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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 YO0574/009/001
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.

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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.

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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.

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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	31.8**	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 3 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.

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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	631.2	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	30.25	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**

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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.

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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.