is 1 mg/kg based on increases in motor activity.**

At 10 mg/kg, treatment-related decreases in motor activity were observed in both males and females treated with chlorpromazine hydrochloride. Total activity was reduced to approximately 48 or 29% of control levels in males and females, respectively. No effects were observed at either 1.0 or 0.1 mg/kg. **The NOEL for chlorpromazine hydrochloride is 1.0 mg/kg and the LEL is 10.0 mg/kg based on decreases in motor activity.**

This study is acceptable as a positive control study for the laboratory in which it was conducted. As a general comment, the animals were examined in the motor activity tests at one hour after dosing and not at any time afterwards. For some other chemicals that have been examined with this test, a response was observed during the first few hours after dosing but then had disappeared by day 8 (the next observation time in the protocol). It was stated by the testing laboratory that the positive response for these chemicals was due to systemic toxicity (not neurotoxicity) because the animals had been tested at levels that were close to the LD_{50}. Since this particular positive control study was terminated after one hour, the test data cannot be compared with any other test data in which the animals were observed beyond one hour (i.e. up to 15 days for an acute neurotoxicity study). Therefore, when using this particular positive control study alone, it is difficult to tell the difference between pharmacological effects, systemic toxicity (i.e. malaise) and neurotoxicity for other chemicals which are being compared to this one.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Amphetamine sulphate or chlorpromazine hydrochloride

Description: Solids
Batch #(s), Other #(s): CTL Ref. No. YO1775/006/002 (amphetamine) and YO2531/002/009 (chlorpromazine)
Purity: 99% w/w (both)
Source: Sigma Chemical Company
Vehicle (if applicable): deionized water

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats
Age: Between 5 and 8 weeks
Weight(s): 174-275g (males); 127-215g (females)
Source(s): Barriered Animal Breeding Unit at ICI Pharmaceuticals, Alderley Park, Macclesfied, Cheshire, UK

3. Procedure:

a. Dosage Preparation: The test materials were weighed out and added to an appropriate amount of deionized water.

Frequency of preparation: Only one time each.

Storage conditions: The test materials were stored at ambient temperature in the dark.

Stability Analyses: The Supplier had stated that the test materials were stable for at least one year under the conditions of the storage used.

Homogeneity Analyses: Not applicable.

Concentration Analyses: Acute study - not conducted.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of studies published in the literature and also, of results from studies previously conducted in this laboratory with this particular strain of rat.

c. Animal Assignment and Dose Levels: The rats were dosed on day 1 of the study, by gavage at 1 ml/100g bodyweight.

Test Group	Dose Administered mg/kg	Main Study male	female
	Amphetamine Sulphate		
Contr.	0	10	10
1	0.1	10	10
2	1.0	10	10
3	10.0	10	10
	Chlorpromazine Hydrochloride		
Contr.	0	10	10
1	0.1	10	10
2	1.0	10	10
3	10.0	10	10

d. Measurement of Motor Activity: One hour following dosing, each rat was allocated to an activity monitor and tested for motor activity. Each animal was assessed for ten 5 minute periods up to 50 minutes.

Clinical observations were recorded immediately prior to dosing for each animal and no abnormalities were recorded. No other measurements were conducted.

e. Statistical Analyses: Motor activity measurements for each 5 minute period and overall minutes (1-50) were considered at each measurement time by analysis of variance. Differences from the control values were statistically tested by comparing each treatment group least square mean with the control group least square mean using a two-sided Student's t-test, based on the error mean square in the analysis.

B. RESULTS:

Measurement Motor Activity

For amphetamine sulphate, the report stated that during day -1, motor activity in all groups, including controls was highest during the first 5 minutes of the measurement period and attenuated thereafter. The decline in activity reached asymptomatic levels after 30 (males) to 40 (females) minutes. Amphetamine sulphate induced a dose-dependent increase in motor activity in both sexes. Effects were observed at both 10 and 1 mg/kg. In the high dose, activity for both sexes remained at a high level throughout the study (3-6 fold above controls). At 1 mg/kg, activity scores were approximately twice the control values. Males showed in increase in activity during the first 35 minutes, whereas females showed an increase primarily during minutes 26-50.

At 10 mg/kg, treatment-related decreases in motor activity were observed in both males and females treated with chlorpromazine hydrochloride. Total activity was reduced to approximately 48 or 29% of control levels in males and females, respectively.

The following tables, taken directly from the report summarize the results.

Intergroup Comparison of Motor Activity - Amphetamine Sulphate

Minutes	Dose Levels (mg/kg)			
	0	0.1	1.0	10.0
		Males		
Pre Dosing				
1-5	73.6	70.5	74.5	73.9
6-10	70.0	58.4	72.3	65.0
11-15	40.5	37.2	62.4*	52.6
36-40	2.1	1.1	1.8	0.1
1-50	214.6	199.6	252.5	243.0
Post Dosing				
1-5	54.5	62.3	69.2	59.8
6-10	40.2	27.4	55.1	67.5**
11-15	19.1	6.2	42.3**	65.1**
26-30	5.8	5.6	15.9	66.5**
46-50	3.4	1.0	2.1	68.9**
1-50	142.3	114.8	262.9**	652.7**
		Females		
Pre Dosing				
1-5	73.8	71.6	66.5	69.7
6-10	70.8	63.0	64.6	62.6
21-25	42.0	47.5	34.3	32.3
36-40	11.1	14.4	19.6	18.4
1-50	388.8	396.9	344.8	368.9
Post Dosing				
1-5	66.8	67.8	71.9	64.4
6-10	57.8	57.4	61.1	57.4
11-15	42.2	46.7	58.4	57.9
26-30	17.1	28.8	41.9	62.3**
41-45	11.0	24.4	44.1**	68.2**
1-50	278.1	393.6	490.0*	640.0**

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

Intergroup Comparison of Motor Activity - Chlorpromazine Hydrochloride

Minutes	Dose Levels (mg/kg)			
	0	0.1	1.0	10.0
		Males		
Pre Dosing				
1-5	78.7	71.4	67.7*	67.7*
6-10	70.7	57.5	55.4	58.5
11-15	60.0	41.0*	32.9**	36.4*
21-25	20.4	13.5	0.4*	3.5
1-50	283.2	215.6*	172.0**	201.6*
Post Dosing				
1-5	67.6	56.8	57.7	6.9**
6-10	49.0	34.2	33.9	5.2**
11-15	27.2	9.5*	19.0	6.5**
21-25	1.5	5.0	3.6	8.5
1-50	161.5	144.3	131.4	77.1*
		Females		
Pre Dosing				
1-5	73.3	71.8	73.8	70.4
6-10	69.4	67.9	70.2	69.4
21-25	20.3	34.1	42.4*	43.7*
1-50	342.0	365.1	371.5	374.3
Post Dosing				
1-5	67.0	73.1	63.6	9.5**
6-10	60.0	54.1	53.9	5.5**
11-15	37.1	48.5	46.2	7.3**
31-35	18.7	13.5	8.3	10.7
46-50	17.4	8.8	8.2	12.4
1-50	299.1	319.2	280.6	87.2**

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability and achieved concentration of the test substance in the vehicle used were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that the motor activity test is a valid test for assessment of either stimulation or inhibition of the central nervous system, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity

of the study. The study shows that either stimulation or inhibition of the central nervous system in the rat by chemicals known to induce those reactions can be measured by using the motor activity test.

011013

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DATA EVALUATION RECORD

STUDY TYPE: Positive Control Study: Subchronic Neurotoxicity in the Rat

ACCESSION NO./MRID NO.: 430133-04

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Trimethyltin Chloride

STUDY NUMBER(S): PRO874

REPORT NUMBER: CTL/P/3658

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmington, Delaware

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Trimethyltin Chloride: Neurotoxicity Study in Rats

AUTHOR(S): S. L. Allen

REPORT ISSUED: 7/30/92

CONCLUSION: Trimethyltin chloride (99%) was tested in a neurotoxicity feeding study in Alpk:APfSD rats for 29 days as a positive control. The following dose levels were administered in the diet: 0, 4 or 8 ppm (0, 0.2 or 0.4 mg/kg/day). Clinical signs of toxicity, body weights, food consumption, functional observational battery, motor activity and microscopic observations were measured and recorded.

At 0.4 mg/kg/day, severe toxicity was observed. As a result, all animals at this dose level were humanely killed prior to the end of the study. Clinical signs of toxicity included piloerection, urinary incontinence, hunched posture, aggression (males), shaking and clonic convulsions in both sexes.
Increases in motor activity were seen in females on day 15 (120-138% over controls). There was pronounced damage to the limbic system. The spinal cord showed minimal/slight vacuolation/degeneration of ventral horn motor neurons. In the peripheral nervous system there was minimal evidence of

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peripheral neuropathy, characterized by Wallerian-type degeneration of peripheral nerve. There also was minimal evidence of degeneration in the sensory roots. The degeneration was confined to the junction of the root with the spinal cord.

The NOEL is 0.2 mg/kg/day and the LEL is 0.4 mg/kg/day based on clinical signs of toxicity and microscopic evidence of neurotoxicity.

This study is acceptible as a positive control study for this particular laboratory.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Trimethyltin chloride

Description: white solid
Batch #(s), Other #(s): CTL YO5954/001/002
Purity: 99%
Source: Aldrich Chemical Company
Vehicle: Ethanol

2. Test Animals

Species and Strain (sexes): Male and female Alpk:APfSD rats
Age: 28 days old upon receipt.
Source(s): ICI Pharmaceuticals at Alderley Park, Macclesfield, Cheshire UK

3. Procedure:

a. Dietary Preparation: The diets were prepared in 10 - 15 kg batches from premixes prepared by adding stock solutions of ethanol containing the appropriate amount of the test substance to 250g of milled diet. The premixes were then rotary evaporated, dried and added to 9.75 or 14.75 kg diet and mixed thoroughly.

Frequency of preparation: Not stated.

Storage conditions: Not stated.

Stability Analyses: Not conducted.

Homogeneity Analyses: Not conducted.

Concentration Analyses: Samples from all dietary levels were taken from each batch and retained for future analysis.

b. Basis For Selection of Dose Levels: The dose levels were selected on the basis of results from published literature results and on a range-finding study conducted in the same laboratory.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 29 days male	female
Control	0	12	12
1	4	12	12
2	8	12	12

Six animals/sex in each group were designated for terminal neuropathology.

d. Clinical Signs of Toxicity and Mortality: All rats were examined prior to the start of the study and cageside checks were conducted daily during the study for clinical signs of toxicity, behaviour changes and mortality. At weekly intervals, each rat was removed from its cage and physically examined for changes in general health status.

e. Body Weight Determinations: Bodyweights were recorded weekly, starting immediately before feeding the experimental diet and then on the same day of each week until termination.

f. Food and/or Water Consumption: Food consumption was recorded continuously and calculated weekly.

g. Functional Observational Battery: The report stated that "detailed clinical observations ...and quantitative assessments of landing foot splay, sensory perception (tail flick test) and muscle weakness (fore and hindlimb grip strength) were made weekly. The clinical observations included, but were not limited to, the following list of measures: assessment of autonomic function (e.g. lachrymation, salivation, piloerection, exophthalmus, urination, defecation, pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behaviour etc; reactivity to stimuli; changes in level of arousal;

sensorimotor responses; [and] alterations in respiration. The observations were made by one observer who was 'blind' with respect to the animal's treatment, and recorded on a computer system by personnel not directly involved in the clinical observations. The observations were carried out in a room separate from that in which the animals were housed and animals were presented to the observer with no indication of the treatment group. The observations were coded and the degree of condition noted (slight, moderate or extreme) where appropriate. This included the recording of no abnormalities detected."

h. Motor Activity: An automated activity recording apparatus was used to measure locomotor activity. The animals were tested on day -1, 15 and 29 of the exposure period. The report stated that "each observation period was divided into fifty scans of one minute duration. Treatment groups were counter balanced across test times and across devices, and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimize disturbances."

i. Neuropathology:
At termination, six animals/sex/group were given full post mortem examinations. The brains were weighed and the length and width were recorded with calipers. The tissues listed below were left in situ and stored in 10% neutral buffered formol saline. These tissues were not microscopically examined.

Six other animals/sex/group were deeply anesthetized with barbituate i.p. and killed by perfusion fixation with modified Karnovsky's fixative. The tissues listed below were removed and brain weight, length and width were recorded. The tissues were microscopically examined. The neuropathological examination was performed on the control and the 8 ppm groups only. All sections were examined by light microscopy. The brain and gastrocnemius muscle were embedded in paraffin wax, cut and stained with H & E stain. The report stated that "the remaining tissues were post-fixed with osmium tetroxide, embedded in resin and semi-thin sections were cut and stained with toluidine blue. The brain was examined in the transverse plane at levels 2, 3, 5, 6 and 7 with sections

submitted including the olfactory bulb, olfactory tuberculum, pyriform cortex, hippocampal formation and amygdaloid nuclei. Spinal cord from the cervical region (C3-C6) and from the lumbar region (L1-L4) was examined in the transverse and longitudinal plane. Spinal roots and dorsal root ganglia were examined from the C3-C6 and L1-L4 levels and the gasserian ganglia from the trigeminal nerve. Transverse and longitudinal sections of the sciatic, sural and tibial nerves were also examined. Samples of the gastrocnemius muscle were examined in the transverse plane."

The following tissues were removed and examined microscopically:

|x| Brain (including forebrain, cerebrum, mid-brain, cerebellum, pons and medulla oblongata)
|x| Spinal cord form cervical region and lumbar region
|x| Gasserian ganglia
|x| Vertebral column including spinal cord
|x| Dorsal root ganglea including spinal roots
|x| Gastrocnemius muscle
|x| Sciatic nerve
|x| Sural nerve
|x| Tibial nerve

j. Statistical Analyses: Body weight gain was analyzed using a two-sided Student's t-test, separately for each sex. Brain weight, brain length and brain width were analyzed by analysis of covariance. Analysis of variance and covariance allowed for the replicate structure of the study design. Motor activity measurements, weekly food consumption, tail flick response, landing foot splay and fore and hindlimb grip strength were all analyzed by analysis of variance. Least squares means for each group were calculated. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

B. RESULTS:

1. Clinical Observations and Mortality: Severe toxicity was observed at the high dose. As a result, all the males were killed on days 22-24 and the females were killed on days 23-25 of the study. As scheduled, however, six/sex were killed by perfusion fixation and

six/sex were exsanguinated under terminal anesthesia. Clinical signs of toxicity were observed at the high dose. These included piloerection, urinary incontinence and hunched posture and did not occur until day 22. Aggression was also observed in males as well as shaking and clonic convulsions in both sexes. The following tables summarize selected clinical observations.

Selected Clinical Signs of Toxicity

Observation	Dose Level (ppm) 0	4	8
Males			
Aggression	0[a]	0	1
Clonic Convulsions	0	0	1
Reduced Foot Withdrawal Reflex	0	1	0
Hunched	0	0	1
Salivation	0	0	1
Response to Sound	0	0	2
Shaking	0	0	24
Reduced Splay Reflex	0	2	0
Subdued	0	0	1
Signs of Urinary Incontinence	0	0	15
Piloerection	0	0	10

[a]Number of animals

Selected Clinical Signs of Toxicity

Observation	Dose Level (ppm) 0	4	8
Females			
Clonic Convulsions	0	0	1
Hunched	0	0	1
Shaking	0	0	23
Reduced Splay Reflex	1	5	2
Signs of Urinary Incontinence	0	0	14

Selected Clinical Signs of Toxicity

	Dose Level (ppm)		
Observation	0	4	8
Piloerection	0	0	5

[a]Number of animals

2. Body Weight Determinations: No treatment-related decreases in body weights or body weight gains were observed. Bodyweight and bodyweight gain were significantly increased in the treated groups. Tables will not be provided in this DER because increases in bodyweight and bodyweight gain are not effects of interest in a positive control study for neurotoxicity.

3. Food and/or Water Consumption: Food consumption was increased in both sexes at the high dose in week 3.

4. Functional Observational Battery:

   Landing Foot Splay No consistent treatment-related effects were observed in landing foot splay measurements for either sex. In high dose females, at week 4, mean landing foot splay was less than controls; however, this was not observed in males or at any other time period.

   Time to Tail Flick No consistent dose-related differences in time to tail flick response were observed in the treated groups when compared to controls for either sex.

   Grip Strength Measurements No treatment-related effects in grip strength measurements were observed for either of the treated groups when compared to controls.

5. Motor Activity: On day 15, the motor activity of high dose females was increased during minutes 11-25 (periods 3-5). On day 29 increases in motor activity was observed in the 4 ppm males during minutes 1-20. The authors stated that this is mainly due to increased activity for a few individual animals. In looking at the individual animal data, it also appears that several of the values for the control group were particularly low for these time periods. The following table summarizes selected results.

Intergroup Comparison of Motor Activity

Minutes	Dietary Concentration (ppm) 0	4	8
	Males		
Day 15			
Minutes 1-5	63.6	64.2	70.1
Minutes 11-15	45.2	43.4	44.3
Minutes 16-20	29.8	21.3	37.6
Minutes 46-50	7.6	3.2	2.2
Minutes 1-50	261.3	205.8	261.5
Day 29			
Minutes 1-5	61.2	73.3	-[a]
Minutes 6-10	58.3	76.9*	-
Minutes 11-15	46.3	66.0*	-
Minutes 16-20	33.7	56.2*	-
Minutes 46-50	32.0	11.7	-
Minutes 1-50	375.0	414.5	-
	Females		
Day 15			
Minutes 1-5	75.3	72.0	75.5
Minutes 11-15	56.0	63.1	67.5*
Minutes 16-20	54.2	60.3	74.8*
Minutes 46-50	49.0	41.0	35.6
Minutes 1-50	504.1	563.8	586.8
Day 29			
Minutes 1-5	74.2	72.3	-[a]
Minutes 6-10	60.3	71.8	-
Minutes 11-15	54.7	62.8	-
Minutes 16-20	56.6	58.0	-
Minutes 46-50	46.9	38.3	-
Minutes 1-50	540.3	502.2	-

*Statistically significant ($p < 0.05$)
[a]Sacrificed prior to this time point.
**Statistically significant ($p < 0.01$)

6. Brain Measurements Both males and females in the high dose group had lower brain weights in the high dose group when compared to the control group. In addition, brain width was slightly less than the control group for the high dose males. However, the authors stated that these animals were sacrificed one week earlier than the other animals, and thus, the differences may reflect the lesser maturity of the rats rather than due to treatment with the chemical. The following tables, taken directly from the report, summarize the results.

Intergroup Comparison of Brain Parameters - Males

Observation	Dietary Concentration		
	0	4	8
Brain Weight (g)	1.99	1.97	1.85**
Brain Length (mm)	26.9	27.8	26.8
Brain Width (mm)	15.3	15.6	14.8*

*Statistically significant ($p < 0.05$).
**Statistically significant ($p < 0.01$).

Intergroup Comparison of Brain Parameters - Females

Observation	Dietary Concentration		
	0	4	8
Brain Weight (g)	1.79	1.78	1.73*
Brain Length (mm)	25.9	26.0	26.3
Brain Width (mm)	14.8	14.7	14.5

*Statistically significant ($p < 0.05$).
**Statistically significant ($p < 0.01$).

7. Neuropathology: In the high dose rats, there was "pronounced damage to the limbic system, characterized by neuronal cell necrosis of the hippocampal formation (CA1, CA3, CA4 and dentate gyrus), pyriform cortex, amygdaloid nuclei and olfactory tuberculum. The degree of necrosis was greatest in the hippocampal formation and pyriform cortex and least in the amygdaloid nuclei and olfactory tuberculum.

The spinal cord of the 8 ppm rats showed minimal/slight vacuolation/degeneration of ventral horn motor neurons (three males, one female).

In the peripheral nervous system there was minimal evidence of peripheral neuropathy, characterized by Wallerian-type degeneration of peripheral nerve, particularly sciatic in both control and 8 ppm trimethyltin chloride treated animals. Surprisingly, there was no Wallerian-type degeneration of the dorsal columns but there was minimal evidence in the 8 ppm trimethyltin chloride treated rats of degeneration in the sensory roots of a few rats. The degeneration was confined to the junction of the root with the spinal cord. No axonal swellings were seen." The following

table, taken directly from the report summarizes the findings.

Intergroup Comparison of Microscopic Findings

Observation	Dose Level (ppm) Males			Females		
	0	4	8	0	4	8
Animals on study	12	12	12	12	12	12
Animals completed	6	0	6	6	0	6
Brain (# Examined)	6	0	6	6	0	6
Neuronal cell necrosis:						
Amygdaloid nuclei	0	-	6	0	-	6
Pyriform cortex	0	-	6	0	-	6
Dentate gyrus	0	-	6	0	-	6
CA1 hippocampus	0	-	6	0	-	6
CA3/CA4 hyppocampus	0	-	6	0	-	6
Tuberculum olfactorium	0	-	6	0	-	6
Dorsal root ganglia lumbar (# Examined)	6	0	6	6	0	6
Occasional eccentric nucleus	0	-	1	0	-	0
Gasserian ganglia (# Examined)	6	0	6	6	0	6
Sensory root degeneration	0	-	1	0	-	0
Sciatic nerve (# Examined)	6	0	6	6	0	6
Nerve fiber degeneration	0	-	2	1	-	4
Sensory spinal root-cervical (# Examined)	4	0	6	5	0	5
Nerve fiber degeneration	0	-	1	0	-	1
Sensory spinal root-lumbar (# Examined)	5	0	6	5	0	6
Nerve fiber degeneration	0	-	0	0	-	1
Spinal cord (# Examined)	6	0	6	6	0	6
Ventral horn cell vacuolation/degeneration	0	-	3	0	-	1
Sural nerve (# Examined)	6	0	5	6	0	6
Nerve fiber degeneration	0	-	0	0	-	1
Axonal degeneration	1	-	0	0	-	0
Tibial nerve (# Examined)	6	0	6	5	0	6
Nerve fiber degeneration	1	0	0	1	-	2

8. Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability, homogeneity and achieved concentration of the test substance in the diet were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that clinical signs of neurotoxicity and neuropathological lesions may be observed in this test system with a known neurotoxicant and since the purpose of the study was

achieved, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity of the study. The study shows that trimethyltin chloride induces neurotoxic effects in rats when administered in the diet for a period of 29 days.

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DATA EVALUATION RECORD

STUDY TYPE: Positive Control Study: Acrylamide Neurotoxicity Study in the Rat

ACCESSION NO./MRID NO.: 430133-05

DP BARCODE/SUBMISSION NO.: D197441

TEST MATERIAL: Acrylamide

STUDY NUMBER(S): PRO705

REPORT NUMBER: CTL/P/2226

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmington, Delaware

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: Acrylamide: Neurotoxicity Study in Rats

AUTHOR(S): M. D. Stonard

REPORT ISSUED: 7/30/90

CONCLUSION: Acrylamide monomer (100%) was tested in Alpk:AP rats as a positive control in a neurotoxicity feeding study for 29 days. It was administered in the diet at 0, 250 or 500 ppm (0, 12.5 or 25 mg/kg/day). Clinical signs of toxicity, body weights, food consumption, motor activity, sensory function, muscle weakness, peripheral nerve function and neuropathology observations were measured and recorded.

At 250 ppm, the animals displayed severe clinical signs of toxicity which included tail erection, tiptoe gait, upward curvature of the spine, piloerection, pinched in sides, abnormal gait, reduced reflex responses, downward curvature of the spine and upward curvature of the spine. Other clinical signs were observed as well. In addition to these, decreases in body weight and body weight gain, food consumption and food efficiency, motor activity, possible motor and sensory nerve conduction velocities and motor and sensory nerve action potential amplitudes were observed. Increases in pull-up time were also observed. Microscopic examinations were not conducted at this dose level.

At 500 ppm, both sexes also showed severe signs of toxicity, although more severely than those mentioned above. Therefore, at this dose level, the test diet was removed after 3 weeks. In addition to the other effects mentioned above, there was "unequivocal histopathological evidence of a peripheral neuropathy characterized by axonal degeneration of Wallerian-type...Mild axonal degeneration of the dorsal columns was also seen at this dose level."

There were effects at both dose levels tested. Therefore, the LEL is 250 ppm based on clinical signs of neurotoxicity, decreased body weights and body weight gains and food consumption, decreases in motor activity and changes in the functional observational battery. In addition, at 500 ppm, there was microscopic evidence of neurotoxicity.

This study is acceptible as a positive control study for this particular laboratory.

A. MATERIALS AND METHODS:

1. Test Compound(s)

Chemical Name: Acrylamide monomer

Description: white solid
Batch #(s), Other #(s): Batch 95822; CTL Y00574/009/001
Purity: 100%
Source: Aldrich Chemical Company
Vehicle: N/A

2. Test Animals

Species and Strain (sexes): Male and female Alpk:AP rats

Age: 6 weeks old upon receipt.
Source(s): ICI Pharmaceuticals at Alderley Park, Macclesfield, Cheshire UK

3. Procedure:

a. Dietary Preparation: The diets were prepared in 10 kg batches from 1000g premixes. It was not stated how the premixes were prepared. It is assumed that the appropriate amount of the test substance was weighed out and added to a measured amount of stock diet.

Frequency of preparation: Weekly.

Storage conditions: Not stated.

Stability Analyses: Not conducted.

Homogeneity Analyses: Not conducted.

Concentration Analyses: Not conducted.

b. Basis For Selection of Dose Levels: It was not stated on what basis the dose levels were selected. However, this particular chemical has extensive literature references.

c. Animal Assignment and Dose Levels:

Test Group	Dose Administered ppm	Main Study 29 days male	female
Control	0	12	12
1	250	12	12
2	500	12	12

d. Clinical Signs of Toxicity and Mortality: All rats were examined prior to the start of the study (day -1) and immediately prior to feeding the experimental diets. Animals were observed at least once daily. Detailed clinical observations were conducted weekly by an observer who was blind with respect to the treatment of each group of animals.

e. Body Weight Determinations: Bodyweights were recorded weekly, starting on day -1, immediately before feeding the experimental diet and then on the same day of each week until termination.

f. Food and/or Water Consumption: Food consumption was recorded weekly.

g. Motor Activity: An automated activity recording apparatus was used to measure locomotor activity. The animals were tested on days -1 and 28 of the exposure period. The report stated that "each observation period was divided into fifty scans of one minute duration. Treatment groups were counter balanced across test times and across devices", and when the trials were repeated on day 28, each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed behind a screen minimize disturbances.

h. Sensory Function Test: Assessment of pain perception was made using the rat tail-flick test on days -1, 8, 15, 22 and 28.

i. Muscle Weakness: Muscle weakness was assessed for all animals on days -1, 8, 15, 22 and 29 using the pull-up test.

j. Peripheral Nerve Function: The report stated that "at the end of the exposure period, all animals had peripheral nerve conduction velocity and amplitude measured using electrophysiological techniques. Each animal was deeply anesthetised by an intraperitoneal injection of barbituate. Motor and sensory nerve conduction velocity and action potential amplitude of the caudal nerves of the tail were measured using needly electrodes connected to a Neurolog electrophysiological amplification and recording system using a method similar to Misumi, 1979."

k. Neuropathology:
All animals requiring euthanasia during the study were anesthetised with halothane and killed by exsanguination using cardiac puncture. These and all animals which were found dead, were given full post mortem examinations. The tissues listed below were processed for microscopic examination.

For those animals surviving to termination, the brains were weighed and the length and width were recorded. Following the assessment of peripheral nerve function and whilst they remained deeply anasthetized, they were killed by perfusion fixation. The tissues listed below were removed and processed for microscopic examination. Nine levels of brain were blocked in paraffin wax and transverse secitons from each block were stained with alum hematoxylin and eosin. In addition, represetative sections were stained with Palmgren's method for nerve fibers, Haltzer's method for glial fibers and Luxol Fast Blue for myelin. Microscopic examinations were limited to 6 male and 6 female rats in the control and 500 ppm dose groups.

The following tissues were removed and examined microscopically:

|x| Brain
|x| Spinal cord form cervical region and lumbar region
|x| Gasserian ganglia
|x| Spinal roots
|x| Dorsal root ganglea
|x| Gastrocnemius muscle
|x| Sciatic nerve
|x| Sural nerve
|x| Tibial nerve

j. Statistical Analyses: Statistical analyses included analysis of variance. Unbiased estimates of the treatment group means were provided by the least square means. Each treatment group mean was compared with the control group mean using a two-sided Student's t-test.

B. RESULTS:

1. Clinical Observations and Mortality: At 250 ppm, the animals displayed severe clinical signs of toxicity after 2 weeks of treatment. These included tail erection, tiptoe gait, upward curvature of the spine, piloerection and pinched in sides. These signs increased in number and severity throughout the course of the study. After 4 weeks, both sexes displayed abnormal gait and reduced reflex responses. Some animals displayed downward curvature of the spine and all animals displayed upward curvature of the spine. Other clinical signs of toxicity included dehydration, decreased activity, piloerection, pinched in sides, reduced stability and ungroomed appearance.

At 500 ppm, both sexes showed severe signs of toxicity. Most had moderate splayed and tiptoe gait, upward curvature of the spine, reduced stability, dehydration, piloerection, pinched in sides and tail erection. Several animals also had reduced righting and splay reflex responses. Therefore, at this dose level, the test diet was removed on day 18 (replicates 1 and 4 - the animals were started on the diets on different days), days 17 and 16 for replicates 2 and 5 and 3 and 6 respectively and replaced with control diet. The clinical condition of the animals continued to decline and 2 males and 1 female either had to be sacrificed in extremis, were found dead or died during observation. By the end of the study, the animals had begun to

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recover. The following tables summarize the most pertinent observations during weeks 4 and 5, where the clinical signs were most prevalent.

Clinical Observations at Weeks 4 and 5 - Males

Observation	Dose Level (ppm)		
	0	250	500[a]
Activity decreased			
Week 4			3
Week 5		5	
Downward curvature of spine			
Week 4			4
Week 5		4	
Reduced righting reflex			
Week 4			6
Week 5		4	1
Splayed gait			
Week 4		7	12
Week 5		12	9
Sides pinched in			
Week 4		2	10
Week 5		3	1
Reduced splay reflex			
Week 4	1		
Week 5	1	1	2
Reduced stability			
Week 4		1	4
Week 5		4	
Tip toe gait			
Week 4		5	12
Week 5		11	10
Upward curvature of spine			
Week 4		10	12
Week 5		12	10

[a]Put on control diet, starting on days 16-18.

Clinical Observations at Weeks 4 and 5 - Females

Observation	Dose Level (ppm)		
	0	250	500[a]
Downward curvature of spine			
Week 4		1	2
Week 5		3	3
Reduced righting reflex			
Week 4			5
Week 5		8	2
Splayed gait			
Week 4		6	13
Week 5		12	9
Sides pinched in			
Week 4		5	7
Week 5		10	6
Reduced splay reflex			
Week 4	4	1	2
Week 5	4	1	3
Reduced stability			
Week 4			9
Week 5		7	2
Tip toe gait			
Week 4		11	12
Week 5		11	10
Upward curvature of spine			
Week 4		12	13
Week 5		12	11

[a]Put on control diet, starting on days 16-18.

2. Body Weight Determinations: Statistically significant decreases in bodyweight and bodyweight gain were observed in both sexes at both dose levels during the study. The following tables summarize bodyweight gain.

Bodyweight Gain (g) - Males

Week	Dose Level (ppm)		
	0	250	500
1	0.0	0.0	0.0
2	49.3	31.6**	7.8**
3	81.2	57.4**	0.1**
4	106.5	62.8**	14.3**
5	129.5	65.7**	47.1**

*Statistically significantly different from controls ($p < 0.05$)
**Statistically significantly different from controls ($p < 0.01$)

Bodyweight Gain (g) - Females

Week	Dose Level (ppm)		
	0	250	500
1	0.0	0.0	0.0
2	22.9	11.0**	-5.0**
3	35.1	16.0**	-17.2**
4	50.7	13.1**	1.0**
5	56.8	13.0**	7.7**

*Statistically significantly different from controls ($p < 0.05$)
**Statistically significantly different from controls ($p < 0.01$)

3. Food and/or Water Consumption: Food consumption was significantly decreased in the high dose group in both sexes for weeks 1-3. At week 4, food consumption was still decreased for high dose females. For the 250 ppm dose groups, food consumption was significantly decreased in weeks 1 and 4 for males and all weeks for females. Food utilization was also significantly decreased for both treated groups in both sexes, although not at every time point. These tables will not be summarized here since this is a positive control study for neurotoxicity.

4. Pull-Up Test for Detection of Muscle Weakness: At 250 ppm, a statistically significant increase in pull-up time was observed by the end of the third week of treatment in both sexes. By the end of the fourth week, the pull-up time had increased in female rats but in male rats, although the pull-up time was increased, it was not statistically significantly increased when compared to the control group. At 500 ppm, a statistically significant increase in pull-up time was observed in both sexes by the end of 2 weeks. Although the test diet was subsequently withdrawn, a week later these animals took progressively more time to complete the test. Both sexes showed signs of recovery by week 5, but the times were still higher than the control groups. The following table taken directly from the report summarizes the results.

Intergroup Compararison of the Logarithm of Mean Pull-Up Time(s)

Day	Dietary Concentration (ppm)		
	0	250	500
Day -1			
Males	0.692	0.484	0.483
Females	0.371	0.435	0.593
Day 8			
Males	0.209	0.124	0.214
Females	0.108	0.067	0.074
Day 15			
Males	0.069	0.250	0.475**
Females	0.095	0.376	0.511**
Day 22			
Males	-0.120	0.255*	1.02**
Females	0.111	0.510*	1.01**
Day 29			
Males	-0.047	0.260	0.697**
Females	-0.059	0.711**	0.406*

*Statistically significant from control group mean ($p < 0.05$)
**Statistically significant from control group mean ($p < 0.01$)

5. Tail-Flick Test for Assessment of Sensory Perception: There was no evidence for an effect on tail-flick. In males, there was no difference between the treated and control group and in females, the response times were significantly reduced at several time points in the 250 and 500 ppm dose groups when compared to controls.

6. Motor Activity: At 250 ppm, there was a statistically significant decrease in motor activity in females at 2-5 minutes. At 500 ppm, there was a decrease in motor activity in males during the first minute. In females, the activity was reduced at the 16-20 and 46-50 minute intervals. The following table summarizes the results.

Intergroup Comparison of Mean Activity Monitoring Measurements

Period	Dose Level (ppm)					
	0		250		500	
	Day -1	Day 28	Day -1	Day 28	Day -1	Day 28
Period 1						
Males	598.1	479.3	946.7**	312.6	621.2	204.6*
Females	546.6	503.9	576.6	431.1	313.6	507.9
Period 2-5						
Males	104.4	88.1	90.9	93.2	95.8	81.7
Females	82.5	113.3	74.1	80.2*	86.6	84.9
Period 6-10						
Males	77.4	84.7	53.6	81.9	76.4	79.1
Females	63.9	94.9	64.8	79.2	66.3	70.7
Period 16-20						
Males	33.2	35.7	19.5	33.8	24.1	48.7
Females	20.8	63.6	32.9	61.3	29.7	24.9*
Period 46-50						
Males	13.9	20.8	13.8	8.7	3.8	4.4
Females	2.9	35.9	6.7	18.9	4.8	10.8*

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

7. Nerve Conduction Velocity Measurements: The report stated that "on day 30, motor and sensory nerve conduction velocities were statistically significantly reduced in males and females at both dose levels, when compared to control. There was, however, no evidence of a dose dependent effect. Similarly, both motor and sensory nerve action potential amplitudes were reduced in both sexes, although in only the sensory fibers was the reduction statistically significant." The following table summarizes the results.

Intergroup Comparison of Mean Nerve Conduction Measurements (Day 30)

Observation	Dietary Concentration (ppm)		
	0	250	500
	Males		
Motor Nerve Conduction Velocity (m/sec)	43.27	38.81*	37.20**
Motor Nerve Action Potential Amplitude (uv)	35.75	[illegible].25	28.42
Sensory Nerve Conduction Velocity (m/sec)	42.04	31.00**	32.96**
Sensory Nerve Action Potential Amplitude (uv)	25.33	16.33**	15.26**

Intergroup Comparison of Mean Nerve Conduction Measurements (Day 30)

Observation	Dietary Concentration (ppm) 0	250	500
	Females		
Motor Nerve Conduction Velocity (m/sec)	42.43	35.63**	38.11*
Motor Nerve Action Potential Amplitude (uv)	38.67	35.58	31.48
Sensory Nerve Conduction Velocity (m/sec)	39.62	31.04**	31.99**
Sensory Nerve Action Potential Amplitude (uv)	22.00	15.42**	15.98**

*Statistically significant ($p < 0.05$)
**Statistically significant ($p < 0.01$)

8. Brain Measurements: No treatment-related differences between the treated groups and the controls were observed.

9. Neuropathology: At 500 ppm, there was "primary axonal degeneration accompanied by myelin degeneration in a good proportion of fibers from both sural and tibial nerves. As expected in a distal axonopathy degenerative changes in the sciatic nerve were less pronounced than in the more distally distributed sural (sensory) and tibial (sensory-motor) nerves.

Peripheral neuropathy was characterized by axonal degeneration of Wallerian type. In some sections degenerating axons could be seen surrounded by an intact myelin sheath. However, in many sections axonal degeneration was accompanied by demyelination with macrophages evident in myelin ovoids. There was some degree of endoneural edema and fiber loss, especially in sural and tibial nerves...

There was a fairly mild degree of axonal degeneration of the dorsal columns from both cervical and lumbar regions of the spinal cord of treated rats.

Only minor changes were seen in the dorsal root ganglia of treated rats. The gasserian ganglia appeared histologically normal although it was possible to find an occasional chromatolytic neuronal cell body. As expected no degenerative changes were seen in the brain." The following table summarizes some of the pertinent results.

Intergroup Comparison of Microscopic Findings

Observation	Dose - ♂ (ppm)		Dose - ♀ (ppm)	
	0	500	0	500
Dorsal Root Ganglia - Cervical				
# examined	5	6	6	6
Occasional chromatolytic cell body	0	1	0	3
Satellite cell proliferation	0	1	0	0
Dorsal Root Ganglia - Lumbar				
# examined	5	6	-	-
Occasional chromatolytic cell body	0	3		
Satellite cell proliferation	0	1		
Gasserian Ganglia				
# examined	6	6	-	-
Occasional chromatolytic cell body	0	1		
Sciatic Nerve				
# examined	6	6	6	6
Peripheral neuropathy	0	4	0	3
Occasional degenerate fiber	0	2	1	3
Spinal Cord - Cervical				
# examined	5	5	6	5
Axonal degeneration of dorsal columns	0	5	0	5
Occasional degenerate fiber	1	0		
Spinal Cord - Lumbar				
# examined	5	6	6	4
Axonal degeneration of dersal columns	0	3	0	2
Occasional degenerate fiber in dorsal columns	0	3	0	1
Spinal Root - Lumbar				
# examined	5	6	6	6
Thinning of normal myelin	0	1		
Occasional degenerate fiber	0	1		
Peripheral neuropathy			0	1
Sural Nerve				
# examined	6	6	6	6
Occasional degenerate fiber	1	0		
Peripheral neuropathy	0	6	0	6
Tibial Nerve				
# examined	6	6	6	6
Peripheral neuropathy	0	6	0	6
Occasional degenerate fiber	1	0	1	0

10. Quality Assurance Measures: The study was conducted in accordance with Good Laboratory Practice Standards except that there was no documentation that the test substance was characterized in a GLP-accredited laboratory and that the stability, homogeneity and achieved concentration of the test substance in the diet were not determined by analysis.

C. DISCUSSION: Since the purpose of this study was to show that clinical signs of neurotoxicity and neuropathological lesions may be observed in this test system with a known neurotoxicant and since the purpose of the study was achieved, the deviations from the Good Laboratory Practice Standards are not considered to have affected the integrity of the study. The study shows that acrylamide induces neurotoxic effects in rats when administered in the diet for a period of 29 days.