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DATA EVALUATION RECORD

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

ACCESSION NO./MRID NO.: 430133-04

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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

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, behavior 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;

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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

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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

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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

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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.

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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

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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

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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.