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

SEP 15 1995

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: RPA 201772 (Isoxaflutole Herbicide): Request for an Experimental Use Permit to ship RPA 201772 WDG Herbicide to the Growers for Use on Field Corn

P.C. Code: 123000
DP BARCODE: D214214
Submission No.: S484204
ID#: 5G04484
MRID No.: 435732-12 through 435732-30

FROM: Sanjivani Diwan, Ph.D. S. Diwan 9/12/95
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

TO: Joanne Miller/Daniel Kenny/PM-23
Registration Division (7505C)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head Y.M. Ioannou 9/12/95
Review Section I, Toxicology Branch II
Health Effects Division (7509C)
KR 9/14/95

Registrant: Rhone-Poulenc Ag Company

Action Requested: Review of toxicology data to support Experimental Use Permit (EUP) and temporary tolerance for use of RPA 201772 WDG (75% a.i.) on field corn

Recommendation: Toxicology Branch II has determined that the Toxicology data base on RPA 201772 (isoxaflutole; ≥98 a.i.) is adequate to support the EUP for its use on field corn with a proposed temporary tolerance of 0.1 ppm on corn grain and 0.2 ppm for corn forage and fodder.

Background:

Rhone-Poulenc AG Company has submitted a request for an EUP/TT to ship RPA 201772 WDG for use on field corn. It is formulated as water dispersible granules. The Registrant is proposing to ship it to the growers for use on a total of 4990 acres (40 acres per trial) in 14 states, using a maximum of 935.625 lbs of active ingredient (a.i.) with no more than 170.625 lbs of a.i. in any one state. This pesticide will be incorporated into the soil at the rate of 0.1875 lb. a.i./acre (single application) in either an early preplant (15-30 days prior to planting; 2.75 to 4 ounces/acre) or pre-emergence application (2 to 2.5 ounces/acre) to control weeds. The requested temporary tolerance on field corn grain is 0.1 ppm. Temporary tolerance for corn forage and fodder is 0.2 ppm.

Review of Toxicology Data on Technical Material - RPA 201772

Acute Toxicity Studies (Guideline §81-1 through 81-6; MRID # 435732-12 through 435732-17):

- Oral LD_{50} - Rat (MRID No.: 435732-12)

  Male and female (5/sex) Sprague-Dawley rats were orally administered test material as a suspension in 0.5% carboxymethylcellulose at a dose of 5000 mg/kg. The acute oral LD_{50} for RPA 201772 in both sexes of rats was greater than 5000 mg/kg.

  Toxicity Category IV; the study is classified as Acceptable

- Dermal LD_{50} - Rat (MRID No.: 435732-13)

  Male and female (5/sex) New Zealand White rabbits were dermally administered test material at a dose of 2000 mg/kg for 24 hours. The acute dermal LD_{50} for RAP 201772 in both sexes of rabbits was greater than 2000 mg/kg.

  Toxicity Category III; the study is classified as Acceptable

- Inhalation LC_{50} - Rat (MRID No.: 435732-14)

  Male and female (5/sex) Sprague-Dawley rats were exposed to an atmospheric concentration of 5.23 mg/l of test material for four hours. The acute inhalation LC_{50} for RPA 201772 in both sexes of rats was greater than 5.23 mg/l.

  Toxicity Category IV; the study is classified as Acceptable.

- Primary Eye Irritation - Rabbit (MRID No.: 435732-15)

  Approx. 99 mg of test material (0.1 ml) was instilled into the conjunctival sac of one eye of three male and three female New Zealand White rabbits. The other eye served as an untreated control. The study demonstrated that RPA 201772 produces minimal ocular irritation in both sexes of rabbits.

  Toxicity Category IV; the study is classified as Acceptable

- Primary Skin Irritation - Rabbit (MRID No.: 435732-16)

  0.5 g of test material moistened with 0.5 ml of 0.5% carboxymethylcellulose was applied to a clipped skin area of five male and one female New Zealand White rabbits for four hours. The study demonstrated that RAP 201772 produces no dermal irritation in rabbits.

  Toxicity Category IV; the study is classified as Acceptable.

- Skin Sensitization - Guinea pig (MRID No.: 435732-17)

  Using a modified Buehler method, ten male and ten female Dunkin-Hartley albino guinea pigs received nine topical induction doses of 0.25 ml of 50% (w/v) RPA 201772 in propylene glycol for six hours at 2 to 3 day intervals. A challenge topical dose of 0.25 ml of 10% or 50% (w/v) RPA 201772 in propylene glycol was administered to animals, ten days following the last induction dose.

  Not a sensitizer; the study is classified as Acceptable.

Genotoxicity: In a battery of assays (Guideline §84-2; MRID # 435732-21 through 435732-23, and 435880-02), RPA 201772 was found to be non-genotoxic.

- In-vitro cytogenetic assay - Human lymphocytes (MRID No.: 435732-21)

  No evidence of clastogenic effect was seen at 75, 300 or 600 ug/ml, with or without metabolic activation. The study is classified as Acceptable.

- Gene Mutation Assay - TK+/- Mouse Lymphoma system (MRID No.: 435732-22)

  No cytotoxic or mutagenic effects at 37.5, 75 or 150 ug/ml with or without S9 activation. The study

is classified as Acceptable.

- Micronucleus assay - Mouse (MRID No.: 435732-23)

  No overt toxicity to the treated animals or cytotoxicity to the target cell was noted. There was also no evidence of a clastogenic or aneugenic effect at any dose (200, 2000, or 5000 mg/kg/day) or harvest time. The study is classified as Acceptable.

- Microbial Gene Mutation Assay - Salmonella typhimurium (MRID No.: 435880-02)

  No evidence of mutagenic response was noted at doses ranging from 25 to 1000 ug/plate in Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 either with or without S9 activation. The study is classified as Acceptable.

21-Day Dermal Subchronic Toxicity - Rat (Guideline §82-2; MRID # 435732-19)

CD rats (8/sex/group) were treated topically with dosages of either 10, 100 or 1000 mg/kg/day of RPA 201772 eight hours per day for 21 days. The test material was applied on 0.5% w/v methyl cellulose in purified water daily at a volume-dosage of 2 ml/kg bodyweight.

The systemic toxicity LOEL is >1000 mg/kg/day for male and female rats; the systemic toxicity NOEL is ≥1000 mg/kg/day for males and females.

The dermal toxicity LOEL is >1000 mg/kg/day for males and females; the dermal toxicity NOEL is ≥1000 mg/kg/day for males and females. The study is classified as Acceptable.

Developmental Toxicity - Rat (Guideline §83-3a; MRID# 435732-20)

In a developmental toxicity study RPA 201772 was administered to twenty-five female Sprague-Dawley rats by gavage at dose levels of 0, 10, 100, or 500 mg/kg/day from gestational days 6-15, inclusive. The maternal LOEL is 500 mg/kg/day based on increased incidence of salivation; decreased body weight, weight gain, and food consumption during the dosing period. The maternal NOEL is 100 mg/kg/day.

The LOEL for developmental toxicity is 100 mg/kg/day based on growth retardations (decreased fetal body weight; increased incidence of delayed ossification of

sternebrae, metacarpals and metatarsals) and an increased incidence of vertebral and rib anomalies and high incidence of subcutaneous edema. The developmental NOEL is 10 mg/kg/day. The study is classified as Acceptable.

Review of Additional Data (not pertaining to EUP)

To support the proposed EUP/TT, the Registrant also submitted the final report for the 52-week chronic toxicity in dog and the 52-week interim report from the combined chronic toxicity/carcinogenicity study in rats. These studies have not been fully reviewed at present. However, based on a cursory review the following are the tentative conclusions from each study:

52-Week Dietary Chronic Study - Dog (Guideline §83-1; MRID # 435732-18)

Beagle Dogs (5/sex/group) received test material in diet at 0, 240, 1200, 12,000 or 30,000 ppm for 52 weeks. These dietary levels were equivalent to doses of 0, 8.41, 45.33, 498 or 1254 mg/kg/day for females and 0, 8.56, 44.81, 453 or 1265 mg/kg/day for males. At 30,000 ppm, all male dogs were sacrificed at 26 weeks of treatment due to severe anemia and weight loss.

The tentative LEL for both sexes of dogs is 12,000 ppm based on changes in relative organ weight, clinical chemistry and histopathological parameters of the liver, anemia and weight loss; the tentative NOEL was 1200 ppm.

104-Week Combined Oncogenicity and Toxicity Study - 52-Week interim report -Rat (Guideline §83-5; MRID # 435880-01)

Groups of 95 male and 95 female CD rats were administered diet containing test material at nominal doses of 0, 0.5, 2, 20, or 500 mg/kg/day. At 52 weeks, 10 rats/sex/group were sacrificed and another 10 rats/sex/group received untreated diet and served as recovery group.

The tentative LEL, at interim sacrifice, was 20 mg/kg/day based on the presence of corneal lesions and changes in relative organ weight, clinical chemistry and histopathological parameters of the liver. The tentative NOEL was 2 mg/kg/day. The body weight changes at 500 mg/kg/day indicated that this dose was adequate to assess the carcinogenic potential of isoxaflutole in the rat.

Review of Toxicity Data on End-Use-Product - RPA 201772 WDG (a 75% water dispersible granular (WDG) formulation of isoxaflutole, referred to as EXP31130A): The Registrant also submitted six acute

toxicity studies with formulation. They were reviewed by the Precautionary Review Section of the Registration Support Branch of the Registration Division. The following summarizes the conclusions of the studies:

Acute Toxicity Studies: (Guideline §81-1 to 81-6; MRID Nos. 435732-25 through 435732-30)

- Oral LD_{50} - Rat (MRID No. 435732-25)

  Male and female (5/sex) Sprague-Dawley rats were orally administered test material (50% w/v aqueous solution) at a dose of 5000 mg/kg. The oral LD_{50} for the combined sexes of rats is >5000 mg/kg.

  Toxicity Category IV; the study is classified as Acceptable.

- Dermal LD_{50} - Rabbit (MRID No. 435732-26)

  Male and female (5/sex) New Zealand White rabbits were dermally administered test material (0.4/ml) at a dose of 2000 mg/kg for 24 hours. The dermal LD50 for both sexes of rabbits is >2000 mg/kg.

  Toxicity Category III; the study is classified as Acceptable.

- Inhalation LC_{50} - Rat (MRID No. 435732-27)

  Male and female (5/sex) Sprague-Dawley rats were exposed to an atmospheric concentration of 5.26 mg/l of test material for four hours. The inhalation LC_{50} for both sexes of rats is >5.26 mg/l; MMAD = 2.3 um; GSD = 2.6

  Toxicity Category IV; the study is classified as Acceptable.

- Primary Eye Irritation - Rabbit (MRID No. 435732-28)

  A dose of approx. 80 mg of ground test substance (0.1 ml) was instilled into the conjunctival sac of one eye of three male and three female New Zealand White rabbits. The other eye served as an untreated control. Mild corneal irritation was apparent in 2 of 6 animals and iritis was observed in 3 of 6 animals; conjunctival irritation in all 6 rabbits cleared within 48 hours.

  Toxicity Category III; the study is classified as Acceptable.

- Primary Skin Irritation - Rabbit (MRID No. 435732-29)

  0.5 g of test material moistened with 0.2 ml of water was applied to a clipped skin area of 3 male and 3 female New Zealand White rabbits for 4-hours. Very slight erythema/edema in 1-2 test animals at 72 hours post-treatment, which cleared in 7 days.

  Toxicity Category IV; the study is classified as Acceptable.

- Skin Sensitization - Guinea pig (MRID No. 435732-30)

  Using the Buehler test, a 40% w/v solution of formulation in 0.25% w/v aqueous methyl cellulose was applied to shaved backs of guinea pigs (5/sex/group) during induction and challenge phase.

  Not a sensitizer; the study is classified as Acceptable.

Review of Proposed Label

Based on the review of the acute toxicity studies with the technical isoxaflutole and formulation (75%, WDG) all studies met the criteria for Toxicity Category III or IV. Thus, the proposed label should bear the signal word "CAUTION" and not the word "WARNING".

Risk Assessment

Based on the results of the submitted studies, the Registrant also submitted to the Agency preliminary risk assessments for dietary and worker exposure. The Agency agrees with the Registrant that the proposed EUP for use of isoxaflutole in field corn represents a negligible risk to humans.

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 EUP/TT for use on field corn with the proposed temporary tolerances of 0.1 on corn grain and 0.2 ppm for corn forage and fodder.

RPA 201772 21-Day Dermal Study (82-1)

Reviewed by: Sanjivani Diwan, Ph.D. S B. Diwan Date: 9/12/95
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Date: 9/12/95
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: 21-Day Dermal Toxicity/Rats (82-2)

EPA I.D. NUMBERS: DP BARCODE: 214214
P.C. CODE: 123000
MRID NUMBER: 435732-19
SUBMISSION No.: S484204

TEST MATERIAL: RPA 201772
CAS No.: 141112-29-0
Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole; isoxaflutole

STUDY NUMBER: LSR Report No. 93/RHA518/1193

TESTING FACILITY: Pharmaco-LSR Ltd., Suffolk, England

SPONSOR: Rhone-Poulenc Agriculture, Essex, England

TITLE OF REPORT: RPA 201772: 21-day percutaneous toxicity study in CD rats

AUTHOR: H.A. Cummins

REPORT ISSUED: April 12, 1994

EXECUTIVE SUMMARY: In this 21-day dermal toxicity study (MRID # 435732-19), 8 CD rats/sex/group were treated topically with dosages of either 10, 100 or 1000 mg/kg/day of RPA 201772 eight hours per day for 21 days. The test material was applied on 0.5% w/v methylcellulose in purified water daily at a volume-dosage of 2 ml/kg bodyweight.

Treatment-related marginal increase in relative liver weight was observed in both sexes of rats at 1000 mg/kg/day. This finding was considered as an adaptive response to RPA 201772 treatment. There were no differences between the control and treated groups in any of the other parameters measured.

The systemic toxicity LOEL is >1000 mg/kg/day for males and females; the systemic toxicity NOEL is ≥1000 mg/kg for males and females.

The dermal toxicity LOEL is >1000 mg/kg/day for males and females; the dermal toxicity NOEL is ≥1000 mg/kg/day for males and females.

The study is **Acceptable** and **satisfies** the guideline requirements for a 21-day dermal toxicity study (82-2) in the rat.

I. MATERIALS

A. Test Material

Name: RPA 201772
Synonym:5-Cyclopropyl-4-(2-methylsulfonyl-4-fluoromethylbenzoyl)isoxazole; isoxaflutole
Purity: 98.3%
Batch Number: FPI1308
Description: Fine cream, yellow crystalline powder
Storage Conditions: In a cool store (not exceeding 15^0 C) and protected from light

Before commencement of treatment, the suitability of the proposed formulations was determined by a trial preparation. Appendix 8A of the study report contains results of these analyses which show that the concentration of RPA 201772 remained stable.

B. Vehicle: 0.5% Aqueous methylcellulose

C. Administration: dermal

D. Test Animals:

Species: Rat
Species: CD Sprague-Dawley
Source: Charles River Ltd., Kent, England
Age: Females - 4 to 5 weeks; Females - 5 to 7 weeks at arrival
Weight: Males - Approximately 183-261 g; Females - approximately 200-243 g at initiation of dosing
Housing: Individually in stainless steel cages
Environmental Conditions:
Temperature: target of 18-25°C; Relative humidity: target of 40-70%
Photoperiod: 12 hours light/dark
Air changes: 15/hour
Food and Water: Laboratory Rodent Diet (RM1(E)SQC) and tap water *ad libitum*
Acclimation Period: Two weeks

II. METHODS

A. Preparation of Dosing Substance

The formulations of the test material were prepared in 0.5% w/v methyl cellulose in purified water to provide the required dosages at a constant volume-dosage of 2 ml/kg body weight. An aliquot of RPA 201772 was dry ground in an agate mortar and

pestle. An appropriate quantity of vehicle was added gradually to form a paste which was then diluted with the remaining vehicle. The resulting suspension was mixed with a laboratory mixer for two minutes at low speed. The suspension was stirred continuously to maintain the homogeneity at higher concentrations. The test formulations were stable over 1 week.

Chemical analyses were done on the contents of four of eight formulations prepared on Day 1 of treatment and once in week 3 (Day 15).

B. Dosage and Administration

Dosage Groups

The animals were assigned by computer randomization to the following treatment groups:

Test Group	Dose Applied (mg/kg/day)	# Males	# Females
1 (Control)	0	8	8
2	10	8	8
3	100	8	8
4	1000	8	8

Dosages were selected by the sponsor; no rationale was provided.

Administration

One day prior to the initiation of dosing, the hair of each rat was shaved from the dorsal area between the limb girdles and the procedure was repeated weekly thereafter. Twenty-one repeated doses of test material or vehicle were administered to each animal once daily, seven days per week, for three weeks at dose volume of 2 mL/kg; the test material was spread evenly over the shaved area which was 4 x 5 cm in size and covered at least 10% of the animal's body surface area. A gauze patch was held in place by tape and secured with a pad of cotton wool and elastic bandage. The animals were fitted with a collar to prevent ingestion of the test substance and disruption of the wrappings. Approximately 8 hours after the application, the dressings were removed and the application site was washed with warm water and dried with a soft paper tissue. Formulations were prepared fresh, on the day before each administration. The animals were observed daily for signs of dermal reactions or systemic toxicity. Control animals were handled similarly except for the substitution of vehicle for the test chemical. All animals were sacrificed after 21 days of treatment.

C. Experimental Design

The study protocol required the following observations and examinations at the indicated times or frequencies:

Signs of dermal irritation, mortality and morbidity - once daily
Systemic toxicity - at least twice daily (before dosing and after dosing); and a detailed examination weekly
Body weights - prior to treatment, and twice weekly thereafter
Food consumption - weekly intervals during the treatment period
Food efficiency - calculated at weekly intervals
Water consumption - daily
Ophthalmoscopy - prior to treatment and daily during the treatment period
Hematology and clinical chemistry - just prior to euthanasia
Gross necropsy - all animals
Organ weights - skin, kidneys and liver from all animals
Histopathology - skin, kidneys and liver from all animals

D. Pathological Parameters

For hematology and clinical chemistry evaluations, blood was drawn from the retro-orbital sinus. The CHECKED (X) hematology parameters were examined.

X Hematocrit (HCT)*	X Total plasma protein (TP)
X Hemoglobin (HGB)*	X Leukocyte differential count
X Leukocyte count (WBC)*	X Mean corpuscular HGB (MCH)
X Erythrocyte count (RBC)*	X Mean corpuscular HGB conc. (MCHC)
X Platelet count*	X Mean corpuscular volume (MCV)

* EPA guideline requirement

The CHECKED (X) clinical chemistry evaluations were done.

Electrolytes:	Other:
X Calcium*	X Albumin*
X Chloride*	X Blood creatinine*
__Magnesium	X Blood urea nitrogen*
X Phosphorus*	__Cholesterol
X Potassium*	X Globulins
X Sodium*	X Glucose*
	X Total Bilirubin*

Enzymes:
__Alkaline phosphatase
__Cholinesterase
__Lactic acid dehydrogenase
X Serum alanine aminotransferase (also SGPT)*
X Serum aspartate aminotransferase (also SGOT)*
X Total Protein*
__Triglycerides
X Creatine phosphokinase*

* EPA guideline requirement

Approximately 24 hours after the last treatment, the animals were sacrificed by carbon dioxide inhalation. The following CHECKED (X) tissues were preserved. The (XX) organ(s) in addition were weighed.

Digestive System
__Tongue
__Salivary glands
__Esophagus
__Stomach
__Duodenum
__Jejunum
__Ileum
__Cecum
__Colon
__Rectum
XXLiver*
__Gall bladder
__Pancreas
Respiratory System
__Trachea
__Lung

Cardiovasc./Hemat. System
__Aorta
__Heart
__Bone marrow
__Lymph nodes
__Spleen
__Thymus
Urogenital System
XXKidneys*
__Urinary bladder
__Testes
__Epididymides
_Prostate/urethra
__Seminal vesicle
_Ovaries
_Uterus
__Vagina

Neurologic System
__Brain
__Periph. nerve
__Spinal cord
__Pituitary
X Eyes (Optic n.)*
Glandular
__Adrenals
__Lacrimal gland
__Mammary gland
__Parathyroids
__Thyroids
Other
__Bone
__Skeletal muscle
X Skin+
__All gross lesions and masses

* EPA guideline requirement
+ Treated and untreated skin were examined

Histological examinations were done on the above tissues from the control and high dosage groups.

E. Statistical Analyses

The following procedures were utilized in analyzing the numerical data:

- mean body weight change, hematology, and blood chemistry -- Student's t-test

- Organ weights --Bartlett's test, Fisher-Behrens test, Dunnett's test, Fisher Exact Probability test; and two-tailed analyses.

F. Compliance

Signed statements of Quality Assurance and compliance with Good Laboratory Practice regulations were submitted by the testing facility. The sponsor submitted a statement claiming no data confidentiality. Any deviations from the protocol were appropriately reported.

III. RESULTS

A. Administered Dosage

The average doses administered to each group were within ± 17% of the targeted doses except for rats in the 10 mg/kg group. The analysis indicated low inclusion of active ingredient in one of the final week samples and, consequently, the animal received an average daily dose of approximately 22% less than the targeted dose.

B. Mortality/Clinical Observations and Systemic Toxicity

There were no deaths nor signs of systemic toxicity observed during the study. Signs of dermal irritation, including slight erythema and slight exfoliation on Days 3 and 4 in one female at 1000 mg/kg/day. Because of the isolated nature of the incidence this finding was attributed to incidental causes.

C. Body Weight and Body Weight Gain

Mean body weights and body weight gains (Day 0-20) of the treated animals did not significantly differ from those of the controls.

D. Food Consumption and Food Conversion Ratio

There were no significant differences between the treated and control groups in mean daily food consumption or mean food efficiency.

E. Clinical Pathology

There were no treatment-related changes in the hematology or clinical chemistry parameters. The lymphocyte counts of treated animals were generally slightly lower than those of the control animals, the values were within the normal ranges. Because of lack of dose-response, this finding was not considered to be treatment-related.

F. Ophthalmoscopy

No treatment-related effects were noted.

G. Necropsy Findings

Gross Necropsy

There were no treatment-related changes on gross necropsy examination of the animals.

Organ Weights

At 1000 mg/kg/day, the absolute liver weights of female rats (data not shown) and relative liver weights of male and female rats were higher (>10%) than those of the control animals (Table 1). This finding was considered to be treatment-related. Although increases (7%) in the above parameters were also noted at 100 and 10 mg/kg/day, the differences were not statistically significant. No treatment-related effect was not in kidney weights.

Table 1
Relative Liver Weight in Rats Treated with RPA 201772 for 21 Days[a]

	Dosage Levels (mg/kg/day)			
Mean Relative Weight (%)	Control	10	100	1000
Males	4.2	3.9	4.5	4.7*
% of control value	-	93	107	112
Females	4.2	4.5	4.5	4.6*
% of control value	-	107	107	110

[a] Extracted from Table and Appendix 6B (pages 40, and 72-73) of the study report; % calculated by the reviewer
*Significant compared to control group ($p<0.05$)

Histopathology

The only findings on histopathology involved the skin. These were confined to encrustations seen in 1 female rat from 10 mg/kg/day group, 2 females from 100 mg/kg/day group and 1 male from 1000 mg/kg/day group at necropsy. These changes were of slight and minimal degree and showed no relationship to treatment.

G. Conclusion from Study Report

The study report concludes that the Lowest Observed Effect Level (LEL) is 1000 mg/kg/day for male and female rats based on increase liver weights; the No Observed Effect Level (NOEL) is 100 mg/kg/day.

H. STUDY DEFICIENCY

The Reviewers noted the following deficiencies:

The error in the formulation at lowest dosage level indicate that the animals received less than the target dosage. Less than 10 animals/sex were used for this study. However, these deficiencies did not affect the overall results of the study since the highest dosage (limit dose) produced minimal effects in rats.

IV. DISCUSSION/CONCLUSIONS

In this 21-day dermal toxicity study (MRID # 435732-19), eight CD rats/sex/group were treated topically with dosages of either 10, 100 or 1000 mg/kg of RPA 201772 eight hours per day for 21 days. There were no differences between the control and treated groups in any of the parameters measured. At 1000 mg/kg/day, the liver weights of rats were slightly higher than those of the controls. This finding was considered to be an adaptive response to RPA 201772 treatment.

The systemic toxicity LEL is >1000 mg/kg/day for males and females; the systemic toxicity NOEL is ≥1000 mg/kg/day for males and females.

The dermal toxicity LEL is >1000 mg/kg/day for males and females; the dermal toxicity NOEL is ≥1000 mg/kg/day for males and females.

RPA 201772 Developmental Study (83-3a)

Primary Review by: Sanjivani B. Diwan, Ph.D. Sanjivani B. Diwan Date 9/12/95
Review Section I, Toxicology Branch II/HED (7509C)
Secondary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson Date 9/12/95
Head, Review Section I, Toxicology Branch II/HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Teratology - Developmental Toxicity/Rat (83-3a)

DP BARCODE: D214214

P. C. CODE: 123000

MRID No: 43573220

SUBMISSION No.: S484204

TEST MATERIAL: RPA 201772

CAS No.: 141112-29-0

SYNONYMS: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole; isoxaflutole

STUDY REPORT NUMBER: 94/RHA536/1203

SPONSOR: Rhone-Poluenc Agriculture Ltd., Essex, England

TESTING FACILITY: Pharmaco LSR Ltd., Suffolk, England

TITLE OF REPORT: RPA 201772 (active ingredient): Teratology study in the rat

AUTHOR: S.C.J. Reader

REPORT ISSUED: February 6, 1995

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID# 43573220) RPA 201772 (99.2% a.i.) was administered to twenty-five female Sprague-Dawley rats by gavage at dose levels of 0, 10, 100, or 500 mg/kg/day from gestational days 6-15, inclusive.

Maternal toxicity, observed at 500 mg/kg/day, was manifested as an increased incidence of salivation; decreased body weight, weight gain, and food consumption during the dosing period. **The maternal LOEL is 500 mg/kg/day, based on increased incidence of clinical signs and decreased body weights, body weight gains and food consumption. The maternal NOEL is 100 mg/kg/day.**

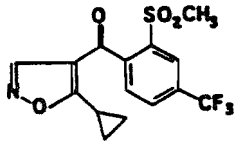
Developmental toxicity, observed at 100 and 500 mg/kg/day, was manifested as growth retardations (decreased fetal body weight; increased incidence of delayed ossification of sternebrae, metacarpals and metatarsals). In addition, an increased incidence of vertebral and rib anomalies and high incidence of subcutaneous edema were observed at 500 mg/kg/day. **The LOEL for developmental toxicity is 100 mg/kg/day, based on decreased fetal body weights and increased incidences of skeletal anomalies.** The developmental NOEL is 10 mg/kg/day.

This study is classified as Acceptable and satisfies the guideline requirement for a developmental toxicity study (83-3a) in rats.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 201772
   Description: Fine white powder
   Batch number: 40 ADM 93
   Purity: 99.2%
   Stability of compound: Demonstrated in earlier study (#93/RHA518/1193)
   Storage Conditions: At room temperature protected from light



2. Vehicle Control: 0.5% w/v Methylcellulose in purified water

3. Test Animals: Species: Rat
   Strain: CD (Sprague-Dawley) rats
   Age and Weight at initiation: 10 to 11 weeks and 211 to 272 g, respectively
   Source: Charles River UK, Ltd., Kent, England
   Housing: Individually in cages
   Diet - Animals were fed commercial laboratory animal diet (LAD1(E)SQC) and watered *ad libitum*
   Environmental Conditions: Temperature: 21°C (range: 19-25°C)
   Relative Humidity: 55% (range: 40%-70%)
   Air changes: 15 per hour
   Photoperiod: 12 hours light/dark
   Acclimation period: Five days
   Males used: Untreated, sexually mature males from the same source and strain

B. PROCEDURES AND STUDY DESIGN:

This study was designed to assess developmental toxicity of RPA 201772 when administered daily by gavage to rats on gestation days 6 through 15, inclusive.

1. Mating: Following 5 days of acclimation, females were mated 1:1 with males of the same strain and source. Females were checked daily for the presence of vaginal sperm or a copulatory plug. In addition, the trays beneath the cages were checked for ejected copulation plugs. The day on which mating was confirmed was designated day 0 of gestation.

2. Animal Assignment and dose selection is presented in table 1. Animals were allocated to four groups in a sequential manner to ensure even distribution amongst the groups as shown below.

TABLE 1. Animal Assignment

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	25
Low dose	10	25
Mid dose	100	25
High dose	500	25

3. Dose Selection Rationale: The dose levels were selected based upon the results of preliminary toxicity studies by the sponsor; no details were provided.

4. Dosing: All doses were in a volume of 10 ml/kg of body weight/day prepared fresh daily, as suspensions in 0.5% aqueous methylcellulose, during the dosing period. Dosing was based on the daily body weight during gestation day 6 through 15. An aliquot of RPA 201772 was dry ground in an agate mortar and pestle; the appropriate quantity of vehicle was gradually added to form a paste. The paste was then diluted with the remaining vehicle and the resulting suspension was mixed with a mixer for two minutes at low speed. The four samples of each test concentration, taken on the first and the last day of treatment, and on six occasions during the treatment period were analyzed to determine the homogeneity and concentration of the test material in the vehicle. The stability at the lowest dose (Batch # JYG 708) was determined over 8 day period. Samples of the dosing solutions were analyzed by HPLC chromatography.

C. OBSERVATIONS:

1. Maternal Observation and Evaluations - The animals were checked any for overt signs of toxicity daily during the study period. Body weights were recorded on gestation day (GD) 0, 3, 6 to 15 inclusive, 16, 18 and 20. Food and water consumption were recorded for the following periods: GD 0-2, 3-5, 6-8, 9-11, 12-15, 16-17, and 18-19. Dams were sacrificed on day 20 of gestation. Examinations at sacrifice consisted of:

- Gross pathology observations for evidence of disease or adverse reaction to treatment and specimens of abnormal tissues were retained

- Individual placental weights

- Number of corpora lutea

- Number of implanation sites; the uteri from apparently nonpregnant animals were stained using the Salewski (1964) technique to detect the implantation sites

- Numbers of resorptions (early and late) and live and dead fetuses

- Number and distribution of fetuses in each uterine horn

2. Fetal Evaluations - The fetuses were examined in the following manner:

- Individual fetal weight and sex

- External anomalies

- Visceral anomalies by dissecting the neck and the thoracic and abdominal cavities of approximately half of each litter

- Skeletal anomalies for all fetuses using the method of Dawson and staining with Alizarin Red S

- Head anomalies (approximately half of the fetal heads) using the technique of Wilson (1965) and fixation in Bouin's solution

3. Historical control data were provided to allow comparison with concurrent controls.

D. STATISTICAL ANALYSIS: The following methods were used.

- Maternal body weight and body weight change, food and water consumption and litter size--One-way analysis of variance and/or t-test

- Fetal weights and placental weights--Nested analysis of variance and weighted t-test

- Corpora lutea, implantations, and early and late resorptions--Mann-Whitney U-test

- Pre- and post-implantation loss, and fetal sex ratios--Chi-square test, Fischer's Exact test or Mann-Whitney U-test

E. COMPLIANCE:

- A signed Statement of No Data Confidentiality Claim, dated February 6, 1995, was provided
- A signed Statement of Compliance with EPA, OECD, UK and Japanese MAFF GLPs, dated January 2 and 31, and February 6, 1995, was provided
- A signed Quality Assurance Statement, dated January 31, 1995, was provided
- A signed Flagging Criteria Statement, dated February 6, 1995, was provided. According to the investigations, the study neither meets nor exceeds any of the applicable criteria.

II. RESULTS

A. TEST MATERIAL ANALYSES

The concentration analysis for the first set of samples for the three dose groups conducted prior to and at the end of the dosing indicated values below the nominal concentration (13-41%). Additional analyses conducted on the three sets of test samples revealed values within ±10% of target, thus showing no degradation. The test compound was homogeneously distributed in the dosing suspension. The reviewers concluded that overall, the stability data were within ± 5% of the target and that the test compound was stable at ambient temperature for up to 8 days.

B. MATERNAL TOXICITY

1. Mortality - No mortality was noted.

2. Clinical observations - Compound-related increased salivation was observed in ten dams at 500 mg/kg/day, within one- and half hour of dosing, beginning from GD 7 through 15. Similar finding was also noted in one dam at 100 mg/kg/day on GD 7 (data not shown).

3. Body weight - Body weight gain data are summarized in Table 2. Compound-related decreases in body weight and weight gain were observed at 500 mg/kg/day. At this dose level, significant decreases in body weight gain were observed for GD 8-12 (19%), 6-15 (19%) and GD 0-20 (6%). During GD 16-20, a slight compensatory increase was noted for this dose group. Maternal body weight (data not shown) was slightly decreased on GD 8 through 15 (1-3%) during treatment period and remained lower (10%) from GD 16 through 20 compared to controls during the post-treatment period.

TABLE 2. Mean Body Weight Gain (g ± S.D.)[a]

Dose Group in mg/kg/day (25 dams/group)	Prior to Dosing Period (GD 0-6)	Dosing Period (GD 6-15)	Post-Dosing Period (GD 15-20)	Gestation Period (GD 0-20)
0	42 ± 7.9	70 ± 7.9	89 ± 12.2	202 ± 23.3
10	45 ± 8.2	69 ± 9.7	88 ± 10.2	203 ± 19.5
100	41 ± 8.7	69 ± 9.1	93 ± 12.9	204 ± 24.9
500	41 ± 8.6	57 ± 11.6***[c]	90 ± 10.2	189 ± 25.9*

[a]Data were extracted from Study No. 94/RHA536/1203, Appendix 3; calculated and analyzed by the Reviewers

[b]Gravid uterine weight was not provided and, therefore, the corrected body weight change could not be calculated.

[c]Body weight gain during GD 8-12 was significantly lower (19%; p=0.0005) compared to control; data analyzed by the Reviewers

*Significantly different from control (p<0.03)
***Significantly different from control (p<0.001)

4. Food consumption - Food consumption data are summarized in Table 3. A compound-related significant decrease (10%, $p< 0.01$) in food consumption was observed at 500 mg/kg/day only during GD 6-8. Significant increase (9-11%, $p<0.01$) in food consumption was noted at 100 and 500 mg/kg/day during post-treatment, on GD 16-17.

5. Water Consumption - No compound-related effects were noted.

6. Gross Pathology - No compound-related gross pathology findings were noted. Incidental observations included one dam in the 100 mg/kg/day group with red fluid in one uterine horn; two dams at 500 mg/kg/day with slight brown staining on head; one dam also at 500 mg/kg/day, had enlarged, pale kidneys, bilateral renal cavitation and bilateral hydroureter.

7. Cesarean section Data - Data are summarized in Table 4. Compound-related effects were observed at 100 and 500 mg/kg/day. Fetal body weights were decreased significantly (8 and 14%, respectively; $p\leq 0.001$) at these dose levels in a dose-related manner when compared to controls. Implantation and fetal survival were unaffected by treatment.

C. DEVELOPMENTAL TOXICITY

Incidences of fetal anomalies with selected external, visceral, and skeletal anomalies are presented in Tables 5 and 6. Compound-related effects were observed at 100 and/or 500 mg/kg/day and were manifested as increased incidences of fetuses/litters with various anomalies. The incidences of these anomalies were higher than the concurrent control values and in some cases exceeded the range for historical controls. Litter incidences, however, were not provided for historical control data. The compound-related anomalies are discussed below.

1. External Examination - There was dose-related increase in the incidence of small fetus and a decrease in the incidence of large fetus at 100 and 500 mg/kg/day (table 5).

2. Visceral Examination - The visceral anomalies were observed at 500 mg/kg/day and included subcutaneous edema, and a slight space between body wall and organs (table 5).

TABLE 3. Mean Food Consumption (g/animal/day ± S.D.)[a]

Study Period in Days	Dose Groups (mg/kg/day)			
	0	10	100	500
Pre-treatment				
0-2	26 ± 1	28 ± 2*	27 ± 2	27 ± 2
3-5	29 ± 2	29 ± 3	29 ± 4	30 ± 3
Treatment				
6-8	30 ± 2	31 ± 2	29 ± 3	27 ± 3**
9-11	31 ± 3	32 ± 3	31 ± 4	29 ± 3
12-15	33 ± 3	33 ± 3	33 ± 3	33 ± 4
Post-treatment				
16-17	35 ± 3	35 ± 3	38 ± 3**	39 ± 4***
18-19	35 ± 4	35 ± 2	36 ± 4	37 ± 4

[a]Data were extracted from Study No. 94/RHA536/1203, Table 2

*Significantly different from control ($p<0.05$).
**Significantly different from control ($p<0.01$).
***Significantly different from control ($p<0.001$)

TABLE 4. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	10	100	500
No. animals assigned	25	25	25	25
No. animals mated	25	25	25	25
No. animals pregnant	25	25	25	25
Pregnancy rate (%)	100	100	100	100
Maternal wastage				
No. died/nonpregnant	0	0	0	0
No. died/pregnant	0	0	0	0
No. nonpregnant	0	0	0	0
No. aborted	0	0	0	0
No. premature delivery	0	0	0	0
Total corpora lutea[b]	439	432	442	432
Corpora lutea/dam	17.6 ± 2.2[c]	17.3 ± 1.5	17.7 ± 2.0	17.3 ± 1.6
Total implantations	422	409	426	414
Implantations/dam	16.9 ± 1.9	16.4 ± 1.8	17.0 ± 1.9	16.6 ± 1.4
Total live fetuses	403	381	410	393
Live fetuses/dam	16.1 ± 2.4	15.2 ± 2.2	16.4 ± 2.0	15.7 ± 1.7
Total resorptions	19	28	16	21
Early	19	28	16	21
Late	0	0	0	0
Resorptions/dam	0.7 ± 0.9	1.1 ± 1.1	0.6 ± 0.8	0.8 ± 0.9
Total dead fetuses	0	0	0	0
Dead fetuses/dam	0	0	0	0
Fetal weight/litter (g)	3.7 ± 0.1	3.7 ± 0.7	3.4 ± 0.1***	3.2 ± 0.1***
Preimplantation loss (%)	4.7 (4.5)[d]	6.2 (6.0)	4.1 (3.9)	5.0
Postimplantation loss (%)	4.5 (4.8)	6.8 (7.2)	3.8 (3.7)	5.1
Sex ratio (% male)	54	51	46	48

[a]Data were extracted from Study No. 94/RHA536/1203, Tables 5 and 6, and Appendix 7.

[b]Data on pregnancies detected by the stain were excluded from analyses.

[c]Mean ± S.D.

[d]Calculated by the Reviewers; discrepancy between summary table and individual data

***Significantly different from control ($p<0.001$)

TABLE 5. Fetal External and Visceral Examination[a]

Findings[b]	Dose Level (mg/kg/day) 0	10	100	500
External Anomalies				
No. fetuses (litters) examined	403 (25)	381 (25)	410 (25)	393 (25)
Small fetus (<2.80 g)	0	5 (4)	9 (7)	25 (11)
Large fetus (>4.10 g)	38 (10)	28 (10)	3 (3)	0
Total No. fetuses (litters) with external malformations	38 (10)	33 (13)	12 (9)	25 (11)
Visceral Anomalies				
No. fetuses (litters) examined	195 (25)	185 (25)	200 (25)	191 (25)
Subcutaneous edema	0	2 (2)	7 (6)[c]	41 (14)
Slight space between body wall and organs	0	2 (2)	2 (2)	5 (3)
Total No. fetuses (litters) with visceral malformations	0	3 (3)	9 (7)	40 (15)

[a]Data were extracted from Study No. 94/RHA536/1203, Tables 7 and 8 and Appendix 9

[b]More than one type of anomaly may be found in one fetus.

[c]Calculated by the Reviewers; discrepancy between summary and individual data

3. Skeletal Examination - The skeletal anomalies seen at 100 mg/kg/day included decrease in sternebral (#s 3, 4 and 5), metacarpal and metatarsal ossification. Additional anomalies noted at 500 mg/kg/day consisted of a lack of ossification of 1st thoracic vertebral centrum, and 1 or more pubic bones, incomplete ossification of caudal vertebrae, and increase in the number of 14th ribs or enlarged 14th ribs, 27th presacral vertebrae and asymmetrical pelvis (table 6). A slight decrease in ossification of pubic bones was noted but the fetal incidence was within the historical control range.

For additional information on fetal anomalies see Attachment to the DER.

III. DISCUSSION:

A. MATERNAL TOXICITY: Compound-related maternal toxicity was observed at 500 mg/kg/day and was manifested as an increased incidence of salivation; decreased body weight, weight gain, and food consumption during the dosing period.

Based on these results, the maternal LOEL was 500 mg/kg/day; the NOEL was 100 mg/kg/day.

B. DEVELOPMENTAL TOXICITY:

1. Deaths/Resorptions: No treatment-related increases in fetal deaths or resorptions were observed.

2. Altered Growth: At 100, and 500 mg/kg/day, the fetal body weights were significantly lower compared to those of the controls and, therefore, this finding was considered to be indicative of a developmental effect. The low fetal weights were associated with low incidence of large fetus and, at 500 mg/kg/day, a higher incidence of small fetus noted was slightly above the background control range. Furthermore, a generalized reduction in skeletal ossification was also observed; these effects were more pronounced in the 500 mg/kg/day dose group.

3. Developmental Anomalies: At 100 and 500 mg/kg/day, there was delayed ossification in the sternebral, metacarpal or metatarsal bones. At 500 mg/kg/day, fetuses had a high incidence of subcutaneous edema. In addition, there was increase in the incidence of vertebral anomalies and asymmetric pelvis, a high incidence of rib anomalies (increase in the number of 14 ribs) and a decrease in ossification of the pubic bones. The number of fetuses and litters with external, visceral, and skeletal anomalies was increased at 100 and/or 500 mg/kg/day. The percentage of fetuses affected was outside the historical range and the incidence of fetuses/litters

TABLE 6. Fetal Skeletal Examination[a]

Findings[b]	Dose Level (mg/kg/day) 0	10	100	500
No. fetuses (litters) examined	208 (25)	196 (25)	210 (25)	202 (25)
Sternebrae and Ribs				
Sternebra(e), unossified - #3	18 (12)	27 (12)	49 (17)	70 (23)
- #4	4 (3)	1	13 (8)	26 (13)
- #5	2 (2)	0	5 (4)	6 (4)
Ribs - 13/14	16 (10)	16 (14)	34 (15)	46 (22)
- 14/14	23 (10)	16 (7)	16 (10)	66 (23)
14th rib or rib enlarged	2 (2)	3 (1)	1	13 (8)
Vertebrae, Limbs and Girdles				
1st thoracic vertebral centrum, unossified	2 (2)	1	3 (3)	19 (9)
27th presacral vertebrae	1	0	3 (3)	16 (11)
Caudal vertebrae, incomplete ossification (<5 ossified)	2 (2)	3 (3)	12 (8)	26 (15)
Pubic bones, one or more, incompletely ossified or unossified	7 (3)	3 (3)	13 (7)	24 (13)
Metacarpals and/or metatarsals incompletely ossified or unossified	1	2 (2)	12 (8)[c]	22 (11)
Asymmetric pelvis	2 (2)	1	0	7 (7)

[a]Data were extracted from Study No. 94/RHA536/1203, Table 9 and Appendix 10

[b]More than one type of anomaly may be found in one fetus.

[c]Calculated by the Reviewers; discrepancy between summary and individual data

was above the concurrent control. Therefore, the above analomalies were considered to be treatment-related.

Based on the findings of altered growth and increased incidence of developmental anomalies, the developmental NOEL and LOEL were 10 and 100 mg/kg/day, respectively.

C. STUDY DEFICIENCIES:

- Gravid uterine weights were not reported; therefore, the corrected body weight change could not be calculated. This deficiency does not negatively impact upon the outcome of the study.

D. CORE CLASSIFICATION: Acceptable

ATTACHMENT

Isoxaflutole Review 123000

Page_____ is not included in this copy.

Pages 32 through 55 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

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Reviewed by: Sanjivani B. Diwan, Ph.D. Sanjivani B Diwan 9/7/95
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. J M Ioannou 9/7/95
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Oral Toxicity/Rats (81-1)
EPA ID NUMBERS:	DP BARCODE: D214214 P. C. CODE: 123000 MRID NUMBER: 435732-12 SUBMISSION No.: S484204
TEST MATERIAL:	RPA 201772 CAS Number: 141112-29-0 Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-isoxazole; isoxaflutole
STUDY NUMBER:	Project No. 238/33R
TESTING FACILITY:	Safepharm Laboratories Ltd., Derby, U.K.
SPONSOR:	Rhone-Poulenc Agriculture Ltd., Essex, England
TITLE OF REPORT:	RPA 201772: Acute oral toxicity (limit test) in the rat
AUTHOR:	D.J. Allen
REPORT ISSUED:	November 3, 1994

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID # 435732-12), a group of five male and five female Sprague-Dawley rats were orally administered RPA 201772 as a suspension in 0.5% carboxymethylcellulose at a dose of 5000 mg/kg. The animals were observed for mortality and clinical signs of toxicity for 14 days post-dosing. There were no deaths during the study. No clinical signs of toxicity were observed during the treatment and observation period. **The acute oral LD_{50} for RPA 201772 was greater than 5000 mg/kg.**

The study is classified as **Acceptable** with a **Toxicity Category IV** and **satisfies** the requirements (81-1) for an acute oral toxicity study in rats.

I. MATERIALS

A. Test Material

Name: RPA 201772
Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole; isoxaflutole
Purity: 98.7%
Lot/batch number: 21 ADM 93
Description: Yellow powder
Storage Conditions: At room temperature protected from light

For dosing, the test material was freshly prepared as a suspension at the appropriate concentration in 0.5% carboxymethylcellulose and homogenized using Silverson homogenizer.

B. Test Animals

Species: Sprague Dawley albino rats
Source: Charles River UK Ltd., Kent, England
Age: 5-8 weeks when received
Weight: Males - 149 to 168 g; Females - 136 to 156 g when dosed
Housing: Up to five rats of same sex per cage
Environmental Conditions: Temperature: 21-23^0 C
Relative Humidity: 54-60%
Photoperiod: 12 hours light/dark
Food and Water: Rat and Mouse Expanded Diet No.1 and water *ad libitum*
Acclimation Period: Five days

II. METHODS

After an overnight fast, five male and five female rats were dosed with 5000 mg/kg RPA 201772 via gavage using a metal cannula attached to a graduated syringe. The dosing volume was adjusted to give 10 ml of dose per 1000 g of body weight. The animals were observed for mortality and clinical signs of toxicity at 0.5, 1, 2, and 4 hours on Day 1 (day of dosing) and once daily for the remainder of the 14-day observation period. Body weights were recorded prior to dosing and at 7 and 14 days post-dosing. At the end of the observation period, all animals were sacrificed and necropsied.

III. RESULTS

None of the animals died during the study. No deaths, clinical or systemic signs of toxicity were observed during the treatment and observation period. All the animals gained weight over the course of the study. There were no lesions on gross necropsy. The acute oral LD_{50} was greater than 5000 mg/kg.

IV. COMPLIANCE

The following compliance documents were submitted: 1) signed statement by the sponsor indicating that the study was conducted in accordance with GLP Regulations; 2) signed Quality Assurance statement by the testing facility; 3) signed statement by the sponsor claiming no data confidentiality.

V. CONCLUSIONS

The acute oral LD_{50} for RPA 201772 in rats was greater than 5000 mg/kg.

The study is classified as **Acceptable** with a **Toxicity Category IV** and **satisfies** the requirements (81-1) for an acute oral toxicity study in rats.

Reviewed by: Sanjivani B. Diwan, Ph.D. Sanjivani B Diwan 9/7/95
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y M Ioannou 9/7/95
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity/Rabbits (81-2)

EPA ID NUMBERS: DP BARCODE: D214214
P. C. CODE: 123000
MRID NUMBER: 435732-13
SUBMISSION No.: S484204

TEST MATERIAL: RPA 201772
CAS No.: 141112-29-0
Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl) isoxazole; isoxaflutole

STUDY NUMBER: Project No. 238/34R

TESTING FACILITY: Safepharm Laboratories Ltd., Derby, U.K.

SPONSOR: Rhone-Poulenc Agriculture Ltd., Essex, England

TITLE OF REPORT: RPA 201772: Acute dermal toxicity (Limit test) in the rabbit

AUTHOR: D.J. Allen

REPORT ISSUED: November 3, 1993

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID # 435732-13), five male and five female New Zealand White rabbits were dermally administered RPA 201772 at a dose of 2000 mg/kg for 24 hours. The animals were observed for mortality and clinical signs of toxicity for 14 days post-dosing. There were no deaths or clinical signs of toxicity during the study. Very slight erythema was observed in two male and one female rabbit. **The acute dermal LD_{50} for RPA 201772 was greater than 2000 mg/kg.**

The study is classified as **Acceptable** with a **Toxicity Category III** and **satisfies** the requirements (81-2) for an acute dermal toxicity study in rabbits.

I. MATERIALS

A. Test Material

Name: RPA 201772
Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole; isoxaflutole
Purity: 98.7% a.i.
Lot/batch number: 21 ADM 93
Description: Yellow powder
Storage Conditions: At room temperature protected from light

B. Test Animals

Species: New Zealand White rabbits
Source: David Percival Ltd., Cheshire, U.K.
Age: 12 to 20 weeks
Weight: Males - 2.13 to 2.46 kg; Females - 2.12 to 2.24 kg at dosing
Housing: Individually in cages
Environmental Conditions: Temperature: 18-23^0 C
Relative Humidity: 55-66%
Photoperiod: 12 hours light/dark
Food and Water: RABMA Rabbit Diet and water *ad libitum*
Acclimation Period: Five days

II. METHODS

One day prior to dosing, fur was clipped from skin area of the trunk. On the day of dosing, pre-weighed amount of the test substance (2000 mg/kg) was applied uniformly to the test area which had previously been moistened with 0.5% (v/v) carboxymethylcellulose. The treated area was then wrapped with gauze, adhesive strapping, and secured by an elastic corset for 24 hours. After the 24-hour exposure period, the wrappings were removed. Observations of toxicity and mortality were recorded 0.5, 1, 2, and 4 hours after dosing and subsequently once daily for 14 days. Body weights were recorded prior to dosing and at 7 and 14 days post-dosing. At the end of the observation period, all animals were sacrificed and necropsied.

III. RESULTS

The amount of test substance/area covered approximately 16 cm x 18 cm (equivalent to 10% of the total body surface). None of the animals died nor were there any clinical signs of toxicity during the study. Only very slight erythema was noted at the application site in two male and one female rabbit 1 day post-treatment; this reaction persisted in one male until day 2. All animals gained weight during the study and there were no lesions on gross necropsy. The acute dermal LD_{50} was greater than 2000 mg/kg.

IV. COMPLIANCE

The following compliance documents were submitted: 1) signed statement by the sponsor indicating that the study was conducted in accordance with GLP Regulations; 2) signed Quality Assurance statement by the testing facility; 3) signed statement by the sponsor claiming no data confidentiality.

V. CONCLUSIONS

The acute dermal LD_{50} for RPA 201772 in rabbits was greater than 2000 mg/kg.

The study is classified as **Acceptable** with a **Toxicity Category III** and **satisfies** the requirements (81-2) for an acute dermal toxicity study in rabbits.

Reviewed by: Sanjivani B. Diwan, Ph.D. Sanjivani B. Diwan 9/7/95
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M. Ioannou 9/7/95
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation/Rats (81-3)

EPA I.D. NUMBERS: DP BARCODE: D214214
P. C. CODE: 123000
MRID NUMBER: 435732-14
SUBMISSION NO.: S484204

TEST MATERIAL: RPA 201772
CAS No.: 141112-29-0
Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole;isoxaflutole

STUDY NUMBER: Report No. RNP 435/932339

TESTING FACILITY: Huntingdon Research Centre Ltd., Cambridgeshire, England

SPONSOR: Rhone-Poulenc Agriculture Ltd., Essex, England

TITLE OF REPORT: RPA 201772: Acute inhalation toxicity in rats 4-hour exposure

AUTHOR(S): G.C. Jackson

REPORT ISSUED: February 24, 1994

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID # 435732-14), five male and five female Sprague Dawley rats were exposed to an atmospheric concentration of 5.23 mg/l of RPA 201772 for four hours. The concentration of the test material in the atmosphere was determined gravimetrically five times during the exposure. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the particles were determined once during the exposure. The nominal concentration of the test material was calculated from the amount of RPA 201772 dispersed and the total volume of air supplied to the exposure system. The animals were observed for mortality and clinical signs of toxicity during the exposure and the 14-day post-exposure observation period. No animals died during the exposure or observation period. The only clinical signs observed were during the exposure and included partial closure of the eyes and presence of test material on the fur in both sexes. The nominal concentration of the test material was 15.5 mg/l. The mean gravimetric concentration was 5.23 (±0.50) mg/l. The mean MMAD was 3.1 μ with a GSD of 1.95. **The acute inhalation LC_{50} for RPA 201772 was greater than 5.23 mg/l.**

The study is classified as **Acceptable** with a **Toxicity Category IV** and **satisfies** the requirements (81-3) for an acute inhalation study in rats.

I. MATERIALS

A. Test Material

Name: RPA 201772
Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl) isoxazole; isoxaflutole
Purity: 98.3% a.i.
Lot/batch number: FPI1308
Description: Fine cream colored powder
Storage Conditions: Ambient temperature protected from sunlight

B. Test Animals

Species: Sprague Dawley rats
Source: Charles River UK Ltd., Kent, England
Age: 6-8 weeks at arrival
Weight: males - 227 to 248 g; females - 212 to 232 g on day of exposure
Environmental Conditions: Temperature: 10-24°C
Humidity: 30-54%
Photoperiod: 12 hours light/dark
Housing: Five per cages except during exposure when housed individually
Food and Water: SDS RM1 diet and tap water *ad libitum* except during exposure
Acclimation Period: Five days

II. METHODS

Exposure Chamber

The perspex made exposure chamber had a volume of approximately 120 liters. Air flow through the chamber was 25 l/min. The theoretical time required for the chamber to reach 90% of the target concentration was 11 min.

Atmosphere Generation and Monitoring

The test material was packed into Wright dust generator using hydraulic bench press and a force of 0.7 tons weight. The test atmosphere was generated by suspending material scraped from the surface of a compressed powder in a stream of dry air. The speed controller of the generator was set to give an expected concentration of dust in excess of 5 mg/l of air. The test material was mixed with compressed air in the nozzle and introduced via an inlet in the base of the chamber. The test atmosphere was introduced through a passage at the base of the chamber and passed out through small holes in the lower edge of the square section. The exposure chamber was situated inside a cabinet equipped with an exhaust fan that controlled the airflow from chamber to the

atmosphere through a collection filter.

The concentration of the chemical in the test atmosphere was determined gravimetrically on five occasions during the four-hour exposure period. Samples were collected through a glass fiber filter at a flow rate of approximately 4 l/min.

The nominal concentration of test substance in the exposure chamber was calculated from the amount of the test substance dispersed in the generator and the total air flow through the generator.

The Marple cascade impactor was used to determine the particle size distribution of the test atmosphere twice during the exposure period. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the particles were calculated.

The temperature and humidity within the chamber were recorded at the start of exposure and then at 30-minute intervals during the 4-hour exposure.

Animal Treatment

Five male and five female rats were administered a four-hour whole-body exposure at a target concentration of 5.23 mg/l (limit dose). Observations for mortality and clinical signs of toxicity were made every 30 minutes during the exposure and then twice daily during the 14-day observation period. The animals were weighed daily from the day of arrival until the end of observation period. At the end of the study, all rats were subjected to a complete gross necropsy examination. The samples of lungs, liver, and kidneys were collected and preserved for possible microscopic examination.

III. RESULTS

Test Atmosphere

The nominal concentration of the test material was 15.5 mg/l. The mean gravimetric concentration based on five samples was 5.23 (± 0.50) mg/l. The mean MMAD was 3.1 μ with a GSD of 1.95.

The mean chamber temperature and relative humidity were 24±0.3^0 C and 53±2.9%, respectively.

No animals died during the exposure or observation period. The only clinical signs observed during and after the exposure for both sexes were partial closure of the eyes and presence of test material on the fur; all animals recovered by day 1 of the observation period. Following exposure, the rate of body weight gain was lower for 1 day in male rats and for 2 days in female rats. All the animals gained weight over the course of the study. No treatment-related gross findings were noted in any animal. On

necropsy, one male had slightly congested lungs which was considered to be an incidental finding. No microscopic findings were reported.

IV. COMPLIANCE

Signed statements of Quality Assurance and compliance with the Good Laboratory Practice regulations were submitted by the testing facility. The sponsor submitted a statement claiming no data confidentiality.

V. CONCLUSIONS

The acute inhalation LC_{50} for RPA 201772 in rats was greater than 5.23 mg/l. The mean MMAD was 3.1 μ with a GSD of 1.95.

The study is classified as **Acceptable** with a **Toxicity Category IV** and **satisfies** the requirements (81-3) for an acute inhalation study in rats.

011675

Reviewed by: Sanjivani B. Diwan, Ph.D. Sanjivani B. Diwan 9/7/95
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. J.M. Ioannou 9/7/95
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Primary Eye Irritation/Rabbits (81-4)
EPA ID NUMBERS:	DP BARCODE: D214214 P. C. CODE: 123000 MRID NUMBER: 435732-15
TEST MATERIAL:	RPA 201772 CAS Number: 141112-29-0 Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl) isoxazole; isoxaflutole
STUDY NUMBER:	Project No. 238/36R
TESTING FACILITY:	Safepharm Laboratories Ltd., Derby, U.K.
SPONSOR:	Rhone-Poulenc Agiculture Ltd., Essex, England
TITLE OF REPORT:	RPA 201772: Acute Eye Irritation Test in the Rabbit
AUTHOR:	D.J. Allen
REPORT ISSUED:	November 3, 1993

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID # 435732-15), 0.1 ml (approx. 99 mg) of RPA 201772 was instilled into the conjunctival sac of one eye of three male and three female New Zealand White rabbits. The other eye served as an untreated control. The eyes were examined for signs of irritation and scored at 1, 24, 48 and 72 hours post-dosing. The only positive score (according to the Draize grading scale) for iritis was in one female at 1 hour post-dosing. Minimal to moderate conjunctival irritation was noted in all six rabbits at 1 hour post-dosing; all eyes appeared normal at the 24-hour observation. **The study demonstrated that RPA 201772 produces minimal ocular irritation in rabbits.**

The study is classified as **Acceptable** with a **Toxicity Category IV** and **satisfies** the requirements (81-4) for a primary eye irritation study in rabbits.

I. MATERIALS

A. Test Material

Name: RPA 201772
Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole; isoxaflutole
Purity: 98.7% a.i.
Reference Number: 21 ADM 93
Description: Yellow powder
Storage Conditions: At room temperature protected from light

B. Test Animals

Species: New Zealand White rabbits
Source: David Percival Ltd., Cheshire, U.K.
Age: 12 to 20 weeks
Weight: Males - 2.54 to 3.20 kg; Females - 2.48 to 2.62 kg at dosing
Housing: Individually in cages
Environmental Conditions: Temperature: 19-22^0 C
Relative Humidity: 58-63%
Photoperiod: 12 hours light/dark
Food and Water: RABMA Rabbit Diet and water *ad libitum*
Acclimation Period: Five days

II. METHODS

Within 24 hours of dosing, the eyes of three male and three female rabbits were examined using fluorescein stain. A dose weight (approx. 99 mg) equivalent to a volume of 0.1 ml of RPA 201772 was then instilled into the conjunctival sac of one eye of each animal. The other eye served as an untreated control. The eyes were examined for evidence of irritation and scored at 1, 24, 48 and 72 hours post-dosing. A copy of the grading scale is attached to the DER. Fluorescein staining was used for the examinations beginning on day 1.

III. RESULTS

The only positive score for iritis was in one female at one hour post-dosing. No corneal effects were noted. Minimal to moderate conjunctival irritation was observed in all six rabbits at 1 hour post-instillation (conjunctival redness in six rabbits; chemosis in three rabbits; and discharge in four rabbits). All treated eyes appeared normal at the 24-hour observation. The maximum group mean score for occular irritation was 5.8. Thus, the study demonstrated that RPA 201772 produced minimal, transient ocular irritation in rabbits.

IV. COMPLIANCE

The following compliance documents were submitted: 1) signed statement by the sponsor indicating that the study was conducted in accordance with GLP Regulations; 2) signed Quality Assurance statement by the testing facility; 3) signed statement by the sponsor claiming no data confidentiality.

V. CONCLUSIONS

The study demonstrated that RPA 201772 produces minimal, transient ocular irritation in rabbits.

The study is classified as **Acceptable** with a **Toxicity Category IV** and **satisfies** the requirements (81-4) for a primary eye irritation study in rabbits.

APPENDIX I

DRAIZE SCALE FOR SCORING OCULAR IRRITATION

1. CONJUNCTIVAE

(A) **Redness** (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

(B) **Chemosis**

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids half closed to completely closed	4

(C) **Discharge**

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs a considerable area around the eye	3

THE TOTAL SCORE = (A + B + C) x 2 MAXIMUM TOTAL = 20

2. IRIS

(D) **Values**

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

THE TOTAL SCORE = D x 5 MAXIMUM TOTAL = 10

3. CORNEA

(E) **Degree of Opacity** (most dense area used)

No opacity	0
Scattered or diffuse areas, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4

(F) **Area of Cornea involved**

One quarter (or less) but not zero	1
Greater than one quarter but less than half	2
Greater than half but less than three quarters	3
Greater than three quarters, up to whole area	4

THE TOTAL SCORE = (E x F) x 5 MAXIMUM TOTAL = 80

MAXIMUM TOTAL SCORE POSSIBLE = 110

MODIFIED KAY AND CALANDRA INTERPRETATION OF EYE IRRITATION TEST

MAXIMUM MEAN SCORE	PERSISTENCE OF SCORE	DESCRIPTION RATING (AND CLASS)
0.0 to 0.5	Group mean score at 24 hours = 0	Non-irritating (1)
	Group mean score at 24 hours > 0	Practically non-irritating (2)
0.5 to 2.5	Group mean score at 24 hours = 0	Non-irritating (1)
	Group mean score at 24 hours > 0	Practically non-irritating (2)
2.5 to 15	Group mean score at 48 hours = 0	Minimal irritant (3)
	Group mean score at 48 hours > 0	Mild irritant (4)
15 to 25	Group mean score at 72 hours = 0	Mild irritant (4)
	Group mean score at 72 hours > 0	Moderate irritant (5)
25 to 50	Group mean score at 7 days ___ 20 or less	{More than half of the individual {total scores at 7 days 10 or less — Moderate irritant (5)
	Group mean score at 7 days ___ 20 or less	{More than half of the individual {total scores at 7 days > 10 but {no individual total score at 7 {days > 30 — Moderate irritant (5)
	Group mean score at 7 days ___ 20 or less	{More than half of the individual {total scores at 7 days > 10 and {any individual score at 7 days {> 30 — Severe irritant (6)
	Group mean score at 7 days > 20	Severe irritant (6)
50 to 80	Group mean score at 7 days ___ 40 or less	{More than half of the individual {total scores at 7 days 30 or {less — Severe irritant (6)
	Group mean score at 7 days ___ 40 or less	{More than half of the individual {total scores at 7 days > 30 but {no individual total scores at 7 {days > 60 — Severe irritant (6)
	Group mean score at 7 days ___ 40 or less	{More than half of the individual {total scores at 7 days > 30 and {any individual total score at 7 {days > 60 — Very severe irritant (7)
	Group mean total score at 7 days > 40	Very severe irritant (7)
80 to 100	Group mean total score at 7 days 80 or less ___	{More than half of the individual {total scores at 7 days 60 or {less — Very severe irritant (7)
	Group mean total score at 7 days 80 or less ___	{More than half of the individual {total scores at 7 days > 60 but {no individual total score at 7 {days > 100 — Very severe irritant (7)
	Group mean total score at 7 days 80 or less ___	{More than half of the individual {total scores at 7 days > 60 and {any individual total score at 7 {days > 100 — Extremely severe irritant (8)
	Group mean total score at 7 days > 80	Extremely severe irritant (8)
100 to 110	Group mean total score at 7 days 80 or less	Very severe irritant (7)
	Group mean total score at 7 days > 80	Extremely severe irritant (8)

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Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. J.M. Ioannou 9/7/95
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Primary Dermal Irritation/Rabbits (81-5)
EPA ID NUMBERS:	DP BARCODE: D214214 P. C. CODE: 123000 MRID NUMBER: 435732-16 SUBMISSION NO.: S484204
TEST MATERIAL:	RPA 201772 CAS No.: 141112-29-0 Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole;isoxaflutole
STUDY NUMBER:	Project No. 238/35R
TESTING FACILITY:	Safepharm Laboratories Ltd., Derby, U.K.
SPONSOR:	Rhone-Poulenc Agriculture Ltd., Essex, England
TITLE OF REPORT:	RPA 201772: Acute dermal irritation test in the rabbit
AUTHOR:	D.J. Allen
REPORT ISSUED:	November 3, 1993

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID # 435732-16), 0.5 g of RPA 201772 moistened with 0.5 ml of 0.5% carboxymethylcellulose was applied to a clipped skin area of three male and three female New Zealand White rabbits for four hours. The treated areas were examined for signs of dermal irritation (edema and erythema) and scored at 1, 24, 48 and 72 hours post-treatment. Very slight erythema was observed in one male at 1 hour after patch removal. All treated skin sites appeared normal at the 24-hour observation. **The study demonstrated that RPA 201772 produces no dermal irritation in rabbits.**

The study is classified as **Acceptable** with a **Toxicity Category IV** and **satisfies** the requirements (81-5) for a primary dermal irritation study in rabbits.

I. MATERIALS

A. Test Material

Name: RPA 201772
Synonym: 5-amino-1-(2,6-dichloro-4-trifluoromethyl-4-trifluoromethylbenzoyl)isoxazole; isoxaflutole
Purity: 98.7% a.i.
Lot/batch number: 21 ADM 93
Description: Yellow powder
Storage Conditions: At room temperature protected from light

B. Test Animals

Species: New Zealand White rabbits
Source: David Percival Ltd., Cheshire, U.K.
Age: 12 to 18 weeks
Weight: Males - 2.04 to 2.51 kg; Female - 2.10 kg at dosing
Housing: Individually in cages
Environmental Conditions: Temperature: 20-23^0 C
Relative Humidity: 64-68%
Photoperiod: 12 hours light/dark
Food and Water: RAMBA Rabbit Diet and water *ad libitum*
Acclimation Period: Five days

II. METHODS

The day before dosing, the dorsal area of the trunk of three male and three female rabbits was clipped. On the day of dosing, 0.5 g of RPA 201772 moistened with 0.5 ml of 0.5% (v/v) carboxymethylcellulose was applied to the skin under a gauze patch and secured with adhesive tape. The trunk of each animal was wrapped in an elastic corset during the exposure period. At the end of the exposure, the corset and patches were removed and any residual test material was removed from the treated sites. The areas were examined for signs of dermal irritation and scored at 1, 24, 48 and 72 hours post-dosing. A copy of the grading scale is attached to the DER.

III. RESULTS

Very slight erythema was observed in one male at 1 hour post-dosing. All treated skin sites appeared normal at the 24-hour observation. The primary irritation index for RPA 201772 was 0.0. The study demonstrated that RPA 201772 produces no dermal irritation in rabbits.

IV. COMPLIANCE

The following compliance documents were submitted: 1) signed statement by the sponsor indicating that the study was conducted in accordance with GLP Regulations; 2) signed Quality Assurance statement by the testing facility; 3) signed statement by the sponsor claiming no data confidentiality.

V. CONCLUSIONS

The study demonstrated that RPA 201772 produces no dermal irritation in rabbits.

The study is classified as **Acceptable** with a **Toxicity Category IV** and **satisfies** the requirements (81-5) for a primary dermal irritation study in rabbits.

4. PROCEDURE (contd)

One hour following the removal of the patches, and approximately 24, 48 and 72 hours after patch removal, the test sites were examined for evidence of primary irritation and scored according to the following scale i.e. Draize J.H. (1959) Association of Food and Drug Officials of the United States, Austin, Texas, "The Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics":-

EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	*Value*
No erythema	*0*
Very slight erythema (barely perceptible)	*1*
Well-defined erythema	*2*
Moderate to severe erythema	*3*
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	*4*

Oedema Formation	
No oedema	*0*
Very slight oedema (barely perceptible)	*1*
Slight oedema (edges of area well-defined by definite raising)	*2*
Moderate oedema (raised approximately 1 millimetre)	*3*
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	*4*

Any other skin reactions, if present, were also recorded.

5. INTERPRETATION OF RESULTS

The scores for erythema and oedema at the 24 and 72-hour readings were totalled for the six test rabbits (24 values) and this total was divided by 12 to give the primary irritation index of the test material. The test material was classified according to the following scheme:

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Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Y.M. Ioannou 9/7/95
Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization/Guinea Pigs (81-6)

EPA ID NUMBERS: DP BARCODE: D214214
P. C. CODE: 123000
MRID NUMBER: 435732-17
SUBMISSION No.: S484204

TEST MATERIAL: RPA 201772
CAS No.: 141112-29-0
Synonym:5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole;isoxaflutole

STUDY NUMBER: Report No. 92/RHA481/0673

TESTING FACILITY: Life Science Research Ltd., Suffolk, England

SPONSOR: Rhone-Poulenc Agrochimie, Lyon, France

TITLE OF REPORT: RPA 201772: Delayed contact hypersensitivity study in guinea-pigs

AUTHOR: P.B. Rees

REPORT ISSUED: October 22, 1992

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID # 435732-17) using a modified Buehler method, ten male and ten female Dunkin-Hartley albino guinea pigs received nine topical induction doses of 0.25 ml of 50% (w/v) RPA 201772 in propylene glycol for six hours at 2 to 3 day intervals. A challenge topical dose of 0.25 ml of 10% or 50% (w/v) RPA 201772 in propylene glycol was administered to animals, ten days following the last induction dose. The control group (five/sex/group) was treated only during the challenge phase.

Application of the highest dose of the test material produced intermittent very faint erythema throughout the induction period; no dermal response was noted at the application sites in the test animals following challenge with 10% or 50% (w/v) RPA 201772. The control animals were negative after the challenge application.

For positive controls, the study author provided historical data on a similar study with formaldehyde tested using identical study procedures.

The results of this study indicate that RPA 201772 is not a sensitizer in guinea pigs.

The study is classified as **Acceptable** and **satisfies** the requirements (81-6) for a dermal sensitization study in guinea pigs.

I. MATERIALS

A. Test Material

Name: RPA 201772
Synonym: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)isoxazole;isoxaflutole
Purity: 99.4%
Lot Number: GAR 292
Batch Number: JYG707
Description: White, crystalline powder
Storage Conditions: at room temperature protected from light

A fresh formulation of the test material was prepared daily as suspension in propylene glycol. For the preliminary trials, concentrations of 5, 10, 30 or 50% (w/v) of RPA 201772 were used. Pre-trials indicated that concentrations higher than 50% (w/v) contained insufficient vehicle to suitably mobilize the test material for administration. For positive controls, the study authors provided historical data from a study using formaldehyde in purified water at 10% (w/v) for the induction phase and at 1 or 3% (w/v) for the challenge phase.

B. Test Animals

Species: Albino guinea pigs, Dunkin-Hartley strain
Source: Olac Ltd., Oxfordshire, England
Age: 6-8 weeks before treatment on day 1
Weight: Males - 300 to 382 g; Females - 305 to 357 g at dosing
Housing: Individually in stainless steel cages
Food and water: Guinea Pig F.D.1. Diet and tap water *ad libitum*
Environmental Conditions: Temperature: 15-23^{0}C
Relative Humidity: 40-70%
Photoperiod: 12 hours light/dark
Acclimation Period: 11 days

II. METHODS

The study was conducted using a modified Buehler method.

Preliminary Test

In a preliminary test, the primary irritancy potential of RPA 201772 in propylene glycol was tested at four sites on each of the four animals per dose formulation. The sites of application of the four formulations were rotated on each successive animal to minimize the variability in responsiveness due to location of the test site. On the day prior to application, the application sites were clipped. On the day of dosing, each site was treated by applying an absorbent patch saturated with 0.25 ml of appropriate test formulation. The patch was held against the skin by an occlusive dressing for six hours. At the end of the exposure period, the application sites were washed with propylene glycol.

At 1 day after the application, each site was clipped and depilated using cream of calcium thioglycolate. The treated areas were evaluated and scored for signs of erythema at 24 and 48 hours using the following scale.

Grade	Reaction to Treatment
0	No reaction
±	Very faint erythema, usually non-confluent
1	Faint erythema, usually confluent
2	Moderate confluent erythema
3	Severe confluent erythema

In the preliminary trial, 5, 10, 30, and 50% (w/v) RPA 201772 in propylene glycol were tested. No propylene control group was maintained. Topical application of 5% (w/v) RPA 201772 produced very faint erythema in one male 24 hours after the exposure period. There was no evidence of dermal irritation with either the 10, 30 or 50% test formulations at 24 or 48 hours, therefore, the 50% formulation was selected for both the induction and challenge doses in the main study.

Main study

The study was performed as follows:

Induction Phase

The animals were assigned to four groups as summarized below.

Group	Number of Animals Male	Female	Induction Dose Concentration	Challenge Dose Concentration
50% w/v RPA 201772 in propylene glycol (Treated)	5	5	50%	50%
10% w/v RPA 201772 in propylene glycol (Control)	5	5	50%	10%
Propylene glycol (control)	5	5	-	50%
Propylene glycol (control)	5	5	-	10%

An induction dose was applied for six hour period on days 1, 3, 5, 8, 10, 12, 15, 17, and 19 using the same procedure described in the

preliminary test. For the test material, all nine induction applications were made to the left scapular region of each test animal. Evaluations for signs of dermal irritation were made at 24 and 48 hours post application.

Challenge Phase

Ten days following the last induction dose, the challenge doses of the test and control chemicals were applied to the right scapular region of each treated and control animal using the same procedure as in the induction phase as follows: occluded topical application of 0.25 ml propylene glycol to one site, 0.25 ml of 10% w/v RPA 201772 in propylene glycol to a second site and 0.25 ml of 50% w/v RPA 201772 in propylene glycol to a third site. After six-hour exposure period, the test sites were washed with propylene glycol. The application sites were examined at 24 and 48 hours post-dosing. Significant erythematous reaction (Grade 1 or above) in at least 15% of the animals was considered to be positive.

Compliance

Signed statements of Quality Assurance and compliance with Good Laboratory Practice regulations were submitted by the testing facility. The sponsor submitted a statement claiming no data confidentiality.

III. RESULTS

The 50% (w/v) RPA 201772 caused intermittent very faint erythema (Grade ±) throughout the induction period. During the first week of induction, one male and one female developed very faint erythema. During the second week, faint erythema was noted in one female and very faint erythema was observed in six females and ten males; during the last week, very faint erythema was noted in two males and one female. No evidence of irritation was seen in the treated or control animals after the challenge dose of 10 or 50% (w/v) RPA 201772.

Very faint to severe erythema was observed in all positive control animals treated with 10% w/v formaldehyde in purified water following the second and third induction doses. A grade of 1 or more was considered positive because no reactions in the concurrent control animals were greater than ± (very faint erythema). By 48 hours, the challenge dose of 3% (w/v) formaldehyde produced very faint to severe erythema in seven of ten treated animals; one male also had edema and eschar formation.

IV. CONCLUSIONS

The results of this study indicates that RPA 201772 is not a sensitizer.

The study is classified as **Acceptable**. The study **satisfies** the requirements (81-6) for a dermal sensitization study in guinea pigs.

011675

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Reference:	DP Barcode D215036
Subject:	EPA File Symbol: 000264-EUP-OO
Chemical:	123000 (Isoxaflutole, RPA 201772)
Test Material:	EXP31130A, 75% Water Dispersible Granule
From:	S.E. Oonnithan June 13/95 Precautionary Review Section Registration Support Branch (H7505W) Registration Division
To:	Joanne I. Miller, PM 23 Fungicide-Herbicide Branch (H7505C) Registration Division
Applicant:	Rhone-Poulenc Ag Company P.O. Box 12014 Research Triangle Park, NC.

FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
RPA 201772 (Isoxaflutole)	75.0
Inert Ingredient(s)	25.0
Total	100.0

BACKGROUND

Rhone-Poulenc Ag Company submitted acute oral toxicity (MRID No. 435732-25), acute dermal toxicity (MRID No. 435732-26), acute inhalation toxicity (MRID No. 435732-27), primary eye irritation (MRID No. 435732-28), primary skin irritation (MRID No. 435732-29), and dermal sensitization (MRID No. 435732-30) studies on RPA 201772 WDG herbicide. The acute toxicity studies were performed by Bushy Run Research Center, Export, PA.

RECOMMENDATION

1. Acute Oral: Category IV. The submitted study is acceptable.

2. Acute Dermal: Category III. The submitted study is acceptable.

3. Acute Inhalation: Category IV. The submitted study is acceptable.

4. Eye Irritation: Category III. The submitted study is acceptable.

5. Skin Irritation: Category IV. The submitted study is acceptable.

6. Dermal Sensitization: Not a sensitizer. The submitted study is acceptable.

LABELING

The appropriate signal word is **CAUTION.**

Recommended Precautionary Statements

Causes moderate eye irritation. Harmful if absorbed through skin. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling.

Recommended Statements of Practical Treatment

IF IN EYES: Flush eyes with plenty of water. Call a physician if irritation persists.

IF ON SKIN: Wash with plenty of soap and water. Get medical attention.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§81-1)

Product Manager: 23
MRID No.: 435732-25
Author: S.M. Christopher
Testing Facility: Bushy Run

Reviewer: S. Oonnithan
Report No.: 94N1402A
Report Date: 07/28/94

Species: Rat, Sprague Dawley
Age: Young adults
Weight: Males 235-260 g; Females 203-233 g
Source: Harlan Sprague Dawley, Inc., Indianapolis, IN.

Test Material: EXP31130A in water (50%; w/v).
Dosage: 1 ml/100 g body wt.

Conclusion:
LD_{50}: **Males:** 5 g/kg; **Females:** 5 g/kg
Combined: 5 g/kg
Tox. Category: IV
Classification: Acceptable

Quality Assurance (40 CFR §160.12): Included
Procedure (Deviations from §81-1): None

Results:

Dosage	Number Dead/Tested		
	Males	Females	Combined
5 g/kg	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:
The observed signs of toxicity included diarrhea and a brown stain on the perianal fur. All animals gained weight during the observation period. In survivors, no gross lesions were evident at necropsy.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (§81-2)

Product Manager: 23
MRID No.: 435732-26
Author: S.M. Christopher
Testing Facility: Bushy Run

Reviewer: S. Oonnithan
Report No.: 94N1402B
Report Date: 07/28/94

Species: Rabbit, New Zealand White
Age: Young adult (12-18 weeks)
Weight: Males 2.7-3.0 kg; Females 2.5-3.0 kg
Source: HRP Inc., Denver, PA.

Test Material: EXP31130A
Dosage: 2 g/kg
Test method: The test substance was moistened with water (at the rate of 0.4 ml/g) and applied to the dorsal surface at 81-82 mg/cm^2 surface area. The test area was occluded with a double layer of gauze sheeting, wrapped with polyethylene, and secured with plastic ties or rubber bands.

Conclusion:
LD_{50}: **Males:** >2 g/kg; **Females:** >2 g/kg
Combined: >2 g/kg
Tox. Category: III
Classification: Acceptable

Quality Assurance (40 CFR §160.12): Included
Procedure (Deviations from §81-2): None

Results:

Dosage	Number Dead/Tested		
	Males	Females	Combined
2 g/kg	0/5	0/5	0/10

Symptoms & Gross Necropsy Findings:
Moderate dermal irritation (erythema, edema, and ecchymoses) and a brown chemical residue and stain were observed in all animals for 1-14 days. There were no signs of systemic toxicity, except for rapid breathing. Some animals exhibited a weight loss for up to 7 days, but recovered by 14 days. Necropsy revealed light or bright red lungs, tan kidneys, pitted kidney surface, and red adrenal glands.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (§81-3)

Product Manager: 23
MRID No.: 435732-27
Author: D.J. Nachreiner
Testing Facility: Bushy Run

Reviewer: S. Oonnithan
Report No.: 94N1401
Report Date: 08/10/94

Species: Rat, Sprague Dawley
Age: Young adult (8 weeks)
Weight: Males 265-275 g; Females 177-190 g
Source: Harlan Sprague Dawley, Inc., Indianapolis, IN.

Test Material: EXP31130A; air-milled by the registrant.
Dosage: 21.0 mg/l (Nominal concentration)
Test Conditions: In a plexiglass/stainless steel rectangular chamber (≈120 liter volume), test animals were exposed in a stainless steel wire mesh cage (5/cage). Chamber concentration was measured 8 times gravimetrically and particle size distribution was measured twice using a cascade impactor. The chamber air flow was maintained at 29.5 l/min (14.8 air exchange/hr).

Conclusion:
LD_{50}: **Males:** >5.26 mg/l; **Females:** >5.26 mg/l
Combined: >5.26 mg/l
Tox. Category: IV
Classification: Acceptable
Quality Assurance (40 CFR §160.12): Included
Procedure (Deviations from §81-3): None

Results:

Exposure Concentration	Number Dead/Tested		
	Males	Females	Combined
5.26 mg/l MMAD = 2.3 μm; GSD = 2.6	0/5	0/5	0/10

The registrant reported that approximately 19% of the dust particles were ≤1 μm and 80% of the dust particles were ≤5 μm, based on particle mass distribution data.

Symptoms & Gross Necropsy Findings:
The clinical signs observed during exposure were blepharospasm and that during post-exposure were perinasal encrustation and unkempt fur. At necropsy one male rat had a dark red punctate color change of the lungs (judged as incidental); no other gross pathologic findings were observed.

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (§81-4)

Product Manager: 23
MRID No.: 435732-28
Author: S.M. Christopher
Testing Facility: Bushy Run

Reviewer: S. Oonnithan
Report No.: 94N1402D
Report Date: 07/27/94

Species: Rabbit, New Zealand White
Age: 12-18 weeks
Weight: 2.0-3.3 kg
Source: HRP Inc., Denver, PA.

Test Material: EXP31130A
Dosage: ≈80 mg; 0.1 ml
Test Conditions: The test substance was ground with a mortar and pestle and 0.1 ml of the powder was placed into the conjunctival sac of one eye. Fluorescein staining was performed on day one and on subsequent examinations.

Summary: Mild irritation cleared in 72 hours.
Tox. Category: III
Classification: Acceptable

Quality Assurance (40 CFR §160.12): Included
Procedure (Deviations from §81-4): None

Results:

Observations		Number "positive"/tested at			
		1 Hr	24 Hrs	48 Hrs	72 Hrs
Cornea Opacity		2/6	0/6	1/6	0/6
Iris		3/6	3/6	0/6	0/6
Conjunctivae:	Redness	1/6	3/6	0/6	0/6
	Chemosis	2/6	1/6	0/6	0/6
	Discharge	6/6	1/6	0/6	0/6

Comments:
Minor corneal irritation was apparent in two animals; in one, the irritation lasted for 48 hours. In three animals, iritis was observed for up to 24 hours. All conjunctival irritation cleared in 48 hours.

DATA REVIEW FOR SKIN IRRITATION TESTING (§81-5)

Product Manager: 23
MRID No.: 435732-29
Author: S.M. Christopher
Testing Facility: Bushy Run

Reviewer: S. Oonnithan
Report No.: 94N1402C
Report Date: 07/27/94

Species: Rabbit, New Zealand White
Age: 12-19 weeks
Weight: 2.0-3.5 kg
Source: HRP Inc., Denver, PA.

Test Material: EXP31130A
Dosage: 0.5 g
Test method: The test substance was moistened with water (0.2 ml/0.5 g) and applied directly to an 1 inch square gauze patch. The patch was placed over the dose site, and secured by adhesive tape. Polyethylene sheeting was wrapped loosely around the trunk and secured.

Summary: Very slight irritation cleared in 7 days.
Tox. Category: IV
Classification: Acceptable

Quality Assurance (40 CFR §160.12): Included
Procedure Deviations from §81-5: None

Results: At 72 hour post-treatment, 1-2 test animals had very slight erythema/edema, which cleared in 7 days.

DATA REVIEW FOR SKIN SENSITIZATION TESTING (§81-6)

Product Manager: 23
MRID No.: 435732-30
Author: S.M. Christopher
Testing Facility: Bushy Run

Reviewer: S. Oonnithan
Report No.: 94N1403
Report Date: 09/07/94

Species: Guinea Pig, Dunkin Hartley Albino
Age: 6.0-7.5 weeks
Weight: Males: 367-425 g; Females: 402-487 g
Source: HRP Inc., Denver, PA

Test Material: EXP31130A

Summary: The product formulation tested at 40% (w/v) indicated that it is not a sensitizer to guinea pigs.
Classification: Acceptable

Quality Assurance (40 CFR §160.12): Included
Procedure Deviations from §81-6: None

Test Procedure:
The Buehler test was used for this study. Based on a preliminary skin irritation test using 65%, 50%, and 40% (w/v) solutions of the test substance in 0.25% (w/v) aqueous methyl cellulose, the 40% concentration was selected for induction and challenge tests. Even though there was no erythema evident at 24 and 48 hours posttreatment with 40% (w/v) concentration, this dose was picked, because higher concentrations of the test substance caused the non-woven cotton pad (used for loading the test material), to disintegrate and dehydrate during the 6-hour exposure period.

As a positive control, dinitrochlorobenzene (DNCB) was used at 0.3% (w/v) and 0.1% (w/v) concentrations for induction challenge tests, respectively. The DNCB solutions were prepared in 0.25% (w/v) aqueous methyl cellulose. A vehicle control was not included in the test, because of the availability of extensive data at Bushy Run, demonstrating the lack of any skin irritation with 0.25% aqueous methyl cellulose.

Groups of 10 guinea pigs (5 male and 5 female) were used for the tests. Each test animal was subjected to an induction treatment, once a week for three weeks. Following a two week rest period, a single challenge application was made. The test animals were examined at 24 and 48 hours after induction and challenge tests. Two naive control tests were also performed by applying a single application of the challenge dose of the test substance or positive control.

Results:
Summary of the induction and challenge test.

Observations		Number positive/tested at 24 and 48 hours			
		EXP31130A		DNCB	
Induction	1st Application	1/10	2/10	5/10	4/10
	2nd Application	0/10	3/10	10/10	10/10
	3rd Application	4/10	4/10	10/10	10/10
Challenge		2/10	1/10	10/10	10/10
Naive control		3/10	2/10	6/10	5/10

After three consecutive induction treatment with the test material, 4/10 animals had slight to solid erythema at 48 hours. Following challenge application, 1/10 had slight patchy erythema at 48 hours. At the 24 and 48 hour readings, red to brown foci and/or excoriations (not given in the table) were observed on/around the test site of 5 test animals, extending beyond the test area in 2 animals. The application of 40% (w/v) test substance to naive control animals, resulted in slight patchy erythema in 3/10 and 2/10 animals at 24 and 48 hours, respectively. Red to brown foci and/or excoriations (not given in the table) were also observed on/around the test site of 5/10 animals.

The three induction treatments with positive control indicated slight patchy erythema to moderate erythema in 10/10 animals. Edema with desquamation at the dose site was also evident in 6/10 animals. The challenge application produced moderate to severe erythema in all test animals; 3/10 had eschar and 5/10 had slight edema at the dose site. The application of 0.1% (w/v) DNCB to naive control animals, resulted in slight to solid erythema in 6/10 and 5/10 animals at 24 and 48 hours, respectively.

ACUTE TOX ONE-LINER

1. **DP BARCODE:** D215036

2. **PC CODE(S):** 123000 Isoxaflutole (RPA 201772)

3. **CURRENT DATE:** June 12, 1995

4. **TEST MATERIAL:** EXP31130A, 75% Water dispersible granule

Study/Species/ Lab/Study#/Date	MRID No.	Results	Tox. Cat.	Core Grade [a]
81-1, Rat, Bushy Run, 94N1402A, 07/28/94	435732-25	LD_{50} >5 g/kg	IV	A
81-2, Rabbit, Bushy Run, 94N1402B, 07/28/94	435732-26	LD_{50} >2 g/kg	III	A
81-3, Rat, Bushy Run, 94N1401, 08/10/94	435732-27	LD_{50} >5.26 mg/l	IV	A
81-4, Rabbit, Bushy Run, 94N1402D, 07/27/94	435732-28	Mild irritation cleared in 72 hours.	III	A
81-5, Rabbit, Bushy Run, 94N1402C, 07/27/94	435732-29	Very slight irritation cleared in 7 days.	IV	A
81-6, Guinea Pig, Bushy Run, 94N1403, 09/07/94	435732-30	Not a sensitizer	--	A

[a] Core Grade Key: A = Acceptable, S = Supplementary, and U = Unacceptable.

RPA 201772 IN VITRO CYTOGENETICS

MUTAGENICITY STUDIES

EPA Reviewer: Nancy McCarroll Signature: Nancy L. McCarroll
Review Section III,
Toxicology Branch II/HED 7509C Date: 8/10/95
EPA Section Head: Yiannakis M. Ioannou, Ph.D. Signature:
Review Section I,
Toxicology Branch II/HED 7509C Date: 9/2/95

DATA EVALUATION REPORT

STUDY TYPE: Mammalian cells in culture cytogenetic assay in human lymphocytes (84-2)

DP BARCODE: D214214

SUBMISSION NO.: S484204

PC CODE: 123000

MRID NUMBER: 435732-21

TEST MATERIAL: RPA201772

CAS NUMBER: 141112-29-0

SYNONYM(S): 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-isoxazole; isoxoflutole

STUDY NUMBER(S): Report No. 93/RHA515/0815

SPONSOR: Rhône-Poulenc Agriculture, Essex, England

TESTING FACILITY: Pharmaco-Life Science Research, Ltd., Suffolk, England

TITLE OF REPORT: In vitro assessment of the clastogenic activity of RPA201772 in cultured human lymphocytes

AUTHOR(S): C.A. Dance

REPORT ISSUED: Study completion date: September 24,1993

EXECUTIVE SUMMARY: In an in vitro cytogenetic assay (MRID No.435732-21), 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 μg/ml) and 16 or 40 hours after a 3-hour exposure to comparable S9-activated levels of RPA201772 (98.7%). Only lymphocytes treated with 600 μg/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 substance was delivered to the lymphocyte cultures in acetone.

Compound precipitation was seen at levels ≥300 μg/ml -S9 and at 600 μg/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($p<0.001$) increases in structural chromosome aberrations at all harvest intervals.

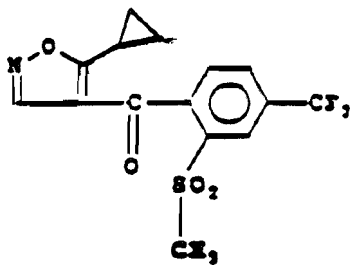
STUDY CLASSIFICATION: Acceptable.

The study is classified as **Acceptable** and satisfies the guideline requirements for an in vitro cytogenetic assay (§84-2).

A. MATERIALS:

1. Test Material: RPA201772

Description: Fine yellow powder
Lot/ batch number: 21 ADM 93
Purity: 98.7% a.i.
Receipt date: May 17, 1993
Stability: Not provided
Structure:



Solvent used: Acetone
Other comments: The test material was stored at room temperature, protected from light. Dosing solutions were prepared fresh on the day of use; actual concentrations were not verified analytically and dosing solutions were not adjusted to 100% a.i.

2. Control Materials:

Negative: None
Solvent/final concentration: Acetone/10 μl/culture

Positive: Nonactivation (concentrations, solvent): Chlorambucil (CB) was prepared in ethanol to yield a final concentration of 2 μg/ml.

Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in purified water to yield a final concentration of 6 μg/ml.

3. Activation: S9 derived from young male CD (≈200 g)

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The rat liver homogenate was prepared by the performing laboratory and used within 1 year of preparation.

S9 mix composition:

Component	Quality (ml)
0.1 M Phosphate buffer (pH 7.4)	7.4
0.4 M $MgCl_2$/1.65 M KCl	0.2
0.1 M NADP	0.4
0.1 M Glucose 6-Phosphate	0.5
S9	1.5

4. Test Compound Concentration Used:

(a) Preliminary cytotoxicity assay: Five doses (75, 150, 300, 450 and 600 μg/ml) were tested in the presence and absence of S9 activation using both a 19- and a 43-hour cell harvest.

(b) Cytogenetic assay:

(1) Nonactivated conditions:

Initial assay: 75, 300 and 600 μg/ml (19- and 43-hour sampling times). Metaphases were examined from all treatment groups harvested at 19 hours and from cultures sampled 43 hours postexposure to 600 μg/ml.

Confirmatory assay: As above with a 19-hour sampling time only

(2) S9-activated conditions:

Initial assay: As above for the initial nonactivated assay.

Confirmatory assay: As above for the confirmatory nonactivated assay.

5. Test Cells: Human lymphocytes were obtained from the blood of one healthy, nonsmoking male volunteer. Lymphocyte cultures were initiated in RPMI 1640 medium containing L-glutamine, HEPES, 10% fetal calf serum, heparin, antibiotics and phytohemagglutinin (concentration not specified). Cells were cultured at 37°C for 48 hours prior to use.

Properly maintained? Yes.

Cell line or strain periodically checked for mycoplasma contamination? Not applicable.

Cell line or strain periodically check for karyotype stability? Not applicable.

B. TEST PERFORMANCE:

1. Cell Treatments:

Cells exposed to test compound, solvent or positive controls for:
19 or 43 hours (nonactivated) 3 hours (activated)

2. Cell Harvest:

Cells harvested at the end of treatment (nonactivated)
Cells harvested 16 and 40 hours after removal of the test material (activated)

3. Preliminary Cytotoxicity Assay: Forty-eight hours after initiation, duplicate cultures were exposed to the selected test material doses or the solvent control (acetone) in both the presence and absence of S9 activation. Under nonactivated conditions, lymphocytes were treated for 19 or 43 hours and harvested at the end of treatment; in the presence of S9 activation, cultures were dosed for 3 hours, centrifuged, resuspended in fresh culture medium and reincubated for an additional 16 or 40 hours. Colcemid (final concentration, 0.4 μg/ml) was added 3 hours before all cultures were harvested. Metaphase cells were collected, swollen in 0.56% KCl, fixed in glacial acetic acid: methanol (1:3) and stained with 10% Giemsa. Approximately 1000 lymphocytes per culture were counted to determine the mitotic index (MI). The level of the test material that caused a depression in mitotic activity was selected as the high dose for the cytogenetic assay.

4. Cytogenetic Assay:

(a) Treatment: The cytogenetic assay was performed in a similar manner with the following exceptions: (1) positive controls (2.0 μg/ml CB -S9 and 6.0 μg/ml CP +S9) were included and, (2) metaphases were examined for chromosome aberrations.

(b) Metaphase analysis: Two hundred metaphase plates (100 cells/culture) from each dose group, the solvent, and positive control groups were scored for chromosome aberrations; gaps were recorded and aberration frequencies were presented with and without gaps. The MI was determined by counting at least 1000 cells per culture. Polyploid and endoreduplicated cells were recorded separately. Slides were coded prior to analysis.

(c) Statistical methods: The data from the experimental groups were evaluated for statistical significance at p values of 0.05 and 0.001 by the Fisher's exact test.

(d) Evaluation criteria:

Assay validity: The assay was considered acceptable if: (1) the percentage of aberrant cells (excluding gaps) in the solvent control cultures was ≤5%; (2) the positive controls induced a statistically and biologically significant increase in the frequency of aberrant cell; (3) a sufficient number of metaphases were available for analysis of each treatment group; and (4) "sufficient toxicity" was demonstrated in the cytogenetic assay.

Positive response: The test material was considered active in this test system if it induced a reproducible and significant increase in the frequency of cells with aberrations compared to both the concurrent and historical negative control data.

C. REPORTED RESULTS:

1. Solubility Determination: RPA201772 formed a fine, homogenous suspension at 600 mg/ml in acetone and was soluble at ≈300 mg/ml. Accordingly, the preliminary cytotoxicity test was initiated with a high dose of 600 mg/ml delivered to the treatment medium in 10-µl volumes yielding an applied concentration of 600 µg/ml. No apparent compound precipitation was observed upon addition of the test material to the culture medium: however, the study author stated that small particles (sedimented/precipitated test material) were seen at harvest in all cultures treated with ≥150 µg/ml -S9 and ≥450 µg/ml +S9.

2. Preliminary Cytotoxicity Assay. Results from the preliminary cytotoxicity assay indicated that the continuous exposure of lymphocytes to the nonactivated test material (75-600 µg/ml) for either 19 or 43 hours had no adverse effect on the MIs. Similar results were obtained with lymphocytes exposed to comparable S9-activated doses for 3 hours followed by a 16- or 40-hour recovery period. Based on these findings, the levels of RPA201772 selected for further study were 75, 300 and 600 µg/ml -/+S9 with cell harvests at 19 and 43 hours.

2. Cytogenetic Assays:

Nonactivated conditions: Representative results from the nonactivated cytogenetic assays with RPA201772 are presented in Table 1. The test material was insoluble at doses ≥300 µg/ml. In agreement with the preliminary findings, RPA201772 was not cytotoxic either after 19 or 43 hours of continuous treatment. There was also no evidence of an adverse effect on chromosome structure or number following 19 hours of exposure to 75-600 µg/ml or 43 hours of exposure to 600 µg/ml. The lack of a clastogenic effect was confirmed in the repeat assay using comparable

doses and a 19-hour cell harvest. By contrast, the nonactivated positive (2 μg/ml CB) induced significant ($p<0.001$) clastogenesis at both harvest times and in both assays.

S9-activated conditions: Similar evidence of compound insolubility but only at the high dose (600 μg/ml), noncytotoxicity and nonclastogenicity was obtained when the assays were performed in the presence of S9 activation using equivalent doses and sampling times. In addition, there was no appreciable increase in the frequency of cells with numerical aberrations. The sensitivity of the test system to detect a clastogenic response was, however, clearly demonstrated by the significant ($p<0.001$) increases in the yield of lymphocytes with structural chromosome aberrations in cultures treated with 6 μg/ml CP.

Based on the overall results, the study author concluded that RPA201772 was not clastogenic in this in vitro human lymphocyte cytogenetic assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that RPA201772 was evaluated to insoluble levels in both the presence and absence of S9 activation and failed to induce a cytotoxic or clastogenic effect in human lymphocytes. The sensitivity of the test system to detect clastogenesis was adequately demonstrated by the significant ($p<0.001$) increases in abnormal chromosome morphology produced by the nonactivated (2.0 μg/ml CB -S9) and S9-activated (6.0 μg/ml CP +S9) positive controls.

We conclude, therefore, that the study provided acceptable evidence that RPA 201773 did not increase the frequency of structural aberrations in human lymphocytes.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated September 24, 1993).

F. APPENDIX ATTACHED: NO.

TABLE 1. Representative Results from the Nonactivated Human Lymphocyte In Vitro Cytogenetic Assays with RPA 201772

Substance	Dose/mL	Harvest Time (Hr)	Mitotic Index (%)[a]	No. of Cell Scored	Total No. of Structural Aberrations[b]	Percent Cells with Structural Aberrations[b]	Biologically Significant Aberrations (No./Type)[c]	Cells with Numerical Aberrations[d]
Solvent Control								
Acetone	10 µl	19[e]	17.3	200	0	0.0	--	1
	10 µl	19[f]	13.8	200	0	0.0	--	1
	10 µl	43[e]	5.3	200	0	0.0	--	1
Positive Control								
Chlorambucil	2 µg	19[e]	15.7	200	66	28.0*	40TB; 3TF; 20SF; 3TE	2
	2 µg	19[f]	9.1	200	47	20.0*	28TB; 4TF; 10SF; 5TE	2
	2 µg	43[e]	6.3	200	29	13.5*	7TB; 2TF; 15SF; 2TE; 3SE	5
Test Material								
RPA 201772	600 µg[g]	19[e]	18.7	200	3	1.0	3TB	5
	600 µg	19[f]	11.1	200	3	1.5	1TB; 2SF	4
	600 µg	43[e]	7.4	200	2	1.0	1TB; 1SF	3

[a]Number of metaphases per ≈1000 cells scored in duplicate cultures

[b]Gaps excluded; only diploid cells were analyzed.

[c]Abbreviations used:

TB = Chromatid break TF = Chromatid fragment TE = Chromatid exchange
SB = Chromosome break SF = Chromosome fragment SE = Chromosome exchange

[d]Number of polyploid and/or endoreduplicated cells observed while scoring 200 metaphases for structural aberrations.

[e]Results from the initial assay; only metaphases recovered from high-dose cultures harvested at 43 hours were examined.

[f]Results from the confirmatory assay.

[g]Highest assayed dose; compound precipitation was seen at levels ≥300 µg/ml. Findings for lower doses (75 or 300 µg/ml --19-hour harvests) did not suggest a positive response.

*Significantly higher ($p<0.001$) than the solvent control by Fisher's exact test.

Note: Data were extracted from the study report pp. 26, 28, 30, 32-36, 38, 40-42.

TABLE 2. Representative Results from the S9-Activated Human Lymphocyte In Vitro Cytogenetic Assays with RPA 201772

Substance	Dose/mL	Harvest Time (Hr)	Mitotic Index (%)[a]	No. of Cell Scored	Total No. of Structural Aberrations[b]	Percent Cells with Structural Aberrations[b]	Biologically Significant Aberrations (No./Type)[c]	Cells with Numerical Aberrations[d]
Solvent Control								
Acetone	10 µl	19[e]	15.5	200	2	1.0	1TB; 1SF	1
	10 µl	19[f]	8.9	200	1	0.5	1TB	9
	10 µl	43[e]	4.9	200	4	2.0	1TB; 3SF	3
Positive Control								
Cyclophosphamide	6 µg	19[e]	14.2	200	54	24.5*	29TB; 3TF; 14SF; 8TE	3
	6 µg	19[f]	7.2	200	46	19.5*	22TB; 3TF; 14SF; 7TE	7
	6 µg	43[e]	8.4	200	55	17.0*	16TB; 5TF; 28SF; 6TE	10
Test Material								
RPA 201772	600 µg[g]	19[e]	16.0	200	1	0.5	1SF	7
	600 µg	19[f]	9.5	200	2	1.0	1TB; 1SF	6
	600 µg	43[e]	5.6	200	6	2.5	2TB; 3SF; 1TE	5

[a]Number of metaphases per ≈1000 cells scored in duplicate cultures

[b]Gaps excluded; only diploid cells were analyzed.

[c]Abbreviations used:

TB = Chromatid break TF = Chromatid fragment TE = Chromatid exchange
SB = Chromosome break SF = Chromosome fragment

[d]Number of polyploid and/or endoreduplicated cells observed while scoring 200 metaphases for structural aberrations.

[e]Results from the initial assay; only metaphases recovered from high-dose cultures harvested at 43 hours were examined.

[f]Results from the confirmatory assay.

[g]Highest assayed dose; compound precipitation was seen at this level. Findings for lower doses (75 or 300 µg/ml --19-hour harvests) did not suggest a positive response.

*Significantly higher ($p<0.001$) than the solvent control by Fisher's exact test.

Note: Data were extracted from the study report pp. 27, 29, 31-35, 37, 39-42.

MUTAGENICITY STUDIES

EPA Reviewer: Nancy McCarroll Signature: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED 7509C Date: 8/10/95
EPA Section Head: Yiannakis M. Ioannou, Ph.D. Signature: Yiannakis M. Ioannou
Review Section I,
Toxicology Branch II/HED 7509C Date: 9/7/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay (84-2)

DP BARCODE: D214214

SUBMISSION NO.: S484204

PC CODE: 123000

MRID NUMBER: 435880-02

TEST MATERIAL: RPA201772

CAS NUMBER: 14112-29-0

SYNONYM(S): 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-isoxazole; isoxaflutole

STUDY NUMBER(S): SA 93134

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: RPA201772: Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: August 16, 1993

EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 435880-02), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to five doses of RPA201772 (98.7%) ranging from 50-1000 µg/plate (initial assay) or six doses of 25-1000 µg/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 system in acetone.

The test material was insoluble at levels ≥500 µg/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.** All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

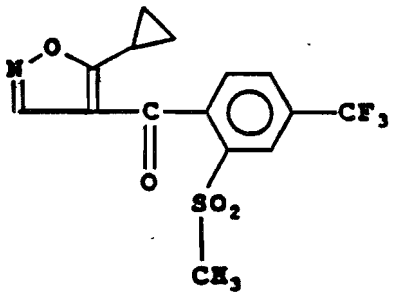
CLASSIFICATION: Acceptable

The study is classified as **Acceptable** and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

A. MATERIALS:

1. Test Material: RPA 201772

Description: Yellow powder
Lot/ batch number: 21 ADM 93
Purity: 98.7% a.i.
Receipt date: Not listed
Stability: Not provided
Structure:



Solvent used: Acetone
Other comments: The test material was stored at room temperature, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically.

2. Control Materials:

Negative: None
Solvent/final concentration: Acetone--0.05 ml/plate

Positive: Nonactivation:

Sodium azide	1 µg/plate	TA100, TA1535
2-Nitrofluorene	1 µg/plate	TA98, TA1538
9-Aminoacridine	50 µg/plate	TA1537
Other:		

Activation:
2-Aminoanthracene (2-Anthramine) 2 µg/plate all strains.

3. Activation: S9 derived from Sprague-Dawley OFA male (unspecified weight or age)

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The rat liver S9 homogenate (Lot no. 32) was obtained commercially from Iffa Credo, France; protein and cytochrome P450 content were determined but not reported. The composition of the S9-cofactor mix was as follows:

Component	Concentration
Sodium phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	33 mM
S9	10%

4. Test Organism Used: S. typhimurium strains
_____ TA97 __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 __x__ TA1538; list any others:

Test organisms were properly maintained: Yes .
Checked for appropriate genetic markers (rfa mutation, R factor): Yes.

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500 and 5000 μg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.

(b) Mutation assays:

Initial assay: Five doses (50, 100, 250, 500 and 1000 μg/plate) were evaluated with and without S9 activation using all tester strains. Triplicate plates were prepared per dose per strain per condition.

Confirmatory assay: As above for the initial assay with the exception that a sixth dose (25 μg/plate) was added.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
__x__ Standard plate test
_____ Pre-incubation (____) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (described).

(a) Preliminary Cytotoxicity / Mutation Assays: Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture (10^9-10^{10} cells/ml) of the appropriate tester strain and 0.05 ml of the appropriate test material dose or solvent, or 0.1 ml of the appropriate positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9-cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for ≈60 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9-cofactor mix and the highest test material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (1) The S9-cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained 10^9-10^{10} viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; and (5) the number of histidine revertants (his^+) induced by the positive controls were within the expected ranges of the reporting laboratory.

(2) Positive response: The test material was considered positive if it caused a reproducible, dose-related ≥2-fold increase in revertant colonies of any strain.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Levels of 1 to 5000 μg/plate +/-S9 were evaluated for cytotoxic effects on strain TA100. Compound precipitation was seen on plates containing test material doses ≥500 μg/plate with or without S9 activation. Revertant colony counts were, however, not affected by treatment with RPA 201772. Based on these findings, the initial mutation assay was performed with five test material doses ranging from 50-1000 μg/plate +/-S9.

2. Mutation Assays: Data from both trials of the mutation assay were in good agreement with the preliminary results and indicated that RPA 201772 was insoluble at concentrations ≥ 500 μg/plate but was neither cytotoxic nor mutagenic in the presence or absence of S9 activation. By contrast to the uniformly negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that RPA 201772 was negative in this microbial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. RPA 201772 was tested to the limit of solubility (500 μg/plate) with no indication of a cytotoxic or mutagenic effect in a well-controlled study. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated August 16, 1993).

F. APPENDIX ATTACHED: No.

TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with RPA 201772

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]				
			TA535	TA1537	TA1538	TA98	TA100
Solvent Control							
Acetone	-	0.05 ml	17±6	10±4	12±2	35±8	125±8
	+	0.05 ml	16±4	14±2	26±5	40±2	116±6
Positive Control							
Sodium azide	-	1 µg	531±56	--	--	--	763±92
2-Nitrofluorene	-	1 µg	--	--	475±67	422±37	--
9-Aminoacridine	-	50 µg	--	350±61	--	--	--
2-Anthramine	+	2 µg	329±11	211±58	1975±200	2131±93	2460±141
Test Material							
RPA 201772	-	250 µg[b]	23±5	11±4	12±3	32±10	116±6
	-	500 µg[c]	21±8	7±2	14±5	36±9	117±10
	+	250 µg[b]	19±8	13±2	18±6	39±5	131±18
	+	500 µg[c]	17±3	11±3	18±2	36±6	116±13

[a]Means and standard deviations of counts from triplicate plates.
[b]Results for lower doses (50 or 100 µg/plate +/-S9) did not suggest a mutagenic effect.
[c]Plates containing the highest assayed level (1000 µg/plate) could not be counted due to heavy compound precipitation; compound precipitation was also seen at 500 µg/plate.

Note: Data were extracted from the study report pp, 27-29.

TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with RPA 201772

Substance	Activation	Dose per plate	Revertants per Plate of Bacterial Tester Strains[a]				
			TA535	TA1537	TA1538	TA98	TA100
Solvent Control							
Acetone	-	0.05 ml	20±5	11±1	14±2	32±8	142±6
	+	0.05 ml	13±5	15±3	22±2	36±9	122±18
Positive Control							
Sodium azide	-	1 µg	570±32	--	--	--	822±49
2-Nitrofluorene	-	1 µg	--	--	432±37	348±48	--
9-Aminoacridine	-	50 µg	--	298±113	--	--	--
2-Anthramine	+	2 µg	288±26	173±8	1983±218	1897±71	2141±292
Test Material							
RPA 201772	-	250 µg[b]	21±7	13±1	13±5	34±11	145±1
	-	500 µg[c]	21±6	12±3	13±5	33±8	143±1
	+	250 µg[b]	13±2	7±3	22±3	34±1	132±15
	+	500 µg[c]	14±1	13±3	23±11	38±6	128±11

[a]Means and standard deviations of counts from triplicate plates.
[b]Results for lower doses (25, 50 or 100 µg/plate +/-S9) did not suggest a mutagenic effect.
[c]Plates containing the highest assayed level (1000 µg/plate) could not be counted due to heavy compound precipitation; compound precipitation was also seen at 500 µg/plate.

Note: Data were extracted from the study report pp, 31-33.

MUTAGENICITY STUDIES

EPA Reviewer: Nancy McCarroll Signature: Nancy E. McCarroll
Review Section III,
Toxicology Branch II/HED 7509C Date: 8/10/95
EPA Section Head: Yiannakis M. Ioannou, Ph.D. Signature: Y.M. Ioannou
Review Section I,
Toxicology Branch II/HED 7509C Date: 9/2/95

DATA EVALUATION REPORT

STUDY TYPE: In vivo micronucleus assay in mice (84-2)

DP BARCODE: D214214

SUBMISSION NO.: S484204

PC CODE: 123000

MRID NUMBER: 435732-23

TEST MATERIAL: RPA201772

CAS NUMBER: 141112-29-0

SYNONYM(S): 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-isoxazole; isoxoflutole

STUDY NUMBER(S): Report No. 93/RHA517/0875

SPONSOR: Rhône-Poulenc Agriculture, Essex, England

TESTING FACILITY: Pharmaco-Life Science Research, Ltd., Suffolk, England

TITLE OF REPORT: RPA 201772: Mouse Micronucleus Test to comply with O.E.C.D. Guideline 474 (1983)

AUTHOR(S): C.N. Edwards

REPORT ISSUED: Study completion date: November 23, 1993

EXECUTIVE SUMMARY: In an in vivo mouse micronucleus assay (MRID No. 435732-23), groups of five male and five female CD-1 mice received 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) at all harvest times. Only cells collected 24-hours postadministration 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.

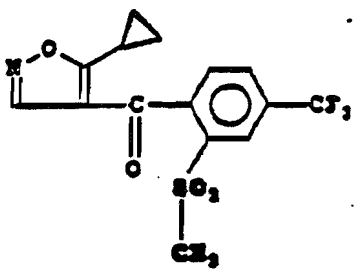
CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a mouse micronucleus assay (84-2).

A. MATERIALS:

1. Test Material: RPA 201772

Description: Fine yellow powder
Lot/ batch number: 21 ADM 93
Purity: 98.7% a.i.
Receipt date: May 17, 1993
Stability: Not provided
Structure:



Vehicle used: Corn oil
Other comments: The test material was stored at room temperature, protected from light. Dosing suspensions were prepared on the day of use; actual concentrations were not verified analytically and dosing suspensions were not adjusted to 100% a.i.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: Corn oil (dosing volume of 15 ml/kg) was administered by oral gavage.

Positive/final concentration/route of administration: Chlorambucil (CB), prepared in aqueous 10% ethanol, was administered by oral gavage at 30 mg/kg.

3. Test Compound:

Route of administration: Oral gavage

Dose levels used:

- Preliminary toxicity test: 625, 1250, 2500 and 5000 mg/kg
- Micronucleus assay: 200, 1000 and 5000 mg/kg

4. Test Animals:

(a) Species: mouse Strain: CD-1 Age (at receipt): 4-5 weeks
Weight range (at dosing): 22.3-29.9 g (males); 19.6-24.8 g (females)
Source: Charles River Breeding Laboratories (UK), Kent, England

(b) Number of animals used per test dose:

- Preliminary toxicity test: 2 males; 2 females, per group
- Micronucleus assay: 15 males; 15 females, per vehicle and test material dose groups
  5 males; 5 females per positive control group

Note: Dosing was based on individual body weights.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound and vehicle control
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr x 48 hr x 72 hr

(b) Positive control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): x 24 hr _____ 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

x bone marrow _____ others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: minimum of 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: minimum of 1000

Note: Bone marrow slides were prepared for mid- and low-dose mice at all sacrifice times; however, only cells recovered from the 24-hour sacrifice of these groups were scored.

3. Details of Slide Preparation: At 24, 48, and 72 hours after administration of the test doses or the vehicle, the appropriate groups of animals were sacrificed by CO_2 asphyxiation and cervical dislocation. Sacrifice time for the positive control group was 24 hours. Animals from the preliminary toxicity test that survived 72 hours postdosing were similarly sacrificed. Bone marrow cells were flushed from both femurs with fetal calf serum and centrifuged. Supernatants were discarded; pellets were resuspended in the remaining supernatant and spread onto slides. Prepared slides were fixed in methanol, stained with 5% Giemsa, coverslipped and scored. Slides prepared from the micronucleus assay were coded prior to scoring.

4. Statistical Methods: The results were evaluated for statistical significance using the Mann-Whitney test.

5. Evaluation Criteria:

Assay validity: The study was considered valid if: (1) the incidence of micronucleated polychromatic erythrocytes (MPEs) was no more than 5 MPEs per 1000 PCEs in all vehicle control animals; (2) the positive control induced a significant ($p<0.05$) increase in the frequency of MPEs; (3) sufficient cells are available for analysis at each dose level; and (4) "sufficient toxicity is demonstrated".

Positive response: The test material was considered positive for micronuclei induction if a significant increase ($p<0.05$) in MPEs compared to the vehicle control was seen in at least one treatment group.

C. REPORTED RESULTS:

1. Preliminary Toxicity Test: Groups of 2 male and 2 female mice received single oral gavage administrations of 625, 1250, 2500 or 5000 mg/kg RPA 201772. Animals were observed daily for mortality and clinical signs; body weights were recorded immediately prior to dosing and daily thereafter for 72 hours. Bone marrow cells were collected from animals surviving to the scheduled sacrifice and examined for cytotoxic effects (PCE:NCE ratios). Results of the preliminary toxicity test indicated that no deaths, clinical signs of toxicity or cytotoxic effects on bone marrow cells were observed in any treatment group. Based on these findings, doses of 200, 1000 and 5000 mg/kg were selected for further study in the micronucleus assay.

2. Micronucleus Assay:

(a) Animal observations: With the exception of a single high-dose animal showing piloerection and hunched posture immediately before sacrifice (24 hours postdosing), no adverse reactions to treatment

were noted. Sporadic weight loss was seen throughout the study but was not considered to be an effect of treatment.

(b) Micronucleus assay: Representative findings from the micronucleus assay are shown in Table 1. RPA 201772 was neither cytotoxic to the target organ nor caused a significant increase in the frequency of MPEs in bone marrow cells harvested from male or female mice 24, 48, or 72 hours postexposure to the high dose (5000 mg/kg). Similar results were obtained for low-(200 mg/kg) and mid-(1000 mg/kg) dose animals at the 24-hour sacrifice time. By contrast, the positive control (30 mg/kg CB) induced significant ($p<0.01$) clastogenic effects in both sexes.

From the overall results, the study author concluded that RPA 201772 was negative in this in vivo assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess, in agreement with the study author, that RPA 201772 was not clastogenic or aneugenic in this in vivo assay when tested to a limit dose. Additionally, the sensitivity of the test system to detect a genotoxic response in male and female mouse bone marrow cells was clearly demonstrated by the significant ($p<0.01$) results obtained with the positive control (30 mg/kg CB). We conclude, therefore, that RPA 201772 was adequately tested and found to be not genotoxic in this in vivo micronucleus assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated November 23, 1993.)

F. APPENDIX ATTACHED: NO.

TABLE 1. Representative Results of the Micronucleus Assay in Mice Treated with RPA 201772

Substance	Dose/kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	Mean MPEs/1000 PCEs ±S.D.	Mean PCE/NCE Ratio
Vehicle Control								
Corn oil	15 mL	24	M	5	5211	6	1.2±1	0.9
			F	5	5458	4 (10)[b]	0.7±1 (0.9±1)[b]	1.1
		48	M	5	5216	8	1.5±1	0.9
			F	5	5971	7 (15)	1.2±1 (1.4±1)	1.1
		72	M	5	5297	6	1.2±1	0.9
			F	5	5680	8 (14)	1.4±1 (1.3±1)	1.1
Positive Control								
Chlorambucil	30 mg	24	M	5	5131	352	68.5±17*	0.8
			F	5	5644	332 (684)	58.3±20*(63.4±18)	1.0
Test Material								
RPA 2011772	5000 mg[c]	24	M	5	5213	4	0.8±1	0.8
			F	5	5958	5 (9)	0.8±1 (0.8±1)	1.0
		48	M	5	5285	8	1.5±2	0.9
			F	5	5957	2 (10)	0.3±0.4 (0.9±1)	1.2
		72	M	5	5178	5	1.0±1	1.0
			F	5	5946	5 (10)	0.9±1 (0.9±1)	1.2

[a]Time after compound administration by oral gavage
[b]Values in () are the combined results for both sexes.
[c]Results for the low- and mid-dose groups sacrificed 24 hours postexposure to 200 or 1000 mg/kg, respectively, did not suggest a positive effect.

*Significantly higher ($p<0.01$) than the corresponding vehicle control by the Mann-Whitney test.

Abbreviations used:

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes
Note: Data were extracted from the study report, pp 19-27.

RPA 201772 MAMMALIAN CELLS IN CULTURE GENE MUTATION

MUTAGENICITY STUDIES

EPA Reviewer: Nancy McCarroll Signature: Nancy E. McCarroll
Review Section III,
Toxicology Branch II/HED 7509C Date: 8/29/95
EPA Section Head: Yiannakis M. Ioannou, Ph.D. Signature:
Review Section I,
Toxicology Branch II/HED 7509C Date: 9/7/95

DATA EVALUATION REPORT

STUDY TYPE: Mammalian cells in culture gene mutation assay in mouse lymphoma cells (84-2)

DP BARCODE: D214214

SUBMISSION NO.: S484204

PC CODE: 123000

MRID Number: 435732-22

TEST MATERIAL: RPA201772

CAS NUMBER: 141112-29-0

SYNONYMS: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-isoxazole; isoxoflutole

STUDY NUMBER(S): Report No. 93/RHA516/0789

SPONSOR: Rhône-Poulenc Agriculture, Essex, England

TESTING FACILITY: Pharmaco-Life Science Research, Ltd., Suffolk, England

TITLE OF REPORT: RPA201772: Investigation of mutagenic activity in the $TK^{+/-}$ mouse lymphoma cell system

AUTHOR(S): P. Strang

REPORT ISSUED: Study Completion Date: September 24, 1993

EXECUTIVE SUMMARY: In two independently performed *in vitro* mammalian cell forward gene mutation assays (MRID No. 435732-22), L5178Y mouse lymphoma cells were exposed to five doses (37.5-600 μg/ml) of RPA201772 (98.7%) in both the presence and absence of S9 activation. The S9 fraction was derived from Aroclor 1254 induced CD rat livers and the test material was delivered to the test system in acetone.

RPA201772 was not cytotoxic at insoluble (≥150 μg/ml +/-S9) or soluble (37.5 or 75 μg/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.

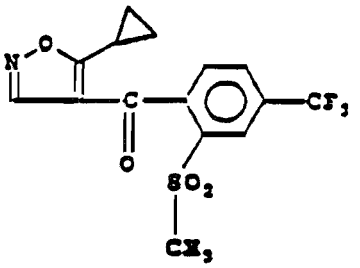
CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a gene mutation study (84-2).

A. MATERIALS:

1. Test Material: RPA201772

Description: Fine yellow powder
Lot/ batch number: 21 ADM 93
Purity: 98.7% a.i.
Receipt date: May 17, 1993
Stability: Not provided
Structure:



Solvent used: Acetone
Other comments: The test material was stored at room temperature, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically and doising solutions were not adjusted to 100% a.i.

2. Control Materials:

Negative: None

Solvent/final concentration: Acetone/0.2%

Positive:

Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in purified water at a final concentration of 500 µg/ml. The promutagen, 7,12-dimethylbenzanthracene (DMBA) was also included in the nonactivated assays. DMBA was prepared in dimethyl sulfoxide (DMSO) to yield a final concentration of 5.0 µg/ml.

Activation (concentrations, solvent): DMBA, prepared as described, was tested at a final concentration of 5.0 μg/ml.

3. Activation: S9 derived from young male CD (≈200 g)

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The rat liver homogenate was prepared by the performing laboratory and used within 1 year of preparation.

S9 mix composition:

Component	Quality (ml)
0.1 M Phosphate buffer (pH 7.4)	8.15
0.4 M $MgCl_2$/1.65 M KCl	0.2
0.1 M NADP	0.4
0.1 M Glucose 6-Phosphate	0.5
S9	0.75

4. Test Cells: Mammalian cells in culture

x mouse lymphoma L5178Y cells
_____ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

x thymidine kinase (TK)
Selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)
4 μg/ml trifluorothymidine (TFT)

_____ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentration) _____ 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
Selection agent: _____ ouabain
(give concentration)

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

(a) Cytotoxicity assay: Ten doses (1.17, 2.34, 4.69, 9.38, 18.75, 37.5, 75, 150, 300 and 600 μg/ml) were evaluated in the presence and absence of S9 activation.

(b) Mutation assays:

(1) Initial assay: Five doses (37.5, 75, 150, 300 and 600 μg/ml) were tested with and without S9 activation. Mutation frequencies (MFs) were determined for all evaluated levels.

(2) Confirmatory assay: As above for the inital assay.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells were exposed to the test compound or negative, solvent, or positive controls
4 hours (nonactivated) 4 hours (activated).

(b) After washing, cells were cultured for 3 days (expression period) before cell selection.

(c) After expression, cells seeded at $5x10^5$ cells/plate (3 plates) were cultured for 11-12 days in selection medium to determine the numbers of mutants; cells seeded at 200 cells/plate (3 plates) were cultured for 11-12 days without selection medium to determine cloning efficiency (CE).

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if (1) the MF of the solvent control cultures was ≤12 mutants per 10^5 survivors (≤120 $x10^{-6}$); (2) the MFs of the positive control cultures were at least 3-fold higher than the MF of the concurrent solvent control and (3) "sufficient toxicity" was demonstrated in the mutation assay.

(b) Positive result: The test material was considered positive if it induced a "significant", dose-related and reproducible increase in the MF compared to the solvent control.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The cytotoxicity test was conducted with ten concentrations of RPA201772 ranging from 1.17 to 600 μg/ml with or without S9 activation. Compound precipitation was reported at the three highest levels (150, 300 and 600 μg/ml). No appreciable reduction in total suspension growth was, however, noted at any nonactivated or S9-activated treatment level. Based on these results, the mutation assays were conducted with five doses (37.5-600 μg/ml +/-S9).

2. Mutation Assays: In agreement with the preliminary findings, the test material was insoluble at concentrations ≥150 μg/ml. Results from the two trials of the mutation assay were as follows:

   Nonactivated conditions: Representative data from the initial and confirmatory nonactivated mutation assays with RPA201772 are presented in Table 1. As shown, the test material was niether cytotoxic nor mutagenic at any dose in either trial of the nonactivated assays.

   S9-activated conditions: Similar results were obtained in the S9-activated phase of testing, therefore, confirming the nonactivated findings that RPA201772 failed to induce either a cytotoxic or mutagenic response in mouse lymphoma cells. Although a sight increase in mutation at the TK locus was noted in cultures exposed to 75 μg/ml (confirmatory trial), the increase was not dose related or reprodcible (Table 2).

By contrast to the uniformly negative results with the test material, the nonactivated (500 μg/ml EMS) and S9-activated (5 μg/ml DMBA) positive controls induced marked increases in the MF in all trials. Based on the overall results, the study author concluded that RPA201772 was not mutagenic in this in vitro mammalian cell test system.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We agree with the author's interpretation of the results and conclude that RPA201772 was evaluated up to insoluble levels and failed to elicit either a cytotoxic or genotoxic response in cultured mouse lymphoma cells. No rationale was provided for extending the postexposure expression time to 3 days, particularily since the test material was not cytotoxic and sufficient cells were available for cloning after the second day of expression. It is doubtful, however, that the prolonged expression time altered the outcome of the study. In addition, the study author did not comment on the high CEs that were obtained in this study with both solvent- and test material-treated cultures. While many of the CEs were beyond the acceptable range (10-120%)[1], we further assess that since actual mutant colonies were generally comparable in treatment and solvent control groups, repeating the assay would not substantively change the results. Additionally, the response to the positive controls (EMS -S9 and 7,12-DMBA +S9) indicated that the assay had an adequate level of sensitivity to detect a mutagenic response. We assess, therefore, that the study provided sufficient evidence to support a negative conclusion for RPA201772 in this in vitro test system.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated September 24, 1993.)

F. APPENDIX ATTACHED? No.

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[1]Caspary, W.J., Lee, Y.J., Poulton, S., Myhr, B.C., Mitchell, A.D., Rudd, C.J. (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality-control guidelines and response categories. Environ Mol Mutagen 12, Supplement 13:19-36.

TABLE 1. Representative Results of the Nonactivated Mouse Lymphoma Forward Mutation Assays with RPA201772

Substance	Dose	Percent Relative Suspension Growth[a] (Posttreatment)	Average Mutant Colonies[b] (Selection) Plates)	Average Viable Colonies[b] (Nonselection Plates)	Absolute Cloning Efficiency[c]	Mutation Frequency x10^{-6}[d]
Solvent Control						
Acetone	0.2%	100[e]	40	254	1.24	64.8
	0.2%	100[f]	57	252	1.26	90.5
Positive Controls[g]						
Ethyl methanesulfonate	500 µg	67[e]	233	178	0.89	523.6
	500 µg	54[f]	243	224	1.12	433.9
Test Material						
RPA201772	75 µg[h]	145[e]	41	259	1.30	63.3
	600 µg[i]	99	42	243	1.22	69.1
	75 µg[h]	104[f]	58	281	1.41	82.6
	600 µg[i]	102	62	288	1.44	86.1

[a]Relative to the corresponding solvent control

[b]Duplicate cultures were prepared for all test groups. Three plates/culture were used to determine viable and mutant colonies.

[c]Absolute Cloning Efficiency (CE) = $\frac{\text{Average Colonies/Plate (Nonselection Plates)}}{\text{Cells/Plate (200)}}$; calculated by our reviewers.

[d]Mutation Frequency (MF) = $\frac{\text{Average Mutant Colonies on Selection Plates}}{\text{Average Colonies on Nonselection Plates}}$ x $4\text{x}10^{-4}$; calculated by our reviewers.

[e]Results from the initial assay.

[f]Results from the confirmatory assay.

[g]7,12-dimethylbenzanthracene at 5 µg/ml was weakly positive in the initial assay and negative in the confirmatory assay; these data were not selected as representative.

[h]Highest soluble concentration; results for the lowest dose (37.5 µg/ml) did not suggest a mutagenic effect.

[i]Highest assayed dose; compound precipitation was reported at levels ≥150 µg/ml. Results for the intermediate doses (150 and 300 µg/ml) did not suggest a mutagenic response.

Note: Data were extracted from the study report, pp.21, 23, 25 and 27.

TABLE 2. Representative Results of the S9-activated Mouse Lymphoma Forward Mutation Assays with RPA201772

Substance	Dose	Percent Relative Suspension Growth[a] (Posttreatment)	Average Mutant Colonies[b] (Selection Plates)	Average Viable Colonies[b] (Nonselection Plates)	Absolute Cloning Efficiency[c]	Mutation Frequency $x10^{-6}$[d]
Solvent Control						
Acetone	0.2%	100[e]	74	249	1.25	118.9
	0.2%	100[f]	40	230	1.15	69.6
Positive Controls						
7,12-dimethylbenz-anthracene	5 µg	10[e]	136	116	0.58	469.0
	5 µg	7[f]	141	108	0.54	522.2
Test Material						
RPA201772	75 µg[g]	111[e]	60	225	1.13	106.7
	600 µg[h]	105	81	262	1.31	123.7
	75 µg[g]	103[f]	65	200	1.00	130.0
	600 µg[h]	105	46	231	1.16	79.7

[a]Relative to the corresponding solvent control

[b]Duplicate cultures were prepared for all test groups. Three plates/culture were used to determine viable and mutant colonies.

[c]Absolute Cloning Efficiency (CE) = $\frac{\text{Average Colonies/Plate (Nonselection Plates)}}{\text{Cells/Plate (200)}}$; calculated by our reviewers.

[d]Mutation Frequency (MF) = $\frac{\text{Average Mutant Colonies on Selection Plates}}{\text{Average Colonies on Nonselection Plates}} \times 4x10^{-4}$; calculated by our reviewers.

[e]Results from the initial assay.

[f]Results from the confirmatory assay.

[g]Highest soluble concentration; results for the lowest dose (37.5 µg/ml) did not suggest a mutagenic effect.

[h]Highest assayed dose; compound precipitation was reported at levels ≥150 µg/ml. Results for the intermediate doses (150 and 300 µg/ml) did not suggest a mutagenic response.

Note: Data were extracted from the study report, pp.22, 24, 26 and 28.