RPA 201772 Subchronic Neurotoxicity (82-7)

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity Study in Rats
[OPPTS 870-6200, OPP 82-7]

DP BARCODE: D224202 SUBMISSION No.: S501233
P. C. CODE: 123000 MRID NUMBER: 43904805

TEST MATERIAL (PURITY): Isoxaflutole (99.2%)

SYNONYM: RPA 201772

CITATION: Mandella, R.C. (1955). A subchronic (3-month) neurotoxicity study of RPA 201772 in the rat via dietary administration. Pharmaco LSR Inc., Toxicology Services Worldwide, East Millstone, NJ. Pharmaco LSR Report No.: 94-4512, August 29, 1995. Report amended on October 13, 1995. MRID No. 43904805. Unpublished.

SPONSOR: Pharmaco LSR, Inc., East Millstone, NJ.

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID # 43904805), RPA 201772 (99.2%) was administered to CD rats (10/sex/group) at dietary levels of 0, 25, 250 and 750 mg/kg/day for 90 days.

Treatment-related effects observed in high-dose males consisted of decreases in body body weight (6-13% of control) and body weight gain (18-30% of control). No neurobehavioral effects were observed in neuropathology, motor activity and functional observational battery assessments.

LOEL was established at 750 mg/kg/day in males based on decreases in body weight and body weight gain; LOEL in females was >750 mg/kg/day.

NOEL was 250 mg/kg/day in males and 750 mg/kg/day in females.

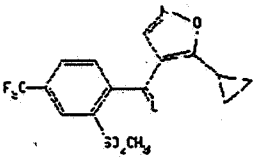
This study is classified as **Acceptable** and **satisfies** the §82-7 guideline requirement for a subchronic neurotoxicity study in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging Statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Isoxaflutole
   Chemical Name:4-(2-Methylsulfonyl-4-trifluro-methylbenzoyl)-5-cyclopropyl isoxazole; RPA 201772
   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: Basal diet

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: 43 days
   Weight at start of dosing: Males: 132.1 (118.7 - 145.1 g)
   Females: 111.3 (97.8 - 128.9 g)
   Housing: Individually in stainless steel cages during study period
   Diet: Certified Rodent Diet® #5002 Meal (PMI® Feeds Inc., St. Louis, MO) ad libitum
   Water: Tap water ad libitum
   Environmental conditions: Temperature: 20-26°C (68-79°F)
   Humidity: 20-70%
   Air changes: not stated.
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period: 14 days

B. STUDY DESIGN:

1. Study Dates: Start: November 1, 1994
   End: January 31, 1994

2. Animal Assignment:

Following a period of acclimation, the rats were assigned to study groups via a body weight-dependant randomization scheme (Table 1); the individual body weights were within ±20% of the mean body weight for each sex.

Table 1. Animal Assignment to Study Groups.

Dose Group	Dose (mg/kg)	Number Assigned Male	Female
Control	0	10	10
Low	25	10	10
Mid	250	10	10
High	750	10	10

2. Justification for dose level selection

The rationale for the selection of dose levels was not provided in the study report.

3. Diet preparation and analysis

Appropriate amounts of the test substance were mixed with basal diet to achieve the desired dietary concentrations which were then adjusted weekly based on body weight and food consumption data of the previous week to provide the desired doses. Test diets were prepared on a weekly basis. Animals received appropriate diets *ad libitum* throughout the study. Homogeneity of the test diets was confirmed before the initiation of the study; the assays were performed on blends of low- and high-dose test diets (three samples each from the top, middle and bottom portions). The stability analyses were conducted on duplicate samples each from the lowest and highest concentration at 5, 7 and 14 days intervals after preparation. The concentration analyses were performed on samples of each diet prepared weekly for the first 4 weeks and monthly, thereafter.

C. METHODS:

1. Observations:

Animals were observed at least twice daily during the study period for mortality and signs of toxicity. A detailed physical examination was performed twice prior to initiation of study and weekly during the study period.

2. Body Weights/Food Consumption:

Body weights of animals were recorded twice prior to dosing, at weekly intervals during the study period and then at study termination. Food consumption was recorded weekly, beginning one week prior to initiation of

treatment. The achieved dosages for each sex were calculated.

3. Neurobehavioral Tests: Neurobehavioral tests consisted of the Motor Activity and Functional Observational Battery (FOB) tests. These tests were performed before commencement of treatment, and during weeks 5, 9, and 13 of treatment. Animals were presented in a blind manner to trained observers.

   a. Motor activity: The locomotor activity was assessed using automated Photobeam Activity System by measuring the number of beam breaks. Animals were monitored individually over a 60 minute session, consisting of 12, five minute intervals.

   b. Functional Observational Battery: The following parameters were evaluated:

Home Cage Observations:	Handling Evaluations:
Posture	Ease of removal
Vocalizations	Ease of Handling
Palpebral closure	Chromodacryorrhea
	Lacrimation/Salivation
Reflex Response Assessments:	Coat
Approach response	
Pupil response	Open field Observations:
Finger snap response	Gait/Locomotion
Tail pinch response	Arousal/Piloerection
Air Righting reflex	Exophthalmia
	Fecal boluses/Urine
Abnormal Movements:	
Convulsions/tremors	Measured Response.:
	Landing foot splay
	Fore/hindlimb grip strength

4. Sacrifice and Pathology: At terminal sacrifice, all animals were subjected to complete gross pathological examination; abnormal tissues were preserved in 10% neutral buffered formalin. Five animals/sex from higgh-dose groups selected for neuropathological evaluations were anesthetized and sacrificed by perfusion fixation. Animals from the control and high-dose groups were subjected to neuropathological examinations of the central and peripheral nervous system. All abnormal tissues were preserved in 4% paraformaldehyde and glutaraldehyde in phosphate buffered saline. 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 system were embedded in plastic, sectioned, and then stained with toluidine blue:

Sciatic, tibial and sural nerves (left side, both transverse and longitudinal sections at distal and proximal ends)

D. DATA ANALYSIS

1. Statistical Analyses: Body weight, body weight change and food consumption values, as well as motor activity counts, grip strength and landing foot splay values were analyzed statistically as follows:

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.

2. 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.

II. RESULTS

A. Analytical Chemistry: Analyses of the samples of the low-and high-dose formulations demonstrated that RPA 201772 was homogeneously distributed in the diet when prepared in the manner described (% recovery: 98.7-101% and 100-102% of nominal, respectively). The stability analyses performed, on the low- and high-dose diets, on Day 5, 7, and 14, revealed values within

acceptable range (93.9%, 103%; 92.6%, 107%; and 94.6%, 98.7% for the low- and high -dose diets, respectively) and demonstrated stability for up to 14 days when stored at ambient temperatures. The concentration analyses of all test samples indicated values within ±5% (range: 91.2-105%; 96.7-104% and 97.9-105% for the low-, mid- and high-dose levels, respectively) of the target concentrations.

B. Mortality and Clinical Signs: No treatment-related deaths occurred. At 250 mg/kg/day, one male was sacrificed moribund on Day 46 of the study. This animal had clinical signs of toxicity including hypothermia, prostration, labored breathing and poor health; necropsy revealed calculi in the kidney and ureter. This isolated finding was considered to be incidental. There were no treatment-related clinical signs of toxicity observed in treated animals.

C. Body Weight: Body weight and body weight gain data are summarized in Table 2. The mean body weights for high-dose males were consistently lower (6-13%) compared to controls throughout the study period (Week 1-13) resulting in overall decrease in body weight gain of 19% over 13 weeks. For high-dose females, the mean body weight and body weight gains were comparable over 13 weeks with the exception of slight decrease in Week 2 and Weeks 11-13 resulting in overall decrease in body weight gain of 9% over 13 weeks. Increases in body weight and body weight gain at low- and mid-dose females were not considered to be toxicologically significant.

D. Food Consumption and Test Substance Intake: The food consumption data are summarized in Table 3. At mid- and high-dose levels, increases in food consumption were noted in males during Weeks 4-13; for high-dose females decrease in food consumption was noted only during Week 2. These findings were not considered to be toxicologically significant. The overall (Weeks 1-13) test substance consumption data are summarized in Table 4.

E. Neurobehavioral Evaluations

1. Functional Observation Battery (FOB): FOB findings observed during the 13-weeks treatment period are summarized in Table 5. Decreases in hind limb grip strength in Week 13 were noted in treated males from all dose groups and forelimb grip strength at 750 mg/kg/day. These decreases were slight and were not seen in both trials, indicating that the observed effects were equivocal in nature. Additional findings consisted of moderate difficulty in handling, piloerection, and absence of a tail pinch response seen prior to treatment and/or with similar frequency in the control group. The decrease in landing foot splay in females at 25 mg/kg/day in Week 9 was considered incidental. These FOB findings were observed sporadically during treatment or lacked dose-response relationship and were not suggestive of treatment-related effects.

Table 2. Mean Body Weight and Body Weight Change Data (g)[a]

Interval	0 mg/kg	25 mg/kg	250 mg/kg	750 mg/kg
Males				
Body Weight (g)				
Week 0	194± 9	196± 9	191± 10	192± 9
Week 1	256± 11	257± 14	246± 13	242± 9*
Week 5	412± 25	407± 24	390± 27	367±24**
Week 13	559± 45	542± 38	523± 44	487±36**
Body Weight Gain (g)				
Weeks 0 to 13	364± 39	346± 33	331± 38	295±30**
% change from control	--	-5	-9	-19
Females				
Body Weight (g)				
Week 0	146± 9	153±10	155± 7	154± 7
Week 1	166± 12	175±12	177±10	169± 9
Week 5	219± 17	242±28*	237±19	222±12
Week 13	261± 17	300±36**	281±24	258±19
Body Weight Gain (g)				
Weeks 0 to 13	114±16	147±27**	126± 0	105±15
% change from control	--	+29	+19	-9

a Data were extracted from Study No. 94/4512, Table 2 and 3; pages 37-40, 42 and 44.

* Significantly different from control value, $p \leq 0.05$

** Significantly different from control value, $p \leq 0.01$

Table 3. Mean (±S.D.) Food Consumption (g/kg/day) [a]

Interval	0 mg/kg	25 mg/kg	250 mg/kg	750 mg/kg
Males				
Food Consumption(g):				
Week 0	141± 6	139± 6	141± 7	143± 6
Week 1	116± 4	118± 5	116± 5	111± 5
Week 5	67± 2	69± 5	74± 5*	76± 4**
Week 13	53± 2	51± 2	60± 8	60± 2**
Week 1 to 13	1082	1078	1126	1130
% Change from Control[b]:				
Week 1 to 13	--	-0.4	+4	+4
Females				
Food Consumption(g):				
Week 0	136± 10	141± 9	144± 11	145± 8
Week 1	110± 5	111± 9	111± 6	105± 7
Week 5	79± 4	77± 3	79± 5	77± 4
Week 13	71± 4	73± 4	67± 5	73± 4
Week 1 to 13	1123	1157	1138	1121
% Change from Control:				
Week 1 to 13	--	+3	+1.3	-0.2

[a] Data were extracted from Study No. 94/4512, Table 4; pages 45-48.

[b] Calculated by the reviewer

Table 4. Group Mean Test Substance Intake (mg/kg/day) from Week 1 to 13[a]

Sex	25 mg/kg	250 mg/kg	750 mg/kg
Males	25.0	253.0	756.9
Females	25.1	249.3	746.1

[a] Data were extracted from Study No. 94/4512, Table 5, p.49-52; calculated by the reviewer.

Table 5. 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	5	9	13	Pre-test	5	9	13	Pre-test	5	9	13
Control	Trial 1	396	619	880	993	255	315	727	639	4.6	7.5	6.7	6.3
	Trial 2	369	619	787	901	230	270	753	734	4.7	7.2	6.4	6.4
25	Trial 1	359	712	821	913	196	325	766	498	5.2	6.9	5.9	5.9
	Trial 2	449	625	581	731	232	254	674	504**	5.5	7.1	6.4	5.7
250	Trial 1	420	689	704	779	269	283	776	586	5.3	7.4	6.3	5.4
	Trial 2	426	643	719	740	244	272	701	562*	5.5	7.2	6.7	5.7
750	Trial 1	329	639	818	670**	267	280	797	476*	5.1	6.4	6.3	6.4
	Trial 2	414	614	669	765	235	258	695	583	5.1	6.8	6.6	6.4

[a]Grip strengths measured in grams of force; Landing foot splay measured in centimeters
Data are extracted from Study No.94/4511, Table 7, p. 66-69.

2. Motor Activity Data:

Motor activity was unaffected by the treatment.

E. Postmortem Data

1. Macroscopic Pathology: No compound-related macroscopic findings were noted in any dose group. Incidental finding consisted of calculi in the kidney and urinary bladder in one male which were possibly associated with the moribund condition of the animal.

2. Microscopic Pathology: No treatment-related lesions of the nervous system were noted in high-dose animals.

III. DISCUSSION

A. Investigator's/Reviewer's Conclusions

Dietary administration of RPA 201772 to male and female CD rats at levels of 0, 25, 250 or 750 mg/kg/day resulted in statistically significant decrease in mean body weight (6-13% of control) and body weight gain (18-30% of control) in males at 750 mg/kg/day; no adverse effects were noted in females.

There were no effects on survival, clinical examination and food consumption. There were no significant differences in the motor activity, functional observational battery as well as gross and microscopic neuropathological findings among the control and treated animals.

The LOEL is 750 mg/kg/day in males and >750 mg/kg/day in females, based on decrease in body weight, and body weight gain in males only. The NOEL was 250 mg/kg/day in males and 750 mg/kg/day in females).

This study is classified as **Acceptable** and **satisfies** the §82-7 guideline requirement for subchronic neurotoxicity study in rats.

B. Study Deficiencies: None noted.