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DATA EVALUATION RECORD

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

ACCESSION NO./MRID NO.: 430133-03

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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

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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.

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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 ($p < 0.01$)

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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

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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.