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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

JAN 5 1990

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Application for Experimental Use Permit and Petition for Temporary Tolerance Use of Fenethanil on Stonefruit

CASWELL NO. 723Q
HED Project No. 9-1381A
Iden Nos: 707-EUP-RER
9 G 3746

Record Nos: 244519
241993

FROM: Sidney Stolzenberg, Ph.D. S Stolzenberg 11/29/89
Section I, Tox Branch II - HFAS (H7509C)

TO: S. Lewis, PM 21
Registration Division (7509C)

THRU: Yiannakis M. Ioannou, Ph.D. Y.M. Ioannou 12/13/89
Section Head, Section I
Tox Branch II - HFAS (H7509C)

and

Marcia van Gemert, Branch Chief
Tox Branch II - HFAS
Health Effects Division (H7509C)

Registrant: Rohm and Haas
Philadelphia, PA 19105

Action Requested:

Review toxicology data in support of an application for an EUP and a petition for temporary tolerance use on stonefruit.

In addition to an application for an EUP and a petition for temporary tolerance uses of fenethanil on stonefruit, this data package also contains 22 new animal safety studies for review by EPA in support of the use of this new fungicide.

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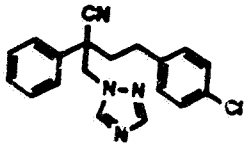
A. BACKGROUND INFORMATION

Compound Name: Fenethanil

Structure and Chemical Name:



2-(2-(4-chlorophenyl)ethyl)-2-phenyl-3-(1H-1,2,4-triazole)-1-propanenitrile

Other Names

RH-57,592
RH-7592

CAS Reg. No. 114369-43-6

Supplier of the Compound: Rohm & Haas.
Philadelphia, PA 19105

B. Application for Experimental Use Permit and Petition for Temporary Tolerance in Stonefruit.

Prepared by: P.K. Chan, Ph.D., D.A.B.T.

Dated: January 30, 1989

Petition # 9 G 3746
Caswell No. 729 Q

Project No. 9-1381 A
MRID: 410312-29
410312-30

Proposed uses: As a systemic, foliar fungicide. It is claimed that the compound has "protectant, curative and eradicant properties" against fungal diseases which attack many fruits, vegetables, cereal crops, turf, ornamentals and tree crops. The diseases include blossom blight (Monilinia supp.), fruit brown rot (Monilinia Supp), rust (Tranzschelia sp.), and other diseases.

Test Product: RH 7592-2F. Contains 24% fenethanil a.i. by weight, or 3.84 oz a.i./lb.

To be tested on: Stonefruits, including apricots, cherries, nectarines, peaches, plums, prunes, etc.

Application rate: Maximum of 0.125 lb a.i./acre per application with 8 applications per season. Maximum

application rate will not exceed 1 lb a.i./acre/season. The average size of each stonefruit trial will be 2.5 acre, with a maximum of about 738 acres, requiring 1116 lb a.i. in a 2 year trial period, or 558 lb a.i./year.

Residues found in previously conducted studies. With treatment-to-harvest intervals of 14-15 days, detectable residues up to 0.796 ppm for cherries, 0.101-0.473 ppm for peaches and 0.022-0.119 ppm for plums, were found.

Proposed Tolerance: A residue of 1.0 ppm is being proposed for the crop group.

C. TOXICOLOGY SUMMARY

1. Introduction

Although this application and petition are for use on stonefruits, fenethanil is being developed for use on fruit trees, vegetables, cereal crops, turf and ornamentals. Therefore, residues on fruits, vegetables, cereal food and possibly other types of food such as dairy and meat products may ultimately be expected.

2. Composition of RH-57,592 technical used in the animal studies.

Pure RH-57,592 is a white, crystalline solid, m.p. of 125-127°C. Technical RH-7592 is an off-white solid. The following two lot numbers of technical fenethanil were used in the animal tests reviewed in this package.

	Purity (%)
Lot # EG-1442	96.4
[illegible]	[illegible]

It is claimed that no significant differences in purity and composition of impurities of these 2 lots were evident.

3. Composition of RH-7592 2F Formulation.

This formulation is the end-use product which is a flowable liquid containing 24% a.i. (RH-7592) by weight.

The following two different lot numbers of EP were used in animal studies.

	Purity %
Lot No. EG - 1452	24.7
Lot No. EG - 1584	24.2

A "Confidential Attachment", which shows the composition of both lot numbers of the end-use product preparation is included in MRID 410312-30. It is concluded by applicant, "There is essentially no difference beween the two lots."

4. Summary of data

a. Acute studies

Low acute oral toxicity was shown in rats for technical fenethanil (Tox category III) and for the end-use product (Tox category IV). Acute dermal toxicity was low for technical fenethanil (Tox category IV) and for the end-use product (Tox category III). An acute inhalation study for technical product is being waived because of difficulties in producing an aerosol, with the end use product, acute inhalation toxicity was low (Tox category III). Primary eye irritation caused by either the technical or end-use product was low (Tox category IV). Neither technical fenethanil nor the end-use formulated product was irritating to the skin of rabbits nor did either of them cause delayed hypersensitization on guinea pig skin.

b. Subchronic

Three 90-day oral, dietary dose subchronic studies, with the mouse, rat and dog, have been submitted. In all three species, hepatomegaly was observed at the higher dose levels in both sexes, which was associated with enlargement of hepatocytes, and hepatocyte vacuolation noted in rats and dogs. Serum enzymes generally associated with liver changes, such as ALP, SGPT and GGT, tended to be elevated at doses that were hepatotoxic. In the rat, an increase in thyroid follicle cell size was also observed. The hepatomegalic effects were considered to be the result of liver enzyme induction, whereas the thyroid follicle cell enlargement in rats was considered to be secondary to hepatomegaly and liver enzyme induction.

c. Chronic

No chronic toxicity studies were submitted in this package. These are not presently required for an EUP or a temporary tolerance permit.

d. Oncogenicity

No oncogenicity tests appeared with this submission. These studies are presently not required for an EUP or temporary tolerance.

e. Developmental Toxicity and Reproduction

In a rat developmental toxicity test, no teratogenic effect was observed. There was an increase in early and late resorption with a decrease in number of live fetuses per dam at 75 and 150 mg/kg/day and a decrease fetal weight at 150 mg/kg/day. However, these two doses were also associated with toxicity to the dams, based on a decrease in body weight compared to controls.

No developmental toxicity test in rabbits has been performed. A 2-generation reproduction test in rats is presently in progress and an interim summary of the results was submitted. These studies are not presently required for an EUP or temporary tolerance.

f. Mutagenicity

A battery of five mutagenicity studies were performed with technical fenethanil. This included two Ames tests, a test for induction of mutation at the HGPRT locus in Chinese hamster ovary cell cultures, an in vivo cytogenetics assay using rat bone marrow cells, and an unscheduled DNA synthesis test using a rat primary hepatocyte culture. No indication of mutagenicity was observed in all five tests. The two Ames tests were classified as unacceptable.

g. Absorption, Retention, Metabolism and Excretion

No studies were submitted but are presently not required for a temporary tolerance use permit.

D. TOXICOLOGY PROFILE

1. Fenethanil (RH-57, 592) technical, 96.4% purity

81 Series Acute Toxicity and Irritation Studies. Sufficient data are available to indicate that fenethanil is of low acute toxicity.

81-1 Actue Oral (MRID 410312-07 for male and female rats and MRID 410312-09 for male rats). The LD50 for male and female rats was greater than 2000 mg/kg but less than 5000 mg/kg in both studies; Toxicity category III. (Core Guideline, both studies).

81-2 Acute dermal (MRID 410312-08). The LD50 in rats of both sexes was greater than 5000 mg/kg; Toxicity category IV. (Core Guideline).

81-3 Acute inhalation. This study has been waived because of technical difficulties in producing an aerosol with technical fenethanil. An acute inhalation study for the end-use product with fenethanil was performed, the results of which are summarized below.

81-4 Primary eye irritation (MRID 410312-11) Under the conditions of this study, 0.1 g of this material was not irritating to the unwashed eyes of rabbits. Toxicity category IV. (Core Guideline).

81-5 Primary dermal irritation (MRID 410312-12). This substance was non-irritating to the skin of male, New Zealand white rabbits. (Core Guideline).

81-6 Acute dermal sensitization (MRID) 410312-13). In guinea pigs, by the Buehler method, this compound did not cause delayed hypersensitivity. (Core Minimum).

82 Series Subchronic Studies

82-1 Subchronic oral. The requirement for oral feeding studies in two species, a rodent and non-rodent, has been completed. Two studies in rodents and one in the dog were performed.

a. Rodent, mouse, 3 month, diet (MRID 410735-03). Doses tested of 96.4% purity were 0, 20, 60, 80 and 540 ppm, which came to 3.8, 11.1, 28.6 and 99.1 mg/kg/day in males, 5.7, 17.6, 50.4 and 139.2 mg/kg/day in females. Increases in liver weight were observed in males at 180 and 540 ppm and in females at 540 ppm. A dose related increased incidence and severity of centrilobular or diffuse hepatocyte hypertrophy was observed in males at 60 ppm and higher doses, and in

females at 180 and 540 ppm. Other changes in liver histopathology were observed. Increases in serum enzymes associated with liver toxicity including SGOT and SGPT, were observed in males at 180 and 540 ppm and in females at 540 ppm. The investigators considered these changes to be associated with liver enzyme induction. The LEL was defined as 60 ppm and the NOEL as 20 ppm. (Core Minimum).

b. Rodent, rat, 3 month, diet (MRID 410735-02). Doses of technical substance tested were 0, 20, 80, 400 and 1600 ppm, which came to 1.3, 5.1, 25.3 and 103.0 mg/kg/day in males, and 1.5, 6.3, 31.1 and 123.9 mg/kg/day in females. The primary effects were on the liver where increased size, lobularization and and histopathology changes were noted. Microscopic changes that were dose related in incidence and severity in both sexes at 80, 400 and 1600 ppm were hepatocellular hypertrophy with vacuolation. Other effects possibly associated with the liver changes were plasma decreases in triglycerides, increases in cholesterol in both sexes at 1600 ppm, and an increase in GGT at 1600 ppm only in the males. Increases in thyroid follicle cell size at 1600 ppm in both sexes was considered by the investigators to be secondary to hepatomegaly and liver enzyme induction. The LEL was 80 ppm and the NOEL was 20 ppm. (Core Guideline).

c. Non-rodent, dog, 3 month, diet (MRID 410735-04). Doses tested were 0, 30, 100, 400 and 1600 ppm, which came to 1.0, 3.3, 13.3 and 50.4 mg/kg/day in males and 1.1, 3.7, 14.8 and 53.3 mg/kg/day in females. The primary effect was on the liver, where an increase in weight was seen in both sexes at 400 (n.s.) and 1600 ($P< 0.05$) ppm. Also in both sexes, diffuse hepatocellular hypertrophy was noted, for which the incidence and severity were dose related. In all 4 males of the 1600 ppm group, multifocal vacuolation was seen in the enlarged hepatocytes. Clinical chemistry changes noted included those usually associated with liver toxicity including increases in ALP, SGPT and GGT at 400 or 1600 ppm. Decreases in albumin, globulin and total protein were generally observed at the highest dose level. The LEL was 400 ppm and the NOEL was 100 ppm. (Core Minimum).

83 Series Chronic Toxicity in 2 Species, Oncogenicity Tests in 2 Species, Developmental Toxicity in 2 Species and 2-Generation Reproduction in the Rat. This group of requirements have not been completed.

83-1 Chronic Feeding in a Rodent and in a Non-Rodent. No such studies have been submitted.

83-2 Oncogenicity Studies in 2 Species. No studies have been submitted.

83-3 Developmental Toxicity in Two Species. Only one study in rats has been completed. No test with rabbits was included.

In a study with rats, no teratogenic effect was observed. At 75 and 150 mg/kg/day, a dose related increase in both early and late resorptions and a decrease in live fetuses per dam was observed. A decrease in mean fetal weight was observed only at the 150 mg/kg/day dose group. Both doses at which embryo toxicity was observed were associated with maternal toxicity, based on decreased body weight of the dams following the dosing period.

83-4 Reproduction, 2-Generation. Only a summary interim report of an experiment that is still in progress was submitted.

84 Series Mutagenicity Tests. No indication of mutagenicity was observed in a battery of five tests of this series. Two of these tests, Both Ames tests, were classified Unacceptable.

84-2 Gene Mutation. Two Ames tests were performed. No evidence of mutagenic responses with bacteria for frame shift or point mutations were noted. Both tests were classified as Unacceptable.

84-2 Gene Mutation. A test for induction of gene mutation at the HGPRT locus in Chinese hamster ovary cells was performed. No evidence gene mutation at the HGPRT locus was observed. (Acceptable).

84-2 Structural Chromosomal Aberration. An in vivo cytogenetics assay using bone marrow from treated rats was performed. No increase in number of cells with aberrations or in aberrations per cell were noted. (Acceptable).

84-2 Other Genotoxic Effects. No increase in unscheduled DNA synthesis in a rat primary hepatocyte culture, was observed. (Acceptable).

85 Series Special Testing.

No studies in this series have been submitted.

2. Fenethanil (RH-7592 2F) End-Use Product, 24%

81-1 Acute, oral (MRID 410312-21 for male rats and MRID 410312-22 for female rats). The LD50 in rats of both sexes is greater than 5000 mg/kg; Toxicity category IV. (Core Guideline for male and female rats).

81-2 Acute dermal (MRID 410312-23 for male rats and MRID 410312-24 for female rats). The LD50 in rats of both sexes was greater than 5000 mg/kg; Toxicity category IV. (Core Guideline for both studies).

82-3 Acute inhalation (MRID 410312-25). The LC50 was greater than 2.1 mg/liter. Tox category III. (Classified core Supplementary because particle size generated was too large to be respirable).

82-4 Primary eye irritation (MRID 410312-26). [illegible] indication of eye irritation. Tox category IV. (Core Guideline).

82-5 Primary dermal irritation (410312-27). RH-57,592 2F was not irritating to the skin of rabbits. Tox category IV. (Core Guideline).

82-6 Acute dermal sensitization (MRID 410312-28) RH-57,592 2F did not cause delayed hypersensitization in guinea pigs. (Core Guideline).

E. DATA GAPS

The following Guideline Toxicology studies can be required for registration of technical fenethanil for terestrial, food crop use.

81-1	Acute oral toxicity	(R)
81-2	Acute dermal toxicity	(R)
81-3	Acute inhalation toxicity, rat	(R)
81-4	Primary eye irritation, rabbit	(R)
81-5	Primary dermal irritation	(R)
81-6	Dermal sensitization	(R)
82-1	90-day feeding studies; 2 spp. rodent and non rodent	(R)
82-2	21-day dermal	(R)
82-4	90-day inhalation	(R)
83-1	Chronic feeding 2 spp. rodent and non-rodent	(R)
83-2	Oncogenicity	(R)
83-3	Developmental toxicity, 2 spp.	(R)
83-4	Reproduction, 2-generation	(R)
84-2	Gene mutation	(R)
84-2	Structural chromosomal aberration	(R)
84-2	Other genotoxic effect	(R)
85-1	General metabolism	(R)

(R) = Required

Acute delayed neurotoxicity studies and 90-day neurotoxicity studies are not required because this is not an organophosphate, it is not expected to depress acetyl cholinesterase activity, and evidence of neurotoxicity was not seen in any of the above studies performed.

The following studies listed above were not performed.

82-2 21-day dermal
83-1 Chronic toxicity, 2 spp
83-2 Oncogenicity, 2 spp
83-3 Developmental toxicity, rabbit
83-4 Reproduction, 2-generation*
85-1 General Metabolism

* A brief summary-interim report of a 2-generation reproduction study presently in progress was

submitted. The study will be core classified by EPA upon submission of the data after completion.

These five categories of studies, required for registration of the technical grade of a compound, are not required for a temporary tolerance permit.

The following Guideline Toxicology studies can be required for registration of the end-use product with fenethanil for terrestrial, food crop use, providing the requirements for technical fenethanil are completed.

81-1	Acute oral toxicity	(R)
81-2	Acute dermal toxicity	(R)
81-3	Acute inhalation toxicity, rat	(R)
81-4	Primary eye irritation, rabbit	(R)
81-5	Primary dermal irritation	(R)
81-6	Dermal sensitization	(R)

All of the above studies with the formulation for an end-use product have been performed.

F. TOXICOLOGICAL ISSUES

Based on the NOEL of 1 mg/kg/day (90-day rat feeding study) and a safety factor of 1000, the provisional ADI value was calculated to be 0.001 mg/kg/day. The average TMRC (based on the requested tolertance of 1 ppm) was calculated to be 0.000364 (for U.S. population). The percent of PADI utilized is approximately 36.4.

G. RECOMMENDATIONS

Toxicology Branch II (HFAS) recommends against granting the EUP and temporary tolerance for the use of fenethanil on stonefruit until the registrant submits an acceptable acute inhalation LC50 study, with the end-use product (RH-57592 2F).

Reviewed By: Sidney Stolzenberg, Ph.D.
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 12-13-88
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

EPA Identification Nos: EPA MRID (Accession) No.: 410735-05 and 410312-14
EPA ID No.: 707-EUP-RER
Caswell No.: 723Q
HED Project No.: 9--1381A

Accession No. 410312-14 consists of corrections for this study due to errors in the original report (Accession No. 410735-05).

Test Material: Fenethanil

Synonyms: RH-7592

Study Number: 87R-065

Sponsor: Rohm & Haas Company
Philadelphia, PA 19105

Testing Facility: Rohm & Haas
Toxicology Department
Spring House, PA 19477

Title of Report: RH-7592: Oral (Gavage) Developmental Toxicity Study in Rats

Authors: H.M. Solomon, Ph.D., Study Director and M.F. [illegible]

Report Issued: February 8, 1988

Conclusions: (Summary of findings)

RH-7592 caused a decrease in body weight and body weight gain of pregnant rats at doses of 75 and 150 mg/kg/day but no observable effect at 30 mg/kg/day. No teratogenic effect was observed, but at 75 and 150 mg/kg/day an increase in resorption sites and a decrease in number of live fetuses per dam were seen; both effects apparently dose related. There was also a decrease in fetal weight in the high dose group.

Maternal toxicity: LEL = 75 mg/kg/day
NOEL = 30 mg/kg/day

Developmental toxicity LEL = 75 mg/kg/day
NOEL = 30 mg/kg/day

No teratogenic effect was evident.

A/D = 1

Core Classification: Minimum (Satisfies data requirement)

A preliminary study, dated February 17, 1987, appears in Appendix M of the report. Lot No. used was W5-9128 (different than in the main study). Doses given between days 6-15 of gestation were 0, 50, 100, and 150 mg/kg in a constant volume of 10 mL/kg for all doses. Group sizes were 11 in controls, 10, 11, and 10 in low, mid and high dose, respectively. All fetuses were examined for external alterations but only controls and high dose were examined for visceral and skeletal anomalies.

A decrease in body weight among dams treated with 100 and 150 mg/kg was seen, but it was transient in the 100 mg/kg group. An increase in incidence of alopecia and scant feces was seen for 100 and 150 mg/kg treated dams. No deaths occurred in any treated dams or controls. No effect on pregnancy rate was evident.

There was an increased incidence in fetal skeletal variations in the high dose group, predominantly of 14 rudimentary rib and retarded skeletal development with unossified sternebrae. There was also a suggestion of teratogenicity in the 150 mg/kg group, based on increased incidence of malformed fetuses; 5 affected litters with 1 malformed fetus in each of them. These included the total of external, soft tissue and skeletal anomalies found. In contrast, no anomalies were scored in the control group for any of these 3 categories.

MAIN STUDY

A. Materials:

1. Test Compound: Purity: 96.4%, Description: White solid, Lot No.: EG1442 (technical), Contaminant: list in CBI Appendix.

2. Vehicle: 0.5 % aqueous methylcellulose.

3. Test Animals: Species: Rat (female), Strain: CRL:CD B, Source: Charles River, Stone Ridge, NY, Age: 66-75 days old when received, Weight: 184-229 g when received. An acclimatization period of 7 days was allowed before mating.

B. Study Design:

This study was designed to assess the developmental toxicity potential of RH-7592 when administered by gavage on gestation days 6 through 15, inclusive.

Mating -The females were mated with males of the same strain, maintained at the testing facility for breeding purposes.

Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	25
Low Dose	30	25
Mid Dose	75	25
High Dose	150	25

Dosing - All doses were in a volume of 10 mL/kg of body weight/day prepared daily during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on the most current body weight during gestation, obtained on days 0, 6, 8, 10, 13, 16, and 20.

Observations - The animals were checked twice daily for mortality or abnormal condition from day 0 to 19. Dams were sacrificed on day 20 of gestation. Examinations at sacrifice consisted of weighing gravid uterus, number and position of live and dead fetuses, corpora lutea count, detection of early resorption with ammonium sulfide.

The fetuses were examined in the following manner: Staples' technique was used for soft tissue evaluation and Dawson's method using alizarin red for skeletal examination. All fetuses were examined for skeletal changes, half (alternate fetuses) for soft tissue changes.

Historical control data were not provided to allow comparison with concurrent controls

Statistical Analysis - A pairwise test between control and treated groups was applied for each parameter measured. For comparisons, Fisher's exact test, ANOVA with Dunnett's test or Mann-Whitney's test were used for the various parameters measured.

Compliance - A signed Statement of Confidentiality Claim was provided.

A signed Statement of compliance with EPA GLP's was provided.

A signed Quality Assurance Statement was provided.

Results

Maternal Toxicity

Mortality: None in any group due to treatment. One on high dose died due to intubation error.

Clinical Observations: Increased incidence of alopecia at 75 and 150 mg/kg doses, dose related, was noted, especially between days 6-15 of gestation. Increased incidence of scant feces was seen at 75 and 150 mg/kg.

Body Weight:

The investigators supplied the following data:

Table I: Mean Body Weight Gain ± S.E. (grams)[a]

Group:	N=	Days 0-6 Pre-dosing Period	Days 6-16 Dosing Period	Days 16-20 Post-Dosing Period
Control	24	28±1.6	56±2.1	69±1.7
LDT	22	27±1.2	57±2.6	71±1.7
MDT	23	26±2.0	42±4.0*	66±2.3
HDT	21	26±1.9	23±3.0*	61±3.9

[a] Data extracted from Table 4 of report.
* P<0.05.

Decreases in mean body weight gain were observed in the mid and high dose groups during the dosing period. Body weight gains were similar in all groups following the dosing period.

Food Consumption: No data.

Cesarean Section Observations:

Table III Cesarean Section Observations

Dose:	Control	LDT	MDT	HDT
#Animals Assigned				
#Animals Mated/Inseminated	25	25	25	25
Number Pregnant	24	22	23	23[b]
Pregnancy Rate (%)	96	88	92	96
Maternal Wastage	No indications of maternal wastage in any group was noted			
Corpora Lutea/Dam	18.5	18.1	16.8	15.8*
Implantations/Dam	15.6	15.3	13.7*	13.9*
Live Fetuses/Dam	14.8	14.7	12.5*	10.6*
Total Resorptions	0.8	0.6	1.3	3.3*
Early	0.8	0.5	1.0	2.4*
Late	0.0	0.0	0.3	0.9*
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	3.5	3.6	3.4	3.0*
Preimplantation Loss (%)	14.5	14.4	16.7	9.7
Postimplantation Loss (%)	5.1	3.9	9.5	23.8
Sex Ratio (% Male)	44.6	48.3	51.2	53.8

[a] = Data extracted from the report.
[b] = One pregnant animal died due to intubation error. Therefore, pregnancy rate is based on 24 animals in this group.
* = P<0.05.

A decrease in corpora lutea count in the high dose group was noted (P<0.05). There were decreases in total implantations per dam in both mid and high dose groups (P<0.05), but no increase in percentage of preimplantation loss. There was a substantial increase in total resorption sites, especially in the high dose group (P<0.05) which for the most part reflected early resorbs, although late resorb sites were also statistically significantly increased. As a result, total live fetuses per dam were decreased in mid and high dose groups (P<0.05) and was apparently dose related. Nevertheless, no dead fetuses were found in any treated or control dams. The decrease in total live fetuses per dam appeared to

be a reflection of postimplantation loss. Mean fetal weight was reduced in the high dose treated dams ($P<0.05$). However, there was no change in sex ratio of fetuses on Day 20 of pregnancy.

Developmental Toxicity:

Table IV: External Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#Pups (litter) examined	355(24)	324(22)	286(23)	222(21)
#Pups (litters) affected	1(1)	0(0)	1(1)	1(1)
(Individual observation with both fetal and litter incidences)	1(1)[a]	0(0)	1(1)	1(1)

(+) Some observation may be grouped together.
(a) Fetal [litter] incidence.

No effects on external malformations were evident.

Table IV: Visceral Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#Pups (litter) examined	186(24)	170(22)	153(23)	127(21)
#Pups (litters) affected	3(3)	0(0)	2(2)	3(3)
(Individual observation with both fetal and litter incidences)	3(3)[a]	0(0)	2(2)	3(3)

(+) Some observation may be grouped together.
(a) Fetal [litter] incidence.

No effect on soft tissue malformations was evident.

Table IV: Skeletal Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#Pups (litter) examined	354(24)	322(22)	285(23)	218(21)
#Pups (litters) affected	1(1)	0(0)	0(0)	1(1)
(Individual observation with both fetal and litter incidences)	1(1)[a]	0(0)	0(0)	4(1)

(+) Some observation may be grouped together.
(a) Fetal [litter] incidence.

No effect on skeletal malformations were noted. In addition, there was no indication of increased incidence of fetal retarded skeletal development due to treatment.

Skeletal Variants - An increase incidence of fetal skeletal variants, both number of fetuses and number of litters affected, was observed at 150 mg/kg dose, predominantly with a 14th rudimentary rib.

Discussion:

Doses of technical, 96.4% of RH-7592 given by oral gavage were 0, 30, 75, and 150 mg/kg/day. No deaths to the dams was seen at any dose levels but increased incidence of alopecia and scant feces was observed in dams treated with 75 and 150 mg/kg. There was a decrease in body weight and body weight gain of dams in the mid and high dose groups.

There was no effect on pregnancy rate at any dose level. There was a substantial increase in resorption sites, especially in the high dose group. Probably reflecting this was a decrease in live fetuses per dam in both mid and high dose groups, apparently dose related. No dead fetuses were found in dams of any treated or control group. Mean fetal weight was reduced in the high dose treated group. No indication of teratogenicity was seen, but there was an increased incidence in skeletal variants, predominantly fetuses with a 14th rudimentary rib.

Maternal toxicity — LEL = 75 mg/kg/day
NOEL = 30 mg/kg/day

For developmental toxicity — LEL = 75 mg/kg/day
NOEL = 30 mg/kg/day

A/D = 1

Reviewed By: Sidney Stolzenberg, Ph.D.
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 12/18/89
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

Study Type: 3-Month Oral, Rat Caswell No.: 723Q

Accession No.: 410735-02 HED Project No.: 9-1381A

Test Material: Fenethanil

Synonyms: RH-7592

Study Number: 87R-103

Sponsor: Rohm & Haas Company
Spring House, PA 19477

Testing Facility: Rohm & Haas
Toxicology Department

Title of Report: Three-Month Dietary Toxicity Study in Rats

Authors: H.J. Bernacki, Jr. and G.A. Hazelton

Report Issued: Completed July 26, 1988

Conclusions:

Doses of fenethanil in the diet tested were 0, 20, 80, 400, and 1600 parts per million (ppm). The primary effects were on the liver, where increased size, lobularization, and histopathology changes were observed. The main histopathology change at 400 and 1600 ppm in both sexes was hepatocellular hypertrophy, and vacuolation of hepatocytes at 80, 400 and 1600 ppm in males and at 400 and 1600 ppm in females. Other effects which may be associated with the liver changes were decreases in plasma triglycerides, increases in cholesterol of both sexes at 1600 ppm, and increases in GGT at 1600 ppm in males. Increases in thyroid follicle cell size at 400 and 1600 ppm in males and 1600 ppm in females were considered to be secondary to the hepatomegaly and liver enzyme induction due to treatment.

- LEL = 80 ppm (5.1 mg/kg/day in males, 6.3 mg/kg/day in females)

- NOEL = 20 ppm (1.3 mg/kg/day in males, 1.5 mg/kg/day in females)

Classification: Core Guideline

A. Materials:

1. Test Compound: RH-7592, Description: Off whitesolid Lot No.: EG1442, Purity: 94.6%

2. Test Animals: Species: Rat, Age: 6 weeks, Source: Charles River Kingston, Stone Ridge, NY.

B. Study Design:

1. Animal Assignment - Animals were assigned 1 per cage to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 3 Months Male	Female
1 Cont.	0	10	10
2	20	10	10
3	80	10	10
4	400	10	10
5	1600	10	10

Doses were based on a 2-week range finding study.

2. Diet Preparation - Diet was prepared biweekly and stored at unspecified temperature. Samples of treated food were analyzed for stability and concentration at weeks 1-2, 4, 8 and 12.

Results - Average for homogeneity and stability after 2 weeks of storage ranged between 96 to 106 percent of target doses in all four diets at all four time [illegible]

3. Animals received food and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Analysis of variance or covariance was used after inspection of homogeneity of variance across treatment groups and sampling times for virtually all parameters except hematology. In evaluating hematology parameters, the values were transformed using a square root function prior to such analyses. Group means were compared using least square means and Dunnett's t-test.

5. Compliance

- A signed statement of Confidentiality Claim was included.
- A signed statement of compliance with EPA's GLP was provided.
- A signed Quality Assurance Statement was provided.

C. Methods and Results:

1. Observations - Animals were inspected daily for signs of toxicity and mortality.

Toxicity/Mortality (survival)

No deaths, no clinical signs indicative of toxicity were seen.

2. Body Weight - Animals were weighed weekly starting from 1 week prior to dosing.

Mean body weight was decreased only in the 1600 ppm group starting after the first week through the 13th week ($P<0.05$ between weeks 1-10 for males and $P<0.05$ between weeks 1-13 for females).

3. Food Consumption and Compound Intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data. Feed intake was decreased only in the 1600 ppm group with both sexes starting at week 1 to about week 10 or 11 in both sexes ($P<0.05$ between weeks 1-8 in males, and 1-9 in females).

Feed efficiency was decreased for both sexes in the 1600 ppm group only during week 1 of the study. Compound intake averaged over the entire 13 weeks of the study was as follows:

Dose ppm	Compound Intake (mg/kg/day) Males	Fema.
20	1.3	1.5
80	5.1	6.3
400	25.3	31.5
1600	103.0	123.9

3 months on animals. Signed statements by Lionel F. Rubin, V.M.D., one dated March 22, 1987 (pretest) and the other dated June 10, 1987, were included.

No effect of compound treatment was evident.

5. Blood was collected before treatment and at 3 months for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood Clotting Measurements (Thromboplastin time) (Clotting time) (Prothrombin time)		
X	Red blood cell morphology		

*Required for subchronic and chronic studies.

Small increases in females of high dose groups were seen for erythrocyte count ($P<0.05$), platelet count ($P<0.05$), MCV ($P<0.05$) and MCH ($P<0.05$) were seen. The toxicological significance of those effects is considered equivocal by the investigators.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
	Potassium*	X	Globulins
	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Serum Protein*
	Cholinesterase	X	Triglycerides
	Creatinine phosphokinase*		Serum protein electrophoresis
	Lactic acid dehydrogenase	X	A/IG ratio
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	gamma glutamyl transferase (GGT)		
	glutamate dehydrogenase		

*Required for subchronic and chronic studies.
In the 1600 ppm group, decrease in triglycerides of

In the 1600 ppm group, decrease in triglycerides of both sexes, substantial in males ($P<0.05$ in males), increases in cholesterol of both sexes ($P<0.05$ in females), decrease in albumin only in females ($P<0.05$), increase in globulin only in females ($P<0.05$) therefore an increase in A/G in females ($P<0.05$) were seen. Also at highest dose, increases in BUN ($P<0.05$ in females) were seen. The changes in albumin, globulin and AIG in group 5 were considered secondary to the decrease in body weight and feed intake by the investigators.

6. Urinalysis* - Urine was collected from fasted animals at 3 months. The CHECKED (X) parameters were examined.

X		X	
	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*		Urobilinogen

*Required for chronic studies

No effects were observed.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	Digestive System
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
X	Liver*
X	Gall bladder*
X	Pancreas*
	Respiratory
X	Trachea*
X	Lung*
	Nose
	Pharynx
	Larynx

X	Cardiovasc./Hemat.
X	Aorta*
XX	Heart*
	Bone marrow*
X	Lymph nodes*
X	Spleen*
	Thymus*
	Urogenital
XX	Kidneys*
X	Urinary bladder*
XX	Testes*
X	Epididymides
X	Prostate
X	Seminal vericle
XX	Ovaries*
X	Uterus*
X	Vagina

X	Neurologic
XX	Brain*
X	Periph. nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (optic n.)*
	Glandular
XX	Adrenals*
	Lacrimal gland
X	Mammary gland*
X	Parathyroids*
X	Thyroids*
	Other
X	Bone*
X	Skeletal muscle*
X	Skin*
X	All gross lesions and masses*

* Required for subchronic and chronic studies.

a. Organ weight - Increases in liver weight of both sexes at 400 and 1600 ppm (dose related in both sexes; relative to body weight at 400 ppm, absolute and relative at 1600 ppm) appeared to be the only compound related effect. A decrease in adrenal weight of males but an increase in adrenal weight of females in the highest dose group appeared to be a sporadic effect. Ovarian weight in the 1600 ppm group was increased ($P<0.05$ for both relative and absolute) but was considered of no toxicological significance because of no histopathology changes. Increase, in relative kidney and brain weights of females were considered to be secondary to the decrease in body weight.

Organ	Dose	Absolute Weights(g) Male	Absolute Weights(g) Female	Relative Weight Male	Relative Weight Female
Liver	0	13.621 ± 2.427	7.263 ± 1.094	2.64	2.50
	20	13.386 ± 1.048	7.160 ± 0.557	2.52	2.56
	80	14.172 ± 1.884	7.623 ± 0.781	2.61	2.65
	400	15.598 ± 2.551	7.874 ± 1.046	2.94*	2.95*
	1600	19.349 ± 2.574	10.227 ± 0.483*	3.90*	4.20*

* P<0.0.05

b. Gross Pathology - The only compound related effects apparently only at highest dose, were an increased incidence of prominent lobular architecture of liver (both sexes) and an increased incidence of foci or diffuse brown discoloration in the liver.

Table I

Sex	Male					Female				
Dosage (ppm)	0	20	80	400	1600	0	20	80	400	1600
Number of Rats/Group:	10	10	10	10	10	10	10	10	10	10
LIVER:										
- discoloration, diffuse	0	0	0	0	0	0	0	0	0	4
- discoloration, focus/foci	0	1	1	0	2	1	3	0	1	2
- prominent lobular architecture	3	3	4	4	6	0	0	1	1	7

c. Microscopic Pathology - All organs listed above, from control and highest dose treated groups, were examined for histopathology, but tissues of all dose groups from kidneys, liver, lung, thyroid, testes, and ovaries were also examined. The only compound related changes noted were in the livers and thyroids as seen from the data abstracted from the reports.

Table II

Sex	Males					Females				
Dose (ppm)	0	20	80	400	1600	0	20	80	400	1600
Number of Animals/Group:	10	10	10	10	10	10	10	10	10	10
LIVER:										
NO. EXAMINED	10	10	10	10	10	10	10	10	10	10
NO. NORMAL	2	3	0	0	0	2	3	1	0	0
-hypertropy, hepatocytes, centrilobular, minimal	0	0	1	8	0	0	0	0	5	2
-hypertropy, hepatocytes, centrilobular, slight	0	0	0	1	6	0	0	0	1	7
-hypertropy, hepatocytes, centrilobular, moderate	0	0	0	0	4	0	0	0	0	1
Total Incidence	0	0	1	9	10	0	0	0	6	10
-angiectasis, focal	0	0	1	0	0	0	0	0	0	0
-cellular alteration, focus/foci, basophilic	0	0	0	0	0	0	0	1	0	0
-infiltration, mono-nuclear-cell, multifocal	7	7	7	7	3	7	7	6	9	3
-lipidosis, tension, focal	1	1	3	0	2	0	1	3	2	2
-necrosis, centrilobular	0	0	0	2	0	0	0	0	0	0
-necrosis, focal	1	0	3	0	0	0	0	0	0	0
-proliferation, bile duct	1	0	0	0	1	0	0	0	0	0
-vacuolation, hepatocytes, centrilobular/midzonal	0	0	3	4	6	0	0	0	7	3
-vacuolation, hepatocytes, multifocal	2	1	0	0	0	1	3	3	0	0
-vacuolation, hepatocytes, periportal/perilobular	0	0	1	0	0	1	0	0	2	6
THYROID:										
NO. EXAMINED	10	10	10	10	10	10	10	10	10	10
NO. NORMAL	6	8	9	1	1	7	9	9	8	9
cyst(s)	0	0	0	0	0	1	0	0	0	1
-ectopic thymic tissue	0	1	1	0	0	2	0	1	0	0
-hypertropy, follicular epithelium	4	0	1	9	8	0	0	0	2	10
-ultimobroanchial ruminant/cyst	2	1	0	0	3	0	1	3	1	0

Liver changes consisted of increased incidence and severity centrilobular hypertrophy and vacuolation of hepatocytes, ranging from 1/10 males and 0/10 females at 80 ppm with minimal effect, 9/10 males and 6/10 females at 400 ppm with minimal or slight effect, but 10/10 males and 10/10 females predominantly with slight or severe effects at 1600 ppm. This is obviously a dose related effect for both incidence and severity.

In the text of the report by the pathologist, it is indicated that "the hypertrophied cells were enlarged with an abundant amount of a dense, eosinophilic cytoplasm. In some of the enlarged cells, the nucleus also was enlarged and densely basophilic."

In the thyroid, an increased incidence of follicular cell hypertrophy was seen at the 400 and 1600 ppm treated rats of both sexes. The investigators suggested that the thyroidal effect was most likely a secondary effect resulting from the liver changes. Compounds which cause liver enzyme induction have been known to increase the turnover of plasma thyroxin, resulting in a stimulation of TSH.

D. Discussion:

The primary effect due to fenethanil in the diet of rats was an increased incidence and severity of hepatocellular hypertrophy and vacuolation, dose related for severity and incidence in both sexes. The minimal dose for this effect to occur in males was 80 ppm and in females it was 400 ppm. Accompanying this change was an increase in size of the livers indicated by increased weight at the 400 and 1600 ppm doses in both sexes and "prominent lobular architecture" at 1600 ppm in both sexes with brown discoloration in the females at 1600 ppm. Other effects apparently associated with liver toxicity at the 1600 ppm dose were decreased triglycerides and increased cholesterol in both sexes, increased gamma glutaryl transferase in males. In the thyroids increased incidence of follicle cell hypertrophy was seen in both sexes treated with 400 and 1600 ppm of the compound. These thyroidal effects were considered to be secondary to the hepatocellular hypertrophy and the liver enzyme inducing effects.

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DATA EVALUATION REPORT

Study Type: 3-Month Dietary, Mouse
Caswell No.: 723Q

Guideline: 82-1
HED Project No.: 9-1381A

Test Material: Fenethanil
MRID No.: 410735-03

Synonyms: RH-7592
Iden. No.: 707-EUP-RER

Study Number: 87R-090

Sponsor: Rohm & Haas Company
Spring House, PA 19477

Testing Facility: Rohm & Haas
Toxicology Department

Title of Report: Three-Month Dietary Toxicity Study in Mice

Authors: J.C. Harris, Principal Investigator, G.A. Hazelton, Ph.D., Study Director

Report Issued: July 8, 1988

Conclusions and Recommendations: (Summary)

Technical grade RH-7592 of 96.4% purity was administered for 3 months at doses of 0, 20, 60, 180, and 540 ppm. No mortalities or decrease in body weight gain was seen at any dose level. Increases in serum enzymes associated with liver toxicity, including SGOT and SGPT were observed in males receiving 180 and 540 ppm and in females receiving 540 ppm. Increases in liver weight were recorded for males at 180 and 540 ppm and in females at 540 ppm. A dose related increased incidence and severity of centrilobular or diffuse hepatocyte hypertrophy was seen in males at 60 ppm and higher and in females at 180 and 540 ppm. Other changes in liver histopathology were also noted. The investigators considered these liver changes to be associated with "enzyme induction."

- NOEL = 20 ppm (3.8 mg/kg/day in males, 5.7 mg/kg/day in females)

- LEL = 60 ppm (11.1 mg/kg/day in males, 17.6 mg/kg/day in females)

Based on centrilobular or diffuse hepatocytes hypertrophy.
Core Classification: Minimum

We are of the opinion that a MTD for a mouse carcinogenicity study cannot be selected from the data of this 3-month mouse study.

A. Materials:

1. Test Compound: RH-7592 (RH-57,592), Description: Off white solid, Lot No.: EG-1442, Purity: 96.4%,
2. Test Animals: Species: Mouse, Strain: CRL:CD-1 (ICR)BR Age: 6 weeks, Weight: 21.8 to 26.2 g, Source: Charles River, Kingston, NY.

B. Study Design:

1. Animal Assignment - Animals were assigned 1 per cage to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 3 Months Male	Female
1 Cont.	0	10	10
2	20	10	10
3	60	10	10
4	180	10	10
5	540	10	10

2. Diet Preparation - Diet was prepared biweekly and stored at room (75°F) temperature. Samples of treated food were analyzed for stability of the first diets mixed 18 days after preparation and storage at room temperature. Determinations of concentration in all four diets were [illegible] test on all four diets was performed only for the first preparation (week 1) by analysis of dietary samples collected from top, middle, and bottom of feed containers.

   Results - Dietary levels found by analyses were in agreement with nominal concentrations in all four diets, ranging between 92 to 112 percent of target at all six time periods when performed. In the homogeneity test, the levels found at analyses were between 94 to 105 percent of nominal concentration in all four diets.
3. Animals received food and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Blood cell counts such as white cells, differential data etc., body weights, feed consumption, and clinical chemistry data were transformed using a square root function prior to analysis. Analysis of variance (or covariance) was used to assess presence or absence of an overall treatment effect. Group means were compared using least square means, and if significant, comparisons between control and exposed group were calculated by Dunnett's t-test using $P<0.05$ or less.

5. Compliance

   - A signed statement of Confidentiality Claim was included.

   - A signed statement of compliance with EPA's GLP was provided.

   - A signed Quality Assurance Statement was provided.

C. Methods and Results:

1. Observations - Animals were inspected daily for signs of toxicity and mortality. Physical examinations were performed weekly starting one week before treatment.

   Toxicity/Mortality (survival)

   No deaths occurred. No treatment related signs were observed during the entire 3 months of the study.

2. Body Weight - Animals were weighed weekly during the entire study.

   No compound related differences were seen at anv dose level throughout the study.

3. 3.Food Consumption and Compound Intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data. No compound related effect on feed intake at any dose level was evident and there was no effect on food efficiency.

Mean compound intake calculated over the entire 3 months of this study was summarized as follows:

Group	Dietary Conc. ppm	3 Month Mean Intake (mg/kg/day) Males	Females
2	20	3.8	5.7
3	60	11.1	17.6
4	180	28.6	50.4
5	540	99.1	139.2

4. Ophthalmological Examinations - Were not performed.

5. Blood was collected at termination from the orbital sinus for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood Clotting Measurements (Thromboplastin time) (Clotting time) (Prothrombin time)	X	Red blood cell morphology

*Required for subchronic and chronic studies.

No effect was evident for either sex at any dose level.

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
X	Calcium*	X	Albumin*
	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorous*	X	Cholesterol*
	Potassium*	X	Globulins
	Sodium*	X	Glucose*
	Enzymes:	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Serum Protein*
	Cholinesterase	X	Triglycerides
	Creatinine phosphokinase*		Serum protein electrophoresis
	Lactic acid dehydrogenase	X	A/G ratio
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	gamma glutamyl transferase		
	glutamate dehydrogenase		

*Required for subchronic and chronic studies.

BUN was elevated in treated males at 20 (P<0.05) and 540 (P<0.05) ppm but not in treated female groups. There was no dose response relationship and no histopathology changes in the kidney, therefore the suggestion that this is not treatment related was made. SGOT levels were elevated in males at 180 (P<0.05) and 540 (P<0.05) ppm and in females at 540 (n.s.) ppm, apparently dose related and SGPT was elevated in 540 ppm treated males (P<0.05) and females (n.s.).

6. Urinalysis* - Urine was collected from fasted animals 1 week prior to necropsy. The CHECKED (X) parameters were examined.

X		X	
	Appearance*, color, clarity	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*		Urobilinogen
X	Color/clarity		

*Required for chronic studies.

No effects were noted.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed. Organ weights were presented as absolute and relative to body weights. Means ± SD were given in Table 6 of the report.

X		X		X	
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	XX	Adrenals*
X	Cecum*	XX	Kidneys*		Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*	X	Parathyroids*
X	Liver*	X	Epididymides	X	Thyroids*
X	Gall bladder*	X	Prostate		

|X|	Pancreas*	|X |	Seminal vesicle		Other
	Respiratory	|XX|	Ovaries*	|X |	Bone*
|X|	Trachea*	|X |	Uterus*	|X |	Skeletal muscle*
|X|	Lung*	|X |	Vagina	|X |	Skin*
	Nose			|X|	All gross lesions and masses*
	Pharynx				
	Larynx				

*Required for subchronic and chronic studies.

a. Organ weight - The data were presented as grams for each organ weight with only three decimals. Even the adrenals, with group mean absolute weights as low as 0.002 g in males and 0.009 g in females, were obtained in grams with only three decimals. We consider the weights obtained for adrenals and ovaries as invalid. These organs with such low absolute weights in mice should have been presented in milligrams with one or two decimals or in grams with four or five decimals.

The only organ weight that appeared to have been affected was the liver where there was an increase in both absolute and relative weights in the males receiving 180 ($P<0.05$) and 540 ($P<0.05$) ppm, and in the females receiving 540 ($P<0.05$) ppm. This effect in males appeared to be dose related as shown in the table which follows.

Liver Weights After 3 Months of Dosage

Dose ppm	Males		Females	
	Absolute ± SD	Relative	Absolute	Relative
0	2.003 ± 0.224	5.15	1.615 ± 0.159	5.39
20	2.111 ± 0.202	5.42	1.595 ± 0.256	5.36
60	2.079 ± 0.202	5.44	1.607 ± 0.157	5.37
180	2.258 ± 0.286*	5.56*	1.[illegible] ± [illegible]	[illegible]
[illegible]	2.308 ± 0.177*	6.00*	2.029 ± 0.159*	6.52*

b. Gross Pathology - No gross changes were evident in either sex.

c. Microscopic Pathology - Complete histopathology on organs listed above was performed for all 10 control and 540 ppm treated animals on test. In addition, kidneys, livers, lungs, and testes of animals in all five groups were examined microscopically. Uteri of two to four animals in the groups receiving lower doses were examined.

Compound related changes were observed only in the livers of the 60, 180, and 540 ppm treated males and in the 180 and 540 ppm treated females. The following is extracted from Table 8 of the applicant's report which summarizes the liver changes observed by the pathologist.

Sex	Males					Females				
Dose (ppm)	0	20	60	180	540	0	20	60	180	540
LIVER:										
NO. EXAMINED	10	10	10	10	10	10	10	10	10	10
No. NORMAL	7	8	6	5	0	8	10	7	6	0
-hypertrophy, hepatocytes, centrilobular, minimal	1	0	3	4	0	0	0	0	3	1
-hypertrophy, hepatocytes, centrilobular, slight	0	1	0	0	5	0	0	0	0	7
-hypertrophy, hepatocytes, centrilobular, moderate	0	0	0	1	4	0	0	0	0	0
-hypertrophy, hepatocytes, centrilobular, marked	0	0	0	0	1	0	0	0	0	0
Total Incidence	1	1	3	5	10	0	0	0	3	8
-hypertrophy, hepatocytes, diffuse	0	0	0	0	0	0	0	0	0	2
-extramedullary hematopoiesis, focal	1	0	0	0	0	0	0	0	0	0
-infiltration, mixed inflammatory cell, multifocal	0	0	0	0	0	0	0	0	0	1
-infiltration, mono-nuclear-cell, multifocal	2	2	0	0	2	1	0	3	1	3
-micrograniuloma(s)	0	0	1	0	0	0	0	0	0	0
-necrosis, focal	0	0	0	0	2	0	0	0	0	1
-necrosis, single-cell	0	0	0	1	1	0	0	0	0	0
-proliferation, bile duct, focal	0	0	0	0	0	1	0	0	0	0
-vacuolation, hepatocytes, periportal/perilobular	0	0	0	0	3	0	0	0	0	7

Chief findings in the liver were centrilobular or diffuse hepatocellular hypertrophy with a dose related increased incidence and severity in males at 60 ppm and higher in females at 180 and 540 ppm. In the 540 ppm group, three males and seven females had periportal and perilobular hepatocyte vacuolation which the pathologist on the study considered to be associated with hypertrophy. Other effects noted were focal necrosis in two males and one female on

540 ppm and single cell necrosis in one male at 180 ppm and one male at 540 ppm.

The pathologist on the study was reported to be W. Ray Brown, D.V.M., Ph.D., veterinary pathologist from Veterinary Pathologists Service. The pathology report was dated August 24, 1987.

Supplementary Information

Appendix J of the submitted report contains detailed summary results of a 2 week range-finding study.

Doses administered to five mice of each sex per group for 2 weeks were 0, 100, 250, 500, and 1000 ppm. Blood collected at necropsy was examined only for SGPT. Liver weights and liver histopathology were evaluated.

No change in body weight (or decrease in food intake) attributable to treatment were observed over the 2 weeks. Mean liver weights, expressed in absolute and relative weights as percent of control, were increased at the two highest dose levels in both sexes (no statistics). Although an increase in SGPT was observed in 250 ppm treated females, this finding was considered incidental since this was the only group with such an increase and no dose-response relationship.

Gross Pathology - At 1000 ppm, all five males and two females had enlarged livers with "prominent lobular architecture" and/or tan in color. Such changes were also seen in two males on the 500 ppm dose.

Histopathology - Liver changes predominantly seen was hypertrophy of centrilobular hepatocytes moderate to severe in all five males of the highest dose, and mild to moderate in four males at 500 ppm. In females, these liver changes were also seen in all five receiving 1000 ppm and in three out of five receiving 500 ppm but in both groups they were classified as "minimal to mild" in both groups. Other changes were also seen in the females such as necrosis in individual hepatocytes, characterized by liver cells with intensely eosinophilic cytoplasm and pyknosis or karyorrhexis of the nucleus. Periportal vacuolation of hepatocytes was increased in the 500 and 1000 ppm treated males and females, also seen in two males and one female at 250 ppm. Liver changes were considered to be more severe in males than in females of the same treatment group.

The NOEL in this preliminary range finding study was 250 ppm (20 mg/kg/day in males, 37 mg/kg/day in females).

D. Discussion:

The liver appeared to be the only organ for which compound related changes were evident. This included increases in liver weight at 180 and 540 ppm in males (dose related) and an increase in liver weight at 540 ppm in females. Microscopic changes were also seen in liver that were compound related with a dose-response relationship in incidence and severity in males between 60 and 540 ppm and in females at 180 and 540 ppm. Details of the liver histopathology changes are described above under Microscopic Pathology (page 7).

Other indications of liver toxicity included increases in SGOT in males at 180 and 540 ppm and in females at 540 ppm. SGPT was also elevated in males and females of the 540 ppm treated group. Males appear to be more sensitive to liver changes than females.

There appeared to be no other compound related effect in this study; not even in body weight or food intake.

The NOEL suggested by the applicant was 60 ppm. However, liver changes were still evident in male rats at 60 ppm, observed microscopically (see page __ of this DER). We therefore conclude the following based on liver histopathology:

- NOEL = 20 ppm (3.8 mg/kg/day in males, 5.7 mg/kg/day in females)
- LEL = 60 ppm (11.1 mg/kg/day in males, 17.6 mg/kg/day in females)

The investigators on the study suggested that the liver changes observed in this study were typical of that which is associated with "enzyme induction".

We do not believe that a MTD for a mouse carcinogenicity test can be adequately selected from this study. Although hepatic changes were observed, there was no indication of decreased body weight gain or "life threatening toxicity".

007677

Reviewed By: Sidney Stolzenberg, Ph.D.
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D.
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)

12/11/87

DATA EVALUATION REPORT

Study Type: 3-Month Dog

Caswell No.: 723Q

Accession Number: 410735-04

HED Project No.: 9-1381A

Test Material: Fenethanil

MRID No.: 410735-04

Synonyms: RH-7592

Study Number: 87R-127

Sponsor: Rohm & Haas Company
Philadelphia, PA 19105

Testing Facility: Rohm & Haas
Toxicology Department
Spring House, PA 19477

Title of Report: Three-Month Dietary Toxicity Study in Dogs

Authors: William D. Shade, B.S. and G.A. Hazelton, Ph.D.,
Study Director

Report Issued: September 8, 1988

Conclusions:

Doses administered in the diet were 0, 30, 400 and 1600 ppm. The main effect was an increase of weight of the liver of both sexes at 400 (n.s.) and 1600 ($P<0.05$) ppm. Diffuse hepatocellular hypertrophy in both sexes was observed at 400 and 1600 ppm, [illegible] which incidence and severity were dose related. In the 1600 ppm treated males, multifocal vacuolation was seen in the enlarged hepatocytes of all four dogs of the group. Other effects included increases in enzymes associated with liver toxicity such as SGPT, ALP and GGT. Decreases in serum protein measurements (total protein, albumin, globulin) were seen at 1600 ppm in males (n.s.) and females ($P<0.05$). Triglycerides were increased at 1600 ppm.

- LEL = 400 ppm (13.3 mg/kg in males, 14.0 mg/kg in females)

- NOEL = 100 ppm (3.3 mg/kg in males, 3.5 mg/kg in females) Primarily based on hepatocellular hypertrophy.

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In a dose range-finding study, doses for 2 or 4 weeks were 200, 400, 800, 1600 and 3200 ppm. There was 1 or 2 dogs per treatment group. At 200 and 400 ppm, no effect was observed. At 800 ppm, the only effect claimed was an increase in serum ALP. At 1600 ppm, increased SGPT, ALP and decreased cholesterol, and a transient loss in body weight during the first week coupled with a decrease in feed intake, were observed. At 3200 ppm, which included two animals fed for 2 hours (usual regimen) and an additional two animals fed for 6 hours per day, there was a 1000-1400 g loss in weight in 2 weeks and a 60 to 70 percent reduction in feed intake. The decrease in feed intake persisted for 4 weeks. The 3200 ppm dose was considered "not tolerable," apparently based on the decreased feed intake and body weight loss during the 2 and 4 week period.

A. Materials:

1. Test Compound: RH-7592, Description: Off white solid, Lot No.: EG1442, Purity: 96.4%,

2. Test Animals: Species: Dog, Strain: Beagle, Age: 5 months, Weight: 6.3 to 9.6 kg, Source: White Eagle Labs, Doylestown, PA.

B. Study Design:

1. Animal Assignment - Animals were assigned to the following test groups:

Test Group	Dose in diet (ppm)	Male	Female
1 Cont.	0	4	4
2	30	4	4
3	100	4	4
4	400	4	4
5	1600	4	4

2. Diet Preparation - Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration at 2, 4, 8, and 12 weeks.

Results - In both tests for homogeneity (samples of diet obtained from top, middle, and bottom of storage container) and stability over 11 days, analytical values ranged between 87 to 114 percent of target concentrations. Homogeneity was tested for the 30, 100, and 1600 ppm diets and stability for all four diets.

3. Animals received food 2 hours daily and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: ANOVA or analysis of covariance was used to assess the presence or absence of an effect for all parameters. Group means were compared by using least square means. When a significant treatment effect was found, comparisons to control were calculated by Dunnett's "t" test, with $P<0.05$ signifying statistical significance.

5. Compliance

   - A signed statement of Confidentiality Claim was included.

   - A signed statement of compliance with EPA's GLP was provided.

   - A signed Quality Assurance Statement was provided.

C. Methods and Results:

1. Observations - Animals were inspected daily for signs of toxicity and mortality.

   Toxicity/Mortality (survival)

   No deaths occurred. No treatment related clinical signs of toxicity were evident in any group.

2. Body Weight - Animals were weighed weekly for the duration of the study starting at week -3.

   Group 5 (1600 ppm) treated males and females lost weight the first 2 weeks but subsequently weight gains were similar to other groups, including controls. Over the 13 weeks, males gained 15 percent less and females 55 percent less than controls.

3. 3. Food Consumption and Compound Intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data. A decrease in feed consumption ($P<0.05$) was noted during the first 2 weeks in males and females receiving the 1600 ppm dose. Although feed intake by both males and females in the 1600 ppm group remained below controls during the combined 2-8 and 8-13 weeks, the differences were not statistically significant. The decrease in feed intake was attributed to decreased palatability of the diet. No effects were

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seen at the lower dose levels.

Food Consumption/Food Efficiency/Compound Intake - A decline in feed efficacy was seen in the 1600 ppm group during weeks 1 and 2 of the study for both males and females (no statistical comparisons).

Compound intake averaged over the entire 3 weeks of the study, in mg/kg/day, were as follows:

Dietary Conc. ppm	3 Month Mean Intake* (mg/kg/day) Males	Females
30	0.97 ± 0.06	1.05 ± 0.05
100	3.30 ± 0.19	3.48 ± 0.22
400	13.27 ± 0.65	13.98 ± 0.78
1600	50.40 ± 3.22	53.27 ± 4.83

*Each value represents mean compound intake ± SD over 3 months.

4. Ophthalmalogical Examinations - Performed before treatment and during week 13 on all animals on tests by indirect ophthalmoscopy, by Lionel F. Rubin, V.M.D.

No compound related effect was evident.

5. Blood was collected twice before treatment and at 4 and 13 weeks for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements	X	Red blood cell morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies.

The effects noted were a slight increase in MCV and MCH at 1600 ppm in both sexes ($P<0.05$) at week 13, an increase in platelet count at week 13 only in females receiving 1600 ppm ($P<0.05$). There were decreases in erythrocyte count in both sexes, statistically significant only in the females, in the 1600 ppm

group at week 13. Decreases in HCT and HGB were also noted in the highest dose group at week 13 but not statistically significant in either sex. Increases in platelet count was seen in females receiving 1600 ppm at 1 and 13 weeks.

No effects were noted at 30, 100 and 400 ppm.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes:	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Serum Protein*
	Cholinesterase	X	Triglycerides
	Creatinine phosphokinase**		Serum protein electrophoresis
	Lactic acid dehydrogenase	X	A/G ratio
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	gamma glutamyl transferase (GGT)		
	glutamate dehydrogenase		

* Required for subchronic and chronic studies.

No effects were noted at 30 and 100 ppm dose levels.

ALP: At 400 ppm, increases in both sexes were evident at 13 weeks but not statistically significant. At 1600 ppm, very large increases in both sexes at 4 and 13 weeks ($P<0.05$).

SGPT: In males, small increase at 1600 ppm at 13 weeks (n.s.). In females, substantial increases at 1600 ppm during weeks 4 and 13, even at 400 ppm.

Total Protein: Decreases in males (n.s.) and females ($P<0.05$) at 1600 ppm during week 13.

Alb: Decreases in males (n.s.) and females ($P<0.05$) at 1600 ppm during week 13.

Globulins: At 1600 ppm, at 13 weeks, a decrease in males (n.s.) and females ($P<0.05$) was seen.

Cholesterol: At 1600 ppm, in males, decreases at 4 (n.s.) and 13 (n.s.) weeks; in females, decreases at 4

A2

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta*	XX	·Brain*
X	Salivary glands*	X	Heart*	X	Periph. nerve*
X	Esophagus*		Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*		
X	Duodenum*	X	Spleen*	X	·Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic n.)*
X	Ileum*		Urogenital		Glandular
X	Cecum*	XX	Kidneys*	XX	·Adrenals*
X	Colon*	X	Urinary bladder*	X	Lacrimal gland
X	Rectum*	XX	Testes*	X	Mammary gland*
XX	Liver*	X	Epididymides	XX	·Parathyroids*
X	Gall bladder*	X	Prostate	XX	·Thyroids*
X	Pancreas*		Seminal vesicle		Other
	Respiratory	XX	Ovaries*	X	Bone*
X	Trachea*	X	Uterus*	X	Skeletal muscle*
X	Lung*	X	Vagina	X	Skin*
	Nose			X	All gross lesions and masses*
	Pharynx				
	Larynx				

* Required for subchronic and chronic studies.

a. Organ weight - Only the liver had a compound related increase. There was an obvious increase in both sexes at 400 ppm for both absolute and relative (to body) liver weights but not statistically significant. At 1600 ppm, the increases in liver weights were even greater ($P<0.05$) in both sexes.

(P<0.05) and 13 (P<0.05) weeks were seen. Curiously, at 400 ppm in females but not in males, increases in serum cholesterol were seen at weeks 4 (P<0.05) and 13 (P<0.05).

GGT: At 1600 ppm, in females but not in males, increases were observed at 4 (P < 0.05) and 13 (P<0.05) weeks.

Triglycerides: At 1600 ppm, in males but not in females, an increase at 13 weeks (P<0.05) was seen.

6. Urinalysis* - Urine was collected from fasted animals four day prior to necropsy. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*		Urobilinogen

* Required for chronic studies.

Bladder urine samples were obtained from about half the dogs in each group of both sexes on the second day of necropsy, using a needle and syringe. They were examined for sperm, red and white blood cells, epithelial cells, hyaline casts, crystals, bacteria. The purpose of such samples was to exclude the possibility of an upper urinary tract infection, because increased numbers of white blood cells were found in about 50 percent of all the dogs in all treated and control groups, both sexes.

No compound related effect was claimed to be evident in the test. (Table 5 of the report for urinalysis values appears to be incomplete but we have no basis for these claims based on individual animal values in Appendix 9 and 9A. From Appendix 9A, there appeared to be bacteria in urine samples collected at necropsy by needle and syringe, present in controls and all treatment groups, suggesting upper urinary tract infections in these dogs. There was no discussion of this finding.)

MALES

Target Organ	GROUP 1 (0 ppm) Mean (G)	Std. Dev.	% of Bdywt.	GROUP 2 (30 ppm) Mean (G)	Std. Dev.	% of Bdywt.
Body weight	9643	783		9925	634	
Liver	270.29	17.24	2.83	281.10	29.29	2.84

Target Organ	GROUP 3 (100 ppm) Mean (G)	Std. Dev.	% of Bdywt.	GROUP 4 (400 ppm) Mean (G)	Std. Dev.	% of Bdywt.
Body weight	9765	883		9718	378	
Liver	289.05	18.27	2.98	314.88	44.05	3.25

Target Organ	GROUP 5 (1600 ppm) Mean (G)	Std. Dev.	% of Bdywt.
Body weight	9286	1432	
Liver	425.10*	48.01	4.62*

FEMALES

Target Organ	GROUP 1 (0 ppm) Mean (G)	Std. Dev.	% of Bdywt.	GROUP 2 (30 ppm) Mean (G)	Std. Dev.	% of Bdywt.
Body weight	8944	1429		8652	946	
Liver	251.07	32.22	2.83	259.38	23.19	3.01

Target Organ	GROUP 3 (100 ppm) Mean (G)	Std. Dev.	% of Bdywt.	GROUP 4 (400 ppm) Mean (G)	Std. Dev.	% of Bdywt.
Body weight	9029	728		9003	1196	
Liver	254.40	28.64	2.83	319.04	28.36	3.59

Target Organ	GROUP 5 (1600 ppm) Mean (G)	Std. Dev.	% of Bdywt.
Body weight	7951	1133	
Liver	360.53*	58.06	4.57*

b. Gross Pathology - No treatment related effect was observed for any organ.

c. Microscopic Pathology - Hepatocellular hypertrophy occurred in males and females at 400 and 1600 ppm and in the males, multifocal vacuolization was seen in the hypertrophied cells of the 1600 ppm group. The intensity and incidence of these liver changes were dose related. The table below is extracted from Table 8 of the report which summarizes the liver changes observed.

Incidence of Histopathomorphic Observations

Sex	Males					Females				
Dose (ppm)	0	30	100	400	1600	0	30	100	400	1600
LIVER:										
NO. EXAMINED	4	4	4	4	4	4	4	4	4	4
NO. NORMAL	2	2	2	1	0	2	3	3	1	0
-accessory liver lobe	0	0	1	0	0	0	0	0	0	0
-congestion	0	0	0	1	0	0	0	0	0	0
-hypertrophy, hepatocytes, diffuse	0	0	0	1	4	0	0	0	3	4
-infiltration, mono-nuclear-cell, multifocal	0	1	0	1	0	0	0	0	1	0
-microgranuloma(s)	2	1	1	0	2	2	1	1	0	1
-vacuolation, hepatocytes, multifocal	0	0	0	0	4	0	0	0	0	0
-vacuolation, hepatocytes, periportal	0	0	0	0	0	0	0	0	1	0

D. Discussion:

Doses administered in the diet of the 3-month dog study were 0, 30, 100, 400, and 1600 ppm. The main effect was on the liver where dose related increased incidence and intensity of diffuse hepatocellular hypertrophy occurred in males and females at 400 and 1600 ppm. In the males, multifocal vacuolization of the enlarged cells was evident. Associated with this phenomenon were increases in liver weight of both sexes, 15 percent in males and 27 percent in females at 400 ppm, 57 percent in males and 44 percent in females at 1600 ppm. The increase in liver weight was statistically significant only in the 1600 ppm treated males and females. Also associated with these changes were increases in ALP, SGPT and GGT activities. Possibly the decrease in cholesterol and increase in triglycerides at 1600 ppm were also associated with the liver changes. Decreases in total protein, albumin and globulins were observed in the highest dose group, particularly in the females. Small hematology changes were also observed at the 1600 ppm dose, including decreases in erythrocyte count, hematocrit and hemoglobin but small increases in MCH and MCV.

A decrease in body weight gain was observed predominantly during the first 2 weeks in males and females receiving the 1600 ppm dose. This was accompanied by a depression in feed intake at the same dose level, predominantly during that period.

Guideline Series 84: MUTAGENICITY

Reviewed By: Sidney Stolzenberg, Ph.D. 12-11-89
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)
Secondary Reviewer: John Hou-Shi Chen, D.V.M.
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

Chemical: Fenethanil
RH-57,592

Caswell No.: 723Q
HED Project No.: 9-1381A

Study Type: Salmonella/mammalian activation gene mutation assay.

Accession Number: 410312-16

Synonyms/Cas No.:

Sponsor: Rohm & Haas Company
Philadelphia, PA 19105

Testing Facility: Rohm & Haas
Toxicology Department
Spring House, PA 19477

Title of Report: RH-57,592; Microbial Mutagenicity Assay

Authors: J.L. Sames, Assoc. Toxicologist and J.P. Frank, D. Sci., Study Director

Study Number(s): 87 R-044

Report Issued: November 10, 1988

Conclusion(s) - Executive Summary:

RH-57,592 was not mutagenic for Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 both with and without the presence of rat liver S9 activation.

Classification: Unacceptable

The reasons for this classification are given in the Discussion, last paragraph.

A. Materials:

1. Test Material: Fenethanil
Description: Technical, off white color.
Lot No.: EG 1442, TD #87-47; Purity: 96.4%
Solvent Used: DMSO

2. Control Materials:

Negative: DMSO
Solvent/final concentration:
Positive: Non-activation: No positive control
Sodium azide ___-___ ug/plate TA100, TA1535
2-Nitrofluorene ___-___ ug/plate TA98, TA1538
9-Aminoacridine ___-___ ug/plate TA97, TA1537
Other (list):

Activation:
2-Aminoanthroacene (2-anthramine) 10 ug/plate usually all strains for strains TA100, TA1535, and TA1537
Other (list): 2-acetamidofluorene (2-AAF) 50 ug/plate, for strain TA98 with activation

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

If other, describe below
Describe S9 mix composition (if purchased, give details).

NADP	$4X10^{-3}M$
Glucose-6-phosphate	$5X10^{-3}M$
Magnesium chloride	$8X10^{-3}M$
Potassium chloride	$33X10^{-3}M$
Sodium phosphate buffer, pH 7.4	$100X10^{-3}M$
Liver homogenate (S-9) from	10%

Aroclor 1254 induced rats

4. Test Organism: S. typhimurium strains
___ TA97 X TA98 X TA100 ___ TA102 ___ TA104
X TA1535 X TA1537 ___ TA1538; list any others:
Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes/No (circle one)

5. Test Compound Concentrations Used: 007677

Non-activated conditions: See Below

Activated conditions: See below

SALMONELLA

B. Test Performance:

1. Type of Salmonella Assay:
   - X standard plate test
   - ___ pre-incubation (__ minutes)
   - ___ "Prival" modification (i.e., azo reduction method)
   - ___ spot test
   - ___ other (describe in a.)

a. Protocol (brief description, or attach copy to Appendix, if appropriate; e.g., include mediums used, incubation times, assay evaluation):

The tester strains were subcultured overnight in Difco nutrient broth. Each test system was immoculated with 10^6-10^7 cells; all 4 strains. Each concentration with all 4 tester strains were tested in triplicate. In addition, plates were supplemented with histidine to assess toxicity at each concentration for each tester strain. All plates were incubated for 72 hours at 37 degrees. Each concentration with all 4 tester strains was tested with an without S9. For controls with each tester strain, 16 plates were incubated both with and without S9.

2. Preliminary Cytotoxicity Assay (include concentration ranges, activation and nonactivation; strain(s) used; reported results, e.g., cytotoxicity indices (effect on background lawn; reduction in revertants) and solubility):

5000, 2000, 500, 200. and 50 ug/plate were initially tested against all 4 strains listed above both with and without metabolic activation. Concentrations tested were adjusted for impurities.

There was no indication of an increased colony count, therefore no mutagenic response at any dose level both with and without S9 for all 4 tester strains. However, a toxic response was detected at all 5 concentrations greater than 500 ug/plate in TA98 and TA100 without S9, and at 5000 ug/plate in TA1535 with S9. In addition, precipitates formed at 200 ug/plate in TA98 and TA1535 with S9, and TA98, TA1535, and TA1537 without S9;

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concentrations exceeding 500 ug/plate in TA100 and TA1537 with S9 and TA100 without S9.

3. Mutagenicity Assay (reported results, e.g., induction of revertants - individual plate counts and/or summary given; appropriateness of positive and background (concurrent and/or historical) revertant levels; number of concentration levels used; number of cultures per concentration; include representative table, if appropriate):

In strains TA1535 and TA1537, concentrations tested were 300, 160, 90, 50, and 30 ug/plate. In TA98, concentrations tested were 20, 5, 2, 0.5, and 0.2 ug/plate. In TA100, concentrations tested were 1600, 900, 500, 300, and 160 ug per plate. All of the above tests were carried out both with and without S9 activation at the indicated concentrations with each strain. All tests were in triplicate, 16 solvent controls. Additional plates supplemented with histidine were used to assess toxicity.

Under the conditions of this test, nc mutagenic response was seen for any of the 4 tester strains.

Positive controls were highly responsive in the test systems in the presence of S9.

4. Reviewer's Discussion/Conclusions (include, e.g., rationale for acceptability or not; necessity for repeat, if appro-priate; address any discrepancies with author conclusions):

The criterion for positive response in any test system was a doubling of the colony count. However, in all of the tests performed, there was no increase in colony count. A slight decrease in colony count was more likely to occur at the highest concentrations probably due to residual toxicity.

Positive mutagenic responses were seen with 2-amino-anthracene in the presence of S9 for TA1535, TA1537, and TA100. Positive responses were seen with 2-AAF for TA98 in the presence of S9. Positive mutagenic responses were not seen in the absence of S9 with either of the 2 positive control substances for any of the 4 tester strains. These results indicate that all 4 tester strains were responsive to mutagenic test materials, indicating the validity of the test systems.

There was no positive mutagenesis contols to confirm the

reversion properties of each specific strain under the non-activated system. In addition, specific procedures for confirming the genotypes of tester strains described by Ames et al. (Mutation Res. 31:347-364, 1975) were not presented in this study. Therefore, this test is classified as Unacceptable.

5. Was test performed under GLPs)? Yes

Guideline Series 84: MUTAGENICITY

Reviewed By: Sidney Stolzenberg, Ph.D. S Stolzenberg 11/30/89
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)
Secondary Reviewer: John Hou-Shi Chen, D.V.M. John H Chen 12/11/89
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

Chemical: Fenethanil

Caswell No.: 723Q
HED Project No.: 9-1381A

Study Type: Salmonella/mammalian activation gene mutation assay.
Accession Number: 410312-17

Synonyms/Cas No.: RH 7592

Sponsor: Rohm & Haas Company
Philadelphia, PA 19105

Testing Facility: Rohm & Haas
Toxicology Department
Spring House, PA 19477

Title of Report: RH-57,592; Salmonella Typhimurium Gene Mutation Assay

Authors: J.P. Frank, D. Sci., Study Director and J.L. Sames

Study Number(s): 88 R-009

Report Issued: June 10, 1988

Conclusion(s) - Executive Summary:

RH-57,592 was not mutagenic for Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100 both with and without the presence of rat liver S9. Concentrations tested with each strain both with and without activation were 30 to 300 ug/plate.

Classification: Unacceptable

See Discussion, last paragraph, for reasons.

A. Materials:

1. Test Material: Name: RH-7,592
Description: Technical
Lot No.: BBP-3-1786R; Purity: 96.4%
Solvent Used: DMSO

2. Control Materials:

Negative: DMSO
Solvent/final concentration:
Positive: Non-activation:
Sodium azide _____ ug/plate TA100, TA1535
2-Nitrofluorene _____ ug/plate TA98, TA1538
9-Aminoacridine _____ ug/plate TA97, TA1537
Other (list):

Activation:
2-Aminoanthroacene (2-anthramine) 10 ug/plate usually all strains for strains TA100, TA1535, and TA1537
Other (list): 2-acetamidofluorene (2-AAF), 50 ug for strain TA98 with activation

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

If other, describe below

Describe S9 mix composition (if purchased, give details):

NADP	$4X10^{-3}M$
Glucose-6-phosphate	$5X10^{-3}M$
Magnesium chloride	$8X10^{-3}M$
Potassium chloride	$33X10^{-3}M$
Sodium phosphate buffer, pH 7.4	$100X10^{-3}M$
Liver homogenate (S-9) from Aroclor 1254 induced rats	10%

4. Test Organism: S. typhimurium strains
___ TA97 X TA98 X TA100 ___ TA102 ___ TA104
X TA1535 X TA1537 ___ TA1538; list any others:
Properly maintained? Yes/No (circle one)
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes/No (circle one)

5. Test Compound Concentrations Used:

Non-activated conditions: 30, 50, 90, 160, and 300 ug/plate

Activated conditions: Same concentrations as non-activated

B. Test Performance:

1. Type of Salmonella Assay:
   - X standard plate test
   - ___ pre-incubation (__ minutes)
   - ___ "Prival" modification (i.e., azo reduction method)
   - ___ spot test
   - ___ other (describe in a.)

   a. Protocol (brief description, or attach copy to Appendix, if appropriate; e.g., include mediums used, incubation times, assay evaluation):

   Each concentration was tested in triplicate in minimal plates (minimal-glucose agar medium with a trace of histidine). Also, each concentration was also tested in plates supplemented with histidine, used only as a visual aid in confirming a toxic response. DMSO solvent control was tested in 6 replicates in minimal plates and in the supplemental plates.

2. Preliminary Cytotoxicity Assay (include concentration ranges, activation and nonactivation; strain(s) used; reported results, e.g., cytotoxicity indices (effect on background lawn; reduction in revertants) and solubility):

   Concentrations tested were 50, 200, 500, 2000, and 5000 ug/plate with and without S9 activation. Solubility problems were encountered at the 3 highest concentrations. At the highest dose, revertant colony count per plate was reduced in all 4 tester strains both with and without the activation mixture.

3. Mutagenicity Assay (reported results, e.g., induction of revertants - individual plate counts and/or summary given; appropriateness of positive and background (concurrent and/or historical) revertant levels; number of concentration levels used; number of cultures per concentration; include representative table, if appropriate):

   There was no indication of mutagenicity at any concentration with any of the 4 tester strains used both with and without metabolic activation. Means at each concentrations were similar to controls.

   2-Aminoanthracene positive control gave a clear mutagenic

responses in the presence of S9 for tester strains TA1535, TA1537, and TA100.

2-AAF positive control gave a clear mutagenic response in the presence of S9 for tester strain TA98.

4. Reviewer's Discussion/Conclusions (include, e.g., rationale for acceptability or not; necessity for repeat, if appro-priate; address any discrepancies with author conclusions):

Concentrations tested were 30 to 300 ug per plate both with and without S9 activation. Tester strains were TA98, TA100, TA1535, and TA1537. No mutagenic activity was seen both with and without metabolic activation. In an initial test, concentrations were 50, 200, 500, 2000, and 5000 ug/plate. Insolubility was encountered at the 3 highest concentrations. Even at the highest concentrations, no indication of mutagenicity was encountered.

Positive controls caused increased revertant colonies in S9 activated systems, indicating that these tests were valid.

There was no positive mutagenesis contols to confirm the reversion properties of each specific strain under the non-activated system. In addition, specific procedures for confirming the genotypes of tester strains described by Ames et al. (Mutation Res. 31:347-364, 1975) were not presented in this study. Therefore, this test is classified as Unacceptable.

5. Was test performed under GLPs (is a quality assurance statement present)? Yes

Guideline Series 84: MUTAGENICITY

Reviewed By: Sidney Stolzenberg, Ph.D. S Stolzenberg 12/6/89
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)
Secondary Reviewer: John H.S. Chen, D.V.M. John H S Chen 12/11/89
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

Chemical: Fenethanil
RH-57,592

Caswell No.: 723Q
HED Project No.: 9-1318A

Study Type: Mammalian cells in culture gene mutation assay in Chinese Hamster Ovary Cell

Accession Number: 410312-18

Synonyms/Cas No.:

Sponsor: Rohm & Haas Company
Philadelphia, PA 19105

Testing Facility: SITEK Research Labs
Rockville, MD 20852

Title of Report: Test for Chemical Induction of Gene Mutation at the HGPRT Locus in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation.

Authors: A. Thilagar, Ph.D., Study Director

Study Number(s): 0079-2500

Report Issued: April 29, 1988

Conclusion(s) - Executive Summary:

Technical RH-57,592 (fenethanil) was tested twice in the presence Chinese hamster ovary cells for its potential to cause gene mutation at the HGPRT locus. No increase in mutant frequency was seen at the HGPRT locus, both in the presence or absence of S-9 activation.

Classification: Acceptable

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MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. Materials:

1. Test Material: Name:
Description: Technical
Batch No.: 3-1768R; Purity: 96.7
Solvent Used: DMSO

2. Control Materials:
Negative: DMSO
Solvent/Final Concentration:
Positive: Non-activation (concentrations, solvent): Ethyl methanesulfonate (EMS) at 0.5 uL/mL in DMSO solvent.

Activation (concentrations, solvent): 7,12-Dimethylbenz(a)anthracene (DMBA) at 5 ug/mL, dissolved in acetone.

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other		___ other	

If other, describe below

Describe S9 mix composition (if purchased, give details):

One gram rat liver was used to make 3 mL buffered S9. S9 mixture consisted of 50 mM sodium phosphate (pH 7.5) 4 mM NADP, 5 mM glucose-6-phosphate, 30 mM KCl, 10 mM $CaCl_2$ and 100 mL S9 fraction. Prior to use, S9 mix was diluted 1:5 with Hx-free Ham's Folz nutrient medium supplemented with 2 mM L-glutamine.

4. Test Cells: Mammalian cells in culture
___ mouse lymphoma L5178Y cells
X 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? Yes
Periodically "cleansed" against high spontaneous background? Yes/No (circle one)

This test was performed under GLPconditions

MAMMALIAN CELLS IN CULTURE GENE MUTATION

5. Locus Examined:

___ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)
_____ trifluorothymidine(TFT)

_X_ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
selection agent: _____ 8-azaguanine (8-AG)
(give concentration) 10 uM 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:

Non-activated conditions: First test: 50, 40, 30, 20, and 10 ug/mL; Second test: 40, 35, 30, 25, 20, and 15 ug/mL

Activated conditions: First test: 60, 45, 30, and 10 ug/mL; Second test: 60 55, 50, 45, 40, and 35 ug/mL

B. Test Performance:

1. Cell Treatment:

a. Cells exposed to test compound for:
5 hours (non-activated) 5 hours (activated)

b. Cells exposed to positive controls for:
5 hours (non-activated) 5 hours (activated)

c. Cells exposed to negative and/or solvent controls for:
5 hours (non-activated) 5 hours (activated)

d. After washing, cells cultured for 18-24 hours (expression period) before cell selection

e. After expression, cells cultured for 10 days in selection medium to determine numbers of mutants and for 7 days without selection medium to determine cloning efficiency

2. Protocol (brief description, or attach copy to Appendix, if appropriate; include, e.g., number of cell cultures; medium; incubation times; cell density during treatment; number of cells seeded for treatment and selection; subculture and feeding schedules, if necessary):

Each test was performed twice using duplicate cultures seeded with 5×10^5 cells/flask at each dose level and was incubation for 5 hours with the proper concentration of test substance with or without S9. After exposure, the cells were washed and allowed to grow for 18 to 24 hours, then subcultured to determine cytotoxicity and to initiate cultures for expression of mutant phenotype. Cytotoxicity was indicated as RCE, defined below under Preliminary Cytotoxicity Assay. For expression of HGPRT locus mutants, cells from each duplicate culture flask were subcultured in hypoxanthine-free Ham's F-12 medium with other supplements added. The cells were subcultured at 2- to 3-day intervals for 10 days. The cells were then harvested and from each replicate seeded in five plates with 2×10^5 cells/plate. To determine cloning efficiency, 200 cells/60 mm^2 dish were plated in triplicate. The cultures were than incubated for 7 days.

3. Preliminary Cytotoxicity Assay (include concentration ranges, activation and nonactivation; reported results, e.g., cytotoxicity and solubility):

Doses higher than 60 ug/mL were not used because of solubility limit. For range finding studies, doses tested were 60, 30, 15, 7.5, 3.8, 1.9, 0.94, 0.47, 0.23, and 0.12 ug/mL; all doses tested in duplicate, including solvent control. Test cultures seeded about 18 to 24 hours earlier were used. For S9, the medium was removed and 5 mL S9 mix added. For non-S9, the medium was removed and 5 mL serum free culture were washed with HBSS, then cultured for 18 to 24 hours prior to cloning for cytotoxicity. To determine cytotoxicity, 200 cells/60 mL dish were trypsinized and incubated in a specified medium for 7 days. The colonies were washed, fixed with methanol, stained with Giemsa stain and then counted. Relative cloning efficiency (RCE) was determined as follows:

$$\frac{\text{No. colonies in test plates}}{\text{No. colonies in solvent plates}} \times 100$$

No colonies grew at $\geq$ 60 ug/mL in S9 free medium. RCE was 49 percent at 30 ug/mL, 92 percent at 15 ug/mL.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

In S9 system, at 60 ug/mL RCE was 19 percent, at 30 ug/mL it was 75 percent, at 15 percent ug/mL it was 81 percent, at 0.94 ug/mL it was 99 percent.

4. Cytogenetics Assay (reported results, e.g., induction of aberration frequency; types of aberrations, e.g., whether gaps are included in analysis or not, chromatid vs. chromosomal events, complex aberrations; positive and background aberration frequencies; number of cultures per concentration; levels of cytotoxicity obtained, e.g., effect on mitotic index or cell survival, if examined; include representative table, if appropriate):

Results of the first test are shown in tables photocopied from the report; Table 3 - without S9 activation and Table 4 - with S9 activation. Without activation, the 50 and 40 ug/plate doses were too high and resulted in toxicity to the cells. Doses of 30, 20, and 10 ug/plate were non-mutagenic (Table 3). In the presence of S9, doses of 60, 45, 30, and 10 ug/mL were non-mutagenic. The positive controls, EMS without S9 and DMBA in the presence of S9, were highly responsive.

Results from the second test are shown in Table 6 without activation and Table 7 with S9 activation. Doses of 35, 30, 25, and 20 ug/mL with cloning efficiency values of 96 percent or higher, showed no indications of mutagenicity without S9. In the presence of S9 metabolic activation, doses of 60, 55, 50, 45, and 40 ug/mL, with cloning efficiencies averaging 80 percent or higher, showed no indications of mutagenicity. The positive controls, EMS without activation and DMBA with activation, were highly responsive.

5. Reviewer's Discussion/Conclusions (include, e.g., rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

RH-57,592 was tested twice in the Chinese hamster ovary cell, in vitro system at the HGPRT locus. Doses up to 35 ug/mL without S9 activation, and up to 60 ug/mL in the presence of S9 activation did not cause gene mutation at the HGPRT locus.

The positive control compounds, ethyl methanesulfonate (EMS) in the absence of S9 and DMBA in the presence of S9 activation were both highly responsive, causing a substantial response for gene mutation at the HGPRT locus, which supports the validity of this test system.

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Guideline Series 84: MUTAGENICITY

Reviewed By: Sidney Stolzenberg, Ph.D. 11/30/89
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)
Secondary Reviewer: John Hou-Shi Chen, D.V.M. 12/1/89
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

Chemical: Fenethanil
RH-57,592

Caswell No.: 723Q
HED Project No.: 9-1381A

Study Type: In vivo mammalian cytogenetics assay in rats, Sprague-Dawley.

Accession Number: 410312-19

Synonyms/Cas No.: 51-18-3

Sponsor: Rohm & Haas Company
Philadelphia, PA 19105

Testing Facility: SITEK Research Labs
Rockville, MD 20852

Title of Report: RH-57,592 Technical. Acute Test for Chemical Induction of Chromosome Aberration in Rat Bone Marrow Cells In Vivo.

Authors: A. Thilagar, Ph.D., Study Director

Study Number(s): 0079-1531

Report Issued: May 25, 1988

Conclusion(s) - Executive Summary:

RH-57,592 at dose levels of 0.25, 1.25, and 2.5 g/kg by oral gavage, cause no increase in the number of cells with aberrations or in number of aberrations per cell in the bone marrow of rats which were collected 6, 24, and 48 hours after treatment.

RH-57,592 does not appear to be mutagenic in this test system.

Classification: Acceptable

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A. Materials:

1. Test Material: Name: RH-57,592 (fenethanil)
Description (e.g., technical, nature, color, stability):
Technical, white solid
Batch No.: 3-1786R; Purity: 96.7%
Solvent Used: 0.5% methocel

2. Control Materials:

Negative (if not vehicle)/Route of administration: 0.5% methocel; orally administered at 10 ml/kg.

Vehicle/Final volume/Route of administration: 0.5% methocel; 0.1 mg/mL.

Positive/Final dose(s)/Route of administration: Triethylenemelamine (TEM), dosed at 0.5 mg/kg, in sterile, deionized water, administered i.p.

3. Test Compound:

Volume of test substance administered: 1 ml/100g

Route of administration: oral gavage

Dose levels used: 2.5, 1.25, and 0.25 g/kg. LD_{10} is reported to be 2.5 g/kg.

4. Test Animals:

a. Species Rat Strain Sprague-Dawley Age 49 days
Weight male _____ Female _____
Source: Charles River, Raleigh, NC

b. No. animals used per dose: 5 males 5 females for each sacrifice time period.

c. Properly maintained? Yes

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B. Test Performance:

1. Treatment and Sampling Times:

a. Test Compound:

Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): __X__ 6 hr ___ 12 hr
__X__ 24 hr __X__ 48 hr ____ 72 hr (mark all that are appropriate)
_____ other (describe):

b. Negative and/or Vehicle Control:

Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): __X__ 6 hr ___ 12 hr
__X__ 24 hr __X__ 48 hr ____ 72 hr (mark all that are appropriate)
_____ other (describe):

c. Positive Control:

Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): ____ 6 hr ___ 12 hr
__X__ 24 hr ____ 48 hr ____ 72 hr (mark all that are appropriate)
_____ other (describe):

d. Administration of Spindle Inhibitor:

Inhibitor used/dose: colchicine/1.0 mg/kg

Interval administered before animal killed: 2-4 hours

Route of Administration: ___ i.p. ___ other (describe)
Route not specified

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2. Tissues and Cells Examined:

__X__ bone marrow _____ other (list):

No. of cells per animal per treatment group examined: 50 metaphases

No. of cells per animal per control group examined: 50

3. Details of Cell Harvest and Slide Preparation (if appropriate, attach copy of procedures):

Femoral bone marrow was suspended in HBSS, centrifuged, resuspended in 0.075 M KCl at 37 degrees for 25 minutes, centrifuged, then prepared, fixed and stained on slides, on which they were then mounted with a coverslip.

Data for number of cells with aberrations were analyzed statistically in comparison to controls, using a Chi-square analysis.

4. Preliminary Cytotoxicity Assay (reported results, e.g., include dose range, signs of toxicity - e.g., MTD considerations, clinical signs, inhibition of mitosis, alteration in cell cycle kinetics, and/or LD_{50} values; no. animals; rationale for determining harvest times and dose levels):

No preliminary study was performed. Highest dose in the main study represented the LD_{10} level.

5. Aberrations Assay (reported results, e.g., induction of aberration frequency; types of aberrations, e.g., whether gaps are included in analysis or not, chromatid vs. chromosomal events, complex aberrations; positive and background aberration frequencies; levels of cytotoxicity obtained, e.g., effect on mitotic index, where applicable; sex differences (if any); appropriateness of dose levels and route; statistical evaluation; include representative table, if appropriate):

The results of this assay were summarized in Tables 3A (for males) and 3B (for females), which are photocopied from the report (see Appendix). RH-57,592 at dose levels of 0.25, 1.25, and 2.5 g/kg, by oral gavage in rats caused no increase in number of cells with aberrations or number of aberrations per cell in bone marrow from male or female rats collected 6, 24, and 48 hours after treatment. TEM positive control cause a substantial increase in number of

chromatin breaks and gaps per cell and number of cells with such aberrations, which supports the validity of this test.

6. Reviewer's Discussion/Conclusions (include, e.g., rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

The highest dose level, 2.5 g/kg, was claimed to be the LD_{10}, based on previously obtained data in rats. A decrease in body weight gains was observed at the high dose level. The 2.5 g/kg dose appears to adequately represent maximum telerated dose.

The frequency of aberrations in cells of the bone marrow collected 6, 24, and 48 hours postdosing for the three treated groups were similar to controls in both the males and females. Thus, there was no suggestion of a mutagenic response at any dose level. TEM positive control cause a large increase in chromosomal aberrations of bone marrow collected 24 hours after dosing, which indicates the potential responsiveness of this test system.

7. Was test performed under GLPs (is a quality assurance statement present)? Yes

8. CBI Appendix attached: Yes

Abbreviations for the Types of Chromosomal Aberrations Listed in Tables 3A and 3B

tg - Chromatid gap - an achromatic region of a width not greater than that of the single chromatid, occurring anywhere along the length of either of the two chromatids of a chromosome.

Isg - Isochromatid gap - same as above, but occurring in both the chromatids of the chromosome at the same locus.

tb - Chromatid break - an achromatic region of a width greater than that of a single chromatid occurring along the length of either of the chromatids of a chromosome, or a chromatid fragment lying adjacent to but not aligned along the axis of either of the two chromatids.

Isb - Isochromatid break - same as above but occurring in both chromatids of the same chromosome at the same locus

tf - Chromatid fragment - a piece of chromatid without a centromeric region, but not appearing in connection with any chromosome.

Isf - Isochromatid fragment - same as above but appearing in pairs.

d - Dicentric chromosome - chromosome with two centromeres.

r - Ring chromosome - chromatids or chromosomes with the two ends joined together to form a ring. with or without a centromere.

qr - Quadriradial - simple interchanges occurring between chromatids of two chromosomes and resulting in four-armed configurations.

tr - Triradial - simple interchanges occurring between one chromosome and chromatids of another chromosome, resulting in three-armed configurations.

Cr - Complex interchanges - multiarmed configurations resulting from breakage and reunion of two or more chromosomes.

pu - Pulverization - extreme fragmentation of chromosomes and individual chromosomes no longer recognizable.

sd - Severely damaged cell - cells with ten or more aberrations.

pp - Polyploid - chromosome number in multiples of haploid set.

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Page_____ is not included in this copy.

Pages 72 through 73 are not included.

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Reviewed By: Sidney Stolzenberg, Ph.D. S Stolzenberg 11/29/89
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)
Secondary Reviewer: John Hou-Shi Chen, D.V.M. John H Chen 12/11/89
Review Section I, Toxicology Branch II, HFAS/HED (H7509C)

DATA EVALUATION REPORT

Chemical: Fenethanil
RH-57,592

Caswell No.: 723Q
HED Project No.: 9-1381A
MRID No. 410312-20

Study Type: Unscheduled DNA Synthesis in Cultured Rat Primary Hepatocytes

Sponsor: Rohm & Haas Company
Philadelphia, PA 19105

Testing Facility: SITEK Research Labs
Rockville, MD 20852

Report No.: 0079-5100

Title of Report: Test for Chemical Induction of Unscheduled DNA Synthesis in Rat Primary Hepatocyte Culture by Autoradiography

Report Issued: April 13, 1988

Conclusion(s) - Executive Summary:

RH-57,592 was tested for unscheduled DNA synthesis in a test system using cultured rat primary hepatocytes. Concentrations tested were 7.5, 10.0, 12.5, and 15.0 ug/mL. Higher concentrations such as 30.0 and 60.0 ug/mL were toxic to the cells in culture.

There was no evidence of primary DNA damage in the cells due to RH-57,592, based on the fact that there was no increase in unscheduled DNA synthesis. Therefore, the compound does not appear to be mutagenic in this test system up to concentrations of 15 ug/mL.

This assay is considered valid because a culture of these cells incubated with 2-AAF (positive control) caused a substantial and statistically significant response. Also, a sufficient number of cells in the S-phase of replication was found, indicating no inhibition of replicative DNA synthesis.

Classification: Acceptable

A. Materials:

1. Test Substance: RH-57,592 (fenethanil) technical, 96.7% pure, Lot No. 3-1786R, white solid.

2. Control Materials:

   a. Negative Control: DMSO

   b. Solvent: DMSO

   c. Positive Control: 2-AAF dissolved in ethanol.

3. Concentrations of Test Substance in the Assay Procedure: 15, 12.5, 10.0, and 7.5 ug/mL. A freshly prepared solution was prepared for each test.

4. Cell Line: Primary rat hepatocytes prepared according to "modifications" of the procedure of G.M. Williams (Canc. Lett. 1:231, 1977, Chemical Mutagens, Vol. 6, F.J. DeSerres & A. Hollander, Eds. 1979, pp. 71-79, 1979).

5. Test Procedure: Cultures were seeded, after incubation for 2 hours they were washed, then treated with the proper concentration of test substance or control substance.

   For the range finding study, all doses were tested in duplicate at concentrations of 0.12, 0.23, 0.47, 0.94, 1.9, 3.8, 7.5, 15, 30, and 60 ug/plate. Cells seeded per plate was 237,600.

   For the UDS assay, concentrations tested were 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 ug/mL. All doses were tested in triplicate and number of cells per plate was 250,000. In addition, there were two solvent controls - DMSO and alcohol at 20 uL, as well as a nontreated control. The positive control consisted of 2-AAF at 2.0 on 10.0 ug/mL.

   The cultures were incubated with ^{3}H-thymidine (10uCi/ml) for approximately 18 hours.

   Relative toxicities were determined in the range finding study. There were also parallel cytoxicity tests at each concentration in the main UDS study. ^{3}H-thymidine uptake was determined by an autoradiographic technique, using a photographic emulsion to coat each slide and 8 days of exposure, then photographic fixing, followed by staining with H&E.

   Slides were scored "blind." Only cells that appeared "normal and healthy" and did not show a severe cytotoxic

effect such as constricted, irregular shape, dark staining nuclei or less than 4 mm^2 in size, were counted. Grain counts were by an electronic colony counter of 75 randomly selected nuclei if possible.

Incorporation of 3H-thymidine into nuclear DNA was determined by counting the darkened grains localized over the nuclear area. The background incorporation was determined by counting at least three nucleus-size areas of cytoplasm adjacent to each nucleus. The net nuclear grain counts were determined by subtracting the average background count from the nuclear count. For each treatment, the average net nuclear grain count ± standard deviation was calculated and recorded on a summary sheet. The number of nuclei showing five or more net nuclear grain counts in each cover glass was also recorded.

In addition, 300 nuclei per culture were counted at random to determine the percentage of nuclei exhibiting S-Phase DNA synthesis.

B. Evaluation of Test Results:

Results for the test article concentrations were considered significant if the average net nuclear grain count was increased by a least five grain counts over the concurrent solvent and/or untreated controls or more than 25 percent of the cells scored showed a net nuclear grain count of five or more.

Positive Response

The test article is considered to have caused a positive response in this assay if:

1. The test article causes a dose-related response and at least one concentration exhibits a significant increase over its concurrent solvent control.

2. In the absence of a dose response, two successive concentrations exhibit a significant increase over the concurrent solvent control data.

Marginal Positive Response

The test article is considered to have caused a marginal positive response if no indication of a positive dose response is observed, but one of the test concentrations shows a significant positive response.

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Negative Response

The test article is considered to have caused a negative response if no indication of a positive concentrations response is observed and none of the test concentrations show a significant positive response.

Other Considerations

The above criteria are used as guidelines in evaluating the test results. However, the Study Director may take other factors into consideration in evaluating the test results.

C. Results:

Results of the range-finding test is shown in Table 1, which was taken from the report by photocopying. The RCS at 60 and 30 ug/mL were zero percent at both concentrations. At concentration of 15 and 7.5 ug/mL, RCS were 58 and 87 percent, respectively. At all concentration between 0.12 and 3.8 ug/mL, the RCS were at least 100 percent, therefore, the relative toxicities were zero percent. RCS of DMSO and untreated control groups were both 100 percent or greater and the relative toxicities therefore zero percent for both.

Results of the parallel toxicity test in the main UDS study are shown in Table 2, also photocopied from the report. It is obvious that cell toxicity, based on RCS and Relative Toxicity, were seen at concentrations of 15, 12.5, and 10 ug/mL, but not at 7.5, 5.0, or 2.5 ug/mL. The positive control, 2-AAF which was tested at 2 and 10 ug/mL, had RCS values of around 60 percent at both concentrations.

Results of the main UDS study are shown in Table 3, also photocopied from the report. Only the highest four dose concentrations, which included 7.5 ug/mL and higher were scored. The concentration of 7.5 ug/mL, as indicated above was non-toxic whereas concentrations $\geq$ 10 ug/mL were toxic (see Table 2).

None of the four concentrations of RH-57,592 shown in Table showed a significant increase in average net nuclear grain count. The positive control, 2-AAF at 2.0 ug/mL, caused a very substantial and statistically significant increase nuclear grain count.

A sufficient number of nuclei in the S-phase were found, indicating replicative DNA synthesis and no inhibition of DNA synthesis.

It is claimed that with the positive control response and the fact that there was no inhibition of DNA synthesis, the necessary criteria for a valid assay were fulfilled.

D. Discussion:

Based on the results of this study using primary hepatocyte cultures from rats, there was no evidence of primary DNA damage, There was no evidence of an increase in unscheduled DNA synthesis caused by RH-57,592.

This compound does not appear to be mutagenic in this test system with concentrations up to 15 ug/mL.

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Primary Reviewer: Victor Miller Dip. Pharm. 11/3/87
Section I, Tox. Branch II (HFAS)
Secondary Reviewer: Y.M. Ioannou Ph.D. 12/17/87
Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Oral Toxicity Study - Male Rats (81-1)
MRID NO.:	410312-09
TEST MATERIAL:	RH-57,592
STUDY NUMBER:	REPORT NO. 87R-098 PROTOCOL NO.87P-095
HED PROJECT NO.	9-1381A
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Haas Company Toxicology Department 727 Norrison Road Spring House, PA 19477
TITLE OF REPORT:	RH-57,592 Acute Oral Toxicity in Rats
AUTHORS:	K.R. LAMPE', R.D. MORRISON, AND R.C. BALDWIN
REPORT ISSUED:	October 26, 1987
CONCLUSIONS:	The authors concluded that the acute oral LD50 of RH-57, 592 in rats is greater than 2.0g/kg. Toxicity Category III Classification of Data: Core-Guideline

METHODS:

A solid off-white test substance identified as RH-57, 592 Technical (Lot No. EG1442, Product Code No. 9-7702) containing 96.4% of the active ingredient alpha-(2-(4-chlorophenyl)-ethyl)-alpha-phenyl-1H-1,2,4-triazole-1-propanenitrile, was evaluated for acute oral toxicity in male rats, when administered as a single gavage dose. The substance was dispersed in 0.5% aqueous Methocel and administered at a constant volume of 10ml/kg body weight to male rats.

Male Crl: CD BR rats (Charles River Breeding Laboratories, Kingston Facility, Stone Ridge N.Y.) used in this study, were approximately 50 days old, and their body weights ranged from 180 to 204g in the range-finding study, 171 to 202g in the first phase of the definitive study, and from 150 to 168g in the second phase of the definitive study. They were housed 2 to 3 per cage, with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum. All surviving animals were euthanatized and necropsied at the end of the study. The necropsy consisted of a gross examination of the organs in situ.

The animals were subsequently observed at 1, 2 and 4 hours after dosing and once daily thereafter for 14 days, for any signs of toxicity including body weight changes, clinical manifestations and gross pathologic changes. Body weights were recorded on Day 0 (prior to dosing) and on Days 7 and 14.

Initially a range-finding study was conducted in which 4 groups of 3 rats each were treated with 0.0 (control), 0.5, 1.0, and 5.0 g/kg of the test substance. On Days 4 and 5, the 5g/kg animals began exhibiting clinical symptoms that were thought to be treatment-related and by Day 8 all three of the rats dosed at this level had died (no data presented).

On this basis 5g/kg was selected as the only dose for the 1st phase of the definitive study in which 10 male rats were used. A control group of ten male rats was treated with the vehicle only. During the second phase of the definitive study, carried out so as to better define the LD50, ten rats were gavaged with the test substance at 2g/kg body weight, in addition to a control group of five rats dosed with the vehicle only.

RESULTS:

Phase I: Definitive study

In the group of rats dosed at 5g/kg the following set of symptoms were observed: ataxia, salivation, prostration, mucus in stool, diarrhea, and tan-stained front paws. At the completion of the 14 day observation period, four out of the ten rats had died.

Phase II: Definitive study

In the group of rats dosed at 2g/kg, no mortality occurred. However red-stained eyes were observed in the rats treated at this dosage.

No mortality or signs of intoxication were observed in any of the control groups.

The researchers involved in this study listed a series of symptoms observed in both treated groups i.e. 2g/kg and 5g/kg, which were thought to be treatment-related: alopecia, emaciation, passiveness, scant droppings, brown, yellow and/or tan-stained anogenital area. They also noted a dose-related decrease in body-weight gain with the changes for the 2 and 5g/kg groups being statistically less than the changes for the respective control groups.

The necropsies of the decedent and surviving animals in the control and both treatment groups revealed no treatment-related gross changes. However pathological observations noted in the 5g/kg treated groups were manifestations seen while the animal was seen alive e.g. alopecia and stained anal-genital area, or other post-mortem changes thought to be unrelated to treatment e.g. single or multiple tan areas in the liver and a tan fluid-filled stomach.

CONCLUSIONS:

The authors concluded that the acute oral LD50 of RH-57, 592 in rats is greater than 2.0g/kg.

Toxicity Category III

Classification of Data: Core-Guideline

Primary Reviewer: Victor Miller Dip. Pharm. VM. 11/3/87
Section I, Tox. Branch II (HFAS)
Secondary Reviewer: Y.M. Ioannou Ph.D. YMI 12/12/87
Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Oral Toxicity Study - Male and Female Rats (81-1)
MRID NO.:	410312-07
TEST MATERIAL:	RH-57,592
STUDY NUMBER:	REPORT NO. 86R-C01 PROTOCOL NO.87P-304
HED PROJECT NO.	9-1381A
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Haas Company Toxicology Department 727 Norrison Road Spring House, PA 19477
TITLE OF REPORT:	RH-57,592 Acute Oral Toxicity in Male and Female Rats
AUTHORS:	R.J. KRAWESKI, R.D. MORRISON, AND R.C. BALDWIN
REPORT ISSUED:	May 10, 1988
CONCLUSIONS:	Acute Oral LD50 for male and female rats = 2000 to 5000 mg/kg

Classification of data: Core-Guideline

METHODS:

The acute oral toxicity of a solid white test substance, identified as RH-57,592 (Lot No. BPP-3-1-1786R, Sample No. TD87-186) containing 96.7% of the active ingredient alpha-(2-(4-chlorophenyl)-ethyl)-alpha-phenyl-1H-1,2,4-triazole-1-propanenitrile was evaluated in male and female rats. The chemical (suspended in a 0.5% aqueous solution of Methocel) being tested, was administered as a single gavage dose to two groups of ten male and female rats at dosages of 2.0 and 5.0 g/kg at a constant volume of 10ml/kg body weight. The control group of twenty rats (ten male and ten female) received 0.5% Methocel in the same manner.

Male and female Crl: CD BR rats (Charles River Breeding Laboratories, Kingston Facility, Stone Ridge N.Y.), approximately 50 days old, weighing 140 to 164 g (male body weights ranged from 150 to 164 g and female body weights ranged from 140 to 152g), were used in this study. They were housed 2 to 3 per cage, with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum. One day prior to being dosed, healthy rats were selected from a stock population, randomly assigned to groups, and fasted overnight. All surviving animals were euthanatized and necropsied at the end of the study. The necropsy consisted of a gross examination of the organs in situ.

The animals were subsequently observed at 1, 2 and 4 hours after dosing and once daily thereafter for 14 days, for any signs of toxicity including body weight changes, clinical manifestations and gross pathologic changes. Body weights were recorded on Day 0 (prior to dosing) and on Days 7 and 14.

RESULTS:

In either the control or the 2.0g/kg group, no deaths occurred but significant treatment-related reductions in body-weight gain among males and females occurred in the latter group. In the 5.0 g/kg group, nine of the ten males died by Day 6, and all the females died on Days 2 through 6. The authors noted the following treatment-related clinical symptoms in both treated groups: passiveness, emaciation, brown-stained anal genital area. diarrhea, red or tan stained muzzle, red-stained eyes and alopecia.

At the higher dose level of 5.0 g/kg the following additional treatment-related clinical signs were noted: moribundity, prostration, reddened extremities, cool to touch, respiratory noise, abdominal breathing, distended abdomen, emaciation, salivation, arched back, lacrimation, opacity in both eyes, tan-stained muzzle, yellow-stained anal-genital area, scant feces and no feces.

At necropsy treatment-related abnormalities amongst the decedents noted were a distended stomach filled with a white/tan fluid and yellow and/or brown stained anal-genital area. No gross pathological changes were noted amongst the survivors.

CONCLUSIONS: Acute Oral LD50 in rats = 2000 to 5000 mg/kg

Toxicity Category III

Classification of Data: Core-Guideline

Primary Reviewer: Victor Miller Dip. Pharm. 12/11/87
Section I, Tox. Branch II (HFAS)
Secondary Reviewer: Y.M. Ioannou Ph.D. 12/12/87
Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Dermal Toxicity Study - Male and Female Rats (81-2)
MRID NO.:	410312-08
TEST MATERIAL:	RH-57,592
STUDY NUMBER:	TD REPORT NO. 88R-002 PROTOCOL NO. 87P-305
HED PROJECT NO.	9-1381A
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Haas Company Toxicology Department 727 Norrison Road Spring House, PA 19477
TITLE OF REPORT:	RH-57, 592 Acute Dermal Toxicity in Male and Female Rats
AUTHORS:	R.J.KRAJEWSKI, R.D. MORRISON, AND R.C. BALDWIN
REPORT ISSUED:	May 10, 1988
CONCLUSIONS:	The acute dermal LD50 of RH-57, 592 was greater than 5000 mg/kg in male and female rats. Toxicity Category IV Classification of Data: Core-Guideline

METHODS:

The acute dermal toxicity of a solid white test substance identified as RH-57,592 (Lot No. BPP-3-1-1786R, Sample No. TD 87-186) containing 96.7% of the active ingredient alpha-(2-(4-chlorophenyl)-ethyl)-alpha-phenyl-1H-1,2,4-triazole-1-propanenitrile, was evaluated in male and female rats, when applied as a single occluded dermal dose to the clipped skin of male rats.

Male CRL: CD BR rats (Charles River Breeding Laboratories, Kingston Facility, Stone Ridge, N.Y.) approximately 50 days old, weighing 167 to 207 g (male body weights ranged from 177 to 207g and female body weights ranged from 167 to 183 g) were used in this study. They were housed 2 to 3 per cage, with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum. One day prior to being dosed, twelve male and twelve female rats were selected from a stock population. All surviving animals were euthanatized and necropsied at the end of the study. The necropsy consisted of a gross examination of the organs in situ.

The following is the method used as described in the report: "One day prior to the application of the test substance, the hair on the back of each rat was clipped closely with electric clippers. The test substance was moistened with 0.85% saline (1:1 w/v) amd applied dermally to the clipped intact skin of six male and six female rats at a dose of 5.0g/kg. The entire trunk of each animal was wrapped in a polyethylene sheet covered with plastic-lined Elastoplast and PEG elastic bandages and secured in place with adhesive tape. Rats in the control group (twelve rats) were treated in the same manner, except they received 0.85% saline at a volume of 5.0 ml/kg".

"After a 24-hour exposure period (during which time the rats remained in their cages), the cuffs were removed and the application sites wiped with water-soaked paper towels to remove any residual substance. The application sites were then blotted dry. Each animal was then fitted with a cardboard collar, (to minimize preening of the application site), which then remained on throughout the entire observation period".

All animals were observed for signs of mortality or morbidity at 1, 2 and 4 hours post-exposure and once daily for 14 days. Body weights were recorded on Day 0 (prior to dosing and on days 7 and 14). The degree of skin irritation was evaluated daily according to the procedure of Draize et al.

RESULTS:

No mortality or treatment-related morbidity was observed in the treated (5.0g/kg) or control group, throughout the course of the study. The authors of the study attributed the symptoms noted in both the control and treated groups i.e. red-stained muzzle and red-stained eyes to the use of the collars as part of the occluded testing methodology. One male died because the cuff was secured too tightly causing suffocation. There were no significant body-weight changes and no skin irritation was evident. Necropsy revealed no treatment-related gross changes.

CONCLUSIONS:

The acute dermal LD50 of RH-57, 592 was greater than 5000 mg/kg in male and female rats.

Toxicity Category IV

Classification of Data: Core-Guideline

Primary Reviewer: Victor Miller Dip. Pharm. V. M. 11/9/89
Section I, Tox. Branch II (HFAS)
Secondary Reviewer: Y.M. Ioannou Ph.D. Y.M.I. 12/12/89
Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE:	Primary Eye Irritation Study - Rabbits (81-4)
MRID NUMBER:	410312-11
TEST MATERIAL:	RH-57,592
STUDY NUMBER:	TD Report No. 87R - 101 Protocol No. 87P-099
HED PROJECT NUMBER:	9-1381A
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Hass Company Toxicology Department 727 Norristown Road Spring House, PA 19477
TITLE OF REPORT:	RH-57, 592 Eye Irritation Study in Rabbits
AUTHOR:	K.R. Lampe', R.D. Morrison R.C. Baldwin
REPORT ISSUED:	October 26, 1988
CONCLUSIONS:	Under the conditions of the study, RH-57, 592 was not irritating to the unwashed eyes of the rabbits.

Toxicity Category IV

Classification of Data: Core-Guideline

[redacted]

METHODS:

An off-white test solid substane, RH57, 592 (Lot No. EG-1142, Product Code No. 9-7702, Toxicology Department Sample Nc. TD 87-047) containing 96.4% of the active ingredient alpha - (2-(4-chlorophenyl) ethyl) -alpha - phenyl - 1H - 1,2,4-triazole-1-propanenitrile was evaluated for the degree of ocular irritation produced when applied to the corneal surface of rabbits.

Male New Zealand white rabbits, approximately twelve weeks old, (Hazelton Research Animals, Denver PA) weighing 2.5 to 3.1 kg, were used in the study. They were individually housed in stainless steel cages, with temperature, relative humidity and light cycly being regulated. Food and water were supplied ad libitum. One day prior to being dosed, nine healthy rabbits were selected from a healthy population.

RH-57, 592 was ground in a mortar and pestle and 0.1g of the test material was applied to the lower conjunctival sac of the left eye of nine male new Zealand White rabbits. The right eye of each rabbit was not treated and served as a control. The treated and control eyes of three of the nine rabbits were irrigated with distilled water for approximately sixty seconds beginning twenty to thirty seconds after dosing. The eyes of the remaining six animals were not rinsed after treatment.

If no gross lesions were evident on the cornea, the eyes were examined by using a 2% aqueous sodium fluorescein dye. When the dye was used in the treated eye of a rabbit, the control eye of that animal was also examined with the dye. The dye was used until no effects were observed with fluorescein.

The cornea, iris and conjunctiva of the treated and control eyes were examined at 24, 48, 72 hours and 7 days post-instillation. The degree of eye irritation was scored according to the procedure of Draize et al. After the 24 hour observation, the treated and control eyes in the unwashed group were irrigated with water for thirty and sixty seconds.

RESULTS:

No ocular effects were evident in the washed or unwashed treated eyes at any observation time.

CONCLUSIONS: Under the conditions of the study, RH-57, 592 was "inconsequentially irritating" to the unwashed eyes of the rabbits.

Toxicity Category IV

Conclusions: Core-Guideline

Reviewed by: Victor Miller Dip. Pharm. V.M. 11/8/89
Section I, Tox. Branch II (HFAS)
Secondary Reviewer: Y.M. Ioannou Ph.D. Y.M.I. 12/12/89
Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE:	Primary dermal irritation-rabbit (81-5)
MRID NO.:	410312-12
TEST MATERIAL:	RH-57, 592
STUDY NUMBER:	Report No. 87R-100 Protocol No. 87P-097
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Haas Company Toxicology Department 727 Norristown Road, Spring House, PA 19477
TITLE OF REPORT:	RH-57, 592: Rabbit Skin Irritation Study
AUTHORS:	K.R. LAMPE', R.D. MORRISON AND R.C. BALDWIN
REPORT ISSUED:	October 26, 1988
CONCLUSIONS:	It appears that the substance RH-57, 592 is non-irritating to the skin of male New Zealand White rabbits. Toxicity Category IV Classification of Data: Core-Guideline

[redacted]

METHODS:

A solid off-white test substance identified as RH-57, 592 Technical (Lot No. EG-1442, Product Code No. 9-7702) containing 96.4% of the active ingredient alpha-(2-(4-chlorophenyl)-ethyl)-alpha-phenyl-1H-1,2,4-triazole-1-propanenitrile, was evaluated for skin irritation potential in six male New Zealand White rabbits.

Male New Zealand White rabbits (Hazelton Research Animals, Denver, PA), approximately 12 weeks old, weighing 3.1 to 3.2 kg were used in the study. They were individually housed, with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum.

One day prior to being dosed six healthy rabbits were selected from a stock population. Twenty-four hours prior to the application of the test substance, the hair on the back of each rabbit was clipped closely with electric clippers. The test substance was ground into a paste with 1.0 ml of 0.85 saline (1:2 W/V). The paste was applied to the shaved intact skin of six male rabbits, the application sites were occluded for four hours after which the cuffs were removed and the sites were wiped dry with water-soaked paper towels to remove any residual test substance.

The application sites were graded for skin irritation (no erythema or edema), according to the Draize procedure at 1, 24, 48, 72 hours and at 7 days after patch removal. If observed, all other skin reactions were recorded.

RESULTS:

No treatment-related morbidity (i.e. erythema or edema) or mortality was observed throughout the course of the study.

CONCLUSIONS:

It appears that the substance RH-57, 592 2F is non-irrtating to the skin of male New Zealand White rabbits.

Toxicity Category IV

Classification of [illegible]

Primary Reviewer: Victor Miller Dip. Pharm. V M. 11/8/89
Section I, Tox. Branch II (HFAS)
Secondary Reviewer: Y.M. Ioannou Ph.D. Y.M.I. 12/12/89
Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Dermal Sensitization Study (81-6)
MRID NUMBER:	410312-13
TEST MATERIAL:	RH-57,592 Technical
STUDY NUMBER:	TD Report No. 88R-027 Protocol No. 88P-003
HED PROJECT NUMBER:	9-1381A
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Hass Company Toxicology Company 727 Norristown Road Spring House, PA 19477
TITLE OF REPORT:	RH-57, 592 Technical: Delayed Contact Hypersensitivity in Guinea Pigs
AUTHOR:	R. Bonin, W.D. Shade and G.A. Hazelton
REPORT ISSUED:	July 26, 1988
CONCLUSIONS:	It appears that RH-57, 592 did not cause delayed hypersensitivity in guinea pigs.
	Classification of data: Core-Minimum

METHODS:

A test substance RH-57, 592 Technical, a white solid containing 96.7% alpha -(2-(4-chlorophenyl) ethyl) - alpha - phenyl -1H -1,2,4 -triazole -1- propanenitrile (Toxicology Department Sample No. (TD No.) 87-186, Lot No. BPP-3-1786R) was evaluated for its potential to produce delayed contact hypersensitivity., using the Buehler closed patch procedure.

Forty guinea pigs, approximately 5 weeks old, (selected from 150 outbred Hartley guinea pigs [Crl: (HA) BR] obtained from Hazelton Research Animals Denver PA.), were used in the study. They were housed singly in suspended stainless steel cages with temperature, relative humidity and light cycle being regulated. One day prior to the first induction dose, the guinea pigs were randomized into groups using computer generated random numbers. At the initiation of the induction phase, the body weights of the animals ranged from 335 to 453g.

Range-finding study

Initially a range-finding skin irritation test was conducted with eight guinea pigs (selected from excess animals from a group of guinea pigs previously received from Hazelton Research Animals) to determine a slightly irritating concentration (SIC) and the highest non-irritating concentration (HNIC). Four concentrations of RH-57,592 in acetone (25%, 20%, 10% and 5%) were applied to the shaved intact skin of each animal's back in Hill Top Chambers at four different sites shown in a diagram in Table I (p. 17). The four remaining guinea pigs received two concentrations of RH-57, 592 (i.e. 2% and 1%) dissolved in 80% aqueous ethanol. The application site was occluded with rubber dental dam.

The application sites were changed among the guinea pigs for each of the above concentrations to minimize any site-to-site variation in irritation responses. After a six-hour exposure period all wrappings and patches were removed, the test sites gently washed with warm water to remove excess test material. All application sites were depilated prior to scoring. Irritation responses were scored 24 and 48 hours post-treatment.

Induction Phase

Test article

Three six-hour induction doses, each consisting of 0.4 ml of the RH-57, 592 at 25% w/v in acetone, were applied once weekly to the shaved backs of twenty guinea pigs (ten male and ten female) over a three-week period, using the same procedure as described above.

Naive controls

Ten guinea pigs (five male and five female) received no induction treatments and a blank patch was applied.

Positive controls

A group of 10 guinea pigs (five male and five female) were treated with 1 - chloro - 2,4 dinitrobenzene (DNCB) at 1600 ppm in 80% aqueous ethanol in the same manner as described above.

Challenge Phase

Test article

The twenty guinea pigs receiving the RH-57, 592 as the induction treatment were challenged two weeks later with 0.4 ml of a 20% w/v acetone solution of RH-57, 592 .

Naive controls

Two weeks after the induction doses had been administered, ten guinea pigs (five male and five female) were challenged with 0.4 ml of 1 - chloro -2,4 dinitrobenzene (DNCB) at 800 ppm in acetone and 0.4 ml of the undiluted RH-57, 592 2F on two separate dose sites.

Positive controls

The positive control group received 0.4 ml of DNCB at 800 ppm in acetone in the same manner as described above.

Nineteen to twenty-two hours after the challenge application, the backs of the guinea pigs were depilated with [illegible] on the backs for approximately 20 minutes. The animals were rinsed with lukewarm running tap water, blotted dry and returned to their cages. Two to five hours after depilation (24 hours after removal of the challenge patch) the erythema reactions at the application sites were scored.

RESULTS:

Range-Finding Study

Slight erythema was observed with 25% w/v RH-57, 592 in acetone and 2% w/v RH-57, 592 in aqueous ethanol. Minimal to no erythema was observed at all other concentrations tested. Based on this outcome, the researchers chose 25% RH-57, 592 as the induction concentration and 20% w/v RH-57, 592 was chosen as the challenge concentration.

Induction Phase

Test article

No erythema responses (0/20) were observed in guinea pigs induced with RH-57. [The researchers applied the criteria that where grades of + were observed (a score that denoted slight patchy erythema), they were considered to be representative of insignificant erythema responses, thus receiving a zero dedsignation.]

Naive controls

These controls received no treatments during the induction phase.

Positive controls

No results were shown for the induction phase (no data table was found in the study in the appendices). Only in the "Results" section, was it stated that " a 100% incidence (10/10) of erythema was observed in the positive control (i.e. guinea pigs induced and challenged with DNCB) group, verifying that the animals on test were responsive to a known sensitizer").

Challenge Phase

Test article

No erythema responses (0/20) were observed in guinea pigs challenged with RH-57, 592.

Naive controls

Incidences of erythema of 1/10 and 0/10 were

observed in the non-induced naive control group following challenge with RH-57, 592 at 20% w/v in acetone and DNCB at 800 ppm in acetone respectively.

Positive controls

A 100% incidence of erythema was observed (10/10) was observed when challenged with DNCB.

CONCLUSIONS:

It appears that RH-57, 592 did not cause delayed hypersensitivity in guinea pigs.

Classification of data: Core-Minimum

It was stated in the protocol on p.25 of the study that " all guinea pigs will be weighed just prior to the 1st "induction" dose and prior to challenge". No body weights prior to the challenge phase were noted in the study.

007677

Primary Reviewer: Victor Miller Dip. Pharm. V. M. 1/8/89
Section I, Tox. Branch II (HFAS)
Secondary Reviewer: Y.M. Ioannou Ph.D. YMI 12/12/89
Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Oral Toxicity Study -Male Rats (81-1)
MRID NO.:	410312-21
TEST MATERIAL:	RH-57,592 2F
STUDY NUMBER:	TD REPORT NO. 87R-170 PROTOCOL NO.87P-137
HED PROJECT NO.	9-1381A
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Haas Company Toxicology Department 727 Norrison Road Spring House, PA 19477
TITLE OF REPORT:	RH-57, 592 2F Acute Oral Toxicity in Male Rats
AUTHORS:	K.R. LAMPE', R.D. MORRISON, AND R.C. BALDWIN
REPORT ISSUED:	January 27, 1988
CONCLUSIONS:	Acute Oral LD50 for male rats = >5000mg/kg Toxicity Category IV Classification of data: Core-Guideline

METHODS:

A liquid test formulation (Experimental Formulation No. XF86077, Lot No. EG-1452, Sample No. TD 87-52) containing 25% of RH-57, 592 2F (Tech 3-168812, 97.0% purity) was evaluated for oral toxicity in male rats.

Thirty male Crl: CD BR rats (Charles River Breeding Laboratories, Kingston Facility, Stone Ridge N.Y.), approximately 50 days old, weighing 232 to 268 g, were used in this study. They were housed 2 to 3 per cage, with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum. One day prior to being dosed, healthy rats were selected from a stock population, randomly assigned to groups, and fasted overnight. All surviving animals were euthanatized and necropsied at the end of the study. The necropsy consisted of a gross examination of the organs in situ.

The test substance referred to above, was dispersed in 0.5% Methocel and administered as a single gavage dose to two groups of ten male rats at 2000 and 5000 g/kg and one control group of ten male rats. All doses were administered at a constant volume of 10 ml/kg.

All animals were observed for signs of mortality or morbidity i.e. clinical manifestations 1, 2 and 4 hours after dosing and once daily thereafter for 14 days. Body weights were recorded on the day prior to dosing and on Days 7 and 14.

RESULTS:

No signs of toxicity were observed in the control group or the 2000 mg/kg group. In one out of five animals (1/5) of the 5000 mg/kg group, clinical signs were recorded i.e. a tan-stained muzzle and a yellow-stained anal genital area. Mo mortality was recorded and necropsy revealed no further abnormalities in any of the three groups.

CONCLUSIONS:

It appears that administration of the test substance, RH-57, 592 2F did not cause any oral toxicity in any of the male rats tested.

Acute Oral LD50 for male rats = >5000 mg/kg

Toxicity Category IV

Classification of data: Core-Guideline

007677

Primary Reviewer: Victor Miller Dip. Pharm. V. M. 12/19/89
Section I, Tox. Branch II (HFAS)
Secondary Reviewer: Y.M. Ioannou Ph.D. [illegible] 12/19/89
Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Oral Toxicity Study - Female Rats (81-1)
MRID NO.:	410312-22
TEST MATERIAL:	RH-57,592 2F
STUDY NUMBER:	TD REPORT NO. 88R-103 PROTOCOL NO.88P-159
HED PROJECT NO.	9-1381A
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Haas Company Toxicology Department 727 Norrison Road Spring House, PA 19477
TITLE OF REPORT:	RH-57, 592 2F Acute Oral Toxicity in Female Rats
AUTHORS:	K.R. LAMPE', R.D. MORRISON, AND R.C. BALDWIN
REPORT ISSUED:	July 11, 1988
CONCLUSIONS:	The acute oral LD50 of RH-57, 592 2F in female rats was found to be greater than 5.0 g/kg. Classification of data: Core-guideline Toxicity Category: IV

METHODS:

A off-white liquid test formulation (Experimental Formulation No. XF86077, Lot No. EG-1452) containing approximately 25% of RH-57, 592 2F (Tech 3168812, 97% purity) was evaluated for acute oral toxicity when admininstered as a single gavage dose of 5.0 g/kg to rats.

Six female Crl: CD BR rats (Charles River Breeding Laboratories, Kingston Facility, Stone Ridge N.Y.), approximately 50 days old, weighing 181 to 212 g, were used in this study. They were housed 2 to 3 per cage, with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum. One day prior to being dosed, healthy rats were selected from a stock population and fasted overnight. All surviving animals were euthanatized and necropsied at the end of the study. The necropsy consisted of a gross examination of the organs in situ. Necropsies were performed on decedents.

The dispersion was prepared by weighing the appropriate amount of test substance and dispersing it in a 0.5% aqueous solution of Methocel. The dose was administered by gavage at a constant volume of 10 ml/kg.

All animals were observed for signs of mortality or morbidity i.e. clinical manifestations 1, 2 and 4 hours after dosing and once daily thereafter for 14 days. Body weights were recorded on the day prior to dosing and on days 7 and 14.

RESULTS:

Two of the six rats died with the following treatment-related clinical signs being observed: passiveness, moribundity, prostration, scant feces, diarrhea, labored breathing, no visible feed consumption, red-stained muzzle, red-stained eyes and yellow-stained anal-genital area. The authors of this study noted alopecia on the rear quarters of one animal but did not consider this symptom to be treatment-related. Necropsies of the decedents revealed autolysis, with the surviving females showing no gross pathological changes.

CONCLUSIONS:

The acute oral LD50 of RH-57, 592 2F in female rats was found to be greater than 5.0 g/kg.

Classification of data: Core-guideline

Toxicity Category: IV

007677

Primary Reviewer: Victor Miller Dip. Pharm. V.M. 11/8/89
Section I, Tox. Branch II (HFAS)
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DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity Study - Female Rats (81-2)

MRID NO.: 410312-24

TEST MATERIAL: RH-57,592 2F

STUDY NUMBER: TD REPORT NO. 88R-104
PROTOCOL NO.88P-160

HED PROJECT NO. 9-1381A

SPONSOR: Rohm and Haas Company

TESTING FACILITY: Rohm and Haas Company Toxicology Department
727 Norrison Road
Spring House, PA 19477

TITLE OF REPORT: RH-57, 592 2F Acute Dermal Toxicity in Female Rats

AUTHORS: K.R. LAMPE', R.D. MORRISON, AND R.C. BALDWIN

REPORT ISSUED: July 11, 1988

CONCLUSION: It appears that the acute dermal LD50 for female rats for the substance RH-57, 592 2F is greater than 5.0 g/kg.

Toxicity Category IV

Classification of Data: Core-Guideline

METHODS:

A liquid test formulation (Experimental Formulation No. XF86077, Lot No. EG-1452, Sample No. TD88-068) containing approximately 25% of RH-57, 592 2F (Tech 3-168812, 97%) was evaluated for dermal toxicity in female rats.

Female CRL: CD BR rats approximately 50 days old, weighing 206 to 220 g were used in this study. They were housed 2 to 3 per cage, with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum. One day prior to being dosed, healthy rats were selected from a stock population, randomly assigned to groups, and fasted overnight. All surviving animals were euthanatized and necropsied at the end of the study. The necropsy consisted of a gross examination of the organs in situ.

One day prior to being dosed, six female rats were selected from a healthy stock population.

Twenty-four hours prior to the test, the hair on the back of each rat was clipped closely with electric clippers. The undilluted tests substance was applied to the clipped intact skin of the six female rats at 5g/kg.

All animals were observed for signs of mortality or morbidity at 1, 2, and 4 hours post-exposure and once daily thereafter for 14 days. Skin application sites were graded for various skin reactions e.g. erythema, edema, eschar, pocketing and blanching). Body weights were recorded on Day 0 (prior to dosing and on days 7 and 14).

The entire trunk of each animal was wrapped in a polyethylene sheet covered with plastic-lined Elastoplast and PEG elastic bandages and secured in place with adhesive tape.

After a 24-hour exposure period (during which time the rats remained in their cages), the cuffs were removed and the application sites wiped with water-soaked paper towels to remove any residual substance. Each animal was then fitted with a cardboard collar, (to minimize preening of the application site), which then remained on throughout the entire observation period.

RESULTS:

No mortality or treatment-related morbidity was observed throughout the course of the study. The authors of the study attributed the symptoms noted i.e. red-stained muzzle and red-stained eyes to the use of the collars as part of the occluded testing methodology. There were no significant body-weight changes and no skin irritation was evident. Necropsy revealed no treatment-related gross changes.

CONCLUSIONS:

It appears that the acute dermal LD50 for RH-57, 592 2F is greater than 5.0 g/kg.

Toxicity Category IV

Classification of Data: Core-Guideline

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Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE:	Acute Dermal Toxicity Study - Male Rats (81-2)
MRID NO.:	410312-23
TEST MATERIAL:	RH-57,592 2F
STUDY NUMBER:	TD REPORT NO. 87R-109 PROTOCOL NO.87P-144
HED PROJECT NO.	9-1381A
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Haas Company Toxicology Department 727 Norrison Road Spring House, PA 19477
TITLE OF REPORT:	RH-57, 592 2F Acute Dermal Toxicity in Rats
AUTHORS:	K.R. LAMPE', R.D. MORRISON, AND R.C. BALDWIN
REPORT ISSUED:	January 27, 1988
CONCLUSIONS:	It appears that the acute dermal LD50 for male rats is greater than 5 g/kg. Toxicity Category IV Classification of Data: Core-Guideline

METHODS:

A liquid test formulation (Experimental Formulation No. XF86077, Lot No. EG-1452, Sample No. TD 87-052) containing approximately 25% of RH-57, 592 (Tech 3-168812, 97.0% purity) was evaluated for acute toxicity when applied as a single occluded dermal dose to the clipped skin of male rats.

Male CRL: CD BR rats (Charles River Breeding Laboratories, Kingston Facility, Stone Ridge, N.Y.) approximately 50 days old, weighing 205 to 250 g were used in this study. They were housed 2 to 3 per cage, with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum. One day prior to being dosed, twelve healthy male rats were selected from a stock population. All surviving animals were euthanatized and necropsied at the end of the study. The necropsy consisted of a gross examination of the organs in situ.

One day prior to the application of the test substance, the hair on the back of each rat was clipped closely with electric clippers. The test substance was administered to the clipped intact skin of six male rats at a dose of 5.0 g/kg. The entire trunk of each animal was wrapped in a polyethylene sheet covered with plastic-lined Elastoplast and PEG elastic bandages and secured in place with adhesive tape. Rats in the control group (six rats) were treated in the same manner, except they received no test substance.

After a 24-hour exposure period (during which time the rats remained in their cages), the cuffs were removed and the application sites wiped with water-soaked paper towels to remove any residual substance. The application sites were then blotted dry. Each animal was then fitted with a cardboard collar, (to minimize preening of the application site), which then remained on throughout the entire observation period.

All animals were observed for signs of mortality or morbidity at 1, 2, and 4 hours post-exposure and once daily thereafter for 14 days. Body weights were recorded on Day 0 (prior to dosing and on days 7 and 14). The degree of skin irritation was evaluated daily according to the procedure of Draize et al.

RESULTS:

No mortality or treatment-related morbidity was observed in the treated or control group, throughout the course of the study. The authors of the study attributed the symptoms noted i.e. red-stained muzzle and red-stained eyes to the use of the collars as part of the occluded testing methodology. There were no significant body-weight changes and no skin irritation was evident. Necropsy revealed no treatment-related gross changes.

CONCLUSIONS:

It appears that the acute dermal LD50 in male rats is greater than 5 g/kg.

Toxicity Category IV

Classification of Data: Core-Guideline

37677

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DATA EVALUATION REPORT

STUDY TYPE:	Acute aerosol inhalation-rats (81-3)
MRID NO:	410312-25
TEST MATERIAL:	RH-57, 592 2F
STUDY NUMBER(S):	TD Report No. 88R-017 Protocol No. 88P-031
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Haas Company Toxicology Department 727 Norristown Road Spring House PA 19477
TITLE OF REPORT:	RH-57, 592 2F: Acute Inhalation Toxicity Study in Rats
AUTHORS:	H.F. Emmons and J.V. Hagan
REPORT ISSUED:	September 12, 1988

CONCLUSIONS:

It appears that male and female rats exposed to 1.2 mg/l and 2.1 mg/l of RH 57, 592 2F exhibited treatment-related signs of nasal irritation and dyspnea. No deaths occurred and necropsy did not reveal any treatment-related alterations.

ACUTE LC50 FOR RATS = > 2.1 mg/liter

Toxicity Category: III

Classification of Data: Core-Supplementary

METHODS:

A test substance, identified as RH-57, 592 2F (XF-86077, TD Number 88-002; Lot No. EG-1584) was an off-white liquid formulation consisting of 23.2% active ingredient, alpha-(2-(4-chlorophenyl)-alpha-phenyl-1H-1, 2, 4-triazole-1-propanenitrile. The study was conducted so as to determine the effects in rats of a four-hour nose-only inhalation exposure to liquid aerosols of the above-mentioned formulation.

Two groups each consisting of ten male and female Crl: CD BR rats (Charles River-Kingston, Kingston NY) were randomly selected from two populations. Body weight ranges for both groups were 189 to 219 g and 204 to 231 g for the males and females respectively. They were individually housed with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum.

All rats being studied were observed for signs of toxicity and significant weight changes during the exposure period and for 14 days thereafter. All survivors were necropsied at the end of the observation period.

Two different aerosol generation systems were used to generate the test substance. One generator system used the RH-57, 592 2F solution as received (Solution I) and the other generator system was modified using a 1:1 RH-57, 592 2F to water solution (Solution II) in an effort to produce smaller aerosol particles and to minimize spray nozzle clogging.

RESULTS

One group of rats were exposed to a a mean aerosol concentration of [illegible] mg/l based on Solution I, with the resultant aerosol particle size sample being generated having a mass median aerodynamic diameter of 15.2 um and a mean standard geometric deviation of 3.0. Treatment-related symptoms, primarily related to nasal mucosa irritation, were rhinorrhea and nasal exudate.

Pink-spotted dropsheets, which the researchers thought were caused by expired nasal exudate, were observed sporadically throughout the 14-day observation period. Other signs observed: wet-red exudate around the eyes and muzzle, wet abdominal fur, brown stained fur and alopecia (one female developed this symptom at the left rump on Day 5, which persisted throughout the remainder of the observation period), were attributed to the nose-only restraining method.

Mean body weight decreases were observed on post-exposure Day I only, for both males and females. By Day 3 both sexes had exceeded their mean pre-exposure body weight and continued to gain

weight normally throughout the remainder of the 14-day observation period.

The group exposed to a mean aerosol concentration of 2.1 + 0.4 mg/l based on Solution II, with the resutant aerosol particle size sample being generated having a mass median aerodynamic diameter of 14.3 um and a mean standard geometric deviation of 3.0. Treatment-related clinical symptoms were also related to nasal mucosa irritation i.e. rhinorrhea, nasal exudate and white test material on the nose and muzzle area. Dyspneic symptoms were also observed.

Other conditions observed i.e. wet-red exudate around the eyes and muzzle, wet abdominal fur, the tucked posture of the rats in their cages and a lacerated right front digit on one animal, were judged by the researchers to be caused by the conditions of the nose-only restraining method.

Mean body-weight decreases were observed on post-exposure Day I only, for both males and females.

At both concentrations no deaths occurred. Necropsy revealed red foci on the lungs (in 2 out of 10 males) and alopecia on the rump (1 out of 10 females) for the 1.2 mg/l exposure group. Gross necropsy at the 2.1 mg/l exposure level revealed red foci on the lungs (3 out of 10 males and 1 out of 10 females), red spotted thymus (1 out of 10 males) and slightly darkened lungs (2 out of 10 females). All symptoms found at necropsy were adjudged by the study authors to be statistically insignificant when compared to historical controls.

CONCLUSIONS:

It appears that male and female rats exposed to 1.2 mg/l and 2.1 mg/l of Rh 57, 592 2F exhibited treatment-related signs of nasal irritation and dyspnea. No deaths occurred and necropsy did not reveal any treatment-related alterations.

ACUTE LC50 FOR RATS = > 2.1 mg/liter

Toxicity Category: III

Classification of Data: Core-Supplementary

The particle size generated was too large to be respirable.

007677

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Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE:	Primary Eye Irritation Study -Rabbits (81-4)
MRID NO.:	410312-26
TEST MATERIAL:	RH-57,592 2F
STUDY NUMBER:	TD REPORT NO. 87R-167 PROTOCOL NO. 87P-141
HED PROJECT NO.	9-1381A
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Haas Company Toxicology Department 727 Norrison Road Spring House, PA 19477
TITLE OF REPORT:	RH-57,592 2F Eye Irritation Study in Rabbits
AUTHORS:	K.R.LAMPE', R.D. MORRISON, AND R.C. BALDWIN
REPORT ISSUED:	January 27, 1988
CONCLUSIONS:	It appears that the substance RH-57, 592 2F in a formulation, after a single application, is non-irritating to the eyes of rabbits. Toxicity Category: IV Classification: Core-Guideline

METHODS:

An off-white liquid test formulation (Experimental Formulation No. XF86077), containing approximately 25% of RH-57, 592 2F (Tech 3168812, 97% purity, Lot No. EG1452), was evaluated for the degree of ocular irritation produced when applied to the corneal surface of rabbits.

Male New Zealand White rabbits (Hazelton Research Animals, Denver PA) approximately 12 weeks old, weighing 31.6 to 3868 g were used in the study. They were individually housed in stainless steel cages, with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum. One day prior to being dosed, nine rabbits were selected from a stock population, their eyes being grossly examined with an aqueous 2% sodium fluorescein solution. If any abnormalities were noted, they were excluded from the study.

0.1 ml aliquots of the undiluted solution containing RH-57, 592 2F was applied to the corneal surface of the left eye of six rabbits, with the right eye not being treated and used as a control.

The treated and control eyes of three of the nine rabbits were irrigated with distilled water for approximately 60 seconds beginning 20 to 30 seconds after dosing. The eyes of the remaining six rabbits were not rinsed after treatment. After the 24-hour observation, the treated and control eyes in the unwashed group were irrigated with water for 30 to 60 seconds.

If no gross lesions were evident on the cornea, the eyes were examined by using a 2% aqueous sodium fluorescein solution. When the dye was used in the treated eye of a rabbit, the control eye of that animal was also examined with the dye. The degree of ocular irritation was evaluated 24, 48 72 and 7 days post-instillation according to the procedure of Draize et al.

RESULTS:

No adverse corneal, iridal and conjunctival effects were observed in the treated and control eyes of the nine rabbits, except for a 2x2 mm striated hazy area appearing on the cornea of a rabbit treated with the test substance, after the application of 2.0% sodium fluorescein. The authors of the study thought that this symptom was incidental and not treatment-related.

CONCLUSIONS:

It appears that the substance RH-57, 592 2F in a formulation, after a single application, is non-irritating to the eyes of rabbits.

Toxicity Category: IV

Classification: Core-Guideline

007677

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DATA EVALUATION REPORT

STUDY TYPE:	Primary dermal irritation-rabbit (81-5)
MRID NO.:	410312-27
TEST MATERIAL:	RH-57, 592 2F
STUDY NUMBER:	Report No. 87R-168 Protocol No. 87P-139
SPONSOR:	Rohm and Haas Company
TESTING FACILITY:	Rohm and Haas Company Toxicology Department 727 Norristown Road, Spring House, PA 19477
TITLE OF REPORT:	RH-57, 592 2F: Skin Irritation Study in Rabbits
AUTHORS:	K.R. LAMPE', R.D. MORRISON AND R.C. BALDWIN
REPORT ISSUED:	January 27, 1988
CONCLUSIONS:	It appears that the substance tested, 592 2F is non-irritating to the skin of male New Zealand White rabbits. Toxicity Category IV Classification of Data: Core-Guideline

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METHODS:

A liquid test formulation (Experimental Formulation No. XF86077) containing approximately 25% of RH-57, 592 2F (Tech 3-168812, 97% purity, Lot No. EG-1452, Sample No. TD87-52) was assesed by applying 0.5ml of the undiluted test substance to the clipped intact skin of six male New Zealand White rabbits.

Male New Zealand White rabbits (Hazelton Research Animals, Denver, PA), approximately 12 weeks old, weighing 2.5 to 3.1 kg were used in the study. They were individually housed, with temperature, relative humidity and light cycle being regulated. Food and water were supplied ad libitum.

One day prior to being dosed six healthy rabbits were selected from a stock population. Twenty-four hours prior to the application of the test substance, the hair on the back of each rabbit was clipped closely with electric clippers. A patch consisting of a 1-inch square guaze-lined adhesive bandage, to which 0.5 ml of the undiluted test substance had been applied, was placed on the shaven intact skin of the rabbit. The application sites were occluded for 4 hours, after which the cuffs were removed and the sites were wiped with water-soaked paper towels to remove any residual test substance.

The application sites were graded for skin irritation according to the Draize procedure at 1, 24, 48 , 72 hours and at 7 days after patch removal. If observed all other skin reactions were recorded.

RESULTS:

Based on the reported results no erythema and/or edema were observed in any of the treated animals. No treatment-related morbidity or mortality was observed throughout the course of the study.

CONCLUSIONS:

It appears that the substance RH-57, 592 2F is non-irritating to the skin of male New Zealand White rabbits.

Toxicity Category IV

Classification of Data: Core-Guideline

007677

Primary Reviewer: Victor Miller, Dip. Pharm. V.M. 11/3/89
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Section I, Tox. Branch II (HFAS)

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Sensitisation Study (81-6)

MRID NUMBER: 410312-28

TEST MATERIAL: RH-57, 592F

STUDY NUMBER: TD Report No. 88R - 061
Protocol No. 88P-58

HED PROJECT NUMBER: 9-1381A

SPONSOR: Rohm and Haas Company

TESTING FACILITY; Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, PA 19477

TITLE OF REPORT: RH-57, 592 2F: Delayed Contact Hypersensitivity in Guinea Pigs

AUTHOR: R. Bonin and G.A. Hazelton

REPORT ISSUED: July 22,1988

CONCLUSIONS: It appears that RH-57, 592 2F did not cause delayed hypersensitivity in guinea pigs.

Classification of Data: Core-Guideline

METHODS:

A liquid test formulation containing RH-57, 592 2F [Toxicology Department No. 88-002, Lot No. EG-1584 containing 24.2% alpha -(2-(4-chlorophenyl) ethyl) - alpha - phenyl -1H -1,2,4 - triazole -1- propanenitrile as the active ingredient, which was 100% of the nominal concentration] was evaluated for dermal sensitization potential using the Buehler closed patch procedure.

Forty guinea pigs, aged seven weeks, (selected from 150 outbred Hartley guinea pigs [Crl: (HA) BR] using computer generated random numbers, obtained from Hazelton Research Animals Denver PA). were used in the study. They were housed singly in suspended stainless steel cages with temperature, relative humidity and light cycle being regulated. The guinea pigs were observed for general health during a quarantine period of one week. At the initiation of the induction phase, the body weights of the animals ranged from 392 to 517g.

Range-finding study

Initially a range-finding skin irritation test was conducted with eight guinea pigs (selected from excess animals from a group of guinea pigs previously received from Hazelton Research Animals), to determine a slightly irritating concentration (SIC) and the highest non-irritating concentration (HNIC). Aliquots of 0.4 ml of the undiluted test material and five concentrations of RH-57, 592 2F in distilled water (75%, 50%, 25%, 12.5%, 6.25%) were applied to the shaved intact skin of each animal's back in Hill Top Chambers. Six skin sites for dosing were noted on a diagram. The application site was occluded with rubber dental dam. The application sites were changed among the guinea pigs for each of the above concentrations to minimize any site-to -site variation in irritation responses. After a six-hour exposure period all wrappings and patches were removed, the test sites gently washed with warm water to remove excess test material. All application sites were depilated prior to scoring. Irritation responses were scored 24 and 48 hours post-treatment.

Induction Phase

Test article

Three six-hour induction doses, each consisting of 0.4 ml of the undiluted RH-57, 592 2F were applied once weekly to the shaved backs of twenty guinea pigs (ten male and ten female) over a three-week period, using the same procedure as described above.

Naive controls

Ten guinea pigs (five male and five female) received no induction treatments. They did however receive a blank patch.

Positive controls

A group of 10 guinea pigs (five male and five female) were treated with 1 - chloro - 2,4 dinitrobenzene (DNCB) at 1600 ppm in 80% aqueous ethanol in the same manner as described above.

Challenge Phase

Test article

The twenty guinea pigs receiving the RH-57, 592 2F as the induction treatment were challenged with 0.4 ml of the undiluted test material in the same manner as described above.

Naive controls

Two weeks after the induction doses had been administered, ten guinea pigs (five male and five female) were challenged with 0.4 ml of 1 - chloro -2,4 dinitrobenzene (DNCB) at 800 ppm in acetone and 0.4 ml of the undiluted RH-57, 592 2F on two separate dose sites.

Positive controls

The positive control group received 0.4 ml of DNCB at 800 ppm in acetone in the same manner as described above.

Nineteen to twenty-two hours after the challenge application, the backs of the guinea pigs were depilated with Neet lotion hair remover. The depilatory was allowed to remain on the backs for approximately 20 minutes. The animals were rinsed with lukewarm running tap water, blotted dry and returned to their cages. Two to five hours after depilation (24 hours after removal of the challenge patch) the erythema reactions at the application sites were scored.

RESULTS:

Range-Finding Study

No erythema was observed at the concentrations tested i.e. 100%, 75%, 50%, 25%, 12.5% and 6.25% w/v. Based on this outcome the researchers induced and challenged with the undiluted (i.e. 100%) test material.

Induction Phase

Test article

No erythema responses (0/20) were observed in guinea pigs challenged with RH-57, 592 2F during the induction phase. [The researchers applied the criteria that where grades of + were observed, (a score that denoted slight patchy erythema), they were considered to be representative of insignificant erythema responses, thus receiving a zero designation.]

Naive controls

These controls received no treatments during the induction phase.

Positive controls

A 100% incidence of erythema was observed (10/10) in the positive control. (No data table was found in the study in the appendices). Only in the "Results" section, was it stated that " a 100% incidence (10/10) of erythema was observed in the positive control (i.e. guinea pigs induced and challenged with DNCB) group, verifying that the animals on test were responsive to a known sensitizer".

Challenge Phase

Test article

No erythema responses (0/20) were observed in guinea pigs challenged with RH-57, 592 2F.

Naive controls

No erythema responses were observed in the non-induced control group following the challenge with either the undiluted RH-57, 592 2F or DNCB at 800 ppm in acetone (0/10 and 0/10 respectively).

Positive controls

A 100% incidence of erythema (10/10) was observed when challenged with DNCB.

CONCLUSIONS:

It appears that RH-57, 592 2F did not cause delayed hypersensitivity in guinea pigs.

Classification of data: Core-Minimum

It was stated in the protocol on p.25 of the study that "all guinea pigs will be weighed just prior to the 1st "induction" dose and prior to challenge". No body weights prior to the challenge phase were noted in the study.