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RPA 201772 Acute Neurotoxicity (81-8)

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DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity/Rats
[OPPTS 870-6200, OPP §81-8]

DP BARCODE: D224202
P. C. CODE: 123000

SUBMISSION NO.: S501233
MRID NO.: 43904804

TEST MATERIAL (PURITY): Isoxaflutole (99.2%)

SYNONYM: RPA 201772

CITATION: Mandella, R.C. (1995). An acute neurotoxicity study of RPA 201772 in the rat via oral gavage administration. Pharmaco LSR Inc., Toxicology Services North America, East Millstone, NJ. Pharmaco LSR Report No.: 94-4511, August, 15, 1995. MRID No. 43904804. Unpublished.

SPONSOR: Rhone-Poulenc, Research Triangle Park, NC

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID # 43904804), CD rats (10/sex/group) received a single oral gavage administration of RPA 201772 in 0.5% aqueous methylcellulose at doses of 0 (vehicle only), 125, 500 and 2000 mg/kg body weight. The animals were observed for mortality and clinical signs of toxicity for 14 days post-dosing.

No compound-related effects on body weight, body weight gain and food consumption were noted. There were significant decreases in landing foot splay measurements in high-dose males during FOB tests indicating impairment of neuromuscular function. No significant differences in the motor activity as well as gross and microscopic neuropathological findings were seen among the control and treated animals.

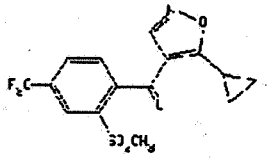
LOEL for systemic toxicity = 2000 mg/kg in males and >2000 mg/kg/day in females; NOEL for systemic toxicity = 500 mg/kg/day in males and 2000 mg/kg/day (Limit dose) in females

This study is classified as **acceptable** and **satisfies** guideline requirements (§81-8) for an acute neurotoxicity study in rats.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 201772
   Chemical Name: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl) isoxazole; isoxaflutole
   Purity: 99.2%
   Batch number: 40 ADM 93
   Description: Beige to tan powder
   Storage Conditions: At room temperature
   CAS NO.: 141112-29-0
   Structure:



2. Vehicle: 0.5% Aqueous carboxymethyl cellulose; Lot No. 123HO589

3. Test animals: Species: Rat
   Strain: Crl:CD®BR VAF/Plus (outbred Albino)
   Source: Charles River Breeding Laboratories, Inc., Stone Ridge, New York
   Age at start of dosing: 49-52 days
   Weight at start of dosing: Males: 239.3 (196.0 - 275.6 g;
   Females: 180.0 (145.8 - 211.3 g
   Housing: Individually in stainless steel cages
   Diet: Certified Rodent Diet® #5002 Meal (PMI® Feeds Inc., St. Louis, MO) ad libitum
   Water: Tap water ad libitum
   Environmental conditions: Temperature: 20-24°C (68-75°F)
   Humidity: 45-72%
   Air changes: not stated.
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period: 20 days

B. STUDY DESIGN AND METHODS:

1. Dose-Selection - The rationale for the selection of dose levels was not provided in the study report.

2. Main Study -

a. Animal assignment and treatment: Animals were assigned to four groups as shown in Table 1 using computer generated randomization procedure; the individual body weights were within ±20% of the mean body weight for each sex. Animals were fasted for 18 hours prior to dosing; the test animals received corresponding doses (10 ml/kg) once by gavage while the control animals received vehicle only. Each animal received a single dose of the test solution on a mg/kg body weight basis.

Table 1. Animal Assignment to Study Groups.

Dose Group	Dose (mg/kg)	Number Assigned Male	Female
Control	0	10	10
Low	125	10	10
Mid	500	10	10
High	2000	10	10

b. Dosing Preparations: The dosing solutions were prepared as a series of graded concentrations in 0.5% aqueous methylcellulose. Fresh formulations of the test material were prepared prior to dosing. The suitability of the mixing procedure was determined by trial procedure.

3. Analytical Chemistry - Prior to initiation of the study, three samples, each from the top, middle, and bottom portions of the low- and high-dose suspensions, were analyzed for homogeneity. Duplicate samples from the above suspensions were assayed for stability after 1, 4, and 7 days following preparation and refrigeration; degradation analyses of RPA 201772 to RPA 202248 were also performed on these samples. Concentration analyses of samples from the three dosing solutions were determined by chromatography.

4. Observations -

a. Clinical signs/Mortality: Animals were observed immediately following the dosing and twice daily, thereafter.

b. Body weights/Food Consumption: Body weight of each animal was recorded twice prior to group assignment, weekly during the study and at terminal sacrifice. Food consumption was recorded prior to dosing and weekly throughout the study.

5. Neurobehavioral Evaluations - Neurobehavioral tests were conducted on 10 animals/sex/group and consisted of Functional Observational Battery (FOB) and evaluation of motor activity. These tests were performed on Day 1 (within 1–2 and 2-4 hrs, respectively, after dosing) and on Days 8 and 15 post-dosing. The evaluation times were based on blood pharmacokinetic data showing a peak concentration of test material at approximately 1 hour after dosing and elimination half-life of approximately 60 minutes. Time to peak assessment is noted as Day 1 throughout the study report.

a. Motor activity: Motor activity was monitored using an automated Photobeam Activity System. This experiment measured the number of beam breaks in an activity box in 12, 5 minute intervals over a 60 minute session. No details of experimental methodology were provided. Treatment groups were counterbalanced across test times.

b. Functional Observational Battery: FOB evaluations were conducted by the technician without prior knowledge of animal's dose group. The following parameters were evaluated for the presence or absence of finding, and were ranked based on severity and degree of effect:

Home Cage Observations:
Posture
Vocalizations
Palpebral closure

Reflex Response Assessments:
Approach response
Pupil response
Finger snap response
Tail pinch response
Air Righting reflex

Abnormal Movements:
Convulsions/tremors

Handling Evaluations:
Ease of removal
Ease of Handling
Chromodacryorrhea
Lacrimation/Salivation
Coat

Open field Observations:
Gait/Locomotion
Arousal/Piloerection
Exophthalmos
Fecal boluses/Urine

Measured Response.:
Landing foot splay
Fore/hindlimb grip strength

6. Positive Control Data - The positive control substance tested consisted of acrylamide (MRID # 440674-01; 436804-14; 1994/LSR031/1172) and Carbaryl (MRID # 436804-15; 95/10332). These studies demonstrated the ability of the performing laboratory to evaluate neurological effects.

7. Sacrifice and Pathology - At the end of the 14 day observation period, 5 animals/sex from the control and high-dose groups were selected for neuropathological evaluations. The animals were anesthetized, sacrificed by perfusion fixation and subjected to neuropathological examinations of the central and peripheral nervous system. Brain and pituitary weights from each animal were determined. Tissues were processed in the following manner:

   The following portions of the central and peripheral nervous systems were processed through paraffin, sectioned and then stained with hematoxylin and eosin, Luxol Fast Blue and Sevier-Munger stains:

   Brain, including all major regions
   Spinal cord, transverse and longitudinal sections at cervical, thoracic, and lumbar levels

   The following portions of the peripheral nervous systems were embedded in resin, sectioned (both longitudinal and transverse sections at distal and proximal regions), and then stained with toluidine blue:

   Sciatic nerve
   Tibial nerve
   Sural nerve

   The remaining animals were anesthetized with sodium pentobarbitone by intraperitoneal injection and sacrificed by perfusion fixation. At necropsy, only abnormal tissues were preserved in 10% neutral buffered formalin.

8. Statistics - Statistical analyses were conducted on body weight, body weight change and food consumption values as well as motor activity counts and FOB variables. The initial assessment was conducted for homogeneity of variance using Bartlett's test followed by one-way ANOVA using the F distribution to assess the significance. Dunnett's test was used to determine which mean values differed from the controls. For heterogeneity of variance the nonparametric procedure such as Kruskal-Wallis test was used followed by summed rank test

(Dunn) to determine the differences between the treated and control groups. Additionally, statistical tests for trend in the dose levels were performed using parametric procedure of standard regression test with trend and lack of fit and nonparametric procedure of Jonckheere's test for monotonic trend. The motor activity scores were repeated using Blom transformed rank data which were used to achieve a normal distribution of the residuals. The residuals were then tested by the Shapiro-Wilk W or the Kolomogrov D test for normality.

When only one treated group was compared to control, the variances between the two groups were tested for equality using F-test. For equal variances, two sample t-test was used; for variances that differed by 1% level of significance, Welch's test was used.

C. Compliance - The following compliance documents were submitted: 1) signed statement by the sponsor indicating that the study was conducted in accordance with EPA GLP Regulations; 2) signed Quality Assurance statement by the testing facility; 3) signed statement by the sponsor claiming no data confidentiality. The flagging criteria were not applied.

II. RESULTS

1. Analytical Chemistry - Analyses showed that the test compound was homogeneously distributed throughout the dosing solution (96.5%-107% and 97.7%-108%, for the 12.5 amd 200 mg/ml trial solutions, respectively; coefficients of variation of 1.13% and 3.53% for the 5 and 60 mg/l trial test solutions, respectively). The stability analyses indicated that the compound was stable in the vehicle over a 7 day period (85.2%-107% of nominal). The degradation analyses revealed that there was little increase in degradation product over 7 days indicating that the parent compound was stable in dosing suspension. The concentration analyses revealed that the achieved concentrations for the 25, 250 and 750 mg/kg dose groups were 106, 95.1, and 92.3% of target concentrations, respectively.

2. Clinical Signs and Mortality - Following dosing no treatment-related clinical signs were observed in animals receiving doses up to 2000 mg/kg. Incidental signs were noted in males from the control and high-dose groups consisted of scabs and cervical ulceration, respectively. No mortalities were detected.

3. Body Weight Gain and Food Consumption - The mean body weight gain for mid- and high-dose males and high-dose females was slightly below control (2% and 4% for males, respectively; 7% for high-dose females)(Table 2A and 2B,

respectively). No changes were observed in males and females at low-dose levels. The body weights for dosed animals were comparable with controls with the exception of mid- and high-dose males and high-dose females in which it was slightly but consistently lower than the controls (1.4-4% for males and 2-4% for females; data not shown) throughout the observation period.

On the day of dosing, the food consumption of mid- and high-dose males was slightly lower (3-4%) compared to controls (Data not presented in this DER). By Day 15, the food consumption of all dose groups was comparable to controls. For high-dose females, the food consumption over the 14-day observation period was comparable with that of controls.

Table 2A: Mean Body Weight and Body Weight Gain (g/rat/day) Data for Male Rats[a]

Day	Dose Level (mg/kg)			
	0	125	500	2000
Body Weight 0	244	239	237	237
8	298	295	288	284
15	352	354	344	347
Body Weight Change- Day 0-15	111	115	107	109
(% change)b	--	+4.0	-4.0	-2.0

[a]Data extracted from Study No.94/4511, Table 3 and 4, pages 34 and 36
[b]Calculated by the reviewer

Table 2B: Mean Body Weight and Body Weight Gain (g/rat/day) Data for Female Rats[a]

Day	Dose Level (mg/kg)			
	0	125	500	2000
Body Weight 0	182	179	182	178
8	198	197	204	194
15	224	221	226	216
Body Weight Change - Day 0-15	42	43	44	39
(% change)b	--	+2.0	+5.0	-7

[a] Data extracted from Study No.94/4511, Table 4, p. 35 and 37
[b] Calculated by the reviewer

Table 3A: Mean Food Consumption Data (g/rat/day) for Male Rats[a]

Day	Dose Level (mg/kg)			
	0	125	500	2000
8	110	106	107	106
% change	--	-4.0	-3.0	-4.0
15	88	87	88	92
% change	--	-1.00	--	+5.0

[a]Data extracted from Study No.94/4511, Table 5, p. 38

Table 3B: Mean Food Consumption Data (g/rat/day) for Female Rats[a]

Day	Dose Level (mg/kg)			
	0	125	500	2000
8	111	112	115	115
% change	--	+0.9	+4.0	+4.0
15	94	92	96	95
% change	--	-1.00	+2.0	+1.0

[a]Data extracted from Study No.94/4511, Table 5, p. 39

4. Motor Activity and Functional Observation Battery (FOB) -

a. Motor activity: There were no significant differences in group motor activity values among the control and treated animals on Days 1, 8 and 15. The decrease in group mean motor activity values for high-dose males on Day 8 compared to controls during the one-hour measurement period was considered to be incidental.

b. Functional Observation Battery: The FOB findings in male and female rats at low-, mid- and high-dose levels were evaluated for the presence or absence of observation, and degree of effect and were ranked based on severity. There was treatment-related effect noted in high dose males during the FOB evaluations. It was evidenced by significant decrease in

landing foot splay measurements on Day 1 and 15 (Table 4). The decreases in landing foot splay measurements was indicative of impairment of neuromuscular function. Significant decrease in mean forelimb grip in mid-dose males on Day 8 and in landing foot splay measurements for mid-dose males on Day 15 were considered incidental. Addition incidental findings noted during the FOB tests consisted of absence of response to a tail pinch in few males and females from various dose groups on Days 1, 8 and 15, absence of pupillary response in 2 mid-dose males on Day 15, and piloerection in 1 or 2 males in the control and each dose group on Day 15.

5. Necropsy - There were no compound-related pathological findings in the central and peripheral nervous system in treated animals examined histopathologically. The brain and pituitary weights between control and treated animals were comparable.

III. DISCUSSION/CONCLUSION

A. In this neurotoxicity study, CD rats (10/sex/group) were orally gavaged once with RPA 201772 at doses of 0 (vehicle only), 125, 500, or 2000 mg/kg.

All animals survived until terminal sacrifice. There were no clinical signs of toxicity observed in treated animals. The body weight gains of high-dose animals were only slightly lower compared to controls, however, food consumption was comparable in all dose groups.

Neurobehavioral test indicated decreases in landing foot splay measurements in high-dose males which were indicative of impairment of neuromuscular function. Neuropathological examinations of the central and peripheral nervous system revealed no compound-related effects.

LOEL for systemic toxicity = 2000 mg/kg in males and >2000 mg/kg/day in females;
NOEL for systemic toxicity = 500 mg/kg/day in males and 2000 mg/kg in females

B. CLASSIFICATION - The study is classified as **acceptable** and **satisfies** guideline requirements (§81-8) for an acute neurotoxicity study in rats.

C. STUDY DEFICIENCIES - No study deficiencies were noted.

Table 4. Effect of RPA 201772 on Functional Observational Battery in Male Rats[a]

Dose Levels (mg/kg)	# of Trials	Mean Grip Strength and Landing Foot Splay Values During various Intervals[a](in Days)											
		Mean Forelimb Grip Strength (g)				Mean Hindlimb Grip Strength (g)				Mean Landing Foot Splay (Cm)			
		Pre-test	1	8	15	Pre-test	1	8	15	Pre-test	1	8	15
Control	Trial 1	319	409	886	564	191	173	499	534	6.0	7.4	6.2	7.8
	Trial 2	702	409	858	501	211	170	588	460	6.5	6.9	6.6	7.8
2000	Trial 1	407	481	778	542	247	121	538	557	5.5	5.1*	5.2	4.7**
	Trial 2	418	422	718	442	201	150	542	433	5.8	5.3	5.5	5.3*

[a]Grip strengths measured in grams of force; Landing foot splay measured in centimeters are extracted from Study No.94/4511, Table 7, p. 53-56.