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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

AUG 06 1997

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Isoxaflutole

FROM: Sanjivani Diwan, Ph.D. *Sanjivani Diwan*
Review Section I *7/30/97*
Toxicology Branch II
Health Effects Division (7509C)

and

Esther Rinde, Ph.D. *E. Rinde*
Manager, Carcinogenicity Peer Review Committee
Science Analysis Branch
Health Effects Division (7509C)

TO: Joanne Miller/Daniel Kenny
Product Manager #23
Herbicide Branch
Registration Division (7505C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on May 14, 1997 to discuss and evaluate the weight-of-the-evidence on isoxaflutole with particular reference to its carcinogenic potential.

In accordance with the EPA proposed Guidelines for Carcinogenic Risk Assessment (April 23, 1996), isoxaflutole was characterized as "likely to be a human carcinogen", based on statistically significant increases in liver tumors in both sexes of mice and rats, and statistically significant increases in thyroid tumors in male rats. Also, the liver tumors in male mice had an early onset.

SUMMARY

Administration of isoxaflutole in the diet to CD-1 mice for 78 weeks resulted in statistically significant increases in hepatocellular adenomas and combined adenoma/carcinoma in both sexes at the highest dose (7000 ppm, equivalent to 977.3 mg/kg/day for males; 1161.1 mg/kg/day for females). There were also positive significant trends for hepatocellular adenomas, carcinomas and combined adenoma/carcinoma in both sexes. In male mice there was also a statistically significant increase in hepatocellular carcinomas at the highest dose with a positive significant trend and, at the 53-week sacrifice, there was evidence of early onset for hepatocellular adenomas. The incidences of hepatocellular tumors exceeded that for historical controls in both sexes.

The CPMC agreed that the highest dose in this study was adequate and not excessive.

Administration of isoxaflutole in the diet to Sprague-Dawley rats for 2 years resulted in statistically significant increases in hepatocellular adenomas, carcinomas and combined adenoma/carcinoma in both sexes at the highest dose (500 mg/kg/day). There were also positive significant trends for hepatocellular carcinomas, adenomas and combined adenoma/carcinoma in both sexes. The incidences of hepatocellular adenomas and carcinomas exceeded that for historical controls in both sexes.

In male rats there was also a statistically significant increase in thyroid follicular cell adenomas, carcinomas and combined adenoma/carcinoma at the highest dose, and positive significant trends for these adenomas and combined adenoma/carcinoma. The incidences of thyroid adenomas and carcinomas exceeded that of historical controls in male rats.

The CPMC agreed that the highest dose in the rat study was adequate and not excessive.

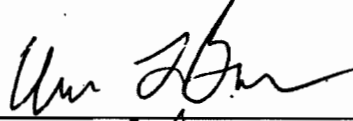
There was no evidence of mutagenicity in the studies submitted and no structurally related analogs could be identified, since isoxaflutole is a member of a new class of chemicals.

The studies submitted by the registrant to show a mechanistic basis for the liver tumors were considered by the CPMC to be suggestive, but not convincing (see Section F.). The CPMC agreed that the mechanistic evidence presented for the thyroid tumors appeared to be scientifically plausible and consistent with EPA current policy.

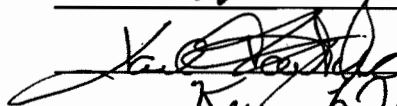
A. **Individuals in Attendance at the meeting:**

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

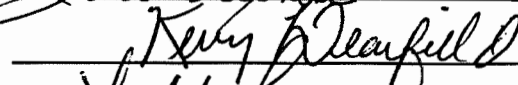
William Burnam



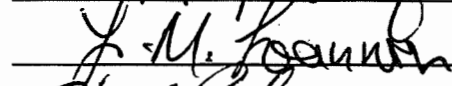
Karl Baetcke



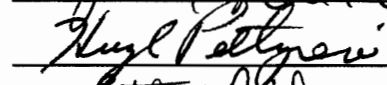
Kerry Dearfield



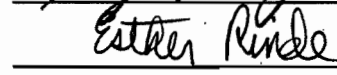
Yiannakis Ioannou



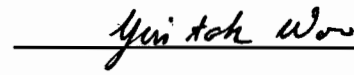
Hugh Pettigrew



Esther Rinde

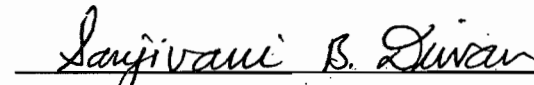


Yin Tak Woo

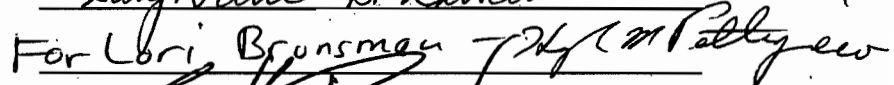


2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

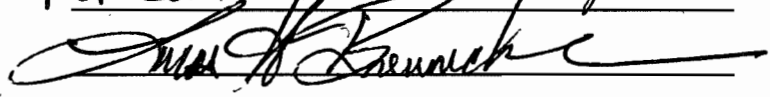
Sanjivani Diwan¹



Lori Brunzman



Lucas Brennecke²
(PAI/ORNL)



3. Other Attendees: Kit Farwell, Kathleen Raffaele, Barbara Madden, Albin Kocialski, Joycelyn Stewart (HED)

¹Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

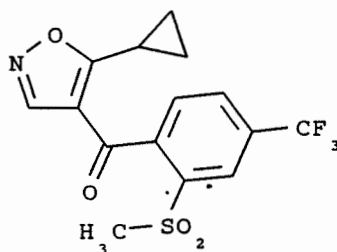
²Signature indicates concurrence with pathology report.

B. Material Reviewed

The material available for review consisted of DER's, data from the literature and other data summaries prepared and/or supplied by Sanjivani Diwan, and tables and statistical analysis by Lori Brunsmann. The material reviewed is attached to the file copy of this report.

C. Background Information:

Chemical Name: isoxaflutole; 5-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxaflutole
Synonym: RPA 201772;
CAS #: 14112-29-0



Isoxaflutole (RPA 201772) is a new experimental herbicide for preplant and preemergence control of grasses and broadleaf weeds in field corn. It is a member of a new class of herbicides, isoxazoles, that disrupts pigment biosynthesis in plants. Application was made to the Agency for an Experimental Use Permit (EUP) and Temporary Tolerances of RPA 201772 as a 75% formulation with proposed temporary tolerances of 0.1 ppm on corn grain and 0.2 ppm for corn forage and fodder. The Toxicology Branch II recommended that the temporary tolerances be granted based on the review of toxicology data submitted by the Registrant. The Registrant has now proposed tolerances of 0.1 ppm for corn grain and 0.4 ppm for corn forage and fodder. The proposed tolerances of 0.2 ppm for liver and 0.03 ppm for kidney in the cow, 0.05 ppm for fat, meat and eggs as well as 0.20 ppm for liver in poultry have also been proposed by the Registrant.

The Toxicology data in this memorandum are based upon review of submitted studies by Rhone Poulenc. The Registrant has conducted mechanistic studies to elucidate the mechanisms involved in the induction of liver and thyroid tumors in mice and rats.

D. Evaluation of Carcinogenicity Evidence:

1. Seventy-eight Week Carcinogenicity study in Mice.

Reference: Chase, K.R. (1995). RPA 201772ai: Oncogenicity study by dietary administration to CD-1 mice for 78 weeks. Study conducted by Pharmaco LSR, Eye, Suffolk IP23 7PX, England, Pharmaco LSR Report No: 95/RHA509/0343, and submitted under MRID # 43904807.

a. Experimental Design

Groups of 64 or 76 CD-1 mice/sex/dose received RPA 201772 ($\geq 98.7\%$ ai) in diet at dose levels of 0, 25, 500, or 7,000 ppm daily (means of 0, 3.2, 64.4, or 977.3 mg/kg/day, respectively, for males; and 0, 4.0, 77.9, or 1161.1 mg/kg/day, respectively, for females). Interim sacrifices were made at 26 weeks (12 mice/sex at the 0 and 7,000 ppm doses) and at 52 weeks (12 mice/sex at all dose levels).

b. Discussion of Tumor Data and comparison with historical control data

An increase in the incidence of hepatocellular tumors was noted at 7,000 ppm in males (adenoma: 58%; adenomas and/or carcinomas combined: 58%) at 53-week sacrifice and in males (adenoma: 55%; carcinoma: 35%; combined adenoma/carcinoma: 78%) at 78-week sacrifice and females (adenoma: 29%; combined adenoma/carcinoma: 35%) at 78-week sacrifice.

At 53-week interim sacrifice, male mice had significant increasing trends ($p < 0.01$), and significant differences in pair-wise comparisons of the 7000 ppm dose group with the controls ($p < 0.05$) in the incidence of liver adenomas and combined adenoma/carcinoma. The CPRC considered the early onset of these tumors to be significant.

At 78-week sacrifice, significant increasing trends ($p < 0.01$) in the incidence of liver adenomas, carcinomas or adenomas and/or carcinomas combined were observed in males and females. There were significant differences in the pair-wise comparisons of the 7000 ppm groups with controls ($p \leq 0.01$) for adenoma, carcinoma (males only) and combined adenoma/carcinoma in both sexes. Tumorigenic evidence observed in this study is shown in Tables 1, 2 and 3.

Historical control data were submitted from 366 male and 366 female mice from 7 studies commenced between October, 1990 and March 1992. The incidence of benign and malignant liver tumors in these animals is presented below.

Historical Control data for Liver Tumors Submitted by Testing Facility¹

Sex		Male	Female
No. Examined		366	366
No. of Livers examined		366	365
No. of adenoma	Mean (%)	55 (15%)	1 (0.27%)
	Range (%)	(3.8-23.1%)	(0-2.0%)
No. of Adenocarcinoma	Mean (%)	23 (6.28%)	2 (0.55%)
	Range (%)	(1.9-11.5%)	(0-2.0%)

¹ The combined total tumor incidence was not provided

The incidences of hepatocellular tumors in this study exceeded that for historical controls in both sexes.

Table 1. Isoxafutole - CD-1 Mouse Study

Male Liver Tumor Rates⁺ and Exact Trend
 Test and Fisher's Exact Test Results (p values)
 EXCLUDING 53-Week Interim Sacrifice Animals

	<u>Dose (mg/kg/day)</u>			
	0	3.2	64.4	977.3
Adenomas (%)	9/47 (19)	10/50 (20)	9 ^a /48 (19)	27/49 (55)
p =	0.000**	0.560	0.584	0.000**
Carcinomas (%)	4/47 (9)	5/50 (10)	8/48 (17)	17/49 (35)
p =	0.000**	0.540	0.188	0.002**
Combined (%)	13/47 (28)	15/50 (30)	14 ^c /48 (29)	38 ^d /49 (78)
p =	0.000**	0.488	0.526	0.000**

*Number of tumor bearing animals/Number of animals examined, excluding those that died before week 47. Also excludes week 27 and week 53 interim sacrifice animals.

^aFirst liver adenoma, excluding 53-week interim sacrifice animals, observed at week 55, dose 64.4 mg/kg/day.

^bFirst liver carcinoma observed at week 47, dose 977.3 mg/kg/day.

^cThree animals in the 64.4 mg/kg/day dose group had both an adenoma and a carcinoma.

^dSix animals in the 977.3 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. See Table 2 for separate analysis of 53-week interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Isoxaflutole - CD-1 Mouse Study

Male Liver Tumor Rates⁺ and Exact Trend
 Test and Fisher's Exact Test Results (p values)
 53-Week Interim Sacrifice Animals ONLY

	<u>Dose (mg/kg/day)</u>			
	0	3.2	64.4	977.3
Adenomas (%)	2/12 (17)	1/11 (9)	0/12 (0)	7/12 (58)
p =	0.001 ^{**}	0.534 ⁿ	0.239 ⁿ	0.045 [*]
Carcinomas (%)	0/12 (0)	0/11 (0)	1/12 (8)	0/12 (0)
p =	0.745	1.000	0.500	1.000
Combined (%)	2/12 (17)	1/11 (9)	1/12 (8)	7/12 (58)
p =	0.002 ^{**}	0.534 ⁿ	0.500 ⁿ	0.045 [*]

⁺Number of tumor bearing animals/Number of animals examined, including ONLY those that were sacrificed at week 53.

ⁿNegative change from control.

Note: ONLY 53-week interim sacrifice animals are included in this analysis.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If ^{*}, then p < 0.05. If ^{**}, then p < 0.01.

Table 3. Isoxaflutole - CD-1 Mouse Study
Female Liver Tumor Rates⁺ and Exact Trend
 Test and Fisher's Exact Test Results (p values)
 EXCLUDING 53-Week Interim Sacrifice Animals

	<u>Dose (mg/kg/day)</u>			
	0	4.0	77.9	1161.1
Adenomas (%)	0/51 (0)	1/50 (2)	1/48 (2)	15 ^a /51 (29)
p =	0.000**	0.495	0.485	0.000**
Carcinomas (%)	0/51 (0)	0/50 (0)	0/48 (0)	4 ^b /51 (8)
p =	0.004**	1.000	1.000	0.059
Combined (%)	0/51 (0)	1/50 (2)	1/48 (2)	18 ^c /51 (35)
p =	0.000**	0.495	0.485	0.000**

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 27 and week 53 interim sacrifice animals.

^aFirst liver adenoma observed at week 77, dose 1161.1 mg/kg/day.

^bFirst liver carcinoma observed at week 60, dose 1161.1 mg/kg/day.

^cOne animal in the 1161.1 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

c. Non-neoplastic lesions and other findings:

RPA 201772 had no significant effect on the survival of mice. Systemic signs of toxicity in the treated groups included: 1) decreased body weight gain in both sexes at 500 ppm (males -15% and females -22%) and 7,000 ppm (males -28% and females -42%); for the 25 ppm group females the total body weight gain was also lower (-16%) compared to controls; 2) food consumption was unaffected; however, food efficiency was lower for both sexes (28% for males and 17% for females) at 7000 ppm during the first 14 weeks of the study; 3) absolute and relative body liver weights were significantly increased in both sexes (up to >200%) at 7,000 ppm; relative liver weight was increased in males at 52 weeks (+19%) and in females at 78 weeks (+13%) at 500 ppm; 4) gross necropsy at 78-week sacrifice revealed increased occurrences of liver masses in both sexes at 7,000 ppm; 5) non-neoplastic lesions of the liver occurred at 52-week sacrifice in males at 500 ppm and in males and females at 7,000 ppm. At termination, the 500 ppm group males exhibited increased incidence of hepatocyte necrosis. At 7,000 ppm, significant increase in non-neoplastic lesions in both sexes included periacinar hepatocytic hypertrophy, necrosis, and erythrocyte-containing hepatocytes. In addition, males at the high dose had pigment-laden hepatocytes and Kupffer cells, basophilic foci, and increased ploidy; extramedullary hematopoiesis in the spleen was noted in both sexes. Based on these findings, the Lowest Observed Effect Level (LOEL) was 500 ppm (64.4 mg/kg/day for males and 77.9 mg/kg/day for females); the No Observed Effect Level (NOEL) was 25 ppm (3.2 mg/kg/day for males and 4.0 mg/kg/day for females). Although body weight was decreased marginally in females at 25 ppm, there were no corroborating findings of toxicity at this dose.

It was noted that the target site for non-neoplastic lesions in both sexes of the mouse (liver) was the same as that for tumor formation.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

At the 7,000 ppm dose level, the mean body weight gain in both sexes was decreased (at 78 weeks, there was 28% decrease in males and 42% decrease in females) compared to controls. During the first 14 weeks of treatment, average weekly food conversion efficiencies for males and females were 72 and 83%, respectively, of the controls. The following evidence of liver pathology was observed: 1) increased incidence of abnormal findings (liver masses, enlarged or swollen livers and liver "areas of change") on gross examination in carcinogenicity phase; 2) increased absolute and/or relative liver weights in the males and females mice in both the toxicity and carcinogenicity phases; 3) increased incidence of periacinar hepatocytic hypertrophy, necrosis of individual

hepatocytes, and pigment laden hepatocytes and Kupffer cells, erythrocytes in hepatocytes in both sexes and basophilic foci of hepatocellular alterations (males), and increased ploidy compared (males) to the controls in the toxicity and/or carcinogenicity phases. In light of these systemic effects, the 7,000 ppm dose level is considered to be an adequate dose for assessing the carcinogenic potential of RPA 201772 in mice. Despite the large body weight gain decrements, the CPRC agreed that the dosing was not excessively toxic, as there was increased survival relative to controls in treated mice.

2. Sprague Dawley Rat Combined Chronic Toxicity/Carcinogenicity Study

Reference: Chase, K.R., (1995) RPA 201772ai: Combined Oncogenicity and Toxicity Study by Dietary Administration to (Sprague-Dawley) CD rats for 104 weeks. Study conducted by Life Science Research Limited, Eye, Suffolk, England, LSR Report 95/0499, and submitted under MRID # 43904806.

a. Experimental Design

In a carcinogenicity study, RPA 201772 (93-99.2% a.i.) was continuously administered to 75 Sprague-Dawley rats/sex/dose at dietary levels of 0, 0.5, 2, 20 or 500 mg/kg/day for 104 weeks. An additional 20 rats/sex/group were treated for 52 weeks, after which 10 rats/sex/group were sacrificed and the remainder were held for a maximum of eight weeks without treatment in order to assess reversibility of treatment-related changes.

b. Discussion of Tumor Data

There were no treatment-related neoplastic lesions detected in the animals at the interim sacrifice. During the 104-week carcinogenicity phase, an increase in the incidence of liver tumors was noted at 500 mg/kg/day in males (adenoma: 26%; carcinomas: 25%; combined adenoma/carcinoma: 46%) and females (adenoma: 42%; carcinomas: 33%; combined adenoma/carcinoma: 63%). An increase in the incidence of thyroid follicular cell adenoma (22%) was noted in males only. Tumorigenic evidence observed in this study is shown in Tables 4-6.

Table 4. Isoxaflutole - CD(SD)BR VAF Plus Rat Study

Male Liver Tumor Rates⁺ and Peto's
 Prevalence Test Results (p values)

	<u>Dose (mg/kg/day)</u>				
	0	0.5	2.0	20.0	500.0
Adenomas (%)	2/41 (5)	3/42 (7)	5/49 (10)	6 ^a /46 (13)	14/54 (26)
p =	0.001**	0.204	0.117	0.076	0.004**
Carcinomas (%)	5 ^b /58 (9)	1/53 (2)	4/62 (6)	2/64 (3)	17/68 (25)
p =	0.000**	-	-	-	0.011*
Combined (%)	7/58 (12)	4/53 (8)	8 ^c /62 (13)	8/64 (12)	31/68 (46)
p =	0.000**	-	0.457	0.494	0.000**

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died before the observation of the first tumor; also excludes week 53 interim sacrifice animals.

^aFirst liver adenoma observed at week 53, dose 500.0 mg/kg/day, in an interim sacrifice animal. Second liver adenoma observed at week 97, dose 20.0 mg/kg/day, in an animal that died on study.

^bFirst liver carcinoma observed at week 85, dose 0 mg/kg/day, in an animal that died on study.

^cOne animal in the 2.0 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There was one liver adenoma in the interim sacrifice group at the 500.0 mg/kg/day dose.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Isoxaflutole - CD(SD)BR VAF Plus Rat Study

Male Thyroid Follicular Cell Tumor Rates⁺
 and Peto's Prevalence Test Results (p values)

	<u>Dose (mg/kg/day)</u>				
	0	0.5	2.0	20.0	500.0
Adenomas (%)	3/66 (5)	1/60 (2)	5 ^a /69 (7)	7/68 (10)	15/69 (22)
p =	0.000**	-	0.271	0.127	0.005**
Carcinomas (%)	0/53 (0)	1/46 (2)	2/59 (3)	1/58 (2)	3 ^b /62 (5)
p =	0.113	0.117	0.159	0.169	0.042*
Combined (%)	3/66 (5)	2/60 (3)	7/69 (10)	8/68 (12)	17 ^c /69 (25)
p =	0.000**	-	0.120	0.081	0.002**

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died before the observation of the first tumor; also excludes week 53 interim sacrifice animals.

^aFirst thyroid follicular cell adenoma observed at week 70, dose 2.0 mg/kg/day.

^bFirst thyroid follicular cell carcinoma observed at week 91, dose 500.0 mg/kg/day.

^cOne animal in the 500.0 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There were no thyroid follicular cell tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then p < 0.05. If **, then p < 0.01.

Table 6. Isoxaflutole - CD(SD)BR VAF Plus Rat Study

Female Liver Tumor Rates⁺ and Peto's
 Prevalence Test Results (p values)

	<u>Dose (mg/kg/day)</u>				
	0	0.5	2.0	20.0	500.0
Adenomas (%)	4/66 (6)	2/59 (3)	1/60 (2)	0/55 (0)	29 ^a /69 (42)
p =	0.000**	-	-	-	0.000**
Carcinomas (%)	0/70 (0)	0/71 (0)	1/69 (1)	0/66 (0)	24 ^b /73 (33)
p =	0.000**	-	0.118	-	0.000**
Combined (%)	4/70 (6)	2/71 (3)	2/69 (3)	0/66 (0)	46 ^c /73 (63)
p =	0.000**	-	-	-	0.000**

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died before the observation of the first tumor.

^aFirst liver adenoma observed at week 76, dose 500.0 mg/kg/day.

^bFirst liver carcinoma observed at week 61, dose 500.0 mg/kg/day.

^cSeven animals in the 500.0 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Significant increasing trends ($p \leq 0.05$; $p < 0.01$) in the incidence of liver adenomas, carcinomas and combined adenoma/carcinoma were observed in males and females and thyroid follicular cell adenoma and combined adenoma/carcinoma ($p < 0.01$) in males only. There were significant differences in the pair-wise comparisons of the 500 mg/kg/day groups with controls ($p < 0.05$; $p < 0.01$) for liver adenoma, carcinoma and combined adenoma/carcinoma in both sexes and in thyroid follicular cell adenoma ($p < 0.01$), carcinoma ($p < 0.05$) and combined adenoma/carcinoma ($p < 0.01$) in males. The above tumor incidences exceeded the historical incidence of these tumors for this strain in this laboratory.

c. Non-neoplastic lesions and other findings:

Administration of RPA 201772 significantly increased survival in both sexes of treated rats. Evidence of systemic toxicity observed in one or both sexes included: 1) abnormal gait, limited use of limbs at 500 mg/kg/day and eye opacity (in males) at ≥ 20 mg/kg/day; 2) lower body weight gains ($\geq 36\%$ in both sexes) and food consumption (12% in females) at 500 mg/kg/day; 3) decreased food efficiency ($\geq 12\%$ in both sexes) at 500 mg/kg/day during the first 14 weeks of the study; 4) elevated cholesterol levels (in both sexes) at 500 mg/kg/day throughout the 104-week study, 5) gross necropsy changes in the liver, and lungs in both sexes at 500 mg/kg/day, and eyes in males at ≥ 20 mg/kg/day; 6) increased absolute and relative liver weights (in both sexes) and thyroid weights in males at 500 mg/kg/day; and 7) increased incidence of peri-acinar hepatocytic hypertrophy, portal tract (senile) bile duct changes, focal cystic degeneration of the liver (in males at ≥ 20 mg/kg/day; in females at 500 mg/kg/day), thyroid cystic follicular hyperplasia (in males at 20 mg/kg/day), corneal lesions (in males at ≥ 20 mg/kg/day), and degeneration of sciatic nerve and thigh muscles (in males at ≥ 20 mg/kg/day; in females at 500 mg/kg/day). Based on these findings, the Lowest Observed Effect Level (LOEL) was 20 mg/kg/day for males and 500 mg/kg/day for females; the No Observed Effect Level (NOEL) was 2 mg/kg/day for males and 20 mg/kg/day for females.

It was noted that there were non-neoplastic lesions in the rat in both the thyroid and liver, which were also target sites for tumor formation.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

At 500 mg/kg/day, the mean body weight gain in both sexes was decreased ($\geq 36\%$ in both sexes) compared to controls; the average weekly food conversion efficiencies for males and females were $\geq 12\%$ compared to controls. Cholesterol levels were elevated in both sexes throughout the study. The following evidence of liver, thyroid and ocular pathology was observed in one or both sexes: 1) increased incidence of gross necropsy changes in the liver (masses,

areas of change and swelling), thyroid enlargement and eye opacity (males only) in toxicity and/or carcinogenicity phases; 2) increased absolute and/or relative liver weights in the male and female rats in both the toxicity and carcinogenicity phases; 3) peri-acinar hepatocytic hypertrophy, portal tract (senile) bile duct changes; thyroid cystic follicular hyperplasia and corneal lesions in males, and degeneration of sciatic nerve and thigh muscles in both sexes. In light of these systemic effects, the 500 mg/kg/day dose level is considered to be an adequate dose for assessing the carcinogenic potential of RPA 201772 in rats. Despite the large body weight gain decrements, the CPRC agreed that the dosing was not excessively toxic, as there was increased survival relative to controls in treated rats.

E. Additional Toxicology Data

1. Metabolism

Reference: Filaquier, C.M. (1994): RPA 201772: Absorption, Distribution, Metabolism and Excretion in the Rat. Study conducted by Rhone-Poulenc Secteur Agro, Sophia Antipolis, France, and submitted under MRID # 43573224

Summary: Disposition and metabolism of ^{14}C -RPA 201772 (98.7%) was investigated in male and female Sprague-Dawley (CD) rats by gavage at a single low oral dose (1 mg/kg), repeated oral dose (1 mg/kg/day as a final dose in a fifteen day repeat dose series), and a single high dose (100 mg/kg). Pharmacokinetics in blood was investigated in male and female rats that received an oral dose of 1 or 100 mg/kg of ^{14}C -RPA 201772.

^{14}C -RPA 201772 was rapidly and extensively absorbed and metabolized. In the two low dose groups, urinary and fecal elimination of RPA 202248, a major metabolite, represented $\geq 70\%$ of the radioactivity excreted. The other minor metabolite, RPA 203328, was more polar. Elimination was rapid and dose-dependent. The mean total recovery ranged from 98.09% to 99.84% (mean 99.21%). Urinary elimination (males: 61.16% to 66.65%, females: 58.80% to 67.41%) was predominant in the two low dose groups while a major portion of radiolabel was excreted via the feces (males: 62.99%, females: 55.23%) in the high dose group. The higher fecal elimination possibly resulted from the saturation of absorption resulting in elimination of unchanged parent compound. The majority of the radiolabel was eliminated in the first 24 and 48 hours for the low and the high dose groups, respectively.

The extensive systemic clearance of the radiolabel was reflected in the low levels of radioactivity found in tissues at 168 hours post-dosing. For the two low dose groups, liver (0.172 to 0.498 ppm) and kidneys (0.213 to 0.498 ppm) accounted for the major portion of

the administered dose found in tissues. In the high dose group, the highest level of radioactivity was found in decreasing order in blood, plasma, liver, and kidney. Sex-related differences were observed in the excretion and distribution pattern among high dose rats. The elimination half-lives were similar among single low and high dose groups, with an estimated mean blood half-life of 60 hours. No sex differences were observed in the metabolism of ¹⁴C-RPA 201772.

2. Mutagenicity

RPA 201772 has not been shown to be mutagenic in vivo and in vitro. These studies are summarized below.

a) Reference: Edwards, C.N. (1993). RPA 201772: Mouse Micronucleus Test to comply with O.E.C.D. Guideline 474. Performed by Pharmaco-Life Science Research, Ltd., Suffolk, England and submitted under MRID No. 43573223:

Summary: In an in vivo mouse micronucleus assay, groups of five male and five female CD-1 mice received a single oral gavage dose of 200, 1000 or 5000 mg/kg RPA 201772 (98.7%). Bone marrow cells were collected 24, 48 or 72 hours posttreatment and preparations from the high-dose males and females were examined for micronucleated polychromatic erythrocytes (MPEs). Only cells collected 24-hours post-administration of the low and intermediate doses were scored for MPEs. The test material was delivered to the test animals as suspensions prepared in corn oil.

RPA 201772 was neither overtly toxic to the treated animals nor cytotoxic to the target cell. There was also no evidence of a clastogenic or aneugenic effect at any dose or harvest time. The positive control induced the expected high yield of MPEs in both sexes.

Classification: Acceptable

b) Reference: Percy, A. (1993). RAP 210772: Salmonella typhimurium Reverse Mutation Assay (Ames Test). Performed by Rhone-Poulenc Centre De Recherche, Sophia Antipolis, France and submitted under MRID No. 43588002.

Summary: In two independent microbial gene mutation assays, Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to five doses of RPA 201772 (98.7%) ranging from 50-1000 ug/plate (initial assay) or six doses of 25-1000 ug/plate (confirmatory assay) in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test systems in acetone.

The test material was insoluble at levels ≥ 500 ug/plate +/-S9 and noncytotoxic. There was also no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. Positive controls induced the expected mutagenic response in the appropriate strains.

Classification: Acceptable

c) Reference: Strang, P. (1993). RPA 201772: Investigation of mutagenic activity in the TK+/- mouse lymphoma cell system. Performed by Pharmaco-Life Science Research, Ltd., Suffolk, England and submitted under MRID No. 43573222.

Summary: In two independently performed *in vitro* mammalian cell forward gene mutation assays, L5178Y mouse lymphoma cells were exposed to five doses (37.5-600 ug/mL) of RPA 201772 (98.7%) in both the presence or absence of S9 activation. The S9 fraction was derived from Aroclor 1254 induced CD rat livers and the test material delivered to the test system in acetone.

RPA 201772 was not cytotoxic at insoluble (≥ 150 ug/mL +/- S9) or soluble (37.5 or 75 ug/mL +/- S9) levels. There was also no evidence of a mutagenic effect at any dose with or without S9 activation. Findings with the positive controls confirmed the sensitivity of the test system to detect mutagenesis.

Classification: Acceptable.

d) Reference: Dance, C.A. (1993). *In vitro* assessment for the clastogenic activity of RPA 201772 in cultured human lymphocytes. Performed by Pharmaco-Life Science Research, Ltd., Suffolk, England and submitted under MRID No. 43573221.

Summary: In an *in vitro* cytogenetic assay, human lymphocytes derived from a single donor were evaluated for chromosome damage 19 or 43 hours postexposure to three nonactivated doses (75, 300 or 600 ug/mL) and 16 or 40 hours after a 3-hour exposure to comparable S9-activated levels of RPA 201772 (98.7%). Only lymphocytes treated with 600 ug/mL +/- S9 were examined from the 43-hour harvest. The assay was repeated using equivalent doses with or without S9 activation and a 19-hour cell harvest. The S9 homogenate was derived from Aroclor 1254-induced CD rat livers and the test substances was delivered to the lymphocyte cultures in acetone.

Compound precipitation was seen at levels ≥ 300 ug/mL -S9 and at 600 ug/mL +S9. The test material was not cytotoxic at any dose or harvest time with or without S9 activation. There was also no evidence of a clastogenic effect at any dose or harvest time with or without S9 activation. The nonactivated and S9-activated

positive controls induced significant increases in structural chromosome aberrations at all harvest intervals.

Classification: Acceptable.

3. Chronic Toxicity Study

Reference: Brooker, A.J. (1994). RPA 201772: Toxicity to Dogs by Repeated Dietary Administration for 52 Weeks. Performed by Huntingdon Research Centre Ltd., Cambridgeshire, England and submitted under MRID No. 43573218.

Summary: In a chronic toxicity study (MRID 43573218), RPA 201772 (isoxaflutole 98.7% a.i.) was administered to five beagle dogs/sex/dose in the diet at dose levels of 0, 240, 1,200, 12,000, or 30,000 ppm (0, 8.56, 44.81, 453, or -- mg/kg/day, respectively, for males; 0, 8.41, 45.33, 498, or 1,254 mg/kg/day, respectively, for females) for 52 weeks. The 52 week mean intake value for males in the 30,000 ppm treatment group was not available because all dogs in that group were sacrificed after 26 weeks due to severe chronic reaction to the test substance.

Dogs in the $\geq 12,000$ ppm treatment groups (both sexes) had lower mean body weights than dogs in the control group; significantly lower in females. Females in these treatment groups showed a significant decrease in red blood cell indices (hematocrit, RBC, and hemoglobin) compared to controls. Males at 30,000 ppm exhibited marked reduction in these parameters. Males (at 12,000 ppm) and females (at $\geq 12,000$ ppm) also exhibited significant concomitant increases in platelet counts. At $\geq 12,000$ ppm, males and females exhibited significantly increased absolute and relative liver weights with friable surfaces and histopathological changes such as hepatocellular swelling, centrilobular clumping and margination of cytoplasmic staining, and centrilobular necrosis and fibrosis. There was an increased incidence of hypertrophy of the thyroid follicular epithelium in males at 12,000 ppm and in males and females at 30,000 ppm. Evidence of prominent hematopoiesis was observed in the sterna and/or femurs and joints of males and females in these treatment groups and an increased degree of extramedullary hematopoiesis was apparent in spleens of males at 30,000 ppm only. These findings correlated well with symptoms of chronic hemolytic anemia.

The LOEL is 12,000 ppm (453 mg/kg/day for males; 498 mg/kg/day for females), based on reduced weight gains compared to controls and intravascular hemolysis with associated clinical chemistry and histopathological findings. The NOEL is 1,200 ppm (44.81 mg/kg/day for males; 45.33 mg/kg/day for females).

Classification: Acceptable.

4. Mechanistic Studies

"MECHANISTIC STUDY FOR THYROID EFFECTS-[RAT]"

a) Reference: Chambers, P.R. (1995). RPA 201772. Effects on the thyroid in male rats after dietary administration for 2 weeks. Performed by Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England and submitted under MRID No. 43904818.

Summary: Male Crl:CD (SD) rats (14/dose), received RPA 201772 (99.7% a.i.) in the diet at dosage levels of 0 or 500 mg/kg/day for 14 days. A third group (positive control) of rats received 80 mg/kg/day sodium phenobarbital by gavage and an untreated diet.

RPA 201772 administration caused more than a two-fold increase in cytochrome P-450 dependent mixed-function oxidase enzymes and p-nitrophenol uridine 5'-diphosphatase-glucuronyltransferase (UDPGT) activity which resulted in increased clearance of ¹²⁵I-thyroxine from the blood as indicated by shorter half-life and decreases in plasma T₄ level. In addition, there were increases in liver and thyroid weights. The plasma T₃ level was unaffected. The significant reduction in the level of circulating T₄ was possibly the result of enhanced glucuronidation by hepatic UDPGT and a rapid systemic clearance of total radioactive ¹²⁵I-thyroxine in RPA 201772 treated group. Following intravenous administration of ¹²⁵I-thyroxine, the thyroid iodine uptake was slightly higher and thyroid weights were significantly higher than controls in RPA 201772 treated rats. The effect of RPA 201772 on thyroxine pharmacokinetics was compared to phenobarbital. The results are summarized in Table 7. They are supportive of the hypothesis that RPA 201772 may have induced thyroid tumors in male rats (MRID# 43904806) through a disruption in the thyroid-pituitary hormonal feedback mechanisms.

Classification: Acceptable

Table 7. Summary of Results

Parameters Measured	Dose Groups ^a		
	Control	RPA 201772	Phenobarbital
Absolute Liver Wt. in g (% of control)	13.9	21.1 (152%)	19.8 (143%)
Relative Liver Wt. in g (% of control)	5.07	7.5 (149%)	7.3 (145%)
Microsomal Protein (mg/g liver) (% of control)	18.1	31.6**(175%)	24.6**(136%)
P-450 (nmol/g liver)	16.1	57.9**	41.3**
Absolute Thyroid Wt. in mg (% of control)	18.7	20.1 (107%)	23.6*(126%)
UDPGT (mmol/hr/g liver)	57	216*	169**
PROD (nmol/hr/g liver)	5.1	137**	104**
T4 (ng/dl)	5.7	3.2*	4.9*
T3 (µg/dl)	74	68	69
125I-Thyroxine Kel (per hr)	0.0407	0.0520***	0.0428
125I-Thyroxine t1/2 (hr)	17.0	13.3	16.2
125I-Thyroxine Clearance (ml/min)	0.038	0.065***	0.049*

^aDose: RPA 201772 = 500 mg/kg/day; Phenobarbital = 80 mg/kg/day; * p<0.05; ** p<0.01; *** p<0.001;

The following two studies were conducted to establish dose-response and to investigate the role of mixed function oxidase system with respect to liver enlargement in RPA 201772 treated mice and rats:

"THE EFFECT OF DIETARY ADMINISTRATION FOR 14 DAYS ON THE LIVER ENZYMES OF MALE SPRAGUE-DAWLEY CD-1 RATS"

b) Reference: Price, S.C. (1994). RPA 201772. Effects of dietary administration for 14 days on the liver enzymes of male Sprague-Dawley CD-1 rats. Performed by Robens Institute of Health and Safety, University of Surrey, U.K. and submitted under MRID No. 43904819.

Summary: In this study, 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.

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.

Classification: Acceptable

c) Reference: Price, S.C. (1994). RPA 201772. Effects of dietary administration for 14 days on the liver enzymes of male CD-1 mice. Performed by Robens Institute of Health and Safety, University of Surrey, U.K. and submitted under MRID No. 43904820.

"THE EFFECT OF DIETARY ADMINISTRATION FOR 14 DAYS ON THE LIVER ENZYMES OF MALE CD-1 MICE"

Summary: In this study, groups of 25 male CD-1 mice received RPA 201772 (99.6% a.i.) in diet at dosage levels of 0, 175, 700, 2800 and 7000 ppm (0, 23, 91, 364 and 910 mg/kg/day, respectively) for 14 days.

RPA 201772 administration caused increase ($\geq 11\%$) 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. The test compound did not induce 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.

5. Structure-Activity Correlations

RPA 201772 is a member of a new class of chemicals known as isoxazole family. Therefore, no structure-activity correlations can be proposed.

F. Weight-of-Evidence Considerations

The Health Effects Division Carcinogenicity Peer Review Committee considered the following toxicology data in determining the carcinogenic potential of RPA 201772:

- 1) In the mouse carcinogenicity study, administration of RPA 201772 in the diet at 7,000 ppm for 78 weeks was associated with a significant increasing trend in hepatocellular adenoma and carcinoma as well as their combined tumor incidence at $p < 0.01$. There were significant differences in the pair-wise comparisons of the dosed groups with the controls in the incidence of adenoma, carcinoma (males only), and adenoma and/or carcinoma combined in both sexes at $p < 0.01$.
- 2) In the rat chronic/carcinogenicity study, administration of RPA 201772 at 500 mg/kg/day in the diet for 104 weeks was associated with a significant increasing trend in hepatocellular adenoma and carcinoma and their combined tumor incidence in males and females ($p \leq 0.05$ or $p < 0.01$). There were pair-wise differences in males and females vs controls in the incidence of hepatocellular adenoma and carcinoma as well as the combined tumor incidence ($p < 0.05$; $p < 0.01$). In males at 500 mg/kg/day, significant increasing trend in the occurrence of thyroid follicular cell adenoma was noted ($p < 0.001$); a pair-wise comparison with the control group indicated a significant difference at $p < 0.01$.
- 3) Significantly increased liver weights, and incidence of liver hypertrophy were noted in both sexes of rats (chronic/carcinogenicity study and reproduction study), mice (carcinogenicity study) and dogs (chronic study). It was noted that there were non-neoplastic lesions in the liver in the mouse, and in both the liver and thyroid in the rat. These were the same target sites for tumors.

Liver enzyme studies in rats and mice suggest that RPA 201772 appears to function as a phenobarbital type inducer of mixed function oxidase enzymes in the liver. The highest dose tested in these studies resulted in maximal enzyme induction and tumor production. Lower doses failed to produce maximal induction of mixed function oxidase enzymes and showed no evidence of induction of tumors. This observation suggests that a threshold may exist for the induction of liver tumors in rodents exposed to RPA 201772.

- 4) In the chronic/carcinogenicity feeding study in rats, statistically significant increase in the incidence of hyperplasia of the follicular epithelium of thyroid was noted in high dose males. Although thyroid amyloidosis was noted in mice thyroid follicular cell hyperplasia was not observed in

the carcinogenicity study. In the chronic dog study, hypertrophy of the thyroid follicular epithelium was observed in males at 12,000 ppm and males and females at 30,000 ppm. The following table summarizes the endpoints, NOELs and LOELs established in these studies:

Study	Endpoint	Sex	NOEL(mg/kg/day)	LOEL(mg/kg/day)
78-Week/Mouse	Hepatocellular tumors	M	64.4	977.3
		F	77.9	1161.1
	Hepatocellular hypertrophy	M	3.2	64.4
		F	77.9	1161.1
	Increased liver weights	M	3.2	64.4
		F	4.0	77.9
104-Week/Rat	Thyroid tumors	M	20	500
		F	500	--
	Thyroid hyperplasia	M	2	20
		F	500	--
	Thyroid weights	M	20	500
		F	500	--
	Hepatocellular tumors	M	20	500
		F	20	500
	Hepatocellular hypertrophy	M	2	20
		F	20	500
	Increased liver weights	M	20	500
		F	20	500
2-Gen. Reproduction study/Rat	Hepatocellular hypertrophy	M	1.74	17.6
		F	1.74	17.6
	Increased liver weights	M	1.74	17.6
		F	1.74	17.6
52-Week/Dog	Thyroid hypertrophy	M	44.81	453
		F	498	1254
	Hepatocellular necrosis/swelling/vacuolation	M	44.81	453
		F	45.33	498
	Increased liver weights	M	44.81	453
		F	45.33	498

- 5) RPA 201772 was not mutagenic in *in vivo* (mouse micronucleus) and *in-vitro* (gene mutation, mouse lymphoma and human lymphocyte) mutagenicity assays.

- 6) RPA 201772 is suggested to act as a phenobarbital type inducer of P-450 2B type microsomal enzymes. In male rats, it causes increases in cytochrome P-450 dependent mixed-function oxidase system and p-nitrophenol uridine 5'-diphosphatase-glucuronyltransferase (UDPGT) activity resulting in increased clearance of ¹²⁵I-thyroxine from the blood as indicated by shorter half-life and decreases in plasma T₄ level. It also increases liver and thyroid weights. RPA 201772 possibly induces thyroid tumors in male rats through a disruption in the thyroid-pituitary hormonal feedback mechanisms secondary to liver enzyme induction.
- 7) RPA 201772 is a member of a new class of chemicals known as isoxazoles. Therefore, no structure-activity correlations can be proposed.
- 8) The Committee considered the possibility of using the threshold model for thyroid neoplasms.

The following discussion has been taken from the Amitrole Peer Review Document (Rinde to Taylor, 11/30/92) and revised for isoxaflutole:

The guidance provided below is given in the Agency's DRAFT Policy Document (Assessment of thyroid follicular cell tumors, SAB Review Draft, April 1996):

"Studies over the last several decades in multiple laboratories and using a number of different treatment regimens (eg., iodine deficiency) have demonstrated the significance of long-term thyroid-pituitary hormonal imbalance in thyroid carcinogenesis. A consistent progression of events is noted: reduction in thyroid hormone concentrations, elevation in TSH levels, cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia. Hyperplasia and sometimes neoplasia of the pituitary may also be seen. A block in any of the early steps acts as a block for subsequent steps including tumor development, and cessation of treatment at an early stage in the progression results in regression toward normal thyroid structure and function. Based on these observations the Agency concludes that:

- a. thyroid follicular cell tumors may arise from long-term disturbances in thyroid-pituitary feedback under conditions of reduced circulating thyroid hormone and elevated TSH levels:
- b. the steps leading to these tumors are expected to show thresholds, such that the risks of tumor development are minimal when thyroid-pituitary homeostasis exists; and
- c. models that assume thresholds may be used to assess the

risks of thyroid follicular cell tumors where there is evidence of thyroid-pituitary hormonal imbalance."

Two basic questions must be addressed before this policy is applied.

"The first is a qualitative issue which addresses whether it is reasonable to presume that the neoplasms are due to thyroid-pituitary imbalance. A corollary issue is the extent to which other carcinogenic mechanisms can be discounted. The second question concerns the procedures to be employed in estimating the risks of these agents."

"The answers to the first question allow one to assign chemicals producing thyroid tumors to one of three categories. The assumption is based upon knowledge as to whether the chemical disrupts thyroid-pituitary feedback, whether tumors other than thyroid follicular cell (and relevant pituitary) tumors are found, and whether mechanisms other than thyroid-pituitary imbalance may apply to the observed tumor response."

Determination of whether neoplasms are due to thyroid-pituitary imbalance

The document goes on to describe the 3 factors which should be considered in making this determination (answering the first question, or "qualitative issue"). These are addressed as they apply to RPA 201772 as follows:

FACTOR I. Consideration of whether the thyroid tumors associated with administration of RPA 201772 can be attributed to disruption of the thyroid-pituitary hormonal balance. (In addressing this factor, the Policy states, 6 indicators should be considered.)

a. Goitrogenic activity in vivo:

There was increase in thyroid weights and thyroid follicular cell hyperplasia in high dose males in the chronic/carcinogenicity rat study. A low incidence of thyroid follicular cell hyperplasia was seen during interim but not during reversibility period. These effects were seen primarily in those animals which died or sacrificed during the treatment period as well as at final sacrifice. Although no thyroid tumors were seen in mice and dogs, goitrogenic activity was seen in mice (thyroid amyloidosis) and dogs (thyroid hypertrophy).

b. Clinical chemistry changes (eg., reduced thyroid hormone

and increased TSH serum concentrations):

In the special thyroid function study in rats, following intravenous administration of ^{125}I -thyroxine, the thyroid iodine uptake was slightly higher. There was increased clearance of ^{125}I -thyroxine from the blood as indicated by shorter half-life and decreases in plasma T_4 level. The plasma T_3 level was unaffected. TSH levels were not measured.

c. Specific evidence of reduced hormone synthesis (eg., inhibited iodine uptake or increased thyroid hormone clearance (eg., enhanced biliary excretion)):

The following effects were observed in the thyroid function studies: two-fold increase in cytochrome P-450 dependent mixed-function oxidase system and p-nitrophenol uridine 5'-diphosphatase-glucuronyltransferase (UDPGT) activity. The increased clearance of ^{125}I -thyroxine from the blood was evident by shorter half-life and decreases in plasma T_4 level. The plasma T_3 level was unaffected. The significant reduction in the level of circulating T_4 was possibly the result of enhanced glucuronidation by hepatic UDPGT and a rapid systemic clearance of total radioactive ^{125}I -thyroxine in RPA 201772 treated group.

d. Evidence of progression (eg., hyperplasia, nodular hyperplasia - neoplasia):

There was evidence of progression (hyperplasia to neoplasia) in rats. In the two-year rat study, increased incidence of cystic follicular hyperplasia in the 20 and 500 mg/kg/day group males and the 500 mg/kg/day group females. Significant increases in thyroid follicular cell adenomas were evident ($p < 0.001$) in males at 500 mg/kg/day, but not in females by the end of the study, although there were positive trends for both sexes ($p \leq 0.05$).

e. Reversibility of effects after exposure is terminated:

In the two-year rat study, the effects on thyroid (hyperplasia and neoplasia) were seen by the end of the study. Therefore, the reversibility of the observed effects could not be determined. Also in the special thyroid function study, no attempt was made to determine whether the following effects were reversible: increased liver and thyroid weights, hepatic T_4 UDPGT activity.

f. SAR to other thyroid tumorigens: No structure-activity comparisons can be made.

Based on the overall judgment of the 6 indicators in Factor I,

it may be concluded that there are sufficient data to determine whether or not there is suggestive evidence that the thyroid tumors in the rat associated with administration of RPA 201772 may be due to a disruption in the thyroid-pituitary status.

FACTOR II. Consideration of the extent to which genotoxicity may account for the observed tumor effects.

The genotoxicity data are negative. There is no indication that genotoxicity plays a role in the tumorigenic activity for this chemical.

FACTOR III. Evaluation of neoplasms other than thyroid follicular cell tumors (and relevant pituitary tumors).

There was an increased incidence of both benign and malignant hepatocellular tumors (adenomas and carcinomas) in male rats in the 500 mg/kg/day dose groups when compared to the controls at the carcinogenicity phase necropsy. Male rats had a significant dose-related increasing trend in both adenoma, carcinoma and their combined tumors incidences at $p \leq 0.05$ and $p < 0.001$. Also there was significant difference in the pairwise comparison with the controls for these tumors at $p < 0.01$ and $p < 0.001$.

Conclusions: As indicated above, based on the overall judgment of the 6 indicators in Factor I, it may be concluded that there are sufficient data to determine whether or not there is suggestive evidence that the thyroid tumors in the male rat associated with administration of RPA 201772 may be due to a disruption in the thyroid-pituitary status. Adding in Factors II and III, this conclusion still stands. All of the criteria for a threshold effect have been met except one: the presence of a second tumor type. The liver tumors were statistically significantly increased in a pairwise comparison with the control group. There is no information on SAR. Nevertheless, other mechanisms of tumor induction by RPA 201772 cannot be excluded.

9) Mechanistic considerations

The Registrant submitted a document entitled "The Carcinogenic Potential and Appropriate Classification of Isoxaflutole". A summary is provided below:

"In view of the similarity in the pattern of enzyme induction and tumor profile produced by isoxaflutole and phenobarbital in rodents, isoxaflutole should be identified as an agent likely to be carcinogenic in rodents under high exposure conditions but not likely to be carcinogenic to humans under anticipated oral, dermal

or inhalation exposure conditions. The weight of evidence is based on (a) liver tumors in mice and rats and thyroid tumors in male rats; (b) the absence of tumors at any other site in mice and rats; (c) the induction of mixed function oxidase enzymes at high but not low exposure; (d) the imbalance of thyroid hormones in rats at high but not low exposures; and (e) the absence of mutagenic activity or structural alerts. A strong mode of action basis exists for the requirements of high doses of isoxaflutole which lead to induction of mixed function oxidase enzymes that in rats resulted in increased clearance of T_4 and a sustained release of TSH as well as the necessity of microsomal enzyme induction and thyroid hormonal imbalance for tumor hazard potential; lower doses of isoxaflutole which failed to produce maximal induction of mixed function oxidase enzymes showed no evidence of tumor induction. Therefore, the dose response should assume nonlinearity."

Recommendations from the cancer peer review of isoxaflutole:

It is the Committee's opinion that the registrant has taken important steps toward demonstrating a mode of action of mouse liver tumor formation. However, these studies have been conducted over short periods of time (14 days), and there is no linkage of precursor events with ultimate tumor formation. It is important that further work be conducted to better establish the events in the carcinogenic process. Repeat dosing studies that include elements of dose response and time action could be designed that would specifically address the various critical events that are part of the process. Such things might include but are not necessarily limited to such effects as liver enlargement, induction of particular microsomal enzymes, development of foci of alteration, growth of foci, development and progression of hepatocellular hyperplasia, with linkage to benign and malignant tumor formation. Stop studies could demonstrate the reversibility of various events.

The registrant claims that isoxaflutole and phenobarbital are working through the same types of microsomal enzyme induction. Data to date show that there are similarities and differences in the spectrum of P450 enzyme isoforms that are induced by the two chemicals. It is important to determine which of these forms are relevant to the proposed carcinogenic mode of action of the compounds, and which ones are not, and why. A qualitative and quantitative comparison should also be made between the murine and human P450 subtypes so that the implications of the rodent findings to humans can be made.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the *EPA proposed Guidelines for Carcinogenic Risk Assessment (April 23, 1996)* for classifying the weight of evidence for isoxaflutole.

The CPRC unanimously agreed to characterize the weight of the evidence for isoxaflutole as "likely" to be carcinogenic to humans by all routes of exposure. This was based on increased incidences of liver tumors in 2 rodent species in adequate long-term studies. The liver tumors occurred in both sexes in both mice and rats, and had an early onset in male mice; there was also an increased incidence of thyroid tumors in male rats.

The studies submitted by the registrant to show a mechanistic basis for the liver tumors were considered by the CPRC to be suggestive, but not convincing (see Section F.). The CPRC agreed that the mechanistic evidence presented for the thyroid tumors appeared to be scientifically plausible and consistent with EPA current policy.

Therefore, the CPRC recommended that for the purpose of risk characterization, a non-linear approach - Margin of Exposure (MOE) be applied to the most sensitive precursor lesion in the male rat thyroid, and that a linear low-dose extrapolation be applied for the tumors of the rat liver.