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RPA 201772 Liver Enzyme Study

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DATA EVALUATION RECORD

STUDY TYPE: Special Study-Mice
Nonguideline

DP BARCODE: D224202
P.C. CODE: 123000
MRID NO.: 43904820

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

TEST 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. The effect of Dietary administration for 14 Days on the Liver Enzymes of Male CD-1 Mice. Robens Institute of Health and Safety, University of Surrey, Surrey, U.K. Report No. RI 94/TOX/031; Study No. 51/92/TX. September 9, 1994. MRID NO. 43904820. (Unpublished)

SPONSOR: Rhöne-Poulenc Agriculture, Essex, England

EXECUTIVE SUMMARY: This study (MRID# 43904820) 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 mice. Groups of 25 male CD-1 mice received RPA 201772 (99.6% a.i.) in diet at dosage levels of 0, 175, 700, 2800 or 7000 ppm for 14 days.

RPA 201772 administration caused increase (≥ 11%) in absolute and relative liver weights in rats at ≥700 ppm. This increase was attributed to the induction of mixed function oxidase enzymes in the liver. The total cytochrome P-450 levels were increased in a dose-dependent manner. The specific forms of isoenzymes responsible for this increase included PROD and BROD, the induction of which may be attributed to the P-450 2B family. Therefore, RPA 201772 appears to function as a phenobarbital type inducer. There was no significant increase in other P-450 isoenzyme activities including MROD and EROD nor did the test compound induced lauric acid hydroxylases that are associated with peroxisome proliferation.

The LOEL was 175 ppm based on induction of P-450 enzyme, BROD, in male mice. In addition, at ≥700 ppm dose-related increase in liver enlargement and induction of PROD was seen.

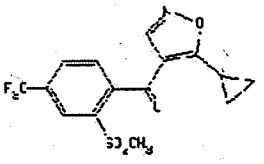
The study is classified as Acceptable (Nonguideline) as it is not a required guideline study. It is acceptable for the purposes for which it was intended as a special study.

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: Mice
   Strain: CD 1
   Age and weight at arrival: Age not reported;
   Males - Approx. 25 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: At least 7 days

B. METHODS/STUDY DESIGN:

1. In Life Dates - start: November 12 and 13, 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[a]

Dose Groups (PPM)	# of Male Mice
Control: 0	25
175	25
700	25
2800	25
7000	25

a Body weight variations were within ±10% of the mean weight. Mice in one cage were dosed for 13 days instead of 14 days.

3. Diet Preparation and Analysis

The premix was prepared by grinding the test substance with basal 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. Liver Enzyme Assays:

At necropsy, individual liver weights were recorded. A section of liver from one mouse per cage was fixed in 10% neutral buffered formalin and stored for possible histopathological examination. The remaining livers from each cage were pooled and homogenized. 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. However, two animals in one cage were eliminated during predosing period because of aggressive behavior found towards the other mouse in this cage. This cage was not used and substituted with a second cage.

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 700 and above, dose-related increases in absolute and relative liver weights were noted. No such effects were noted at 175 ppm.

Table 2

Effect on Body Weight, Food Consumption and Liver Weights in Mice Following Treatment with RPA 201772 for Fourteen Days[a]

Parameters Measured	Dose Levels (ppm)				
	0	175	700	2800	7000
Body Weights (g)					
Day 0:	28.4	27.6	27.4	27.2	27.8
Day 7:	32.0	30.7	31.4	31.4	32.7
Day 14:	35.4	33.7	34.5	34.6	35.3
(% of control)	- -	(96)	(98%)	(99)	(100)
Food Consumption (g/animal/day)					
Week 1:	7.0	5.9	6.0	6.4	6.3
Week 2:	6.2	5.9	6.3	6.0	5.8
(% of control)	- -	(95)	(102)	(97)	(94)
Terminal Body Wt (g)	35.0	33.7	34.4	34.4	35.2
(% of control)	(- -)	(96)	(98)	(98)	(101)
Liver weight (g)	1.9	1.9	2.1*	2.8***	3.9***
(% of control)	(- -)	(100)	(111)	(148)	(202)
Liver/body weight ratio	0.05	0.05	0.06*	0.08***	0.11***
(% of control)	(- -)	(104)	(113)	(150)	(201)

a Extracted from Tables 1, 2a and 2b (pages 19, 20 and 21) of the study no. RI94/TOX/031; *p<0.05; **p<0.01; ***p<0.001

E. Effect on Liver Enzymes: Treatment-related changes in the liver enzyme activities were noted at 700 ppm and above with the exception of PROD and BROD which were seen at all dose levels. These data are summarized in Table 3 and are discussed below.

- Total Cytochrome P-450: Treatment with RPA 201772 resulted in a dose-related increase in total liver P-450 at 700 ppm and above. Total cytochrome P-450 was increased to 136, 168, and 216% of control at the 700, 2800, and 7000 ppm dose levels, respectively.

- Pentoxyresorufin O-dependentylase (PROD) and Benzoresorufin O-debenzylate (BROD): Upon P-450 isoenzyme analysis, a dose-related and significant induction in PROD activity, expressed as absolute activity (≥187% of control) or in relation to total liver P-450 activities

(≥157% of control), were noted at all dose levels. BROD activity was significantly elevated compared to controls in all dose groups (≥310% of control). This enzyme activity remained significant when expressed in terms of total cytochrome P-450 and peaked at 2800 ppm and above.

- Ethoxyresorufin O-deethylase (EROD): A significant increase occurred in absolute EROD activity at 2800 ppm and above. EROD was increased to 206% of control at 2800 ppm and 215% of control at 7000 ppm. In relation to the total liver P-450, significant peak activity was noted in the 2800 ppm group.

- Methoxyresorufin O-demethylase (MROD): MROD activity increased in a dose-dependent manner at 2800 ppm and above when compared with controls. However, in terms of total cytochrome P-450, the MROD activity was comparable to the control values.

- Lauric acid hydroxylase: At 700 ppm and above, a dose-related trend in the induction of lauric acid 11-hydroxylase activity was noted; the 12-hydroxylation of the fatty acid was noted at 700 ppm only. 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, no significant induction of activity was noted for both the 11- and 12-hydroxylase isoforms.

IV. DISCUSSION

A. Reviewer's interpretation of study results:

Dietary administration of RPA 201772 at doses of 700 ppm and above for 14 days caused dose-related increase (≥11%) in absolute and relative liver weights in male CD-1 mice. 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 isoform of P-450, (namely, PROD) and was attributed to P-450 2 family, associated with the B1 isoform. The EROD levels increased significantly at 2800 ppm and above. However, in terms of total cytochrome P-450 content of the liver, this activity did not differ significantly from the controls. While both the MROD and BROD showed induction at ≥700 and ≥175 ppm, respectively, in terms of total P-450, only BROD activity remained significant which is associated with P-450 2B family. Compared to PROD activity, BROD activity is induced at lower doses (175 ppm) which suggests that BROD activity is associated with more than one isoform of the 2B family. Both MROD and

EROD activities are associated with the P-450 1 family, in particular the A2 isoenzyme. Lauric acid 11-hydroxylase and 12-hydroxylase activities were induced at ≥700 ppm; 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 in male mice 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 175 ppm based on BROD induction of P-450 enzymes of 2B family. In addition, at ≥700 ppm above there was increase in liver enlargement was and induction of PROD activity also noted.

B. Study deficiencies: No deficiencies were noted.

Table 3
Effect on Hepatic Enzyme Activity in Mice
Treated with RPA 201772 for Fourteen Days[a]

Hepatic Enzyme	Dose Levels (ppm)b				
	Control	175	700	2600	7000
Total P-450 (nmole/mg protein)	0.91	1.09 (119)c	1.24*(136)	1.53**(168)	1.97*** (216)
EROD (pmoles/min/mg) **EROD:P-450** (pmole/min/nmole P-450)	44.93 48.90	55.01 (122) 52.59 (108)	61.80 (138) 49.81 (102)	92.54* (206) 62.94* (129)	96.50* (215) 48.04 (98)
PROD (pmoles/min/mg) **PROD:P-450** (pmole/min/nmole P-450)	7.61 8.57	14.23*(187) 13.48**(157)	118.95***(1562) 94.95***(1109)	217.66***(2859) 149.47***(1745)	249.40***(3276) 125.30***(1463)
MROD (pmole/min/mg) **MROD:P-450** (pmole/min/nmole P-450)	142.21 154.92	175.74(124) 163.87(106)	245.83* (173) 195.42 (126)	308.12 (217) 211.46 (136)	285.25* (201) 142.75 (92)
BROD (pmole/min/mg) **BROD:P-450** (pmole/min/nmole P-450)	48.51 53.57	150.36**(310) 140.56** (262)	1040.03***(2144) 844.93***(1577)	1547.63***(3190) 1040.19***(1942)	1776.58***(3662) 900.44***(1681)
Lauric acid 11-hydroxylase nmol/min/mg protein **LAH:P-450**	0.99 1.08	1.01 (101) 0.89 (82)	1.18 (119) 0.99 (87)	1.50 (151) 0.99 (91)	2.54*** (256) 1.30 (120)
Lauric acid 12-hydroxylase: nmol/min/mg protein **LAH:P-450**	1.79 1.93	1.89 (106) 1.68 (87)	1.41 (79) 1.13**(59)	1.77 (99) 1.17**(61)	2.90* (162) 1.47 (76)

a Extracted from Tables 3 (pages 22 and 23) of the study no. RI94/tox/031;
*p<0.05; **p<0.01; ***P<0.001
b 25 males/dose/group
c Values in parenthesis represent percent of control.