RPA 201772 Liver Enzyme Study

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DATA EVALUATION RECORD

STUDY TYPE: Special Study-Rats
Nonguideline

DP BARCODE: D224202
P.C. CODE: 123000
MRID NO.: 43904819

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. 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 (Unpublished)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: Rhöne-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 (≥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 ≥100 mg/kg/day liver enlargement was also seen.

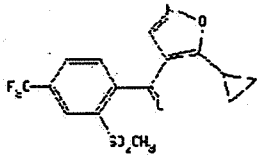
The study is classified as Acceptable (Nonguideline) as it is not a required guideline study. It is acceptable for the purposes for which it was intended as a special study.

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[a]

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

a Body weight variations were within ±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[a]

Parameters Measured	Dose Levels (mg/kg/day)			
	Control	10	100	400
Body Weights (g)				
Day 0:	300	303	310	308
Day 7:	329	336	337	334
Day 14:	380	394	396	390
(% of control)	- -	(103)	(102)	(103)
Food Consumption (g/rat/day)				
Week 1:	30	33	33	28
Week 2:	32	34	32	35
(% of control)	- -	(108)	(100)	(110)
Terminal Body Weight (g)	380	394	396	390
(% of control)	(- -)	(104)	(104)	(103)
Liver weight (g)	15.3	17.3	21.1*	26.1*
(% of control)	(- -)	(113)	(138)	(171)
Liver/body weight ratio	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 (≥128% of control) in total liver P-450 in all 3 treated groups.

- Pentoxyresorufin O-depentylase (PROD) and Benzoresorufin O-debenzylate (BROD): Upon P-450 isoenzyme analysis, marked and significant increases in PROD and BROD, expressed as absolute activity (≥329% of control) or in relation to total liver P-450 activities (≥233% of control), were noted.

- Ethoxyresorufin O-deethylase (EROD): A significant but non-dose related increase (≥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[a]

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