

RPA 201772

Liver Enzyme Study

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Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION RECORD
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STUDY TYPE: Special Study-Rats  
Nonguideline

DP BARCODE: D224202SUBMISSION CODE: S501233P.C. CODE: 123000TOX. CHEM. NO.: [New Chemical]MRID NO.: 43904819TEST MATERIAL (PURITY): RPA 201772 (99.6%)

CHEMICAL NAME: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)  
isoxazole

SYNONYM: Isoxaflutole

CITATION: Price, S.C. (1994). RPA 201772. Effects of Dietary administration for 14 Days on the Liver Enzymes of Male Sprague Dawley CD-1 Rats. Robens Institute of Health and Safety, University of Surrey, Surrey, U.K. Report No. RI 94/TOX/030; Study No. 55/92/TX. September 9, 1994. MRID NO. 43904819. (Unpublished)

SPONSOR: Rhône-Poulenc Agriculture, Essex, England

EXECUTIVE SUMMARY: This study (MRID# 43904819) was conducted to establish the dose response and to investigate the role of mixed function oxidase system with respect to liver enlargement in RPA 201772 treated rats. Groups of 5 male Sprague-Dawley rats received RPA 201772 (99.6% a.i.) in diet at dosage levels of 0, 10, 100, or 400 mg/kg/day for 14 days.

RPA 201772 administration caused an increase ( $\geq 33\%$ ) in absolute and relative liver weights in rats at 100 and 400 mg/kg/day. This increase was attributed to induction of MFO enzymes in the microsomal fraction of the homogenized liver. The total cytochrome P-450 levels were increased in a dose-dependent manner. The specific forms of isoenzymes responsible for this increase were PROD and BROD enzymes, the induction of which may be attributed to the P-450 2B family (i.e., phenobarbital type). Therefore, RPA 201772 appears to function as a phenobarbital type inducer of P-450 2B family. There was no increase in other P-450 isoenzyme levels including MROD and EROD nor did the test compound induced lauric acid hydroxylases that are associated with peroxisome proliferation.

Thus, RPA 201772 appears to be a phenobarbital type inducer of liver enzymes.

The LOEL was 10 mg/kg/day based on induction of P-450 enzymes in male rats. In addition, at  $\geq 100$  mg/kg/day liver enlargement was also seen.

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: RPA 201772

Chemical Name: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl) isoxazole

Synonym: Isoxaflutole

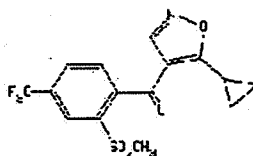
Description: Beige powder\*

Batch #: JYG708

Purity: 99.6%\*

Storage: At room temperature in the dark\*

Structure:



\* From MRID No.: 43904808

#### 2. Vehicle: Basal diet

#### 3. Test Animals: Rat

Strain: Sprague-Dawley CD 1

Age and weight at arrival: 28 days old;

Males - 250 g (at dosing)

Source: Charles River U.K. Ltd, Kent, England

Housing: Five per cage

Diet: CRM SDS standard rodent diet ad libitum

Water: Tap water ad libitum

Environmental conditions: Temperature: 20 ± 3°C;

Relative Humidity: 30-70%;

Air changes: Not reported;

Photoperiod: 12 hours light/dark

Acclimation period: Approx. 7 days

### B. METHODS/STUDY DESIGN:

#### 1. In Life Dates - start: November 19, 1992

End: December 12, 1992

## 2. Animal Assignment

Animals were assigned randomly to the test groups on a weight basis (see Table 1).

Table 1: Study Design<sup>a</sup>

Dose Groups (mg/kg/day)	# of Male Rats
Control: 0	5
10	5
100	5
400	5

<sup>a</sup> Body weight variations were within  $\pm 10\%$  of the mean weight.

## 3. Diet Preparation and Analysis

The premix was prepared by grinding the test substance with a small amount of the basal diet using a pestle and mortar. It was then added to the bulk of the diet and mixed at a low speed using a Hobart paddle mixer. Each diet concentration was mixed for 20 minutes starting with the lowest concentration. The test diet mixtures were stored in color coded plastic bags at 4°C until needed. The stability of the test diet over two weeks was confirmed in previous studies. Therefore, no confirmatory analyses for concentration or homogeneity were carried out.

## 4. Dose Selection

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

## C. METHODS:

### 1. Observations:

Animals were observed once daily during the treatment period for clinical or behavioral signs of toxicity

## 2. Body Weight

Animals were weighed at the beginning of the study, weekly thereafter and at necropsy.

## 3. Food Consumption

Food consumption (g/rat/day) was measured weekly over the exposure period for all treatment groups.

## 4. Clinical Chemistry:

At necropsy, individual body and liver weights were recorded. A section of liver from each rat was fixed in 10% neutral buffered formalin and stored for possible histopathological examination. The remaining liver was homogenized and the following assays were conducted on the relevant liver fraction:

- Total cytochrome P-450 (SOP TX/METH/00108) \*
- Ethoxyresorufin O-deethylase (SOP TX/METH/0031-3)
- Pentoxyresorufin O-depentylase (SOP TX/METH/00128)
- Methoxyresorufin O-demethylase (SOP TX/METH/00170)
- Benzoxyresorufin O-debenzylase (SOP TX/METH/00171)
- Total and microsomal protein (SOP TX/METH/00105)
- Lauric acid hydroxylase (carried out at University of Surrey)

\* indicates the methodology used

## II. RESULTS

### A. Observations :

Observations - No mortalities, clinical or behavioral signs were noted.

### B. Body weight/Food Consumption/Liver Weights:

There were no treatment-related effects on body weight or body weight gain and food consumption in rats from all dose groups (Table 2). At 100 and 400 mg/kg/day, dose-related increases in absolute and relative liver weights were noted in these groups. No such effects were noted at 10 mg/kg/day.

Table 2

Effect on Body Weight, Food Consumption and Liver Weights in Rats Following Treatment with RPA 201772 for Fourteen Days<sup>a</sup>

Parameters Measured	Dose Levels (mg/kg/day)			
	Control	10	100	400
<b>Body Weights (g)</b>				
Day 0:	300	303	310	308
Day 7:	329	336	337	334
Day 14:	380	394	396	390
(% of control)	--	(103)	(102)	(103)
<b>Food Consumption (g/rat/day)</b>				
Week 1:	30	33	33	28
Week 2:	32	34	32	35
(% of control)	--	(108)	(100)	(110)
<b>Terminal Body Weight (g)</b>	380	394	396	390
(% of control)	(- -)	(104)	(104)	(103)
<b>Liver weight (g)</b>	15.3	17.3	21.1*	26.1*
(% of control)	(- -)	(113)	(138)	(171)
<b>Liver/body weight ratio</b>	4.0	4.4	5.3*	6.7*
(% of control)	(- -)	(109)	(133)	(165)

a Extracted from Tables 1, 2a and 2b (pages 18 and 19) of the study no. RI94/TOX/030; \*p < 0.05

E. Effect on Liver Enzymes: Treatment-related changes in the liver enzyme activities were noted at 100 and 400 mg/kg/day. These data are summarized in Table 3 and are discussed below.

- Total Cytochrome P-450: Treatment with RPA 201772 caused dose-related increase ( $\geq 128\%$  of control) in total liver P-450 in all 3 treated groups.
- Pentoxoresorufin O-depentylase (PROD) and Benzo(a)pyrene O-debenzylate (BROD): Upon P-450 isoenzyme analysis, marked and significant increases in PROD and BROD, expressed as absolute activity ( $\geq 329\%$  of control) or in relation to total liver P-450 activities ( $\geq 233\%$  of control), were noted.
- Ethoxoresorufin O-deethylase (EROD): A significant but non-dose related increase ( $\geq 128\%$ ) occurred in absolute EROD activity. EROD

activity in relation to the total liver P-450, was significantly lower at 400 mg/kg/day.

- Methoxyresorufin O-demethylase (MROD): MROD activity was not increased when compared with controls.
- Lauric acid hydroxylase: At 400 mg/kg/day, some induction of lauric acid 11-hydroxylase and 12-hydroxylation of the fatty acid was noted. However, dose dependent trend towards increase was noted only for 11-hydroxylation of the fatty chain and not the 12-hydroxylation which is catalyzed by the P-450 4 family. In terms of total P-450, although a trend towards decrease in activity was noted for both the 11- and 12-hydroxylase isoforms, the decreases were not statistically significant.

#### IV. DISCUSSION

##### A. Reviewer's interpretation of study results:

Dietary administration of RPA 201772 at 100 and 400 mg/kg/day for 14 days caused increase in absolute and relative liver weights in male CD-1 rats. This was attributed to an induction of MFO enzymes in the microsomal fraction of the homogenized liver. The total P-450 levels increased due to dose-related induction of specific isoforms of P-450 such as PROD and BROD. These were attributed to P-450 2B family, associated with the B1 and B2 isoforms. The EROD levels increased significantly in all treated groups but in a non-dose related manner while MROD activity did not increase compared to controls. Both activities are associated with the P-450 1 family, in particular the A1 and A2 isoenzymes, respectively. Lauric acid 11-hydroxylase and 12-hydroxylase activities were induced at the highest dose only; of these only the 11-hydroxylase activity showed a dose-dependent trend towards induction. In terms of total cytochrome P-450, there was no induction of either the 11 or 12-hydroxy form. Overall results of the study shows that RPA 201772 caused a dose-related increase in liver enlargement due to marked elevation of P-450 enzymes of the P-450 2B family, typical of phenobarbital. It does not induce other P-450 isoenzymes significantly nor cause peroxisome proliferation.

The LOEL is 10 mg/kg/day based on induction of P-450 enzymes of 2B family. In addition, at 100 mg/kg/day increase in liver enlargement was also noted.

##### B. Study deficiencies: No deficiencies were noted.

**Table 3**  
**Effect on Hepatic Enzyme Activity in Rats**  
**Treated with RPA 201772 for Fourteen Days<sup>a</sup>**

Hepatic Enzyme	Dose Levels (mg/kg/day) <sup>b</sup>			
	Control	10	100	400
<b>Total P-450:</b> nmole/mg protein	0.78	1.00* (129) <sup>c</sup>	1.44*** (185)	1.69*** (217)
<b>EROD:</b> pmoles/min/mg	15.80	23.29** (148)	20.79* (132)	20.21* (128)
<b>EROD:P-450</b> pmole/min/nmole P-450	21.62	23.36 (108)	14.52 (67)	11.97* (55)
<b>PROD</b> pmoles/min/mg	15.96	55.00** (345)	973*** (6096)	1675*** (10498)
<b>PROD:P-450</b> pmole/min/nmole P-450	20.91	54.33** (260)	682*** (3262)	990*** (4734)
<b>MROD:</b> pmole/min/mg	20.59	33.26 (162)	47.23 (229)	26.88 (131)
<b>MROD:P-450</b> pmole/min/nmole P-450	28.63	33.34 (117)	33.53 (117)	15.99 (56)
<b>BROD:</b> pmole/min/mg	72.37	237.88*** (329)	6238.74*** (3620)	8708.39*** (12033)
<b>BROD:P-450</b> pmole/min/nmole P-450	100.86	234.97** (233)	4375.09*** (4338)	5155.72*** (5112)
<b>Lauric acid 11- hydroxylase</b> nmol/min/mg protein	1.22	1.36 (112)	1.77 (146)	2.27* (186)
<b>LAH:P-450</b>	1.68	1.36 (81)	1.25 (75)	1.34 (80)
<b>Lauric acid 12- hydroxylase:</b> nmol/min/mg protein	1.13	1.31 (116)	1.21 (107)	1.49* (131)
<b>LAH:P-450</b>	1.52	1.30 (86)	0.84 (55)	0.88 (58)

a Extracted from Table 3 (pages 20 and 21) of the study;

\*p<0.05; \*\*p<0.01

b 5 rats/dose groups with the exception of 4 rat for the highest dose group

c Values in parenthesis represent percent of control.