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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUN 11 1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **RPA 201772 *(ISOXAFLUTOLE)* Herbicide:** Review of Toxicity Studies Submitted to Support Registration and Permanent Tolerance Petition

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TASK ID: Submission: S501233, S508413 & S519523 .
DP Barcodes(s): D224202, D227954 & D234283

PC Code:123000 **Common Name: RPA 201772**

CHEMICAL NAME: **5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethyl-benzoyl)-isoxazole**

REGISTRANT: Rhone-Poulenc Agrochimie, Lyon, France

ACTION REQUESTED: Review toxicity studies to support Registration and Permanent Tolerance Petition for Herbicide RPA 201772 for use on field corn. The formulated product is a 75% water dispersible granular (WG) formulation of isoxaflutole, referred to as EXP 31130A or BALANCE™ 75 WDG.

RECOMMENDATIONS: The Registrant has submitted an application for registration of RPA 201772 for preplant and preemergence weed control in field corn at low rates of application (up to 0.18 lbs. a.i. per acre). The Registrant has proposed tolerances of 0.1 ppm for corn grain and 0.4 ppm for corn forage and corn fodder. Studies submitted to support these applications include: Acute toxicity (refer to memo from S. Diwan to J. Miller/D. Kenny/PM-23 dated Sept. 15, 1995), acute and subchronic neurotoxicity, 21-day dermal, 28-day oral toxicity, developmental and reproductive toxicity, chronic and carcinogenicity, mutagenicity, as well metabolism studies. In addition, the Registrant has submitted dermal absorption study, comparative tyrosine tolerance and metabolism as well as mechanistic studies to investigate the underlying mechanisms of ocular, liver and thyroid toxicity with the technical material. Attached to this ***MEMORANDUM*** is the Toxicity Profile, the Executive Summaries and the Data Evaluation Records for the studies reviewed under this submission. The Registrant has satisfied the toxicology data requirements for the Registration of RPA 201772. Therefore, based on the available toxicity data, the Toxicology Branch II recommends approval for Section 3 registration of isoxaflutole or the proposed use.

TABLE 1
TOXICITY PROFILE FOR ISOXAFLUTOLE (RPA 201772 Technical)

§ No.	Study Type	MRID No.	Results	Toxicity Category	Required	Status
81-1	Acute Oral*	43573212	LD50 = >5,000 mg/kg	IV	Yes	Satisfied
81-2	Acute Dermal*	43573213	LD50 = >2,000 mg/kg	III	Yes	Satisfied
81-3	Acute Inhalation*	43573214	LC50 = >5.23 mg/L	IV	Yes	Satisfied
81-4	Primary Eye Irritation*	43573215	Non-irritating	IV	Yes	Satisfied
81-5	Primary Skin Irritation*	43573216	Non-irritating	IV	Yes	Satisfied
81-6	Dermal Sensitization*	43573217	Non-sensitizer	NA	Yes	Satisfied
81-8	Acute Neurotoxicity*	43904804	NOEL = 500 mg/kg/day in males and 2,000 mg/kg (HDT) in females LOEL = 2000 mg/kg/day in males and ≥2,000 mg/kg/day in females	NA	Yes	Satisfied
82-2	21-Day Dermal-Rat*	43573219	NOEL = ≥1,000 mg/kg/day LOEL = Not achieved	NA	Yes	Satisfied
82-7	90-Day Subchronic Neurotoxicity- Rat	43904805	NOEL = 250 mg/kg/day in males and 750 mg/kg/day in females LOEL = 750 mg/kg/day in males based on decreases in body weight and body weight gain; LOEL in females = Not achieved	NA	Yes	Satisfied
83-1 (b)	Chronic Toxicity - Dog	43573218	NOEL = 25 ppm (3.2 mg/kg/day for males and 4.0 mg/kg/day for females) LOEL = 500 ppm (64.4 mg/kg/day for males and 77.9 mg/kg/day for females), based on decreased body weight gains, increased liver weights, and increased incidence of histopathological liver changes. RPA 201772 is carcinogenic at dosage of 500 ppm inducing hepato-cellular adenoma and carcinoma in both sexes.	NA	Yes	Satisfied

Table 1 (Continued)-

§ No.	Study Type	MRID No.	Results	Toxicity Category	Required	Status
83-2(a)	Carcinogenicity - Mouse	43904807	NOEL = 1,2000 ppm (44.81 mg/kg/day for males; 45.33 mg/kg/day for females LOEL = 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.	NA	Yes	Satisfied
83-3 (a)	Developmental - Rat*	43573220	Maternal NOEL = 100 mg/kg/day Maternal LOEL = 500 mg/kg/day based on increased incidence of salivation; decreased body weight, weight gain, and food consumption during the dosing period Develop. NOEL = 10 mg/kg/day Develop. LOEL = 100 mg/kg/day based on growth retardations (decreased fetal body weight; increased incidence of delayed ossification of sternebrae, metacarpals and metatarsals	NA	Yes	Satisfied
83-3 (b)	Developmental- Rabbit	43904808	Maternal NOEL: 20 mg/kg/day Maternal LOEL = 100 mg/kg/day based on increased incidence of clinical signs and decreased body weight gains and food consumption Develop. NOEL = <5 mg/kg/day Develop. LOEL = 5 mg/kg/day based on increased incidence of fetuses with 27th presacral vertebrae	NA	Yes	Satisfied

Table 1 (Continued)-

§ No.	Study Type	MRID NO.	Results	Toxicity Category	Required	Status
83-4	Reproduction - Rat	43904809	Systemic LOEL = 17.6 mg/kg/day based on increased liver weights and hypertrophy in both sexes Systemic NOEL = 1.74 mg/kg/day Develop. NOEL = 1.74 mg/kg/day Develop. LOEL = 17.6 mg/kg/day based on decreased litter viability Repro. LOEL = >1.74 mg/kg/day based on lack of reproductive effects Repro. NOEL = ≥17.6 mg/kg/day	NA	Yes	Satisfied
83-5	Chronic/Carcinogenicity - Rat	43904806	NOEL = 2.0 mg/kg/day LOEL = 20 mg/kg/day based on liver, thyroid, ocular, and nervous system toxicity in males and liver toxicity in females RPA 201772 is carcinogenic at dosages of 500 mg/kg/day inducing hepatocellular adenoma and carcinoma in both sexes and thyroid follicular cell carcinoma in males.	NA	Yes	Satisfied
84-2(b)(1)	In vitro cytogenetic Assay - Human Lymphocytes*	43573221	Negative	NA	Yes	Satisfied

Table 1 Continued -

§ No.	Study Type	MRID No.	Results	Toxicity Category	Required	Status
84-2(b)(2)	Gene mutation Assay - Mouse Lymphoma Assay*	43573222	Negative	NA	Yes	Satisfied
84-2(b)(2)(B)	Micronucleus Assay - Mouse*	43573223	Negative	NA	Yes	Satisfied
84-2(b)(3)	Gene Mutation Assay - Ames*	43588002	Negative	NA	Yes	Satisfied
85-1	Metabolism - Rat	43573224	RPA 201772 is rapidly and extensively metabolized; RPA 202248, a major metabolite represented ≥70% of the radioactivity excreted in the urine and feces. The other minor metabolite, RPA 203328, was more polar.	NA	Yes	Satisfied
85-3	Dermal Absorption- Rat	44044702	RPA 201772 is absorbed and excreted slowly and is mostly retained in carcass. Due to slow excretion, it bioaccumulates over increased duration of exposure.	NA	Yes	Satisfied

* Toxicity summaries for these studies are provided in an earlier memo from S. Diwan to J. Miller/D. Kenny/PM-23 dated Sept. 15, 1995.

Toxicology Issues

1. TES

The data for RPA 201772 was reviewed by the Toxicology Endpoint Selection Committee on 04/29/97. The Committee selected the following endpoints for Acute and Chronic Dietary Risk assessment:

- Acute Dietary Endpoint (One day)- Females 13+ Years: LOEL of 5 mg/kg/day was selected based on increased incidence of 27th pre-sacral vertebrae; a NOEL was not established in this study. Since LOEL was used, an extra modifying factor of 3 was recommended in addition to factor of 100 for inter and intra-species variation.

- Chronic Occupational or Residential Exposure (Several months to Lifetime): A NOEL of 2 mg/kg/day based on liver and thyroid toxicity at 20 mg/kg/day (LOEL) was recommended for non-carcinogenic risk assessment. Since the dose identified is from an oral study, the Committee recommended using dermal absorption rate of 0.2% in risk calculations.

2. RfD

The data for RPA 201772 were presented to the RfD/Peer review Committee for consideration on 04/24/97. The Committee recommended using the NOEL of 2 mg/kg/day based upon a LOEL of 20 mg/kg/day for liver toxicity in a chronic/carcinogencity study in rats. The Committee recommeded an RfD of 0.02 mg/kg/day. An uncertailty factor of 100 for interspecies extrapolation and intraspecies variability was used.

2. Carcinogenicity

The data on carcinogenicity of RPA 201772 in rats and mice was presented to the Cancer Peer Review committe on 06/09/97. The cancer classification for RPA 201772 is currently under consideration by the Committee. The results of *in vivo* and *in vitro* mutagenicity studies were negative. These studies are acceptable and fulfill guidelines 84–2a and 84–4.

3. Toxicology data gaps - None.

Conclusions/Recommendations

Based on the review of the toxicology data, Toxicology Branch II has determined that the data base for RPA 201772 is adequate to support the registration for use as pre-plant and pre-emergence herbicide in field corn.

I. EXECUTIVE SUMMARIES OF TOXICOLOGY STUDIES WITH RPA 201772 TECHNICAL

The executive summaries for acute toxicity studies (§81-1 thru 81-6), 21-day dermal toxicity study in rats (§82-2), developmental toxicity study in rats (§83-3a) as well as mutagenicity studies (§84-2) are provided in an earlier memo from S. Diwan to J. Miller/D. Kenny/PM-23 dated Sept. 15, 1995 and brief summaries are presented in Table 1. The executive summaries of studies included in this submission are as follows:

§81-8. *"AN ACUTE NEUROTOXICITY STUDY OF RPA 201772 IN THE RAT VIA ORAL GAVAGE ADMINISTRATION"*

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID # 43904804), CD rats (10/sex/group) received a single oral gavage administration of RPA 201772 in 0.5% aqueous methylcellulose at doses of 0 (vehicle only), 125, 500 and 2000 mg/kg body weight.
No compound-related effects on body weight, body weight gain and food consumption were noted. There were significant decreases in landing foot splay measurements in high-dose males during the functional observational battery test. indicating impairment of neuromuscular function. No significant differences in motor activity as well as gross and microscopic neuropathological findings among the control and treated animals.

LOEL for systemic toxicity = 2,000 mg/kg/day in males and >2000 mg/kg in females
NOEL for systemic toxicity = 500 mg/kg/day in males and 2000 mg/kg (limit dose) in females

CORE CLASSIFICATION: This study is classified as **acceptable** and **satisfies** guideline requirements (§81-8) for an acute neurotoxicity study in rats.

§82-7. *"A SUBCHRONIC (3-MONTH) NEUROTOXICITY STUDY OF RPA 201772 IN THE RAT VIA DIETARY ADMINISTRATION"*

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID # 43904805), RPA 201772 (99.2%) was administered to CD rats (10/sex/group) at dietary levels of 0, 25, 250 and 750 mg/kg/day for 90 days.

Treatment-related effects observed in high-dose males consisted of decreases in body weight (6-13% of control) and body weight gain (18-30% of control). No neurobehavioral effects were observed in neuropathology, motor activity and functional observational battery assessments.

LOEL was established at 750 mg/kg/day in males based on decreases in body weight and body weight gain; LOEL in females was >750 mg/kg/day.
NOEL was 250 mg/kg/day in males and 750 mg/kg/day in females.

CORE CLASSIFICATION: This study is classified as Acceptable and satisfies the §82-7 guideline requirement for a subchronic neurotoxicity study in rats.

83-1b. ***"TOXICITY TO DOGS BY REPEATED DIETARY ADMINISTRATION FOR 52 WEEKS"***

EXECUTIVE 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 ≥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 ≥12,000 ppm) also exhibited significant concomitant increases in platelet counts. At ≥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 males at 12,000 ppm and 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).

CORE CLASSIFICATION: This chronic toxicity study in the dog is **Acceptable** and does satisfy the guideline requirements for a chronic oral study (§83-1b) in dogs.

§83-2 (a). ***"ONCOGENICITY STUDY BY DIETARY ADMINISTRATION TO CD-1 MICE FOR 78 WEEKS"***

EXECUTIVE SUMMARY: In a 78-week carcinogenicity study (MRID 43904807), RPA 201772 (isoxaflutole, ≥98.7% ai) was fed to 64 or 76 mice/sex/dose at dietary 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 pm doses) and at 52 weeks (12 mice/sex at all dose levels).

RPA 201772 had no significant effect on the survival of animals. Systemic signs of toxicity in the treated groups included: 1) decreased body weight gain in both sexes at 500 ppm (males -16% and females -22%) and 7,000 ppm (males -28% and females -42%); values for the 25 ppm group females at 78 weeks were also decreased (down 15% 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 hemopoiesis in the spleen was noted in both sexes; 6) liver adenoma and carcinoma were observed in both sexes at 7,000 ppm in the 52-week and 78-week studies.

Among scheduled and unscheduled deaths in the 78-week study, there were significant occurrences of adenomas in 27/52 males (52%) and 15/52 females (29%), and carcinomas in 17/52 males (33%) and 4/52 females (8%;non-significant). The incidences of these tumors exceeded the corresponding historical incidence with this species, in this laboratory. Combined liver tumor incidences at 7,000 ppm were 73% for males and 35% for females. At 500 ppm the incidences of 17% adenomas and 15% carcinomas in males and 2% adenomas in females were not statistically significant, but exceeded the means for historical controls. The 52- and 78-week studies revealed a dose-related decrease in the first occurrence of carcinomas in males; the earliest carcinomas were observed at 78, 71, 52, and 47 weeks at the 0 through 7,000 ppm doses. There were no carcinomas in females up to 78 weeks at 0, 25, or 500 ppm, although, the earliest finding at 7000 ppm was at 60 days.

The LOEL for this study is 500 ppm (64.4 mg/kg/day for males and 77.9 mg/kg/day for females), based on decreased body weight gains, increased liver weights, and increased incidence of histopathological liver changes. The NOEL is 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.

Under conditions of this study, RPA 201772 appears to induce hepatocellular adenomas and carcinomas of the liver in male and female CD-1 mice. The chemical was tested at doses sufficient to measure its carcinogenic potential.

CORE CLASSIFICATION: This carcinogenicity study is **Acceptable** and **satisfies** the guideline requirements for a carcinogenicity study (§83-2 [a]) in the mouse.

83-3(b). *"STUDY OF EMBRYO-FETAL TOXICITY IN THE RABBIT BY ORAL (GAVAGE) ADMINISTRATION"*

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID# 43904808), RPA 201772 (99.6 a.i.) was administered to twenty-five female New Zealand White Rabbits by gavage at dose levels of 0, 5, 20, or 100 mg/kg/day from gestational days 6–19, inclusive.

Maternal toxicity, observed at 100 mg/kg/day, was manifested as increased incidence of clinical signs (little diet eaten and few feces) and decreased body weight gain (23%) and food consumption ($\geq$15%) during the dosing period. **The maternal LOEL is 100 mg/kg/day, based on increased incidence of clinical signs, decreased body weight gains and food consumption. The maternal NOEL is 20 mg/kg/day.**

Developmental toxicity, observed at 5 mg/kg/day consisted of increased incidence of 27th pre-sacral vertebrae. Additional findings noted at 20 and 100 mg/kg/day were manifested as increased number of postimplantation loss and late resorptions, as well as growth retardation in the form of generalized reduction in skeletal ossification, and increased incidence of 13 pairs of ribs. At 100 mg/kg/day, an increased incidence of fetuses with incisors not erupted was also observed. **The LOEL for developmental toxicity is 5 mg/kg/day, based on increased incidence of fetuses with 27th pre-sacral vertebrae. The developmental NOEL is <5 mg/kg/day.**

CORE CLASSIFICATION: This study is classified as acceptable and satisfies the guideline requirement for a developmental toxicity study (83-3b) in rabbits (For developmental study in rats refer to memorandum by S.Diwan to J.Miller/D. Kenny/PM-23 dated Sept. 15, 1995).

§83-4. *"TWO GENERATION REPRODUCTION STUDY WITH RPA 201772 IN RATS"*

EXECUTIVE SUMMARY: RPA 201772 (98.7% a.i.) was administered to Crl:CD®BR VAF/Plus® rats (30/sex/group) at dietary levels of 0, 0.5, 2, 20 or 500 mg/kg/day (actual levels in males: 0. 0.45, 1.76, 17.4 or 414 mg/kg/day; for females: 0, 0,46, 1.79, 17.7 or 437 mg/kg/day, respectively) for two successive generations (MRID # 4390489).

Evidence of toxicity observed in the male and female parental rats of both generations at 20 and 500 mg/kg/day consisted of increased absolute and relative liver weights associated with liver hypertrophy; at 500 mg/kg/day (HDT), decreased body weight, body weight gain and food consumption during premating and gestation, and increased incidence of subacute inflammation of the cornea of the eye in F_0 adults as well as keratitis in F_1 adults were reported. The reproductive performance of animals was unaffected. Treatment-related effects were observed in F_1 and F_2 offspring: at 20 and 500 mg/kg/day, reduction in pup survival was noted; at 500

mg/kg/day, decrease in body weights of F_1 and F_2 pups, increased incidence of chronic keratitis, low incidence of inflammation of the iris, as well as retinal and vitreous bleeding in F_2 pups and weanlings were observed. Necropsy of F_1 and F_2 pups revealed an increased number of pups with no milk in the stomach and underdeveloped renal papillae.

Systemic LOEL = 17.4 mg/kg/day for males and females, based upon increased liver weights and hypertrophy
Systemic NOEL = 1.76 mg/kg/day for males and females

Developmental LOEL = 17.4 mg/kg/day, based on decreased litter viability.
Developmental LOEL = 1.76 mg/kg/day

Reproductive LOEL = > 17.4 mg/kg/day, based on lack of reproductive effects
Reproductive NOEL = ≥ 17.4 mg/kg/day

CORE CLASSIFICATION: This study is classified as acceptable/Guideline and satisfies the guideline requirement for a 2-generation reproduction study (§83-4) in rats.

§83-5. *"COMBINED ONCOGENICITY AND CHRONIC TOXICITY STUDY BY DIETARY ADMINISTRATION TO CD RATS FOR 104 WEEKS"*

EXECUTIVE SUMMARY: In a combined chronic toxicity/carcinogenicity study (MRID 43904806), RPA201772, (93-99.2% a.i.) was continuously administered to 75 CD 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.

Evidence of systemic toxicity observed in one or both sexes included: 1) abnormal gait, limited use of limbs (in both sexes) at 500 mg/kg/day and eye opacity (in males) at ≥20 mg/kg/day); 2) lower body weight gains (≥36% in both sexes) and food consumption (12% in females) at 500 mg/kg/day; 3) decreased food efficiency (≥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) changes on gross necropsy 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) at 500 mg/kg/day; and 7) increased incidence of periacinar 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 500 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).

Under the conditions of this study, RPA 201772 induced benign and malignant tumors of the liver in both sexes at 500 mg/kg/day (hepatocellular adenomas in 14/75 in males and 29/74 in females vs. 2/75 and 4/74 in the control group rats and hepatocellular carcinomas (17/75 and 24/74 vs. 5/75 and 0/74 in the controls, respectively). Combined incidences of liver adenoma/carcinoma in males and females were 31/75 and 46/74, respectively, with animals bearing carcinomas in the majority. Thyroid follicular adenomas occurred with increased frequency in 500 mg/kg/day males (15/75 vs 3/74 in controls). The above tumor incidences exceeded the historical incidence of these tumors for this strain in this laboratory.
The study demonstrated that RPA 201772 is carcinogenic to rats at dosage of 500 mg/kg/day. The chemical was administered at dosages sufficient to test its carcinogenic potential.

The chronic LOEL is 20 mg/kg/day based on liver, thyroid, ocular, and nervous system toxicity in males and liver toxicity in females. The chronic NOEL is 2.0 mg/kg/day.

CORE CLASSIFICATION: This study is classified as **acceptable** and **satisfies** the guideline requirements for a chronic toxicity/carcinogenicity study (§83-5) in the rat.

§85-1. *"RPA 201772: ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN THE RAT"*

EXECUTIVE SUMMARY: In a metabolism study (MRID # 43573224), ^{14}C-RPA 201772 (98.7%) was administered to groups (5/sex/dose) of male and female Sprague-Dawley (CD) rats by gavage at a single low oral dose (1 mg/kg), repeated low 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). In addition, pharmacokinetics in blood was investigated using two groups of 10 rats (5/sex/dose) that received a single oral dose of 1 or 100 mg/kg of ^{14}C-RPA 201772.

^{14}C-RPA 201772 was rapidly and extensively absorbed and metabolized. RPA 202248, a major metabolite, represented ≥70% of the radioactivity excreted in the urine and feces from the two low dose groups. 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 major portion of radiolabel was excreted via the feces (males: 62.99%, females: 55.23%) in the high dose group. The majority of radiolabel was eliminated in the first 24 and 48 hours for the low and the high dose groups, respectively. 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 ^{14}C-RPA 201772.

CORE CLASSIFICATION:This is classified as ***acceptable*** and **satisfies** the guideline requirement for a metabolism study (85-1) in *rats*.

§85-2. *"DERMAL ABSORPTION OF ^{14}C-ISOXAFLUTOLE IN MALE RATS"*

EXECUTIVE SUMMARY: Male rats were dosed with RPA 201772 at 0.865, 7.32 and 79.00 μg/cm2 (MRID # 44044702). Four animals per dose were exposed for 0.5, 1, 2, 4, 10 and 24 hours. Only a small portion of the dose was absorbed (11.9, 6.3 and 2.11%, respectively at 24 hours). The results of the study indicate that RPA 201772 is absorbed and excreted slowly and is mostly retained in carcass. Due to slow rate of urinary excretion, the quantity in the carcass increased with increasing duration of exposure. RPA 201772 showed bioaccumulation at all three dose levels.

CORE CLASSIFICATION:This study is classified as ***acceptable*** and **satisfies** the guideline requirement for a metabolism study (85-2) in *rats*.

II. EXECUTIVE SUMMARIES FOR THE MECHANISTIC STUDIES (NONGUIDELINE) ON RPA 201772

The Toxicity Profile for the non-guideline studies is presented in Table 2.
The summaries are provided below.

"COMPARATIVE TYROSINE METABOLISM STUDY- [RAT AND MOUSE]".

EXECUTIVE SUMMARY: In a comparative metabolism study (MRID # 43904815), RPA 201772 (98.3%) was administered to groups (5/species) of male Sprague-Dawley (CD) rats and CD-1 mice by gavage at a single dose (10 mg/kg) followed one hour later with a single oral dose of ^{14}C-Tyrosine (500 mg/kg).

For both species, a major portion of ^{14}C-Tyrosine administered dose was eliminated via urine and expired air. Urinary elimination (mice: 46.79%, rats: 15.70%) was predominant in the mouse while a significant portion of radiolabel was predominantly excreted via the expired air as CO_2 in the rat (mice: 6.47%, rat: 17.04%) during the first 48 hours following administration of ^{14}C-Tyrosine. HPLC analysis of ^{14}C-Tyrosine metabolites found in the urine of both species revealed higher amounts of two major metabolites, HPLA and HPAA, in the mouse than those in the rat. The enzymatic hydrolysis of conjugates indicated that some metabolites were excreted as glucuronides and/or sulfates in urine; these did not include hydroxyphenyl lactic acid (HPLA) and hydroxyphenyl acetic acid (HPAA).

The results of this study indicate species-related qualitative and quantitative differences in the excretion of tyrosine following single simultaneous administrations of RPA 201772 and ^{14}C-Tyrosine to male mice and rats. The findings of the study further suggests differential ability of the two species to alternatively utilize a by-pass metabolic route for the blocked tyrosine pathway via HPLA and HPAA. However, the Registrant should provide additional data as discussed

under deficiencies.

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

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

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

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

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

TABLE 2
TOXICITY PROFILE FOR NON-GUIDELINE STUDIES FOR ISOXAFLUTOLE
RPA 201772 (Technical)

Study Type	MRID No.	Results	Status
Comparative Tyrosine Metabolism - Rat and Mouse	43904815	There are species-related qualitative and quantitative differences in the excretion of tyrosine following single simultaneous administrations of RPA 201772 and ^{14}C-Tyrosine to male mice and rats. The results indicate differential ability of the two species to alternatively utilize a by-pass metabolic route for the blocked tyrosine pathway via HPLA and HPAA. However, the Registrant should provide additional data as discussed under deficiencies.	Unacceptable
Tyrosine: Ocular Toxicity - Rat and Mouse	43904816	Dietary administration of 5% tyrosine produced corneal opacities in 3 of 5 male CD rats and 1/5 Brown rats. These effects were not seen in female rats or mice of either sex.	Acceptable
Comparative Tyrosine Tolerance - Rat	43904817	Both RPA 201772 and NTBC affect the main catabolic pathway for tyrosine by inhibiting 4-HPPDase enzyme.	Acceptable
Mechanistic Study on Thyroid Effects - Rat	43904818	RPA 201772 possibly induces thyroid tumors in male rats (MRID# 43904806) through a disruption in the thyroid-pituitary hormonal feedback mechanisms	Acceptable
14-Day Liver Enzyme Study - Rat	43904819	RPA 201772 caused increased absolute and relative liver weights in rats via induction of MFO enzymes. The specific forms of isoenzymes being responsible were PROD and BROD enzymes, the induction of which may be attributed to the P-450 2B family. Therefore, RPA 201772 appears to function as a phenobarbital type inducer of P-450 2B family.	Acceptable
14-Day Liver Enzyme Study - Mouse	43904820	As in case of rats, RPA 201772 appears to function as a phenobarbital type inducer of P-450 2B family.	Acceptable

"TYROSINE: EXPLORATORY 14-DAY (OCULAR TOXICITY) STUDY IN THE RAT AND MOUSE"

EXECUTIVE SUMMARY: In this exploratory study (MRID # 43904816), groups of 5 male and 5 female CD rats, Brown Norway rats and CD-1 mice received 0, 2 or 5% tyrosine (0, 0.1 or 0.25 mg/kg/day for rats and 0, 0.26 or 0.65 mg/kg/day for mice, respectively) in their diet for 14 days.

Within 48 hours of dietary administration of 5% tyrosine, corneal opacities with superficial keratitis were observed in 3 of 5 male CD rats; by Day 7, corneal opacities developed in all five rats. At study termination, these corneal lesions were found to be associated with elevated plasma tyrosine levels. One of five male Brown Norway rats receiving 5% tyrosine had slight bilateral opacities at 14 days accompanied by a high plasma tyrosine level. Histopathology revealed changes characteristic of corneal opacity involving various corneal layers and ciliary processes. These effects were not seen in female rats or mice of either sex. Dietary administration of 2% tyrosine failed to produce similar effects in any group or in any female rats and both sexes of mice. There were no differences between the control and treated groups in any of the other parameters measured.

"COMPARATIVE TYROSINE TOLERANCE STUDY-[RAT]"

EXECUTIVE SUMMARY: In a comparative tyrosine tolerance study (MRID# 43904817), RPA 201772 (98.7% a.i.) or RPA 200261 (99.8% a.i.; 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1,3-dione or NTBC), a therapeutic agent were administered in the diet to male Sprague-Dawley rats (5/dose) at dosage levels of 0 and 10 mg/kg/day for one week. The animals then received 500 mg/kg/day tyrosine on the day of treatment, and on Days 2, 3 and 8 after the test substance administration. Urine was analyzed for tyrosine metabolites.

Administration of tyrosine to rats pretreated with RPA 201772 or NTBC, increased the urinary excretion of tyrosine metabolites, N-acetyl tyrosine (NAT), 4-hydroxyphenyl acetate (4-HPAA) and 4-hydroxyphenyl lactate (4-HPLA). The effect of RPA 201772 was reversible within 48 hours after administration while that of NTBC was not.

The results of this functional assay suggests that both RPA 201772 and NTBC affect the main catabolic pathway for tyrosine by inhibiting 4-HPPDase enzyme.

"MECHANISTIC STUDY FOR THYROID EFFECTS-[RAT]"

EXECUTIVE SUMMARY: Male Crl:CD (SD) rats (14/dose) (MRID# 43904818), 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 two-fold increase in cytochrome P-450 dependent mixed-function oxidase system and p-nitrophenol uridine 5'-diphosphatase-glucuronyltransferase (UDPGT) activity which resulted in increased clearance of ^{125}I-thyroxine from the blood as indicated by shorter half-life and decreases in plasma T_4 level. In addition, there were increases in liver and thyroid weights. 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. Following intravenous administration of ^{125}I-thyroxine, the thyroid iodine uptake was slightly higher and thyroid weights were significantly higher than controls in RPA 201772 treated rats. The effects observed in this study 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.

CORE CLASSIFICATION: This 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.

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"

EXECUTIVE SUMMARY: In this study (MRID# 43904819), groups of 5 male Sprague-Dawley CD-1 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.

CORE CLASSIFICATION: This 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.

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

EXECUTIVE SUMMARY: In this study (MRID# 43904820), 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. 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.

PROPOSED MECHANISMS INVOLVED IN RPA 201772-INDUCED TOXICITY

The Registrant has proposed the following mechanisms involved in ocular, liver and thyroid toxicity caused by RPA 201772 in animals:

Ocular Toxicity

In chronic/oncogenicity and 2-generation reproduction studies in rats, dietary administration RPA 201772 caused corneal lesions in adults and offspring. These findings appear to be specific to rats as they were not observed in mice as well as dogs of either sex. Dietary administration of tyrosine was also shown to cause corneal opacities with superficial keratitis in male rats. The corneal lesions found in male CD rats and Brown Norway rats were identical. Dietary administration of RPA201772 or tyrosine caused inhibition of 4-HPPDase which is involved in the catabolism of tyrosine. As a result there was increase in plasma tyrosine levels. Thus, ocular toxicity caused by RPA 201772 in rats appears to be related to apparent failure to catabolize tyrosine. However, the Registrant needs to provide additional information to confirm the differential ability of rats and mice to metabolize tyrosine.

Thyroid Toxicity

Dietary administration of RPA 201772 for 14 days caused increase in P-450 dependent mixed-function oxidase system and enhanced UDPGT activity in rats. The increased clearance of ^{125}I-thyroxine from the blood was seen by decrease in T4 level (result of the enhanced glucuronidation by hepatic UDGPT), shorter half-life, and increased liver and thyroid weights. Chronic dietary administration of RPA 201772 caused development of thyroid follicular cell adenoma in male rats. Thus, RPA 201772 possibly causes thyroid tumors through a disruption in the thyroid-pituitary hormonal feedback mechanisms. The occurrence of thyroid tumors appears to be species-specific as no such tumors were observed in mice and dogs.

Liver Toxicity

Chronic dietary exposure of dogs, rats and mice to RPA 201772 caused liver toxicity in all three species. It was evidenced by increased liver weights accompanied by non-neoplastic histopathological changes. Increase in the combined incidence of hepatocellular adenoma and carcinoma was noted in rats and mice; no tumors were induced in dogs. The liver toxicity was caused by induction of mixed function oxygenase enzymes in the liver. As a result the total cytochrome P-450 levels increased in a dose-dependent manner. The specific forms of isoenzymes responsible for this increase were PROD and BROD enzymes. Thus, RPA 201772 appears to function as a phenobarbital type inducer of P-450 2B family.

III. EXECUTIVE SUMMARIES OF TOXICOLOGY STUDIES WITH METABOLITES OF RPA 201772 TECHNICAL, RPA 202248, RPA 200761, AND RPA 203328

The two metabolites of RPA 201772, RPA 202248 and RPA 203328, appear to have low acute oral toxicity and they are non-genotoxic. The available studies are summarized below.

RPA 202248

§81-1. *"RPA 202248. ORAL LD_{50} IN THE RAT"*

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID # 44044701), groups of five male and five female Sprague-Dawley rats were orally administered RPA 202248 in 0.5% aqueous methylcellulose at dose levels of 2000, 2710, 3690 and 5000 mg/kg. Two males and one female at 3690 and one male at 5000 mg/kg died by Day 3. The clinical signs of toxicity observed in both sexes, within few hours of dosing, included piloerection at all dose levels and hunched posture in males at 2710 mg/kg as well as in both sexes at 3690 and 5000 mg/kg/day. Reduced motor activity was noted in both sexes at 3690 and 5000 mg/kg/day. **Based on the estimated acute oral LD_{50} of >5,000 mg/kg in both sexes, RPA 202248 is placed in Toxicity**

Category IV.

CORE CLASSIFICATION: This study is classified as **Acceptable** and **satisfies** the Subdivision F Guideline requirement (§81-1) for an acute oral toxicity study in rats.

§81-1. ***"RPA 202248. ORAL LD_{50} IN THE RAT"***

In another study (MRID # 43904810), groups of five male and five female Sprague-Dawley rats were orally administered RPA 202248 in 0.5% methylcellulose and distilled water (20 ml/kg b.w.) at dose levels of 2000 and 5000 mg/kg. Two males and two females at 5000 mg/kg died by Day 2; the clinical signs of toxicity observed in both sexes on Day 1 included palpebral ptosis, piloerection, reduced motor activity, tremors (females) and coldness to touch (females). **Based on the estimated acute oral LD_{50} of >5,000 mg/kg for both sexes, RPA 202248 is placed in Toxicity Category IV.**

CORE CLASSIFICATION: This study is classified as **Acceptable** and **satisfies** the Subdivision F Guideline requirement (§81-1) for an acute oral toxicity study in rats.

§84-2. ***"RPA 202248: SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY"***

EXECUTIVE SUMMARY: *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and TA102 were exposed to 250, 500, 1000, 2500, or 5000 μg/plate RPA 202248 in the absence or presence of S9 activation (MRID # 43904814). Minimal toxicity was observed at 5000 μg/plate +S9 (plate incorporation method) or 5000 ug/plate -S9 (preincubation method). No evidence of RPA 202248-induced mutagenic response was seen.

CORE CLASSIFICATION: This study is classified as **Acceptable** and satisfies the Subdivision F Guideline requirement (§84-2) for a microbial gene mutation assay.

RPA 203328

§81-1. ***"RPA 203328. ORAL LIMIT TEST IN THE RAT"***

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID # 43904812), a group of five male and five female Sprague-Dawley rats were orally administered RPA 203328 in 0.5% methylcellulose and distilled water at a dose level of 5000 mg/kg. The animals were observed for 15 days post-dosing. No mortalities were noted; the clinical signs of toxicity observed in two males and one female included dyspnea, piloerection, soiled fur, mucoid feces or increased salivation. The female also exhibited reduced motor activity, hunched posture and noisy breathing.

The acute oral LD_{50} for RPA 203328 was >5,000 mg/kg for both sexes.

CORE CLASSIFICATION: This study is classified as **Acceptable** with a **Toxicity Category IV** and **satisfies** the Subdivision F Guideline requirement (§81-1) for an acute oral toxicity study in rats.

§84-2. *"RPA 203328: SALMONELLA TYPHIMURIUM/REVERSE MUTATION ASSAY"*

EXECUTIVE SUMMARY: *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA10O were exposed to 100, 250, 500, 1000, 2500, or 5000 μg/plate RPA 203328 in the absence or presence of S9 activation. Cytotoxicity was observed at levels of ≥2500 μg/plate +S9/-S9. No evidence of RPA 203328-induced mutagenic response was seen.

CORE CLASSIFICATION: This study is classified as **Acceptable** and satisfies the Subdivision F Guideline requirement (§84-2) for a microbial gene mutation assay.

"RPA 203328 (A METABOLITE OF RPA 201772). 28-DAY TOXICITY STUDY IN THE RAT BY DIETARY ADMINISTRATION"

EXECUTIVE SUMMARY: In a 28-day subchronic toxicity study (MRID# 43904813), RPA 203328 (99.7% a.i.) was administered in the diet to male and female Sprague-Dawley rats (10/sex/dose) at dosage levels of 0, 150, 500, 5,000, and 15,000 ppm (0, 11.14,37.57, 376.96 or 1,117.79 mg/kg/day in males and 12.68, 42.70, 421.53 or 1268.73 mg/kg/day in females, respectively) for 28 days.

There were no compound related adverse effects on survival, clinical signs, body weight, food consumption, clinical chemistry, hematology, and gross or microscopic pathology.

The LOEL is >15,0000 ppm (1,117.79 mg/kg/day in males and 1,268.73 mg/kg/day in females).
The NOEL for both sexes is ≥15,000 ppm.

CORE CLASSIFICATION: 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.