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DATA EVALUATION RECORD

STUDY TYPE: Comparative Metabolism Study- *[Rat and Mouse];* nonguideline

DP BARCODE: D224202 SUBMISSION CODE: S501233
P.C. CODE: 123000 TOX. CHEM. NO.: [New Chemical]
MRID NO.: 43904815

TEST MATERIAL (PURITY): RPA 201772 (98.3%)

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

SYNONYM: Isoxaflutole

CITATION: Filaquier, C.M. (1995). RPA 201772: Qualitative Comparison of Metabolism of Tyrosine following a single oral administration of RPA 201772 in the Rat and in the Mouse. Rhône-Poulenc Agrochimie, Sophia Antipolis. Report No. SA 94246; March 15, 1995. MRID NUMBER: 43904815. (Unpublished)

SPONSOR: Rhône-Poulenc Agrochimie, Lyon, France

EXECUTIVE SUMMARY: In a comparative metabolism study (MRID # 43904815), RPA 201772 (98.3%) was administered to groups (5/species) of male Sprague-Dawley (CD) rats and CD-1 mice by gavage at a single dose (10 mg/kg) followed one hour later with a single oral dose of ^{14}C-Tyrosine (500 mg/kg). The total radioactivity in the urine and expired CO_2 was estimated at 0-5, 5-12, 12–24, and 12–48 hour intervals. Metabolite analysis was performed on the urine of the rat and the mouse to analyze the quantitative differences in their ability to utilize a by-pass metabolic route for the blocked tyrosine pathway via hydroxyphenyl lactic acid (HPLA) and hydroxyphenyl acetic acid (HPAA).

For both species, a major portion of ^{14}C-Tyrosine administered dose was eliminated via urine and expired air. Urinary elimination (mice: 46.79%, rats: 15.70%) was predominant in the mouse while a significant portion of radiolabel was predominantly excreted via the expired air as CO_2 in the rat (mice: 6.47%, rat: 17.04%) during the first 48 hours following administration of ^{14}C-Tyrosine. HPLC analysis of ^{14}C-Tyrosine metabolites found in the urine of both species revealed higher amounts of two major metabolies, HPLA and HPAA, in the mouse than those in the rat. The enzymatic

hydrolysis of conjugates indicated that some metabolites were excreted as glucuronides and/or sulfates in urine; these did not include HPLA and HPAA.

This study demonstrated species-related qualitative and quantitative differences in the excretion of tyrosine following single simultaneous administrations of RPA 201772 and ^{14}C-Tyrosine to male mice and rats. The findings of the study further suggests differential ability of the two species to alternatively utilize a by-pass metabolic route for the blocked tyrosine pathway via HPLA and HPAA.

This comparative metabolism study using *rats* and *mice* is classified as ***Unacceptable*** (Nonguideline) and is not a required guideline study. It can be upgraded provided the registrant provides clarification of the following issues:

1. It is not clear why there was an absence of a control group in this study, specifically, that there were groups of rats and mice dosed only with radiolabelled tyrosine. This could have facilitated comparison of disposition observed after dosing with the combination of tyrosine and RPA 201772. The co-administration of tyrosine and RPA 201772 might have also influenced the disposition of tyrosine accounting in part for the observed difference in disposition between rats and mice. This should be explained.

2. It is also unclear why only a single dose of RPA 201772 was used. A series of doses could have better delineated possible differences between rats and mice in sensitivity. The only major difference in this study was noted in the percentage of HPLA and HPAA excreted from 0-5 hours post-dose in rats and mice, where mice showed higher percentages of these metabolites in urine. The differences in percentages of these metabolites at later times of excretion was not major, i.e. from 0.13-0.5% greater in mice vs. rats.

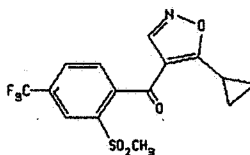
3. There are no individual animal data to verify the summary data on metabolite fractions presented in Tables 5 and 6 of the submitted report, pages 23 and 24. It is unclear what is meant by the term "individual" samples as stated in the heading to Table 5. Individual animal data should be submitted to verify the summary data.

4. There appears to be a shift in the retention time for HPLA and HPAA metabolites using the same HPLC method on individual and pooled samples. In Table 5 of the report, the HPLA and HPAA metabolites are listed as fractions 5 and 6, respectively, while for the pooled samples, the same metabolites are listed as fractions 6 and 8. The reason for this apparent shift needs explanation.

A. MATERIALS:

1. Test Compound:

Non radioactive Compound RPA 201772
Chemical Name: 5-Cyclopropyl-4-(2-methylsulphonyl-4-trifluoromethylethyl benzoyl) isoxazole; isoxaflutole
Purity: 98.3%
Lot/Batch No.: FPI1308
Description: Fine yellow powder, as noted in MRID #435732-19
Storage: at +4°C.
CAS No.: 141112-29-0
Structure:



Radiolabelled Compound L-Tyrosine-(U-^{14}C)
Radiochemical Purity: 98.35%
Batch No.: 94193
Specific Activity: 360 mCi/mmol
Storage: at -20°C

Non-Radiolabelled Compound L-Tyrosine
Batch No.: 68160-123
Storage: at +4°C

2. Vehicle: 0.75% (w/v) Aqueous methyl cellulose

3. Test animals:

Species: Rat; Mouse
Strain: CD, Sprague-Dawley; CD1
Age: Not specified
Weight: Male Rats: 161.93-167.85 g; Male Mice: 30.23–31.93 g at initiation of dosing
Source: Charles River, France
Housing: Initially in metal, wire mesh bottomed cages, and then individually

in metabolism cages
Animals received food: Diet (free protein diet, "Regime carence en Tyrosine" from U.A.R.) and water ad libitum.
Environmental conditions:
Temperature: 20 ± 2°C
Humidity: Not reported
Air changes: Not reported
Photoperiod: 12 hours light/dark
Acclimation period: Not reported

4. Preparation of dosing solutions:

Each dose suspension was prepared fresh on the day of dosing and assayed for active ingredient by High-Performance-Liquid Chromatography [HPLC]) and for radioactivity (by Liquid Scintillation Counting) before, during and after the dosing. Appropriate calculated amount of non-labelled L-Tyrosine added to a 250 ml flask was ground to a fine powder and suspended in a few drops of aqueous methyl cellulose (0.75% w/v). The calculated amount of non-radiolabelled L-Tyrosine was added to the radiolabelled L-Tyrosine and mixed together by grinding to obtain the required dose level and the specific activity. An appropriate amount of methyl cellulose was added to prepare a homogenous dosing suspension of sufficient quantity and concentration to dose animals at a rate of 4 g dose/200 g rat and/or mouse b.w.

The dose suspension of the RPA 201772 was prepared in the same manner in order to obtain a homogeneous suspension of sufficient quantity and concentration to dose the animals at a rate of 1 g dose suspension per 200 g rat and/or mouse b.w. and at the required active ingredient dose level.

B. STUDY DESIGN AND METHODS:

1. Group Arrangements

The animals were assigned on a weight-basis to the test groups as shown in table 1.

TABLE 1: Dosing groups

Test Species	Dose of RPA 201772 and 14C-Tyrosine, mg/kg	Number of males	Remarks
Rat	10 500	5	Single oral administration of non-labelled material followed 1 hour later by single oral dose of labelled material; sacrificed at 48 hours post-dosing
Mouse	10 500	5	Single oral doses of non-labelled material followed 1 hour later by single dose of labelled material; sacrificed at 48 hours post-dosing

2. Dosing and sample collection/Preparation

Twenty-four hours before dosing, the animals were placed in individual metabolism cages; the animals remained in those cages for 48 hours post dosing. Water was allowed ad libitum.

The animals were weighed prior to dosing and the dose required for each animal was calculated, dispensed and administered by gavage. One hour later each animal received an oral administration of ^{14}C-Tyrosine.

Sample collection

Air was drawn through each metabolism cage (at a rate of 0.3–0.4 l/min) and exhaled carbon dioxide was trapped for the first 48 hours following dosing.

The urine and feces were collected over solid carbon dioxide in polystyrene boxes during at 5, 12, 24 and 48 hour intervals following dosing. These samples were protected from light and stored at -20°C before being analyzed

by liquid scintillation counting.

3. Metabolite characterization

Urine and CO_2: Both urine and expired air samples were analyzed by HPLC to investigate ^{14}C-tyrosine metabolic profiles. The individual and pooled urine samples from each individual animals were collected at each time point up to 48 hours post-dosing for each species; the samples were pooled at 0–5 hours, 5-12 hours, 12-24 hours, and 24–48 hours. HPLC Method # ANL/048-94E was used to characterize the presence of three tyrosine metabolites: p-hydroxyphenyl lactate (HPLA), p-hydroxyphenyl acetate (HPAA), and N-acetyl tyrosine (NAT). The elution gradient of the HPLC method was modified. This modified method was applied to pooled urine samples to obtain a better resolution of several polar compounds detected initially in individual urine samples.

To detect glucurono- and sulpho-conjugates in the urine, β-glucuronidase or sulphatase were added to aliquots of pooled urine samples (0.5 ml) from rats and mice. The samples were incubated at 37°C in a waterbath for 4 hours. Following incubation a few drops of methanol were added to precipitate the proteins in the biological media. The samples were centrifuged and the supernatant was analyzed by HPLC.

To aid identification of metabolites, reference standards were used. The presence of three tyrosine metabolites was confirmed by comparing the retention times of HPLC fractions detected in urine samples and those available for chemical standards. HPLC was performed on Waters M600E Multisolvent Delivery System.

4. Statistics:

The percent dose recovery and compound concentrations, including means and standard deviations were calculated using the Microsoft Excel spreadsheet program.

5. Compliance:

Signed and dated GLP, quality assurance and confidentiality statements were provided.

C. RESULTS:

1. Urinary and Pulmonary Elimination:

Based on the cumulative mean recoveries shown in Table 2, it appears that the percent dose of ^{14}C-Tyrosine administered excreted during first 48 hours in the urine of mouse and the rat was 46.79% and 15.70%, respectively; the major portion of the radioactivity was eliminated during first 12 and 24 hours post-dosing in mice and rats, respectively.

The amount of cumulative mean radioactivity detected in the expired air within first 48 hours of dosing for the rat and the mouse was 17.04% and 6.47%, respectively. The major portion of radioactivity eliminated via CO_2 was during 12-24 hours and 24-48 hours for rats and mice, respectively, post-dosing.

TABLE 2. Recovery of radioactivity in the urine and expired air of male rats and mice following administration of ^{14}C-Tyrosine[a].

Time Period in Hours	Cumulative Percent of radioactive dose recovered			
	Rat		Mice	
	Urine	CO_2	Urine	CO_2
0-5	3.03	0.69	22.93	1.43
5-12	9.07	4.05	39.36	3.04
12-24	14.10	12.90	44.55	4.11
24-48	15.70	17.04	46.79	6.47

a = Data extracted from study number SA 94246 and tables 1-4; pages 19-22; MRID# 43904815

2. Metabolite characterization and identification:

A total of 9 metabolites (UMET/1 thru UMET/9) were detected in the individual urine samples from rat and mouse. UMET/9, a more apolar component had the longest retention time. The percentage of each metabolite fraction in terms of administered dose is presented in Table 3A and 3B, respectively. The main urinary metabolites of tyrosine excreted by the mouse were more polar than HPLA and HPAA. N-Acetyl Tyrosine (NAT) was not detected in urine of the rat and the mouse. Both HPLA and HPAA were present in the urine of both species, up to 48 hours and represented less than 1.1% of the administered dose, irrespective of species and collection time.

In order to get a better resolution, the HPLC system was modified and used to quantify the metabolites in the pooled urine samples from both species. A total of 10 metabolites were detected (UMET/1 thru UMET/10); UMET/10 was more apolar and had the longest retention time. As shown in Tables 3A and 3B, the urinary contents of HPLA and HPAA were different in the two species; NAT was not detected. During the first 0-5 hour time interval, the percent dose administered excreted as HPLA and HPAA in the mouse was 4.95% and 1.16%, respectively; these values were higher than those seen for 0-5 hr elimination in the rat. At the 5-12, 12-24, and 24-48 hr intervals, the percentages of HPLA and HPAA in mouse urine were higher than in rat urine, but the differences were not appreciable (0.13-0.5%). Thus, using the modified HPLC system, the amounts of HPLA and HPAA excreted in urine were higher in the mouse than in the rat suggesting a somewhat greater efficiency of this metabolic pathway in the mouse. The total amounts of polar metabolites found in the mouse were greater than for the rat. These results suggests species-specific differences in the metabolic profile of tyrosine. The metabolism of ^{14}C-Tyrosine was qualitatively and quantitatively different in both species. The compound was rapidly and extensively metabolized as seen by the lack of parent material in the urine of both species.

The pooled urine samples subjected to enzymatic hydrolysis with β-glucuronidase or sulphatase produced a total of 10 metabolites (UMET/1 thru UMET/10) similar to those found in the pooled urine samples of male mice and rats subjected earlier to quantification by HPLC. Of these 10 metabolites, UMET/10 was the more apolar component detected having the longest retention time. The results are presented in Table 5. Based on these results, the study author concluded that some metabolites were eliminated as glucuronides and/or sulphates in urine indicating modification in metabolic profiles; no HPLA or HPAA were observed as conjugates.

D. DISCUSSION:

The present study was conducted to examine the possible quantitative differences in the ability of the mouse and the rat to effectively utilize an alternative metabolic route via HPLA and HPAA for the blocked tyrosine pathway.

Excretion data showed significant differences between the two species in the total radioactivity excreted via urine and via expired air. The urinary elimination was predominant in the mouse (46.79% of the administered dose being excreted during first 48 hours compared to 15.70% of the administered dose in the rat); a significant portion of the total radioactivity (39.36%) was excreted within first 12 hours. These results suggests differential rate of excretion in both species. The total amount of radioactivity eliminated as $^{14}C\text{-}CO_2$ differed in both species. During first 48 hours, 6.47% of the administered dose was eliminated as $^{14}C\text{-}CO_2$ in the mouse compared to 17.04% of the administered dose being eliminated in the rat.

eliminated in the rat.

TABLE 3A. Metabolite Profile in Individual and Pooled Urine Samples of Mice Dosed With RPA 201772 and ^{14}C-Tyrosine[a].

Metabolite fractions	Percent of administered dose							
	Individual samples/Collection period in hrs.				**Pooled samples**/Collection period in hrs.			
	0-5	5-12	12-24	24-48	0-5	5-12	12-24	24-48
UMET/1	1.86	12.33	0.57	0.19	1.01	4.16	1.21	0.31
UMET/2	11.77	1.99	3.32	1.31	n.d.	0.50	n.d.	1.49
UMET/3	5.44	0.63	0.51	0.02	0.87	n.d.	n.d.	n.d.
UMET/4	0.22	0.04	0.12	0.05	1.88	3.90	n.d.	n.d.
UMET/5	**0.58**[b]	**0.54**[b]	**0.09**[b]	**0.02**[b]	12.40	4.80	2.22	n.d.
UMET/6	**0.73**[c]	**0.33**[c]	**0.33**[c]	**0.22**[c]	**4.95**[b]	**1.87**[b]	**1.17**[b]	**n.d.**[b]
UMET/7	n.d.	0.31	n.d.	0.08	n.d.	n.d.	n.d.	0.13
UMET/8	0.24	0.26	0.14	0.04	**1.16**[c]	**0.66**[c]	**0.58**[c]	**0.32**[c]
UMET/9	0.23	0.12	0.04	0.01	0.28	0.31	n.d.	n.d.
UMET/10	- -	- -	- -	- -	0.37	0.10	n.d.	n.d.

a = Data extracted from study number SA 94246 and Table 5 and 6; pages 23 and 24

b = HPLA: Hydroxyphenyl lactic acid; c = HPAA: Hydroxyphenyl acetic acid

n.d. = Not detected

TABLE 3B. Metabolite Profile in Individual and Pooled Urine Samples of Rats Dosed With RPA 201772 and ^{14}C-Tyrosine[a].

Metabolite fractions	Percent of administered dose							
	Individual samples/Collection period in hrs.				**Pooled samples**/Collection period in hrs.			
	0-5	5-12	12-24	24-48	0-5	5-12	12-24	24-48
UMET/1	0.34	1.06	0.61	0.47	0.16	0.42	0.64	0.52
UMET/2	0.64	0.78	0.47	0.37	0.40	0.35	0.34	n.d.
UMET/3	0.18	2.04	0.37	0.04	0.12	0.11	n.d.	0.21
UMET/4	1.13	1.73	1.33	0.09	0.15	n.d.	n.d.	0.21
UMET/5	**0.77[b]**	**0.56[b]**	**1.03[b]**	**0.26[b]**	1.00	2.83	1.52	n.d.
UMET/6	**0.11[c]**	**0.45[c]**	**0.41[c]**	**0.18[c]**	**0.48[b]**	**1.37[b]**	**0.86[b]**	**0.24[b]**
UMET/7	0.12	0.02	0.02	n.d.	0.12	n.d.	0.33	n.d.
UMET/8	0.10	0.13	0.29	0.06	**0.28[c]**	**0.40[c]**	**0.44[c]**	**0.19[c]**
UMET/9	n.d.	0.14	0.21	0.06	0.18	0.18	0.32	0.22
UMET/10	- -	- -	- -	- -	0.14	0.38	0.41	n.d.

a = Data extracted from study number SA 94246 and Table 5 and 6; pages 23 and 24

b = HPLA: Hydroxyphenyl lactic acid; c = HPAA: Hydroxyphenyl acetic acid

n.d. = Not detected

TABLE 5. Metabolites Representing Percent Dose Administered in Pooled Urine of Rats and Mice, Before and After Enzymatic Deconjugation[a].

Species/ Time Intervals	Metabolic fractions UMET/..									
	1	2	3	4	5[b]	6	7	8	9[c]	10
Rat										
Non-deconjugated										
0-5 h	0.32	0.78	0.26	0.31	**0.81**	n.d.	n.d.	n.d.	**0.37**	0.18
5-12 h	0.36	0.78	n.d.	n.d.	**2.12**	2.02	n.d.	n.d.	**0.58**	0.18
12-24 h	0.59	0.62	0.34	n.d.	**1.21**	n.d.	0.89	0.24	**0.75**	0.43
24-48 h	0.33	0.38	n.d.	0.15	**0.33**	n.d.	n.d.	n.d.	**0.10**	0.23
Deconjugated										
0-5 h	0.60	1.34	n.d.	n.d.	**0.48**	0.05	n.d.	n.d.	**0.39**	0.19
5-12 h	0.56	3.31	n.d.	n.d.	**1.28**	n.d.	n.d.	n.d.	**0.55**	0.26
12-24 h	0.93	2.17	n.d.	n.d.	**0.93**	0.09	n.d.	n.d.	**0.59**	0.33
24-48 h	0.41	0.31	n.d.	n.d.	**0.34**	n.d.	n.d.	n.d.	**0.26**	0.28
Mouse										
Non-deconjugated										
0-5 h	n.d.	4.36	n.d.	n.d.	**15.02**	n.d.	n.d.	n.d.	**2.82**	0.74
5-12 h	n.d.	12.70	n.d.	n.d.	**2.32**	n.d.	n.d.	n.d.	**1.41**	n.d.
12-24 h	0.26	1.05	1.26	n.d.	**1.89**	n.d.	n.d.	n.d.	**0.57**	0.16
24-48 h	0.35	0.92	0.30	n.d.	**n.d.**	n.d.	n.d.	0.40	**0.11**	0.17
Deconjugated										
0-5 h	1.41	5.15	9.50	n.d.	**4.45**	n.d.	n.d.	n.d.	**1.70**	0.55
5-12 h	1.06	10.63	1.09	n.d.	**2.11**	n.d.	n.d.	n.d.	**1.32**	0.22
12-24 h	0.58	1.89	0.73	n.d.	**1.12**	n.d.	n.d.	n.d.	**0.78**	0.11
24-48 h	0.36	1.04	0.18	n.d.	**0.14**	0.06	n.d.	n.d.	**0.35**	0.11

a = Data extracted from study number SA 94246 and Table 7 and page 26.
b = HPLA: Hydroxyphenyl lactic acid; c = HPAA: Hydroxyphenyl acetic acid
n.d. = Not detected

HPLC analysis of ^{14}C-Tyrosine metabolites revealed that the metabolic profiles were largely different in both species. ^{14}C-metabolites excreted in urine of the mouse were more polar than those excreted in the rat resulting in higher urinary elimination of radiolabel in the mouse. When analyzed by the modified HPLC system, species-related differences were observed in the elimination of radiolabel with amounts of HPLA and HPAA in the pooled urine samples being higher in the mouse than those measured in the rat. The enzymatic deconjugation revealed that some metabolites were eliminated as glucuronides and/ or sulfates in urine; both HPLA and HPAA were not detected as conjugates.

The results of this study demonstrate qualitative and quantitative differences in the elimination process of Tyrosine after oral administration of RPA 201772 followed one hour later by the oral administration of ^{14}C-Tyrosine. The ability of the mouse and the rat to use alternative metabolic route for the blocked Tyrosine pathway via HPLA and HPAA also differed in these species.