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RPA 201772 Tyrosine Tolerance Study

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DATA EVALUATION RECORD

STUDY TYPE: Comparative Tyrosine Tolerance Study-[Rat]

DP BARCODE: D224202
P.C. CODE: 123000
MRID NO.: 43904817

SUBMISSION CODE: S501233
TOX. CHEM. NO.: [New Chemical]

TEST MATERIAL (PURITY): RPA 201772 (98.7%)

CHEMICAL NAME: 5-Cyclopropyl-4-(2-methylsulphonyl-4-trifluoromethylethyl benzoyl) isoxazole

SYNONYM: Isoxaflutole

CITATION: Esdaile, D.J. (1995) RPA 201772, 2-(2-Nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione. Comparative One-Week Tyrosine Tolerance Study in the Rat. Rhöne-Poulenc Agrochimie, Sophia Antipolis. Report No. SA 94277; December 20, 1995. MRID NUMBER: 43904817. (Unpublished)

SPONSOR: Rhöne-Poulenc Agrochimie, Lyon, France

EXECUTIVE SUMMARY: In a comparative tyrosine tolerance study (MRID# 43904817), RPA 201772 (98.7% a.i.) or RPA 200261 (99.8% a.i.; 2-(2-Nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione or NTBC), a therapeutic agent were administered in the diet to male Sprague-Dawley rats (5/dose) at dosage levels of 0 and 10 mg/kg/day for one week. The animals then received 500 mg/kg/day tyrosine on the day of treatment, and on Days 2, 3 and 8 after the test substance administration. Urine collected at 4, 8 and 24 hours was analyzed for tyrosine metabolites.

Administration of tyrosine to rats pretreated with RPA 201772 or NTBC, increased the urinary excretion of tyrosine metabolites, N-acetyl tyrosine (NAT), 4-hydroxyphenyl acetate (4-HPAA) and 4-hydroxyphenyl lactate (4-HPLA). The effect of RPA 201772 was reversible within 48 hours after administration while that of NTBC was not.

The results of this functional assay suggests that both RPA 201772 and NTBC affect the main catabolic pathway for tyrosine by inhibiting 4-HPPDase.

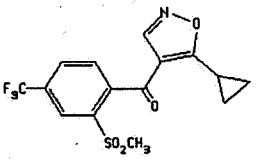
The study is classified as **Acceptable** (Nonguideline) as it is not a required guideline study. It is acceptable for the purposes for which it was intended as a special study.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material 1: RPA 201772
   Chemical Name: 5-Cyclopropyl-4-(2-methylsulphonyl-4-trifluoromethylethyl benzoyl) isoxazole
   Synonym: Isoxaflutole
   Description: A yellow powder
   Batch #: 21 ADM 93
   Purity: 98.7%
   Structure:



   Test Material 2: RPA 200261
   Chemical Name: 2-(2-Nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione
   Synonym: NTBC
   Description: Off-white crystalline powder
   Batch #: SMG 3191
   Purity: 99.8%

   L-Tyrosine (Batch 68160-123; purity 98%)

   Storage: All three chemicals were stored in an air-tight, light-resistant container; RPA 201772 and NTBC were kept room temperature while L-tyrosine was stored at 5°C.

2. Vehicle: 0.05% aqueous methylcellulose

3. Test animals: Rat
   Strain: Crl:CD (SD) BR Sprague-Dawley
   Age and weight at arrival: Approx. 4 weeks;
   Males - 155 to 175 g (at dosing)
   Source: Charles River France, St. Aubin-les-Elbeuf, France
   Housing: Individually in stainless steel cages fitted with urine collecting trays
   Diet: Special powder diet with a very low tyrosine level (UAR,

Villemoisson-sur-Orge, France) ad libitum
Water: Tap water ad libitum
Environmental conditions: Temperature: 22 ± 2°C;
Relative Humidity: 55 ± 15%;
Air changes: 10-15/hour;
Photoperiod: 12 hours light/dark
Acclimation period: Approx. 13 days

B. STUDY DESIGN:

1. In life dates - Start: August 16, 1994; End: September 28, 1994

2. Animal assignment

Animals were assigned to the test groups as shown below (see Table 1).

TABLE 1: STUDY DESIGN[a]

Dietary Concentration (mg/kg/day)	# of Males
Control (0)	5
RPA 201772 (10 mg/kg/day)	5
NTBC (10 mg/kg/day)	5

[a]Rats pretreated with RPA 201772 or NTBC were fed 500 mg/kg/day tyrosine in the diet for one week.

3. Diet preparation and dosing

The dosing formulations were prepared fresh on the day of dosing by suspending the test substance in 0.05% methylcellulose in distilled water to produce the desired concentrations. Rats were administered the test substance or vehicle control formulations (3 ml/kg) by gavage based on the most recently recorded body weight. Approximately 2, 24, and 48 hours and 8 days following test substance administration, all animals received tyrosine (20 ml/kg) by gavage at 500 mg/kg in 0.05% methyl cellulose in distilled water. Test formulations were considered to be sufficiently stable and therefore, no analyses of formulations were performed.

The dose level of RPA 201772 selected for this study were based on the findings of corneal lesions in subchronic and chronic toxicity studies in rats conducted earlier by the sponsor. RPA 201772 exerts its herbicidal action by inhibiting the enzyme, 4-hydroxyphenyl pyruvic acid dioxygenase (4-HPPDase). NTBC, a human therapeutic agent, is used in the treatment of Type I tyrosinemia. Although, this compound inhibits 4-HPPDase, it does not produce corneal lesions in patients.

4. Statistics - No statistical analysis was performed. Only the means and standard deviations were calculated separately for each group at each time period.

C. METHODS:

1. Observations:

Animals were observed twice daily (once during holidays and week-ends) for clinical signs of toxicity, mortality and moribundity. Detailed physical examinations were performed at least once daily.

2. Body weight

Animals were weighed on the first day of dosing and then before necropsy.

3. Urinalysis

Following tyrosine administration, urine was collected for the following durations: 0-4, 4-8 and 8-24 hours. The urine volume was noted and the samples were stored frozen at -20°C. Urinalysis was conducted to identify three metabolites: N-acetyl tyrosine (NAT), 4-hydroxy-phenyl acetic acid (4-HPAA) and 4-hydroxyphenyl lactic acid (4-HPLA). One ml sample of urine was centrifuged at 5500 rpm for 5 minutes and an aliquot of 0.2 ml was mixed with 1.8 ml deionized water. Samples were analyzed by HPLC.

4. Sacrifice and Pathology

After last urine collection, animals from all dose groups were sacrificed by exsanguination under anesthesia (by intraperitoneal injection of pentobarbital). Necropsy was performed and major organs in visceral and cardiac cavities were examined. No microscopic pathology was performed.

II. RESULTS

A. Observations :

1. Toxicity - No treatment-related clinical signs of toxicity were observed.

2. Mortality - No mortalities were noted.

B. Body weight and weight gain:

Following one-week of dietary treatment, decreases in body weights were noted in rats from all dose groups including controls compared to Day 1 of treatment (9, 10, and 16% in control, RPA 201772 and NTBC groups, respectively). When compared with controls the body weight loss (7%) was higher in NTBC treated rats than RPA 201772 treated group (4%). The body weight loss over the entire one-week treatment period was 15, 16 and 26 g in the control, RPA 201772 and NTBC treated rats, respectively. These decrease primarily resulted from low tyrosine diets. Table 2 summarizes the body weights and body weight changes during the study.

Table 2
Mean Body Weights and Body Weight Changes in Rats Treated with RPA 201772 and NTBC for Eight-Days[a]

Parameter	Dose Groups (mg/kg/day)		
Body weight (g):	Control	RPA 201772	NTBC
Day 1	167	162	167
Day 8 % loss compared to Day 1	152 9	146 10	141 16
Body Weight Change (g): Day 1-8	-15	-16	-26
% decrease compared to control value[b]	- -	-4	-7

[a] Extracted from Table 1 (page 26) of the study no. SA 94277; 5 males/dose group; [b] Calculated by the reviewer

C. Urinalysis: Treatment of rats with RPA 201772 or NTBC had no adverse

effects on urine volume (Refer to Table 3). The significant variability in the data resulted from discontinuous nature of urine production and irregular emptying of the bladder.

Table 3. Mean Urine Volume Data (ml) Collected During 24 hours on Various Days From Tyrosine Fed Rats Pretreated With RPA 201772 and NTBC[a]

Day of Urine Collection	Dose Groups		
	Control	RPA 201772	NTBC
Day 1	9.0	15.0	12.0
Day 2	6.0	6.1	6.4
Day 3	7.0	8.0	7.0
Day 8	8.0	7.0	9.0

[a]Data extracted from report number SA 94277 and Table 2, p.27; calculated by the reviewer

D. Urinary Tyrosine Metabolites:

There were treatment-related changes in urinary excretion of three tyrosine metabolites, NAT, 4-HPAA and 4-HPLA, in both RPA 201772 and NTBC treated rats. Mean values for each metabolite in each treatment group, expressed as mg/ml and total concentration (μg) in the urine as well as μg/hour, excreted on Days 1, 2, 3 and 8 of treatment are presented in Table 4, 5 and 6, respectively.

Urinary Excretion of Tyrosine Metabolites:

- N-acetyl tyrosine (NAT): NAT was found in very few control samples. Contrary to these findings, in RPA 201772 pretreated group, NAT was mostly excreted during the first two days of urine collection (4-24 hrs). By day three, the excretion was comparable to that in controls. In NTBC pretreated rats, NAT was increasingly detected in the urine samples during the first three days (twice the amount excreted by the RPA 201772 group). On day 8, NAT excretion was higher than in controls. These increases in RPA 201772 and NTBC groups were considered to be treatment-related.

- 4-Hydroxyphenyl lactic acid (4-HPLA): Urinary excretion of 4-HPLA was seen in few controls samples and only at detectable levels during Day 1.

In RPA 201772 pretreated rats, 4-HPLA level was elevated during first two days (≈ 200 μg/hr/animal). From then on the levels were comparable to that of controls. NTBC treatment caused a marked increase in excretion of 4-HPLA in the urine during first three days and was five-fold higher than the RPA 201772 treated group. By day 8 the levels remained elevated.

- 4-Hydroxyphenyl acetic acid (4-HPAA): In the control group, although the urinary excretion of 4-HPAA was noted during the first two days, it was near the detection limit. This was attributed to induction of tyrosine transaminase (TAT) during the main catabolic pathway of tyrosine. In rats pretreated with RPA 201772, excess 4-HPAA was detected in the urine during first two days; on Day 3 and 8, the levels were comparable with that of controls. In NTBC pretreated rats, 4-HPAA was detected in excess during first three days; the residual elevation in the urine remained the same on Day 8.

Table 4. Tyrosine Metabolites (mg/L) in the Urine Collected Within 24 Hours at Various Intervals From Tyrosine Fed Rats Pretreated With RPA 201772 and NTBC[a]

Metabolite/ Day of Collection	Dose Groups		
	Control	RPA 201772	NTBC
NAT			
Day - 1:	NQ- 23.3	NQ-275	NQ-350
- 2:	NQ- 656	42.7-2921	99.5-2855
- 3:	NQ- 32.2[b]	NQ-28.8	49.3-1436
- 8:	NQ- 41.7	NQ-48.7	8.9-270
4-HPLA			
Day - 1:	NQ-947*	33.9-863	32.7-1404
- 2:	NQ-73.4	122-1476	154-8335b
- 3:	NQ-69.1	31.7-79.2	39-14567
- 8:	NQ	NQ	36.8-504
4-HPAA			
Day - 1:	NQ-275[b]	25-484	19.3-743
- 2:	NQ-191[b]	87.1-902	95.2-1403
- 3:	NQ-17.6[b]	NQ-94.8	99.4-1350
- 8:	NQ-41.7	NQ-48.7	9.1-270

[a]Data extracted from report number SA 94277 and Table 2, p.28-31; NQ = Not quantifiable; [b]Below detection limit; * $p<0.05$

Table 5. Total Concentration (μg) of Tyrosine Metabolites in the Urine Collected Within 24 Hours At Various Intervals from Tyrosine Fed Rats Pretreated With RPA 201772 and NTBC[a]

Metabolite (μg) Day of Treatment	Dose Groups		
	Control	RPA 201772	NTBC
NAT			
Day- 1:	NQ-23.3	NQ-1733	NQ-2374
- 2:	NQ-984	21.4-3654	0-5312
- 3:	NQ-219	NQ-34.6	0-5467
- 8:	NQ-48.8	NQ-42.9	NQ-759
4-HPLA			
Day- 1:	NQ-2937*	77.1-4640	49.1-11482
- 2:	NQ-54.4	75.5-2149	339-19015
- 3:	NQ-130	NQ-110	0-37568
- 8:	NQ	NQ	NQ-1663
4-HPAA			
Day- 1	NQ-689	71.3-4637	58.2*-6803
- 2:	NQ-993	98.8-2246	0-3518
- 3:	NQ-109[a]	NQ-168	0-4368
- 8:	NQ-91.7	NQ-62.4	0-739

[a]Data extraacted from report number SA 94277 and Table 3, p.32-35

NQ = Not quantifiable

[a]Below detection limit

*$p < 0.05$

Table 6. Tyrosine Metabolites (μg/hr) Collected Within 24 Hours at Various Intervals from Tyrosine Fed Rats Pretreated With RPA 201772 and NTBC[a]

Metabolite (μg/hr)/ Day of Treatment	Dose Groups		
	Control	RPA 201772	NTBC
NAT			
Day - 1:	NQ-1.5	NQ-117	NQ-148.4
- 2:	NQ-61.5	5.4-228.4	0-397.3
- 3:	NQ-13.7	NQ-2.2	0-463.3
- 8:	NQ-3.1	NQ-2.7	NQ-47.4
4-HPLA			
Day - 1:	NQ-183.6	19.3-290	12.3-717.6
- 2:	NQ-55.0	18.9-226.8	0-1489.5
- 3:	NQ-24.5	NQ-27.8	0-2348.0
- 8:	NQ	NQ	NQ-157.8
4-HPAA			
Day - 1:	NQ-75.5	15.3-289.8	14.6-425.2
- 2:	NQ-101.3	30.5-172.3	0-486.8
- 3:	NQ-21.2.	NQ-35.5	0-273.0
- 8:	NQ-5.7	NQ-11.6	0-65.0

aData extraacted from report number SA 94277 and Table 3, p.37-39
NQ = Not quantifiable

E. Gross Pathology: No abnormalities were observed in any dose group.

III. DISCUSSION

A. Reviewer's interpretation of study results: The analytical chemistry data indicate that the concentration, stability and homogeneity of RPA 201772 and NTBC were within acceptable limits and the animals received appropriate dosages of the test compound.

In this study, three primary metabolites of tyrosine formed were NAT, 4-HPAA, and 4-HPLA which are normally present at very low levels in urine. Following dietary administration of large doses of tyrosine (approximately 5%) to CD rats pretreated with single doses 10 mg/kg of RPA 201772 or NTBC, there were significant increases in all three urinary metabolites which were attributed to

inhibition of 4-HPPDase enzyme. These metabolites were detected at very low levels in control animals. The increases in the amount of metabolites excreted in the urine were pronounced with NTBC than with RPA 201772 pretreated rats during first two days of tyrosine treatment. On Day 3, the amounts of metabolites excreted by the RPA 201772 group were comparable to controls while NTBC pretreated group continued to show effect on all three metabolites on Day 8. This observation suggests that the inhibition of 4-HPPDase in RPA 201772 pretreated male rats was reversible within 48 hours while it was not completely reversed in NTBC treated rats until Day 8.

The findings of this study demonstrate that in the event of 4-HPPDase inhibition high doses of tyrosine administered to rats are metabolized by alternative route and the resulting metabolites formed are detected in the urine. The results also suggest that RPA 201772 forms a reversible complex with 4-HPPDase with a relatively short halflife.

In previous studies by the Sponsor, dietary administration of tyrosine to CD rats, was found to inhibit 4-HPPDase and increase the plasma tyrosine level resulting in development of corneal lesions within 1-3 days (LSR SA 94100; MRID #43904816). Similar results were obtained in rats pretreated with RPA 201772 and fed diet containing high levels of tyrosine although the lesions developed after 10 days (LSR 93-0906 and LSR 94/0279; MRID #43904806). No corneal lesions have been reported in humans following therapeutic use of NTBC. Therefore, the effect of RPA 201772 on rat cornea appears to be a species-specific effect.

B. Study deficiencies: No study deficiencies were noted: