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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Review of Data for the Technical Fungicide RH-7592

EPA ID NUMBERS: DP Barcode: D171006 and D171015
Submission #: S406678
Caswell No.: 723Q

FROM: SanYvette Williams, D.V.M. 3/23/93
Review Section IV, Tox. Branch II (H7509C)

TO: Dolphine Wilson/Cynthia Giles-Parker PM 22
Registration Division

THRU: Elizabeth Doyle, Ph.D. E. A. Doyle 3/23/93
Section IV, Tox. Branch II (H7509C)

and

Marcia van Gemert, Ph.D., Chief
Toxicology Branch II
Health Effects Division (H7509C) mvangemert 3/23/93

Registrant: Rohm and Haas Company

Action Requested: The registrant, Rohm & Haas, has requested review of the following toxicology data to be used to support requests for EUP's and temporary tolerances.

A. The following studies were reviewed:

Data Considered:

Study	Results
4-Week Oral-dog	Range-Finding
21-Day dermal-rat	NOEL $\geq$ 1000 mg/kg/day (limit dose)
78-Week chronic/onco-mice	NOEL = 1.43 mg/kg/day

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	LOEL = 28.6 mg/kg/day (male 92.9 mg/kg/day (female
Developmental toxicity -rabbit	Maternal NOEL = 10 mg/kg/day Maternal LOEL = 30 mg/kg/day Developmental NOEL = 30 mg/kg/day Developmental LOEL = could not be determined (>30 mg/kg/day)
2-Generation reproduction -rat	Parental NOEL = 4 mg/kg/day (80 ppm) Parental LOEL = 40 mg/kg/day (800 ppm), based on decreased body weight, food consumption, increased # dams not delivering viable or delivering nonviable offspring, and icreases in adrenal and thyroid/parathyroid weights Reproductive NOEL > 40 mg/kg/day (800 ppm)
Dermal absorption-rat	Acceptable
Thyroid Function and Hepatic Clearance	NOEL = 8 ppm LOEL = 800 ppm (based on increased liver and thyroid weights, diffuse thyroid hyperplasia and increased TSH levels)
Metabolism study	Supplementary
Particle size distribution	Supplementary

B. The conclusions for each study are included below:

1. Title of Report: RH-7592: Oral (Gavage) Developmental Toxicity Study in Rabbits EPA MRID (Accession) No.: 418750-14

Conclusions: The results of this study indicate that oral administration of RH-7592, by gavage, during gestation days 7-19 at dose levels of 10, 30 and 60 mg/kg of body weight had a maternal no observable effect level (NOEL) of 10 mg/kg. The NOEL for embryo-fetotoxicity and maternal reproductive lowest observed effect level (LOEL) were 30 mg/kg. Those malformations or variations demonstrated at dose levels up to 30 mg/kg were characterized as incidental. Fetal evaluations were not meaningful in the 60 mg/kg group because only 1/19 (5%) of the pregnant does produced a viable

This reviewer found some discrepancies [illegible] of mean corrected body weights and net weight [illegible] om Day 0 in the control and high dose animals that need [illegible] addressed. The difference with regard to net weight change in the Authors reported value and that calculated by this reviewer is significant. Therefore, the true value of the calculations for each tested group of females can not be properly assessed.

Maternal NOEL = 10 mg/kg/day
Maternal LOEL = 30 mg/kg/day
Developmental Toxicity NOEL = 30 mg/kg/day
Developmental Toxicity LOEL = could not be determined

This study conforms to most of the guideline requirements for a developmental toxicity study according to guideline #83-3.

Classification: Core - supplementary and may be upgraded upon the clarification of said discrepancies.

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2. TITLE OF REPORT: RH-7592: 4-Week Oral (Dietary Administration) Toxicity Study in Beagle (Revised Report). MRID#: 418933-02

CONCLUSION: There were no adverse effects on test animals administered 100 ppm RH-7592. There appears to be a palatability problem in test animals that were fed diets containing 1600 or 3200 ppm of the test material that caused a reduction in their food consumption with subsequent body weight loss. This reviewer agrees that using the effect on body weight and food consumption at dose levels of 1600 and 3200 ppm precludes them from use in subsequent [illegible].

This study was not performed to fulfill any guideline requirements, but to determine the feasibility of certain dosages of RH-7592 in the diet of dogs.

CLASSIFICATION: Core - Supplementary

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3. TITLE OF REPORT: RH-7592: 52 Week Oral (Dietary Administration) Toxicity Study in the Beagle. MRID#: 418750-49

CONCLUSION: Dosing with RH-7592 at concentrations up to 1200 ppm (30 mg/kg bw/day) was associated with reduced body weight gain and adaptive changes in the liver which reflected increased metabolic activity. In males, no changes considered to be toxicologically

significant were seen at the mid-dose level of 150 ppm (3.75 mg/kg bw/day). Therefore, this represents the no effect level (NOEL). The lowest observed effect level (LOEL) for females based on body weight gain was 150 ppm, while the no observed effect level was 15 ppm (0.38 mg/kg bw/day).

This study meets guideline requirements for a one-year dietary toxicity study (#83-1).

CLASSIFICATION: core - guideline

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4. TITLE OF REPORT: RH-7592: Two-Generation Reproduction Study in Rats MRID#: 418750-15

CONCLUSIONS: Administration of RH-7592 to rats for two generations had a no-observable-effect-level for reproductive structure or function of 80 ppm. There were many adverse reproductive effects seen among females treated at 800 ppm in the P1 and P2 generations. These included increases in maternal death during delivery, increases in the number of dams not delivering viable or delivering nonviable offspring, decreases in body weight and food consumption. Systemic toxicity was observed at 80 and 800 ppm.

Parental No-Observed-Effect Level (NOEL) = 4 mg/kg/day (80 ppm)
Parental Lowest-Observed-Effect Level (LOEL) = 40 mg/kg/day (800 ppm), based on decreased body weight and food consumption, increased number of dams not delivering viable or delivering nonviable offspring, and increases in adrenal and thyroid/parathyroid weights
Reproductive No-Observed-Effect-Level (NOEL) > 40 mg/kg/day (800 ppm)

Core Classification: This study conforms to guidelines for a [illegible] Guideline.

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5. Title of Report: RH-7592: Dermal Absorption in Male Rats (Preliminary and Definitive Phases) MRID#: 418750-19

Conclusions: The rate and extent of dermal absorption of 14C-RH-7592 was studied in 78 male rats divided into a control and four treated groups. One group of four male rats was dosed with 14C-RH-7592 at 0.123 mg/kg followed by a post-dose site wash at 10 and 168 hours. The washings were analyzed for total radioactivity. Urine and feces were collected in 24-hour increments up to 168 hours post-dose and also analyzed for total radioactivity.

Three additional groups of 24 animals each were given,

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respectively, 0.125, 1.25 and 125 mg/kg of the test compound. Urine, feces, carcass, skin site and skin washings from 4 animals at each time period were collected at 0.5, 1, 2, 4, 10 and 24 hours post-dose and analyzed for total radioactivity.

The majority of 14C-RH-7592 was not systemically absorbed and could be recovered in the skin wash (77.2 and 110%). The highest dermal absorption was found in animals that had the longest exposure to the dose. The mean percent of the dose absorbed (sum of the percent of dose in the urine, feces, carcass and skin of test site) was 12.35%%, 5.24% and 1.59% of the total dose, respectively, at 0.125, 1.25 and 125 mg/kg. The amount left on or in the skin site ranged from 0.13% to 4.27%. Nondetectable to 8.08% of the total dose was eliminated in the excreta.
Classification: Core - Acceptable

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6. Title of Report: Thyroid Function MRID#: 418750-20

Conclusions: Male rats (10-20/group) were given diets containing 0, 8, 800, 1600 or 3200 ppm of the test substance for 4 or 13 weeks. Additional groups received 1600 or 3200 for 4 weeks followed by 9 weeks of normal diet. The control group received normal rat chow. The study authors calculated the mean daily intake to be 1, 57, 116 and 231 mg/kg/day for 8, 800, 1600 and 3200 ppm dose groups, respectively. These calculations appear to overestimate the intake by about 10% since intake estimates were not used for weeks 6, 7, 9, 10, 11 or 12.

Thyroid function was affected and had the following:

NOEL = 8 ppm no treatment-related effects
LOEL = 800 ppm (based on increased liver and thyroid weights, diffuse thyroid hyperplasia and increased TSH levels)

The increased incidence and severity of effects beginning at 800 ppm [illegible] body weights were decreased at 1600 and 3200 ppm throughout the duration of the study, and serum levels of T4 were decreased at 1600 and 3200 ppm at Week 13. Hepatic clearance of T4 was tested in the control and 3200 ppm groups. The 3200 ppm group had increased biliary excretion of T4, primarily as the glucuronide, and hepatic UDP glucuronosyltransferase activity with T4 was increased.

Classification: Supplementary

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7. Title of Study: Metabolism #85-1 MRID#: 418750-17, 418750-18

Conclusions: The absorption, distribution, metabolism and excretion of RH-7592 were studied in groups of male and female Sprague-Dawley rats administered a single oral gavage dose of 1 or 100 mg/kg [14C]RH-7592, or 1 mg/kg unlabeled RH-7592 in the diet

for 14 days followed by a single gavage dose of 1 mg/kg [14C]Rh-7592 on day 15. An additional group of rats were administered a single IV injection of 1 mg/kg [14C]RH-7592.

[14C]RH-7592 was rapidly absorbed, distributed, metabolized, and excreted in rats for all dosing regimens. The 4-day recoveries were at least 82.6% of the administered dose for all dosing groups. The elimination of radioactivity in the feces (75.6-83.7% of administered dose) and urine (5.46-12.60%) were almost comparable for all oral-dosed groups, with slightly higher radioactivity in the feces of the repeated oral-dosed group than the single-dose groups. The radioactivity in the blood peaked at 3 hours for the low-dose group and 3-6 hours in the high-dose group, indicating biphasic elimination. In the IV group, most of the recovery was in the feces (77.2-91.40% of administered dose). Therefore, the elimination and pharmacokinetic data suggest that absorption of RH-7592 is rapid, bioaccumulation is low, and excretion is primarily in the feces due to biliary excretion. The study also indicates that RH-7592 and/or its metabolites do not bioaccumulate to an appreciable extent following oral or IV exposure since all the tissues contained negligible levels (<1%) of radioactivity at 4 days post-exposure.

Metabolism of RH-7592 appears to be extensive because the unmetabolized parent compound represented a minor amount of the recovered radioactivity in the excreta. Thirteen metabolites of RH-7592 and their conjugates were identified in the high-dose group. The most prevalent radioactive metabolites in the urine were the ketoacid, 3- and 4-phenol conjugates, and sulfate conjugates at 7 days post-exposure. Lactone A and sulfates contained the most radioactivity in the fecal extract. Sex-related differences were found for keto-acid and sulfate metabolites in the urine and feces. However, about 50% and 20% of the total radioactivity in the feces and urine, respectively, were not identified in the study, suggesting the lack of sensitivity of the analytical method used for metabolite analyses. Furthermore, dose-related differences of metabolism could not be determined since the metabolite pattern for low dose oral groups was not evaluated.

Based on the study results, oral absorption and fecal elimination of RH-7592 were not sex- or dose-related. A sex-related difference in the metabolism of a single oral dose of 100-mg/kg [14C]RH-7592 in rats was observed. A dose-related effect for metabolic pathway could not be determined. No apparent treatment-related clinical effects were induced by administration of 1 and 100 mg/kg RH-7592.

Classification: Core-Supplementary, but upgradeable if additional data are provided regarding metabolite analysis for the low-dose oral groups so that a possible dose-related difference in metabolism could be evaluated.

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8. Title of Report: Measurement of the Particle Size Distribution of RH-7592 2F Following Aerosol Generation (#81-3) MRID#: 418750-

Conclusions: The concentration and particle size distribution, throughout the 4-hour generation period, were found to be stable. It was not possible to generate the test material satisfactorily without first diluting it with water.

This study does not meet requirements for guideline #81-3. It was performed to measure the particle size distribution of RH-7592 2F using the same generation system as that currently used at RCC and Consulting Co., AG.

Classification: Supplementary, not a guideline study.

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9. Title of Report: RH-7592 Technical: 78-Week Dietary Oncogenicity Toxicity Study in Mice MRID#: 418933-01

CONCLUSION: Treatment-related findings to test animals administered RH-7592 for 78 weeks included decreases in mean body weight in male mice at 650 ppm, and increases in relative and/or absolute liver weights in males receiving 200 or 650 ppm and in females receiving 650 or 1300 ppm. Evidence of hepatocellular enlargement and vacuolization was obtained from histopathology of the livers of males receiving 200 or 650 ppm, and in females receiving 650 or 1300 ppm. As a result, the lowest observed effect level (LOEL) for RH-7592 in mice is 200 ppm in males and 650 ppm in females. The no observed effect level (NOEL) is 10 ppm in both male and female mice. The Author stated that the Agency recommended using 650 and 1300 ppm as the maximum tolerated dose (MTD) for males and females, respectively. This was based on results from 2-week and 3-month dietary studies in mice (letter from Rohm & Haas dated November 18, 1988 and appended to the DER).

NOEL = 10 ppm/day (1.43 mg/kg/day), male and female mice
LOEL = 200 ppm/day (28.6 mg/kg/day) in males
and 650 ppm/day (92.9 mg/kg/day) in females

This study is classified core - Guideline.

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Reviewed by: SanYvette Williams, D.V.M. SW 4/21/92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 4/22/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Particle size distribution (#81-3)

TOX. CHEM. NO.: 723Q MRID NO.: 418750-12

HED NO.: 1-2499

TEST MATERIAL: RH-7592 2F

LAB PROJECT STUDY ID NO: 263013

SPONSOR: Rohm and Haas

TESTING FACILITY: RCC, Research and Consulting AG, Itingen, Switzerland

TITLE OF REPORT: Measurement of the Particle Size Distribution of RH-7592 2F following Aerosol Generation.

Study Director: Dr. F. Duchosal

Letter Dated: March 1990

CONCLUSION: The concentration and particle size distribution, throughout the 4-hour generation period, were found to be stable. It was not possible to generate the test article satisfactorily without first diluting it with water.

This study does not meet requirements for guideline #81-3. It was performed to measure the particle size distribution of RH-7592 2F for rat inhalation studies using the same generation system as that currently used at RCC, Research and Consulting Company [illegible]

CLASSIFICATION: core - Supplementary

PURPOSE: This pilot study was performed in order to measure the particle size distribution of RH-7592 2F using the same generation system as that currently used at RCC for rat inhalation studies in the Fischer 344 rat.

COMPLIANCE: A signed and dated GLP statement was included on page 3 of the study.
A signed and dated Quality Assurance statement was included on page 4 of the study.

Test compound: RH-7592 2F (lot No. EG-1995; Purity: 23.1% a.i.; liquid, white to cream.

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Test Article Generation:

RH-7592 2F was diluted with distilled water and placed in a nebulizer. The test atmosphere generated by the nebulizer was then diluted with clean air to achieve the highest possible concentration and discharged into a nose-only exposure chamber.

Exposure system monitoring:

Concentration (nominal and gravimetric determination), particle size distribution, oxygen concentration, relative humidity and temperature were measured on test atmosphere samples. Airflow rates were determined during collection of samples for concentration and particle size.

Concentrations (mg/l air) Nominal	Gravimetric	Percentage of particles found on the impactor stages (mean ± S.D.) ≤ 3 um	≤ 1.06 um
4.48	0.86 ± 0.12	67.1 ± 4.5	18.3 ± 2.1

The concentration and particle size distribution, throughout the 4-hour generation period, were found to be stable. It was not possible to generate the test article satisfactorily without first diluting it with water.

This study does not meet requirements for guideline #81-3. It was performed to measure the particle size distribution of RH-7592 2F for rat inhalation studies using the same generation system as that currently used at RCC, Research and Consulting Company AG.

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Reviewed by: Steven L. Malish, Ph.D., S.L. Malish 9/18/92
Review Section, IV; Toxicology Branch II (H7509C)
Secondary reviewer: SanYvette Williams, D.V.M., [illegible] 9/21/92
Review Section, IV; Toxicology Branch II. (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 82-2 Repeated Dose Dermal Toxicity: 21 Day-Rat

MRID NO.: 418750-13

TEST MATERIAL: RH-57,592

SYNONYMS: None

SPONSOR: Rohm and Haas Company

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

LAB STUDY NO. 90R-084

TITLE of REPORT: RH-57,592 2F and Technical Fungicides
Four-Week Dermal Toxicity in Rats
Protocol No. 90P-084 Report No. 90R-084

AUTHORS: K.R. Lampe', B.A. Kulwich and R.C. Baldwin

REPORT ISSUED: February 22, 1991

CONCLUSION:

Six groups of 6 rats per sex were treated dermally, occluded, 6 hours/day, 5 days per week for a total of 21 or 22 doses. Group 1 (sham control) remained untreated. Group 2 received the Formulated Vehicle. Group 3 received the technical grade of RH-57,592 at 1.0 gm a.i./kg as a slurry. Groups 4 and 5 received aqueous dispersions of RH-57,592 2F at 0.625 or 0.25 gm a.i./kg, respectively, and group 6 received the undiluted RH-57,592 test material at 1.0 gm a.i./kg.

The test substance, RH-57,592 produced no systemic toxic effects and was considered to be a non-irritant using the Draize criteria.

The NOEL = 1000 mg a.i./kg/day (highest dose tested and the EPA limit dose).

CLASSIFICATION: CORE: supplementary - upgradeable

This study does not satisfy the guideline requirements 82-2 for a Repeated Dose Dermal Toxicity: 21 Day.

The study can be upgraded by supplying the stability of the formulated and technical test material over the 4 week test period. A flagging statement is required.

QUALITY ASSURANCE:

A quality assurance statement and a statement of compliance with FIFRA Good Laboratory Practice Standards were signed and dated.

FLAGGING CRITERIA

A flagging statement was not included in the report.

A. MATERIALS

1. Test Compound:

a. Technical	Chemical:2-(2-(4-chlorophenyl)-ethyl)-2-phenyl-(1H-1,2,4-triazole)-1-propanenitrile Synonym: RH-57,592 Technical Fungicide Purity: 97.1% w/w Description: off white solid powder Lot No.: BPP-3-1786R; Sample No. TD-90-045
b. Formulated	RH-57,592 2F Fungicide (23.5% w/v) Description: off white liquid Lot No.: SW/89/0673; Sample No. TD-90-046
2. Formulation Vehicle	RH-57,592 2F Formulation (Blank) Vehicle Description: gray translucent water-like liquid Lot No.: CDP1082; Sample No. TD-90-047

Preparation and Analysis of Formulation

Test solutions were prepared from the RH-57,592 Technical Fungicide solid using the RH-57,592 2F Formulation Vehicle. The Group 6, RH-57,592 2F test suspension (1.0 gm a.i./kg) was diluted with the Formulation Vehicle to prepare the Group 4 (0.0625 gm a.i. /kg) and Group 5 (0.25 gm a.i./kg) RH-57,592 2F test formulations. The Group 6, RH-57,592 2F test suspension had a similar percentage of the formulation components (except the active ingredient) as the Formulation Vehicle, Group 2.

Chemical analysis of the aqueous dosing suspensions of RH-57,592 2F used at the beginning of the study indicated that the actual analytical concentrations were 93% and 92% of the expected target concentrations for the 0.0625 gm a.i./kg (Group 4) and 0.25 gm a.i./kg (Group 5) dose groups, respectively. Neither the 1.0 gm a.i./kg technical (Group 3) or Group 6 (1.0 gm a.i./kg formulated)

were analyzed. The authors note that both had previously been characterized. [The reviewer notes that no analysis was made to determine if the test substance and test substance formulations were stable over the 4 week test period nor was there any mention of when the test solutions were prepared].

3. Test animals:

Species: Rat
Strain: Crl:CD®BR
Source: Charles River King ton Facility (Stone Ridge, NY)
Age: 8 weeks old at start of pretest
Weight: 214-246 gm (males);
190-209 gm (females)

B. STUDY DESIGN

Animal Assignments

Animals were quarantined for 7 days and then acclimated to laboratory conditions for another 7 days, identified by uniquely numbered ear-tags, and assigned randomly by weight to one of six treatment groups. All animals were housed individually in suspended stainless steel cages.

Thirty-six (36) rats per sex divided into 6 groups, were treated dermally, occluded, 6 hours/day, 5 days per week for a total of 21 or 22 doses. Group 1 (Sham Control) remained untreated. Group 2 received the Formulated Vehicle. Group 3 received RH-57,592 Technical at 1.0 gm a.i./kg as a slurry. Groups 4 and 5 received aqueous dispersions of RH-57,592 2F at 0.0625 or 0.25 gm a.i./kg, respectively, and Group 6 received RH-57,592 2F [illegible] suspension at 1.0 gm a.i./kg.

Table 1

Animal Test Group Assignments[1]

Group No.	Treatment	Daily Dose (gm a.i. /kg)[2]	Daily Dose (ml/kg)	No. of Rats/Sex
1	Sham Control	0.0	0	6
2	Formulation Vehicle	0.0[3]	3.3[4]	6
3	RH-57,592 Technical	1.0[3]	--	6
4	RH-57,592 2F	0.0625	4.1	6
5	RH-57,592 2F	0.25[3]	4.1	6
6	RH-57,592 2F	1.0[3]	4.1	6

[1]Adapted from the original Report p. 11.
[2]gm active ingredient (a.i.).
[3]EPA Limit dose. Administered as a slurry moistened with saline (1:1 w/v).
[4]The formulation component dose of RH-57,592 Formulation Vehicle was equivalent to that of the formulation components dose (less the active ingredients) received by the Group 6 (high dose 2F) animals.

Diet

Animals received food (Purina Certified Rabbit Chow #5002) and filtered tap water ad libitum.

Preparation of Animal Skin

The fur on the dorsal area of the trunk between the flank and the shoulders was clipped 1 day prior to the start of dosing and as needed during the study. A different area was designated for each dose of each rat to ensure dosing in the same area.

Skin irritation was evaluated immediately before each treatment. Erythema and edema were evaluated according to the method of Draize et al. (J. Pharmcol. Exptl. Therap. 82, 277-390, 1944). All other skin reactions such as scab formation, desiccation, etc. were recorded.

Dose Preparation and Administration

The animals were dosed once per day for 6 hours, 5 days per week (Monday through Friday) for approximately 4 weeks for a total of 21 or 22 daily applications. Logistics prevented all animals from being necropsied the same day. The animals were treated with RH-57,592 Technical moistened with 0.85% saline (1:1 w/v) at 1.0 gm a.i./kg. Aqueous dispersions of RH-57,592 2F were applied in a constant volume of 4.1 ml/kg. The application volume

of the RH-57,592 2F Formulation Vehicle contained the same amount of non-active ingredient received by the Group 6 (1.0 gm a.i/kg) animals. Dose volumes were calculated from the most recent body weights.

Each of the liquids and the moistened solid were applied to a patch which was then applied to the designated application site. The test substance was evenly distributed on the patch using the tip of a glass syringe for the liquids and a spatula for the solid.

The patch was composed of 3 layers of absorbent gauze (4x5 cm) backed by a layer of polyethylene which was held together with a slightly larger piece of Elastoplast™ adhesive and then placed on the designated area on the back of each animal.

The entire trunk of each animal was then wrapped with PEG™ elastic bandages (Becton-Dickinson) and secured in place with adhesive tape. Care was taken to assure that the test substance remained within the designated area.

After the 6-hour exposure, the animals were unwrapped, the application sites were washed with soft tissues saturated with a 1% aqueous solution of Ivory™ Liquid handsoap. The application sites were then rinsed with lukewarm water and then gently blotted dry with additional tissues.

The Sham Control (Group 1) was shaved and treated the same as the other groups, however, Group 1 did not receive any test material or saline.

At the beginning of the fourth week, the size of the patch used for the male rats was increased from 4x5 cm to 5x6 cm in order to retain the increased volume of the test substance required by the increasing body weight of the male rats.

Statistics:

The distribution of body weight and weight gains, mortality, food consumption, hematology and serum chemistry values, urine values and absolute and relative organ weights were inspected for normality and homogeneity of variance across treatment groups.

Transformations to an appropriate metric (log, arcsine, square root, etc.) were utilized when non-normality or heterogeneity of variance were present. When a significant treatment effect was found, four mutually orthogonal contrasts of the treatment means were examined and included:

1. Sham Control vs. Neat Technical Material (Test for Technical Material Effect).

2. Formulation Vehicle vs. 2F low, middle and high concentrations (Test for Overall 2F Effect).

3. Linear Dose-Response in 2F, low, middle and high concentrations (Test for Linear Trend in the 2F concentrations).

4. Quadratic Dose-Response in 2F low, middle and high concentrations (Test for Quadratic (non-linear) Trend in the 2F concentrations.

If the results for Contrasts 2 and 3 or 2 and 4 were significant, comparisons between each 2F concentration and Formulation Vehicle were made using Dunnett's t-test. Statistical significance was indicated when the observed p-value was less than the critical p-value as indicated by Dunnett's procedure.

C. METHODS AND RESULTS:

Observations

Each animal was observed daily for mortality, signs of ill health or reaction to treatment. A complete physical examination was performed weekly on each animal. The physical examination included external structure and appearance, posture, gait, behavior, body orifices and palpation for body masses.

Observations were considered to be unremarkable throughout the 4 week study. The skin effects were discussed below.

Body Weights

Animals were weighed prior to dosing, and weekly thereafter. At the end of the study, fasting terminal body weights were measured prior to sacrifice.

[illegible] were no test substance related effects on body weight or body weight gain in either sex when compared to the corresponding controls.

Food Consumption

Food consumption was measured weekly in all groups.

Food consumption was considered to be not remarkable when compared to the corresponding controls throughout the study.

Ophthalmology

Ophthalmologic examinations of all rats were performed using a binocular indirect ophthalmoscope during the pretest and week 4 of the study.

No ocular abnormalities were noted throughout the 4-week study.

Clinical Pathology

Blood was collected from the abdominal aorta of all animals (fasted) at study termination for hematology and clinical chemistry analyses. The CHECKED (X) parameters were examined.

a. Hematology Parameters

X	Hematocrit (HCT)*
X	Hemoglobin (HGB)*
X	Erythrocyte count*
X	Leukocyte count*
X	Platelet*
X	Leukocyte differential count*
-	Coagulation: thromboplastin time (PT)*
X	Reticulocyte count (RETIC)
X	Red cell morphology
X	Mean corpuscular HGB concentration (MCHC)
X	Mean corpuscular volume (MCV)
X	Mean cell hemoglobin (MCH)

* Recommended by tbe latest guidelines.

No test related hematology effects were noted.

b. Clinical Chemistry:

X	Serum alanine aminotransferase (SGPT)*
X	Serum aspartate aminotransferase (SGOT)*
X	Alkaline phosphatase (ALP)
X	Cholinesterase
X	Creatine phosphokinase
X	Lactic acid dehydrogenase
X	Gamma glutamyltransferase (GGT)
X	Calcium*
X	Phosphorus*
X	Chloride*
X	Sodium*
X	Potassium*
X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Glucose*
X	Total bilirubin*
X	Total protein*
X	Cholesterol
X	Albumin/globulin ratio
X	Globulins
X	Triglycerides
X	Albumin to globulin ratio

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* Recommended by the latest guidelines

No test-substance related clinical chemistry effects were observed in any group. Sporadic increases were occasionally seen but were not considered to be test-compound related.

c. Urinalysis:

Urinalysis was performed on all animals during the fourth week of [illegible] parameters were examined.

X Specific Gravity	X Bilirubin
X Occult blood	X pH
X Protein	X Color/clarity
X Microscopy of sediment	X Glucose
X Ketones	

Among the females, the urinary pH of Groups 3, 4, 5, and 6 were statistically lower than the Group 2, the vehicle control or Group 1, the sham control. Females, showed a decreasing specific gravity with dose in groups 4, 5 and 6 when compared to group 2. No difference was seen between group 1 and group 3 (Table 2).

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Table 2

Mean Urinalysis Values in Female Rats with RH-57,592 2F and Technical Fungicide at the End of 4 Weeks of Treatment[1]

Group No.	Dose (gm a.i./kg)	Spec. Grav.	pH
1	Sham Control	1.033	7.0
2	Formulation Vehicle	1.046	6.9
3	1.0 Technical	1.028	7.7*
4	0.625 2F	1.030*	7.5*
5	0.25 2F	1.022*	7.5*
6	1.0 2F	1.017*	7.3*

[1]Adapted from the original report, p. 49.
*Degree of statistical significance not reported.

Sacrifice and Pathology:

All animals (6 rats/sex/group) were subjected to gross pathological examination at sacrifice. The organs and tissues marked by a (X) were examined microscopically from Groups 1 (Sham Control) and 3 (RH-57,592 1.0 gm a.i./kg) Technical and 6 (RH-57,592 2F at 1.0 gm a.i./kg). Gross lesions and tissues designated by a + were examined from Groups 2, 4 and 5 as noted below.

Digestive System	Cardiovasc./Hemat.	Neurologic
	X Aorta	X Brain^
X Salivary glands	X Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord
X Stomach	X Lymph nodes	X Pituitary
X Rectum	X Spleen^+	X Eyes (optic nerve)
X Colon	X Thymus	
X Cecum		
X Ileum	**Urogenital**	**Glandular**
X Jejunum	X Kidney*^+	X Adrenals^
X Duodenum	X Urinary bladder	X Lacrimal gland
X Liver*^+	X Testes^	X Mammary gland
	X Epididymis	X Thyroids^
X Pancreas	X Prostate	X Parathyroids^
	Seminal vesicle	X Harderian glands
Respiratory	X Ovaries	
X Trachea	X Uterus	**Other**
X Lung+	X Vagina	X Bone (sternum & femur)
		X Skeletal muscle
		X Skin (treated & untreated)*+
		X All gross lesions & masses*+

* Recommended by the latest guidelines.
^ Weighed
\+ Gross lesions and tissues were examined from Groups 2, 4 and 5.

e. Organ Weights

In all animals (6 rats/sex/group) the brain, adrenals, testes, and [illegible] were weighed fresh and the thyroids and parathyroids were weighed after fixation. Relative organ weights were calculated.

Male animals showed a decrease in the absolute and relative adrenal weight in Groups 4, 5 and 6 which were statistically significant in Group 4 (0.0625 2F) and 5 (0.25 2F) when compared to Group 2 [Formulation Vehicle]. The decreases showed a trend toward the control value with increasing dose. Male animals in Group 3 (1.0 Technical) also showed a statistically significant decrease in the absolute and relative adrenal weight when compared to Group 1 [Sham control] (Table 3).

Female animals showed a dose related increase in the relative liver weight in Groups 4, 5 and 6 (0.0625, 0.25 and 1.0 gm a.i./kg 2F) which was statistically significant only at the high dose when compared to Group 2 (Formulation Vehicle). A statistically significant increase in the relative liver weight of Group 3 (1.0 Technical) was also noted when compare to the Group 1 [Sham Control] (Table 3).

Table 3

Mean Absolute/Relative Organ Weight at Necropsy[1]

G	Treatment (gm a.i./kg)	Adrenal Weight[2] Abs.[4]	Adrenal Weight[2] Rel.[5]	Liver Weight[3] Abs.[4]	Liver Weight[3] Rel.[5]
1	Sham Control	0.082	2.194	8.34	316.3
2	Formulation Veh.	0.077	2.187	7.63	314.4
3	1.0 Technical	0.059*	1.680*	8.81	343.8*
4	0.0625 2F	0.055*	1.485*	7.72	324.8
5	0.25 2F	0.061*	1.704*	8.39	334.1
6	1.0 2F	0.065	1.943	8.56	355.0*

[1]Adapted from the original report, p. 44, 46.
[2]males
[3]females
[4]gm
[5]Organ weight x 10,000/absolute weight
*Degree of statistical significance not reported.
G denotes groups

Skin Irritation

Using the Draize criteria for scoring, irritation was not noted in any of the dose groups throughout the study. Albeit, in females, starting on day 12 thru termination on day 22, erythema was minimally increased in Group 6 when compared to Formulation Vehicle (Group 2). Analyses of the data indicated that the erythema appeared to be caused more by the irritant effects of the Formulation Vehicle (and clipping procedure) (Group 2) than by the active ingredient, RH-57,592.

Table 4

Mean Skin Irritation Scores[1,2]

Days	Females Group 2	Females Group 6
0-11	0.0	0.0
12	0.0	0.3
13	0.3	0.3
14	0.3	0.5
15	0.7	0.8
16	0.7	0.8
17	0.3	1.0
18	0.3	1.0
19	0.7	1.3
20	0.8	1.3
21	0.7	1.2
22	0.0[2]	1.0[2]

[1]Adapted from the original report, p. 30 thru 33.
[2]3/6 animals evaluated

f. Gross Pathology:

There were no test-compound related findings at gross necropsy.

The incidence of red areas and/or eschar (scabs) involving the treated skin was generally comparable between Sham Control and Formulation Vehicle and treated groups.

g. Microscopic Pathology:

There were no test-compound related microscopic lesions seen at

Microscopic skin changes were noted in all groups but were associated with the vehicle and/or the clipping procedure and were not caused by the test-compound per se.

The most common significant findings were acanthosis, parakeratosis, eschar/superficial exudate and necrosis of the epidermis which corresponded to the gross observation of red areas and eschar (scabs).

In general, the findings in Group 3 (RH-57,529 Technical) were comparable to the Sham Control Group 1 while Group 6 (1.0 a.i. gm/kg RH-57,592 2F) findings were comparable to the Formulation Vehicle, Group 2. The findings in Groups 4 and 5 were less severe than Group 6 and the Formulation Control, Group 2 (Table 5).

Table 5

Microscopic Pathology of Skin Lesions in Animals Treated Dermally with RH-57,592 for 21-21 Days[1]

	Dose Groups					
	G1[2] M/F	G2[3] M/F	G3[4] M/F	G4[5] M/F	G5[6] M/F	G6[7] M/F
No. of Animals	6/6	6/6	6/6	6/6	6/6	6/6
Lesion						
Acanthosis	3/3	5/5	5/2	4/4	4/5	6/6
Parakeratosis	1/3	3/5	2/1	2/2	4/4	5/5
Eschar/exudate	0/2	3/4	0/1	0/1	1/0	1/6
chronic inflammation	0/3	4/3	1/1	1/3	1/1	2/6
necrosis, epidermis	1/1	0/2	0/1	0/1	2/2	1/2
Total Lesions	5/12	15/19	8/6	7/11	12/12	15/25

[1]Adapted from the original report, p. 240 thru 280.
[2]G1=Sham Control (minimal to slight severity).
[3]G2=Formulation Vehicle (minimal to moderate severity).
[4]G3=RH-57.592 Technical (1 gm a.i./kg) (minimal to slight severity).
[5]G4=RH-57,952 2F (0.0625 gm a.i./kg (minimal to slight severity).
[6]G5=RH-57,952 2F (0.25 gm a.i./kg) (minimal to slight severity).
[7]G6=RH-57.952 2F (1.0 gm a.i./kg) (minimal to moderate severity).

DISCUSSION:

The urinary pH of females in Groups 3, 4, 5, and 6 were statistically lower than the Group 2, the Formulation Vehicle or Group 1, the Sham Control. Females also showed a decrease in specific gravity with dose in Groups 4, 5 and 6 when compared to Group 2. No difference was seen between Group 1 and Group 3 (Table 2). No effect in either parameter was seen in the males. [The reviewer speculates that this effect may be indicative of the dermal absorption of the test-material in the female animals].

Male animals showed a decrease in the absolute and relative adrenal weight in Groups 4, 5 and 6 which was statistically significant in Group 4 (0.0625 2F) and 5 (0.25 2F) when compared to Group 2 (Formulation Vehicle). The decreases showed a trend toward the control value with increasing dose. Since no significant differences existed between Group 2 (Formulation Vehicle) and Group 1 (Sham Control), the facts suggest that the effect may have been caused by the test compound, RH-57,592. Histopathological examination revealed no adrenal pathology due to the test material. (Table 3).

Male animals in Group 3 (1.0 Technical) also showed a statistically significant decrease in the absolute and relative adrenal weight when compared to Group 1 (Sham control) suggesting that effect might have been caused by RH-57,592. Histopathological examination revealed no adrenal pathology due to the test compound (Table 3).

Comparison of Group 3 (1.0 Technical) to Groups 4, 5 and 6, (2F formulations) could not be directly compared because of the formulations differences (Table 3).

Female animals showed a dose related increase in the relative liver weight in Groups 4, 5 and 6 (0.0625, 0.25 and 1.0 gm a.i./kg 2F) which was statistically significant only at the high dose when compared to Group 2 (Formulation Vehicle). Group 2 (Formulation vehicle) showed no difference when compared to Group 1 (Sham control) suggesting the formulation vehicle had no effect on the relative liver weights (Table 3).

Group 3 (1.0 Technical) also produced a statistically significance increase in relative liver weight when compared to Group 1 (Sham Control). Although different formulations were compared, the information suggests that 1.0 gm a.i./kg of the test substance (RH-57,592) as contained in either Group 3 (1.0 Technical) or Group 6 (1.0 2F) produced an increase in the relative liver weight (Table 3).

The relative liver weight increase occurred without a corresponding statistical significant change in the mean absolute liver weight or mean body weight. No alterations in either clinical chemistry or liver histopathology were noted due to the test compound (Table 3).

The skin reaction potential of Group 3 (1.0 Technical) applied at 1.0 gm a.i./kg as a slurry in saline could not be directly compared to the other dosage forms because of the difference in the physical state of the formulation. Although [illegible] produce a lesion incidence less than the combined effects of the Sham Control (Group 1) and the Formulation vehicle (Group 2) as can be seen in Table 5.

In the 2F Groups 4, 5 and 6, the application site lesion incidence increased with increasing dose. In these groups the active ingredient increased as did the formulation vehicle components. In Group 6, however, the amount of the formulation vehicle components applied to the skin were the same as that administered from Group 2 and both groups exhibited similar number of application site lesions (the effects were greater than Group 1 (Sham Control) or Group 3 [1.0 Technical] (Table 5).

As can be seen in Table 4, the above results could be correlated with the minimum skin erythema scores in the females which appeared to be caused more by the irritant effects of the Formulation Vehicle (Group 2)/Sham Control (Group 1) procedure rather than the active ingredient, RH-57,592 (Table 4).

It may be concluded that the Formulation Vehicle (Group 2) and the Sham Control (Group 1) procedure produced more irritation than the active ingredient, RH-57,592 when erythema incidence or microscopic pathology of the skin was evaluated (Table 4, 5).

CONCLUSIONS:

Six groups of 6 rats per sex were treated dermally, occluded, 6 hours/day, 5 days per week for a total of 21 or 22 doses. Group 1 (Sham Control) remained untreated. Group 2 received the Formulated Vehicle. Group 3 received the technical grade of RH-57,592 at 1.0 gm a.i./kg as a slurry. Groups 4 and 5 received aqueous dispersions of RH-57,592 2F at 0.625 or 0.25 gm a.i./kg, respectively, and group 6 received the undiluted RH-57,592 test material at 1.0 gm a.i./kg.

The test substance, RH-57,592 produced no systemic toxic effects and was considered to be a non-irritant using the Draize criteria.

The NOEL = 1000 gm a.i./kg/day (highest dose tested and the EPA limit dose).

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Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 3/25/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 4-Week Oral Toxicity Study in the Beagle Dog

TOX. CHEM. NO.: 723Q MRID NO.: 418933-02

HED NO.: 1-2499

TEST MATERIAL: RH-7592

REFERENCE NO'S: HUK Report #: 6047-616/4R
HUK Project #: 616/4
Rohm & Haas Report #: 88RC-97

SPONSOR: Rohm and Haas Co.

TESTING FACILITY: Hazleton UK; Otley Rd.; Harrogate, North Yorkshire, England, HG3 1PY.

TITLE OF REPORT: RH-7592: 4-week oral (dietary administration) toxicity study in Beagle (Revised Report).

AUTHOR(S): J.F. Richards, B.Sc., M.Sc.

STUDY COMPLETED: Dec. 2, 1988

CONCLUSION: There were no adverse effects in test animals administered 100 ppm RH-7592. There appears to be a palatability problem in test animals that were fed diets containing 1600 or 3200 ppm of the test material that caused a reduction in their food consumption with subsequent body weight loss. This reviewer agrees that using the effect on body weight and food consumption as [illegible] precludes them from use in subsequent studies.

This study was not performed to fulfill any guideline requirements, but to determine the feasibility of certain dosages of RH-7592 in the diet of dogs.

CLASSIFICATION: Core - Supplementary

PURPOSE: This study was performed in order to determine the palatability of diet containing RH-7592 in dogs following oral administration by admixture in the diet for 4 weeks and to demonstrate the effects seen on body weight and food consumption in studies conducted previously by the sponsor.

COMPLIANCE: A signed and dated GLP statement was included on page 3 of the study.

A signed and dated Quality Assurance statement was included on page 6 of the study.

A. MATERIALS:

1. Test compound: RH-7592 (Lot #BPP-3-1786); Purity: 96.7%. It came in the form of a white solid. The control and vehicle was a mixture of acetone and powdered diet.

2. Test animals: Species: canine; Strain: beagle; Average age: 6-7 months at the beginning of dosing (4-5 months when received); Source: Hazleton Research Products Inc., Pennsylvania. After complete physical examinations, they were acclimated to the laboratory environment for at least 7 weeks prior to the start of dietary exposure. Following the acclimation period the beagles were weighed (males: 7-9.4 kg; females (6.2-8.2 kg) and assigned to the various experimental groups.

3. Diet: Test diets containing RH-7592 were found to be stable for at least 2 weeks and homogenous. Throughout the study, each animal was offered 400 g of diet every morning. Any remaining diet was removed, weighed and discarded in the afternoon. During Weeks 2, 3 and 4, food for group 4 animals was left overnight when less than 1/2 of the food offered was not eaten.

4. Statistical evaluation
Only descriptive statistics (group means and standard deviations) were conducted.

B. STUDY DESIGN:

1. Animal assignment:

Animals were assigned randomly to the following test groups:

Test Group	Dose Level (ppm w/w) Active ingredient	Test article#	Number of animals M	F
1 Control	0	0	2	2
2 Low (LDT)	100	103	2	2
3 Mid	1600	1655	2	2
3 High (HDT)	3200	3309	2	2

actual amount of test article in ppm added to diet

C. METHODS AND RESULTS:

1. Observations:

Test animals were checked twice a day during the work week for any

signs of ill health or reaction to treatment. Detailed clinical examinations were made once a week.

Results - All test animals survived to the end of the study. One male and one female in the high dose group were noted to be thin during the last week of the study.

2. Body weight

Body weights were recorded weekly on an individual basis.

Results - Weight loss was evident in animals treated at the medium and high doses. Body weight loss was most marked in the first 2 weeks of treatment.

Table 1*

Individual Body Weights (kg)

Dose (ppm)	day -1	day 7	day 14	day 21	day 28	gain day -1 to 28
0F	6.20 6.50	6.25 6.90	6.35 6.90	6.30 6.90	6.60 7.10	0.40 0.60
100F	8.15 6.60	8.30 6.50	8.30 6.85	8.40 6.85	8.70 7.40	0.55 0.80
1600F	6.80 7.25	6.50 7.25	6.35 7.10	6.45 7.00	6.55 7.20	-0.25 -0.05
3200F	7.20 6.80	6.50 6.15	6.20 5.95	6.10 6.00	5.85 5.80	-1.35 -1.00
0M	8.80 7.00	9.25 7.30	9.40 7.30	9.85 7.30	10.05 7.70	1.25 0.70
100M	7.20 9.40	7.30 9.60	7.30 9.75	7.75 10.05	7.80 10.20	0.60 0.80
1600M	9.30 7.35	8.90 7.15	9.20 7.05	9.30 7.00	9.25 7.05	-.05 -.30
3200M	7.60 8.90	7.10 8.50	6.45 8.00	6.50 7.85	6.35 7.95	-1.25 -0.95

*From pages 28 and 29 of the study.

3. Food consumption and compound consumption

Individual food consumption was determined daily throughout the study from the amount of food left or discarded from the measured quantity offered. Weekly food consumption data were then calculated

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from these measurements. Individual compound consumption was also calculated.

Results - A marked reduction in food consumption was evident from the start of treatment in medium and high-dose animals. Test animals in the high-dose group showed a more severe effect. During weeks 2, 3 and 4 food for the high-dose animals was left overnight so that they had longer access to the food. When compared to the pre-dose and control values, however, there was still a marked reduction in food consumption. When compared to control animals, food consumption in the low-dose group was comparable.

Food conversion efficiencies, however, tended to be negative or very low in the medium and high-dose animals. This was reflected in the body weight losses and poor food consumption of these animals.

Achieved levels of RH-7592 consumed were 3.61-4.94 mg/kg/day for low-dose animals, 39.4-74.51 mg/kg/day for middle dose animals and 42.71-92.01 mg/kg/day for high-dose animals.

Table 2*

Individual food consumption (g/animal/week)

Dose (ppm)	Week -1	Week 1	Week 2	Week 3	Week 4
0M	2800 2030	2800 2390	2700 2180	2680 2010	2630 2310
100M	1990 2610	2190 2800	1910 2800	2440 2620	2210 2800
1600M	2690 2050	1580 1750	1560 2230	1810 2290	2100 2290
3200M	2350 2410	880 1460	1050 1190	1200 1410	900 1590
0F	2770 1400	2370 2600	2210 1570	2280 2290	2080 2540
100F	2350 1970	2530 2170	2120 2060	2160 2370	2190 2390
1600F	2360 2420	1210 1610	1200 1540	1710 1970	1750 1890
3200F	2170 2070	640 710	1040 710	1200 900	930 1000

*From pages 30 and 31 of the study.

4. Sacrifice and Pathology

All animals were sacrificed at the end of the study. A full internal and external examination was made which included the following:

the external surface; all orifices; cranial cavity; carcass; external and cut surfaces of the brain and spinal cord; thoracic, and abdominal and pelvic cavities and their viscera; cervical tissues and organs; opened GI tract; parenchyma of serially sliced liver and spleen

The following CHECKED (X) tissues were collected for histological examination.

Digestive System	Cardiovasc./Hemat.	Neurologic
X Tongue	X Aorta	X Brain
X Salivary glands	X Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord
X Stomach	X Lymph nodes	X Pituitary
X Rectum	X Spleen	X Eyes (optic nerve)
X Colon	X Thymus	
X Cecum		
X Ileum		
X Jejunum	Urogenital	Glandular
X Duodenum	X Kidney	X Adrenals
X Liver	X Urinary bladder	X Lacrimal gland
X Gallbladder	X Testes	X Mammary gland
X Pancreas	X Epididymis	X Thyroids
	X Prostate	X Parathyroids
	Seminal vesicle	X Harderian glands
Respiratory	X Ovaries	
X Trachea	X Uterus	
X Lung	X Vagina	Other
		X Bone (sternum & femur)
		X Skeletal muscle [illegible] & untreated)
		X All gross lesions & masses

Results - There were no gross or microscopic findings noted in any test animal that could be attributed to treatment with RH-7592.

D. DISCUSSION/CONCLUSION:

-There were no mortalities throughout the study period, but 2 animals in the high dose group were thin from Week 3 on.

-Achieved levels of RH-7592 consumed were 3.61-4.94 mg/kg/day for low-dose animals, 39.4-74.51 mg/kg/day for middle dose animals and

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42.71-92.01 mg/kg/day for high-dose animals.

- Test animals in the mid- and high-dose groups lost weight during the treatment period. There was no effect on test animals in the low-dose group.

There were no adverse effects in test animals administered 100 ppm RH-7592. There appears to be a palatability problem in test animals that were fed diets containing 1600 or 3200 ppm of the test material that caused a reduction in their food consumption with subsequent body weight loss. This reviewer agrees that using the effect on body weight and food consumption at doses of 1600 and 3200 ppm precludes them from use in subsequent studies.

This study was not performed to fulfill any guideline requirements, but to determine the feasibility of certain dosages of RH-7592 in the diet of dogs.

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Reviewed by: SanYvette Williams, D.V.M. SW 3/30/92
Section IV, Tox. Branch II (H7509C)
Secondary Reviewer: Elizabeth Doyle, Ph.D. EA Doyle 4/1/92
Section IV, Tox. Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: One-Year Dietary Toxicity Study in Beagle Dogs (#83-1)

TOX. CHEM. NO.: 348B MRID NO.: 418750-49

TEST MATERIAL: RH-7592

HED NO.: 2-0424

SYNONYMS: Fenbuconazole

LAB PROJECT ID NO.: HUK Project No: 616/5

SPONSOR: Rohm and Haas

TESTING FACILITY: Hazleton UK, Otley Rd., Harrogate, North Yorkshire, England, HG3 1PY

TITLE OF REPORT: RH-7592: 52 Week Oral (Dietary Administration) Toxicity Study in the Beagle.

AUTHOR(S): Clare Morgan, B.Sc.(Hons.), Study Supervisor

STUDY COMPLETED: January 11, 1990

CONCLUSION: Dosing with RH-7592 at concentrations up to 1200 ppm (30 mg/kg bw/day) was associated with reduced body weight gain and adaptive changes in the liver which reflected increased metabolic activity. In males, no changes considered to be toxicologically significant were seen at the mid-dose level of 150 ppm (3.75 mg/kg bw/day) Therefore, this represents the no effect level (NOEL). The lowest observed effect level ([illegible] for females based on body weight gain was 150 ppm [illegible] observed effect level was 15 ppm (0.38 mg/kg bw/day).

This study meets guideline requirements for a one-year dietary toxicity study (#83-1).

CLASSIFICATION: core - guideline

PURPOSE: The purpose of this study was to determine the toxicological effects of the test article, RH-7592, in the dog following chronic dietary administration for 52 weeks.

A. MATERIALS:

1. Test compound: RH-7592 (Batch # BPP-3-1786R). The test article was a white powder of 96.7% active ingredient. The control and vehicle

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for this study was basic powdered diet with acetone added. RH-7592 was found to be homogenous and stable for 7 days in the diet.

2. Test animals: Species: canine, strain: Beagle; average age: between 6 and 9 months at initiation of the study. Average weight: 7.20 to 10.35 kg (males) and 6.10 to 8.25 kg (females), Source: Hazleton Research Products Inc., Pennsylvania, 17517, USA.

B. STUDY DESIGN:

1. Animal assignment:

Test animals were acclimated to the laboratory environment at least 30 days before the start of the study. Afterwards, they were assigned randomly to the following test groups:

Table 1

Test Group	Dose Level in diet ppm	mg/kg	Number animals M	F
1 Control	0	0.00	4	4
2 Low (LDT)	15	0.38	4	4
3 Mid (MDT)	150	3.75	4	4
4 High (HDT)	1200	30.00	4	4

2. Diet and test material preparation:

A basal diet of SQC Laboratory Diet A, ground was the vehicle for XRD-498. It and tap water were available ad libitum.

3. Statistics:

Appended from pages 31-33 of the study.

4. A signed quality assurance statement was included.
A signed and dated GLP statement was included.
A flagging statement was not included.
A statement of No Data Confidentiality claim was included.

C. METHODS AND RESULTS:

1. Observations:

Test animals were observed in the morning before feeding and throughout the work day as necessary for any signs of ill health or reaction to treatment or deaths.

Prior to the start of dosing, the eyes of each dog were checked by ophthalmoscopy. They were checked again one week before the scheduled necropsy (Week 52).

All animals had their rectal temperatures recorded twice in pre-dose and in Weeks 12, 26 and 52.

Results - In the high-dose group, 2/4 (50%) males appeared thin from Week 21 up to Week 36 after which they were no longer considered to appear thin. Another high-dose male (25%) and 2/4 females (50%) appeared thin from Week 14 to the end of the study. One female and two low-dose females also appeared thin during the treatment period but only for a short isolated period.

There were no treatment-related changes noted upon ophthalmological or rectal examination.

2. Body weight and body weight gain

All animals were weighed weekly throughout the study.

Results - Males in the low- and high-dose groups gained less weight over the treatment period when compared to controls. However, this was not a statistically significant event.

Mean body weight gain of male test animals in the high-dose group was apparently reduced due to one test animal which gained only 200 g over the 52 week period. There were statistically significant ($p<0.05$) differences in body weight gain of the mid- and high-dose group females. The mid-dose group females did not show body weight gain that was less than that of controls until Week 41-52 while the high dose female values were less than controls over the entire treatment period.

3. Food consumption, compound consumption and feed efficiency

During the twelve-month test period, individual food consumption was recorded daily from the amount of food left or discarded from the measured quantity offered. Weekly food consumption data were calculated from these measurements and individual food conversion efficiency was calculated by dividing the body weight gain value by the amount of food consumed times 100.

Results - A statistically significant decrease in food consumption was evident in high-dose males ($p<0.01$) and females ($p<0.001$) during the first week of treatment. By Week 3, however, food consumption of all treated groups was similar to those of the controls throughout the rest of the study.

There were no significant findings in regard to compound consumption or food efficiency.

4. Blood was collected prior to dosing and in Weeks 13, 26 and 52 after 18 hour fasts. The CHECKED (X) parameters were examined:

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a. Hematology

X Packed cell volume (PCV)*
X Hemoglobin (HGB)*
X Erythrocyte count*
X Leukocyte count*
X Platelet*
X Leukocyte differential count*

* Required for subchronic and chronic studies

Results - The total white blood cell count in high-dose males (weeks 13, 26 and 52) and females (weeks 26 and 52) were reduced due to a reduction in the number of neutrophils. The platelet count was elevated for both male and females in the high-dose groups in Week 13, 26 and 52. The Author states that there is no evidence to indicate that these alterations are of toxicological significance. There were no other significant alterations appearing to be a result of treatment with RH-7592.

b. Clinical Chemistry

Electrolytes

X Calcium*
X Phosphorus*
X Chloride*
X Sodium*
X Potassium*

Enzymes

X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
X Alkaline phosphatase (ALP)

Other

X Blood creatinine*
X Blood urea nitrogen*
X Glucose*
X Total bilirubin*
X Total protein*
X Cholesterol
X Creatinine phosphokinase
X Globulins
X Triglycerides

* Required for subchronic and chronic studies

Results - In Weeks 13, 26 and 52, statistically significant ($p<0.05$ to $p<0.001$) increases in alkaline phosphatase activity were observed in high-dose male and female dogs. Although not

statistically significant, alanine aminotransferase activity was increased in one high-dose female compared to controls in Weeks 13, 26 and 52 and in high-dose males compared to controls ir Week 52 of the study.

Total protein concentrations in high-dose females (Week 26) and males (Weeks 26 and 52) were statistically significantly ($p<0.05$) decreased when compared to controls. A slight decrease in total cholesterol was observed in low- and high-dose males in Week 52, while in high-dose females a statistically significant ($p<0.05$) decrease in cholesterol was evident in Week 26.

In Weeks 13, 26 and 52, high-dose males and females had increased triglyceride concentrations. The significance of these increases is unclear because triglyceride concentrations in the mid- and high-dose males of the pre-dose group were higher compared to controls. There were no other significant findings that could be attributed to treatment with RH-7592.

Mean Clinical Chemistry Results (Male and Female) - 13 Weeks++

Dose ppm	ALP IU/l M/F	TPROTEIN g/l M/F	CHOLES mmol/l M/F	TRIGLY mmol/l M/F	ALT IU/l M/F
0	110/133	59/55	3.3/3.2	.34/.42	28/34
15	117/101	59/57	3.4/3.4	.30/.32	42/35
150	155/152	55/58	3.9/4.2*	.40/.42	29/30
1200	1013*/834*	53/53	2.8/3.0	.64/.59	33/38

* $p<0.05$

Mean Clinical Chemistry Results (Male and Female) - 26 Weeks++

Dose ppm	ALP IU/l M/F	TPROTEIN g/l M/F	CHOLES mmol/l M/F	TRIGLY mmol/l M/F	ALT IU/l M/F
0	116/140	60/58	3.1/3.8	.40/.47	48/37
15	128/110	59/57	2.9/3.9	.42/.56	49/38
150	172/171	57/59	3.4/3.6	.47/.51	43/36
1200	1403*/946***	55/52*	2.8/2.6*	.70/.69	58/65

* $p<0.05$
** $p<0.01$
*** $p<0.001$

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Mean Clinical Chemistry Results (Male and Female) - 52 Weeks++

Dose ppm	ALP IU/l M/F	TPROTEIN g/l M/F	CHOLES mmol/l M/F	TRIGLY mmol/l M/F	ALT IU/l M/F
0	87/178	62/55	3.3/3.9	.48/.45	42/40
15	99/95	60/58	2.9/3.0	.49/.47	43/38
150	136/154	58/58	3.5/3.3	.55/.39	40/32
1200	1433*/854***	54/54	2.6/3.0	.78/.60	63/75

* $p<0.05$
*** $p<0.001$
++ Data excerpted from Table 7 of the study, pages 96-111.

5. Urinalysis

Urine was collected at necropsy from the urinary bladder by transmural aspiration with a syringe and needle. The following parameters were measured:

pH	ketones
bilirubin	occult blood
glucose	urobilinogen
proteins	color
specific gravity	appearance
sediment exam	reducing substances

Results - No treatment-related changes were observed.

6. Sacrifice and Pathology

After one year on study, all surviving dogs were sacrificed and necropsied. Terminal body weights were recorded. The CHECKED (X) tissues were collected for histological examination and stained with hematoxylin and eosin. The double-checked (XX) organs were weighed and the organ weight to final body weight [illegible] were calculated.

Digestive System	Cardiovasc./Hemat.	Neurologic
X Tongue	X Aorta	XX Brain
X Salivary glands	X Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord
X Stomach	X Lymph nodes	X Pituitary
X Rectum	X Spleen	X Eyes (optic nerve)
X Colon	X Thymus	
X Cecum		
X Ileum		
X Jejunum	**Urogenital**	**Glandular**
X Duodenum	XX Kidney*	XX Adrenals
XX Liver*	X Urinary bladder	X Lacrimal gland
X Gallbladder	XX Testes	X Mammary gland

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X Pancreas	X Epididymis	XX Thyroids
	X Prostate	XX Parathyroids
	Seminal vesicle	X Harderian glands
Respiratory	XX Ovaries	
X Trachea	X Uterus	
X Lung	X Vagina	Other
		X Bone (sternum & femur)
		X Skeletal muscle
		X Skin (treated & untreated)*
		X All gross lesions & masses*

* Recommended by Subdivision F (October 1982) Guidelines

Results - Statistically significant differences in absolute ($p<0.01$) and relative ($p<0.001$) liver weights of high-dose males and females were increased when compared to controls. An increase in relative kidney weights was observed in high-dose males and females when compared to controls. The difference for the high-dose males was statistically significant ($p<0.05$).

Absolute and relative adrenal weights were increased in both males and females of the high-dose group. Relative adrenal weights were statistically significantly ($p<0.05$) greater when compared to the control.

Although not significant, high-dose males and females had increased absolute and relative thyroid weights when compared to controls.

Gross pathology - There were no treatment-related findings upon macroscopic examination.

Microscopic pathology - Findings in [illegible] high-dose animals that were considered to be related to treatment, included the presence of hypertrophic hepatocytes. This was characterized by cellular enlargement in the midzonal region of the liver, and cytopolasmic changes of an eosinophilic ground glass nature. The Author stated that this finding was representative of cellular adaptation associated with test substance metabolism or elimination. There was also an increase in pigmentation of the hepatocyte consistent with lipofuscin formation.

D. DISCUSSION/CONCLUSION:

-Primary findings in animals treated with up to 1200 ppm RH-7592 were reduced body weight gain in all high-dose animals and mid-dose females and changes to the liver which were considered to reflect increased metabolic activity. As there was no clear dose-response

in males in regard to body weight, the significance of a reduction of body weight in the low dose animals compared to control seems to be equivocal.

-There were several changes in the liver including increases in absolute and relative liver weights of high-dose animals. This was associated with increases in alkaline phosphatase and, possibly, alanine aminotransferase, and decreases in cholesterol and total protein and albumin levels. Microscopic identification of hepatocellular hypertrophy and an increase in the incidence and severity of pigmentation in hepatocytes, consistent with lipofuscin appears to be associated to treatment. Because there were no degenerative changes in hepatocytes, these findings were considered to be representative of adaptive cellular change associated with metabolism and/or elimination of RH-7592.

-Relative kidney weights of all high-dose animals were increased when compared to controls. Because there were no unusual histopathologic findings, it appears that this increase was possibly a reflection of increased metabolic activity of the kidney associated with elimination of RH-7592.

Dosing with RH-7592 at concentrations up to 1200 ppm was associated with reduced body weight gain and adaptive changes in the liver which reflected increased metabolic activity. In males, no changes considered to be toxicologically significant were seen at the mid-dose level of 150 ppm (3.75 mg/kg bw/day) Therefore, this represents the no effect level (NOEL). The lowest observed effect level (LOEL) for females based on body weight gain was 150 ppm, while the no observed effect level was 15 ppm (.38 mg/kg bw/day).

This study meets guideline requirements for a one-year dietary toxicity study (#83-1) and is classified core - guideline.

FENBUCONAZOLE TOX 01/01/09

Page_____ is not included in this copy.

Pages 39 through 41 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

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____ Sales or other commercial/financial information.

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010109

Primary Review by: SanYvette Williams, D.V.M. 3/23/93
Review Section IV, Tox. Branch II (H7509C)
Secondary Review by: Elizabeth Doyle, Ph.D. E.A. Doyle 3/23/93
Section Head, Review Section IV, Tox. Branch II(H7509C)

DATA EVALUATION REPORT

STUDY TYPE: Dietary Chronic/Oncogenicity (83-2)

TOX. CHEM NO: 723Q ACCESSION NUMBER: 418933-01

TEST MATERIAL: RH-7592 Technical

SYNONYMS: Fenbuconazole

LAB PROJECT ID #: HLA Study No. 417-438

ROHM AND HAAS REPORT NO: 88RC-107

SPONSOR: Rohm and Haas Co.

TESTING FACILITY: Hazleton Laboratories America, Inc.; 1330-B Piccard Drive; Rockville, MD 20850-4373

TITLE OF REPORT: RH-7592 Technical: 78-Week Dietary Oncogenicity Toxicity Study in Mice

AUTHOR(S): Gary W. Wolfe, Ph.D., D.A.B.T.

STUDY COMPLETED: March 20, 1991

CONCLUSION: Treatment-related findings to test animals administered RH-7592 for 78 weeks included decreases in mean body weight in male mice at 650 ppm, and increases in relative and/or absolute liver weights in males receiving 200 or 650 ppm and in females receiving 650 or 1300 ppm. Evidence of hepatocellular enlargement and vacuolization was [illegible] the livers of males receiving 200 or 650 ppm, and in females receiving 650 or 1300 ppm. As a result, the lowest observed effect level (LOEL) for RH-7592 in mice is 200 ppm in males and 650 ppm in females. The no observed effect level (NOEL) is 10 ppm in both male and female mice. The Author stated that the Agency recommended using 650 and 1300 ppm as the maximum tolerated dose (MTD) for males and females, respectively. This was based on results from 2-week and 3-month dietary studies in mice (letter from Rohm & Haas dated November 18, 1988 and appended to this DER).

NOEL = 10 ppm/day (1.43 mg/kg/day), male and female mice
LOEL = 200 ppm/day (28.6 mg/kg/day) in males
and 650 ppm/day (92.9 mg/kg/day) in females

This study is classified core - Guideline.

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PURPOSE: This study was conducted in order to evaluate the oncogenic potential of RH-7592 Technical when administered in the diet to mice for 78 weeks.

A. MATERIALS:

1. Test compound: RH-7592 Tech., Lot #: BPP-3-1786R; Description: white powder; Purity 96.7% and adjusted to 100% for dosing purposes. Reagent grade acetone (Lot # 2440-KCMT) was the vehicle.

2. Test animals: CD-1 (Crl:CD-1(ICR)BR VAF/+) mice, age (after 2-week acclimatization period): 43 days; Weight: males - 23.9 to 32.5 grams and females - 17.8 to 26.8 grams; Source: Charles River Labs, Raleigh, NC. Test animals were maintained under adequate environmental conditions.

B. STUDY DESIGN:

1. Animal assignment

Test animals were randomly assigned to the following test groups:

Table 1

Test Group	# of animals		Dietary Levels ppm a.i.	
	males	females	males	females
1 Control	60	60	0	0
2 Low	60	60	10	10
3 Mid	60	60	200	650
4 High	60	60	650	1300

(Note: An additional 15 mice/sex were used as sentinel animals.)

[illegible] recommended using 650 and 1300 ppm as the maximum tolerated dose (MTD) for males and females, respectively. This was based on results from 2-week and 3-month dietary studies in mice.

The mid-dose level was chosen by taking 1/3 or 1/2 of the MTD in males and females, respectively and the low-dose of 10 ppm was selected to be a no-observed effect level (NOEL).

2. Diet :

After RH-7592 Technical was adjusted to 100 % purity, test diets were prepared biweekly and dietary concentrations were

based on the active ingredient of RH-7592 Tech. The control diet was prepared by mixing acetone with the basal diet. The diet of sentinel animals was the basal diet without acetone. Samples of the prepared test, fortified control and control diets were taken for homogeneity and stability analyses. Test animals were allowed access to food and water ad libitum.

Results - RH-7592 was determined to have an average homogeneity percent of target ranging between 89-105%. It was also found to be stable in the diet up to 21 days.

3. Statistics

Appended from pages 22-24 of the study.

4. Compliance

A signed Quality Assurance statement was included.
A signed GLP statement was included.
A signed Flagging statement was included.
A No Data Confidentiality Claim statement was included.

C. METHODS AND RESULTS:

1. Observations:

Animals were inspected twice a day for signs of mortality and moribundity. Cageside observations were conducted once a day for obvious signs of toxic and pharmacologic effects, while detailed physical examinations were performed at each weighing interval.

Results - There was no effect on the survival rate of test mice treated with RH-7592. The adjusted survival rates for the males were 80%, 80%, 85% and 80% for Groups 1, 2, 3, and 4, respectively. The adjusted survival rates for the females were 75%, 84%, 77% and 78% for Groups 1, 2, 3 and 4, respectively. There were no clinical observations that were [illegible] to test material treatment.

Sentinel animal program - prior to the initiation of the study, 5 mice/sex (from a total of 30) had blood samples taken for assay of viral or mycoplasma antibody titers. Test animals were also examined for parasites (internal and external), had pulmonary washings taken and were necropsied. Both gross and microscopic examinations were performed on the brain, liver, kidneys, lungs, spleen, small intestine (ileum), colon and gross lesions. At Week 72, 5 mice/sex were tested for Mouse Hepatitis Virus and at Week 79 (termination), the procedures performed at initiation were performed on all surviving mice.

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Results - When checked during Weeks 72 and 79, test animals had positive Mouse Hepatitis Virus (MHV) titers. Microscopic evaluation of the livers at terminal sacrifice revealed foci of minimal to moderate chronic-active inflammation in all animals that were consistent with lesions associated with Mouse Hepatitis Virus.

2. Body weight and food consumption:

a. Individual body weights were recorded prior to treatment and weekly for Weeks 1-14 and once every 2 weeks thereafter.

Results - A decreasing trend in mean body weight change (Table 2) was evident in both males and females in all dose groups throughout the study. Group 4 (high-dose male) mean body weight at Weeks 13, 40 and 52 and mean body weight change values for Weeks 0-13, 0-26, 0-40, 0-52 and 0-64 were significantly lower than those of Group 1 (controls). The decrease in mean body weight change as a percentage of control for the Group 4 males, along with significantly lower mean absolute body weights and growth at selected intervals, indicates a treatment-related effect.

Table 2

Mean Body Weight Change as Percent of Control^

	Males				Females			
	Weeks							
Grp	0-13	0-26	0-52	0-78	0-13	0-26	0-52	0-78
2	-5.5	0.0	0.0	3.6	0.0	0.0	7.5	3.7
3	-9.6	-4.1	-7.1	3.6	-9.5	-3.8	-3.2	-4.7
4	-19.2	-16.5	-19.6	-12.6	-7.9	-5.0	-6.4	-1.9

^Table excerpted from page 29 of the study.

[illegible] revealed statistically significant ($p \leq 0.05$) decreases in mean values for the Group 4 males for Weeks 0-13, 0-26 and 0-40 and for the Group 3 females for Weeks 0-13. These findings appear to indicate a treatment-related effect. Mean body weights, mean body weight change and growth were all comparable to controls in the remaining female groups.

b. Food consumption was recorded prior to treatment and weekly for Weeks 1-14 and once every 2 weeks thereafter (termination). Food utilization was calculated weekly for Weeks 1-14 and every 2 weeks thereafter through week 52 (growth phase).

Results: Food consumption was significantly higher in Group 4 females when compared to controls. At Week 4, Group 2 and 4 females mean food consumption values were significantly lower than the control, and at Week 13 Group 2, 3 and 4 male mean food consumption values were significantly lower than controls. Analysis of mean total food consumption in males revealed a decreasing trend in all treated groups throughout the study when compared to controls. This appears to be a treatment-related effect. A statistically significant decrease in mean total food consumption was evident in female mice during the 1-4 week interval. After the 1-26 week interval to the end of the study, mean total food consumption values were equal to or greater than those in the control group. There did appear to be an effect on food-efficiency between groups of the same sex.

3. Clinical Pathology:

Blood was collected from 10 randomly selected mice/sex/group in Groups 1 (control) and 4 (high-dose) at Weeks 53 and 79 to evaluate the differential leukocyte count, cell morphology and M/E ratio.

Results - There were no treatment-related findings in hematology or clinical chemistry.

4. Sacrifice and Pathology

Necropsies were performed on unscheduled deaths, on 10 animals/sex/ at Week 52 for the interim sacrifice and on all remaining animals at terminal sacrifice (Week 78). Terminal body weights were recorded before necropsy. The necropsies included examination of the following: external surfaces, all orifices, carcass, external surface of the brain, nasal cavity and paranasal sinus, thoracic, abdominal, and pelvic cavities and their viscera, cervical tissues and their organs and the cranial cavity.

The following checked (X) tissues from each animal were preserved for histopathological examination and those double-checked (XX) organs were weighed.

Digestive System	Cardiovasc./Hemat.	Neurologic
	X Aorta	X Brain
X Salivary glands	X Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord
X Stomach	X Lymph nodes	X Pituitary

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X Rectum	X Spleen	X Eyes (optic nerve)
X Colon	X Thymus	
X Cecum		
X Ileum		
X Jejunum	Urogenital	Glandular
X Duodenum	XX Kidney	X Adrenals
XX Liver	X Urinary bladder	X Parathyroids
XX Gallbladder	XX Testes	X Mammary gland
X Pancreas	X Epididymis	X Thyroids
	X Prostate	
	Seminal vesicle	
Respiratory	XX Ovaries	
X Trachea	X Uterus	
X Lung	X Vagina	Other
		X Bone (sternum & femur)
		X Skeletal muscle
		X Skin (treated & untreated)*
		X All gross lesions & masses*

* Recommended by Subdivision F (October 1982) Guidelines

Tissues were collected from all interim- and terminal-sacrifice animals in groups 1, 2, 3 and 4. The relative severity of graded non-neoplastic lesions was scored on a 5-step grading system specific to each organ or process (minimal, slight, moderate, moderately severe or severe).

(Livers from all mice, and all tissues from 5 mice/sex from Group 1 and 4 terminal and interim sacrifices and 2 mice/sex from Group 4 unscheduled deaths were submitted for peer review to Pathology Associates, Inc. (Frederick, MD).)

a. Gross pathology results:

There were no significant treatment-related findings [illegible] the 52-week interim necropsy other than a slight increase in the total incidence of enlarged livers in Group 4 males and females.

In Table 3, there appeared to be a slight increase in the total incidence (unscheduled deaths and terminal necropsy) of liver masses for Group 4 females and males. This reviewer disagrees with the Author that this is an equivocal increase that could be due to the unusually low incidence in the concurrent control group. There appears to be a treatment-related effect when the incidence is viewed in light of the total group and compared to controls (i.e. control - 4% vs Group 4 - 14%, and control - 6% vs Group 4 - 16% for males and females, respectively. A slight decrease in the total incidence of liver masses in the Group 2 males was also noted.

Table 3

Incidence of Enlarged Livers^

Necropsy Group	Male				Female			
	1	2	3	4	1	2	3	4
Terminal	1/39	0/39	0/40	3/39	1/35	0/40	0/36	4/39
Unscheduled	1/11	2/11	3/10	4/11	2/15	4/10	3/14	4/11
Total	2/50	2/50	3/50	7/50	3/50	4/50	3/50	8/50

^Table excerpted from page 36 of the study.

b. Organ weight - At interim sacrifice, mean absolute organ weights (Table 4), organ/body (Table 5) and organ/brain liver weight ratios (Table 6) were statistically significantly ($p < 0.05$) increased in both male and female mice (Group 4 males and Group 3 and 4 females).

At terminal sacrifice, a statistically significant dose-dependant increase was observed in mean absolute weights, organ/body, and organ/brain liver weight ratios (Group 3 and 4 males and females) when compared to controls.

Table 4

Absolute organ (liver) weight mean (g)^

Dose (ppm) M / F	BW (g) M / F	Interim Male	Interim Female	BW (g) M / F	Terminal Male	Terminal Female
0/0	35/27	1.60	1.26	35/28	1.68	1.48
10/10	36/27	1.56	1.36	35/28	1.67	1.45
200/650	35/28	1.75	1.68*	35/28	1.84*	1.75*
650/1300	33/27	2.03*	2.01*	34/28	2.32*	2.38*

* Statistically different from control ($p < 0.05$)
^ Excerpted from pages 243, 244, 246 and 247 of the study.

Table 5

Organ/Terminal Body Weight Ratio Means (g)^

Dose (ppm) M / F	Interim Male	Interim Female	Terminal Male	Terminal Female
0 / 0	4.7	4.7	4.8	5.3
10 / 10	4.4	5.0	4.8	5.1
200 / 650	5.1	6.0*	5.3*	6.4*
660 / 1300	6.2*	7.3*	6.8*	8.5*

* Statistically significant from control (0.05)
^ Excerpted from pages 249, 250, 252 and 253 of the study.

Table 6

Organ/Brain Weight Ratio Means (g)^

Dose (ppm) M / F	Interim Male	Interim Female	Terminal Male	Terminal Female
0 / 0	2.9	2.4	3.1	2.8
10 / 10	3.0	2.6	3.1	2.7
200 / 650	3.2	3.1*	3.5*	3.3*
650 / 1300	3.8*	3.8*	4.3*	4.4*

* Statistically significant from control (0.05)
^ Excerpted from pages 255 and 256 of the study.

c. Histopathology:

Treatment-related histomorphologic alterations (Tables 7 and 8) consisting of centrilobular to midzonal diffuse hepatocellular enlargement and a greater incidence and severity of hepatocellular vacuolation were observed in the livers of all mice in Groups 3 and 4. These hepatic changes were present at the interim sacrifice and then at terminal sacrifice. The NOEL for hepatocellular enlargement and vacuolation was 10 ppm (Group 2) for both male and female treated mice.

Table 7

Treatment Related Change - Interim Sacrifice^

Group	1	2	3	4	1	2	3	4
Sex	Male				Female			
Number Examined	10	10	10	10	10	10	10	10
Liver Finding								
Hepatocellular enlargement	1	0	5	10	0	0	5	10
Hepatocellular vacuolation	2	0	4	3	1	1	6	4

^Table excerpted from page 48 of the study.

Table 8

Treatment Related Change - Terminal Sacrifice^

Group	1	2	3	4	1	2	3	4
Sex	Male				Female			
Number Examined	39	39	40	39	35	40	36	39
Liver Finding								
Hepatocellular enlargement	2	3	13	38	0	1	22	35
Hepatocellular vacuolation	0	1	7	28	3	0	14	27

^Table excerpted from page 50 of the study.

There was also a noticeable decrease in the incidence of hepatocellular proliferative lesions (Tables 9 and 7) observed in the Group 2 males. Group 2 females, however, exhibited an increase in the incidence of proliferative hepatocellular lesions, that appears to be of equivocal significance.

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Table 9

Incidence of Hepatocellular Proliferative Lesions in Animals Sacrificed after 78 Weeks^

Group	1	2	3	4	1	2	3	4
Sex	Male				Female			
Number Examined	39	39	40	39	35	40	36	39
Finding								
Hepatocellular Hyperplasia	3	0	0	6	0	1	0	3
Hepatocellular Adenoma	7	1	6	5	0	0	0	3
Hepatocellular Carcinoma	1	1	3	4	0	1	0	1

^Table excerpted from page 51 of the study

Table 10

Incidence of Treatment Related Change in Scheduled and Unscheduled Deaths^

Group	1	2	3	4	1	2	3	4
Sex	Male				Female			
Number Examined*	60	59	60	60	58	60	57	60
Liver Finding								
Hepatocellular enlargement	4	4	22	55	1	1	34	49
Hepatocellular	2	1	11	31	4	1	20	31

*Autolytic tissues are deleted from original number.
^Table excerpted from page 52 of the study.

On page 38 of the study, the Author states that evidence of a low grade disease process was present in a large number of animals at termination from all treatment groups characterized microscopically by the presence of hepatic microgranulomas, foci of subacute inflammation, lymphoid infiltrates, pigment and necrosis. Similar hepatic changes were observed in sentinel animals which were killed at the termination of the study. Since sentinel animals were seropositive for MHV, it was assumed that the infection was concurrently present in the experimental groups, incidental, and not influential on the

mortality, detection of hepatotoxicity, or integrity of the study. This reviewer concurs with the conclusions of the Author on this issue.

D. DISCUSSION/CONCLUSIONS:

-- Overall mean compound consumption for Weeks 1-78 was 1.28, 26.28 and 85.26 mg/kg/day for the group 2, 3 and 4 males, respectively, at dose levels of 10, 200 and 650 ppm. Females dosed at 10, 650 and 1300 ppm had mean compound consumption values of 1.59, 104.64 and 208.84 mg/kg/day for the Group 2, 3 and 4 females, respectively.

-- Survival rates were comparable in all groups at 80, 80, 85 and 80% for Group 1, 2, 3 and 4 males, respectively and 57, 84, 77 and 78% for Group 1, 2, 3 and 4 females, respectively.

-- There were no treatment-related clinical observations noted.

-- Mean body weights were significantly decreased in the Group 4 males for Weeks 13, 40 and 52 when compared to the control. Corresponding mean body weight change was significantly decreased from initiation through Weeks 13, 26, 40, 52 and 64. As a percentage of the mean control body weight change, the mean change for Group 4 males was -19.2, -16.5, -19.6 and -12.6 from initiation to Weeks 13, 26, 52 and 78, respectively. Growth rate was also significantly decreased for the Group 4 males from initiation to Weeks 13, 26 and 40. For the Group 3 males, the mean body weight change as a percentage of control was decreased by -9.6, -4.1 and -7.1 from initiation to Weeks 13, 26 and 52, respectively. There was, however, a percentage of mean body weight change increase (+3.6%) from Week 0 to Weeks 13, 26, 52 and 78, the percentage of mean body weight change in Group 4 females compared to control was decreased by -7.9, -5.0, -6.4 and -1.9.

The growth rate for Group 3 females was significantly decreased from initiation to Week 13. While body weight results suggest some indications of a treatment-related effect, the Author feels that these changes are not believed to be treatment-related due to the small magnitude of the differences and the inconsistency of the findings.

Mean weekly food consumption was significantly decreased for Group 2 and 4 females at Week 4 and for Group 2, 3, and 4 males at Week 13 when compared to respective controls, but were not considered to be related to treatment.

--Results of clinical pathology test indicated that no treatment-related findings were present in the hematology findings of either sex.

--An increase in the incidence of enlarged livers in Group 4 males and females was seen grossly at terminal necropsy, in animals found dead, or killed moribund. The enlarged livers correspond with an increase in absolute liver weights at interim and terminal necropsy. The absolute liver weights were significantly ($p < 0.05$) increased in Group 4 males

and Group 3 and 4 females at the 52-week interim sacrifice and at the terminal necropsy. This corresponded to histomorphological alterations in the livers of the Group 3 and 4 males and females with centrilobular to midzonal hepatocellular enlargement and vacuolization.

-- In treated females, there were no significant differences ($p<0.05$) in the incidence of hepatocellular adenoma or carcinoma. The combined incidence of both adenoma and carcinoma in Group 4 was significantly higher than control (pairwise at 0/60 vs 5/60). In males, statistical analysis of liver tumor incidence revealed a significantly positive trend in the hepatocellular carcinomas, but no significant individual differences between treated groups and control except for significant differences for Group 2.

The incidence of hepatocellular adenoma and combined adenoma and carcinoma were significantly decreased in the Group 2 males compared to concurrent control groups. Although the incidence of liver adenoma in the Group 2 males (1.7%) was slightly lower than the lowest historical control incidence (2%) and the incidence of combined liver adenoma and carcinoma in the Group 4 females (8.3%) was slightly higher than the highest historical control incidence (6.1%), the incidence of liver adenoma and/or carcinoma in all groups, including these 2 groups, of both sexes in the study were basically within historical control ranges .

Because of a) the variability of the tumor results (i.e. marginal increase in Group 4 females but a decrease in Group 2 males), b) all tumor incidence are essentially within historical control ranges, and c) the low (0%) tumor incidence in female control mice, the results are equivocal, and provide insufficient evidence to conclude that RH-7592 is oncogenic in mice.

Treatment-related findings to test animals administered RH-7592 for 78 weeks included decreases in mean body weight in male mice at 650 ppm, and increases in relative and/or absolute liver weights in males receiving 200 or 650 ppm and in females receiving 650 or 1300 ppm. Evidence of hepatocellular enlargement and vacuolization was obtained from histopathology of the livers of males receiving 200 or 650 ppm, and in females receiving 650 or 1300 ppm. As a result, the lowest observed effect level (LOEL) for RH-7592 in mice is 200 ppm in males and 650 ppm in females. The no observed effect level (NOEL) is 10 ppm in both male and female mice. The Author stated that the Agency recommended using 650 and 1300 ppm as the maximum tolerated dose (MTD) for males and females, respectively. This was based on results from 2-week and 3-month dietary studies in mice (letter from Rohm & Haas dated November 18, 1988 and appended to this DER).

NOEL = 10 ppm/day (1.43 mg/kg/day), male and female mice
LOEL = 200 ppm/day (28.6 mg/kg/day) in males
and 650 ppm/day (92.9 mg/kg/day) in females

This study is classified core - Guideline.

PHILADELPHIA PA 19105 U S A TELEPHONE (215) 592-3000
TELEX 845-247 TWX 710-670-5335 TELECOPIER (215) 592-3377

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F. 1,

ROHM AND HAAS COMPANY

December 18, 1988

Mr. Lawrence J. Schnaubelt (PM-21)
Registration Division (TS-767C)
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Dear Mr. Schnaubelt:

SUBJECT: RH-7592 Experimental Fungicide
Meeting with EPA on Dose Selection for an
Oncogenicity Study in Mice

On November 16, Dr. Chan and I met with you, Mr. Fiol, Mr. Burnam and Dr. Copley to consider dose selection for the subject study. The basis for discussion was Dr. Chan's document submitted October 10, 1988 entitled, "RH-7592: Oncogenicity Study in Mice. Rationale for Dose Selection. Laboratory Project ID 88R-196," which presented a summary of the results from a 2-week and two 90-day dietary studies.

The meeting concluded with an agreement that:

1. The estimated MTD for the male is 650 ppm. The mid and low doses will be 200 ppm and 10 ppm, respectively. Should excessive mortality be incurred at the high dose (an event we all consider unlikely), then 200 ppm will be accepted as approximately 1/2 MTD and the study accepted as meeting the criteria of the OPP MTD Position Document of January, 1988.

2. The estimated MTD for the female is between 1000 and 3000 ppm, but closer to 1000 ppm. We agreed that the high dose will be 1300 ppm, the mid dose 650 ppm and the low dose 10 ppm.

Unless we hear otherwise by December 1, we will assume our understanding of the agreement is correct and shall initiate the study immediately.

Sincerely,

Michael A. Morelli

M.A. Morelli, Ph.D.
Product Registration Manager
Agricultural Chemicals Registration
and Regulatory Affairs

010109

Primary Review by: SanYvette Williams, D.V.M. 2-4-93
Review Section IV, Toxicology Branch II/HED (H7509C)
Secondary Review by: Elizabeth Doyle, Ph.D. E.A. Doyle 2/8/93
Review Section IV, Toxicology Branch II/HED (H7509C)

DATA EVALUATION RECORD

Study Type: Developmental Toxicity
Species: Rabbit
Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No.: 418750-14
Caswell No.: 723Q
HED Project No.: 1-2499

Test Material: RH-7592

Sponsor: Rohm and Haas Company

Report Number: 88R-195

Testing Facility: Rohm and Haas Co. Toxicology Department; 727 Norristown Road; Spring House, PA 19477.

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

Author(s): H.M. Solomon and M.F. Lutz

Report Finalized: August 24, 1989

Conclusions: The results of this study indicate that oral administration of RH-7592, by gavage, during gestation days 7-19 at dose levels of 10, 30 and 60 mg/kg of body weight had a maternal no observable effect level (NOEL) of 10 mg/kg. The no observable effect level for embryo-fetotoxicity and maternal reproductive effect was 30 mg/kg. [illegible] variations demonstrated at dose levels up to 30 mg/kg were characterized as incidental. Fetal evaluations were not meaningful in the 60 mg/kg group because only 1/19 (5%) of the pregnant does produced a viable fetus.

This reviewer found some discrepancies in the calculations of mean corrected body weights and net weight changes from Day 0 in the control and high dose animals that need to be addressed. The difference with regard to net weight change in the Authors reported value and that calculated by this reviewer is significant. Therefore, the true value of the calculations for each tested group of females can not be properly assessed.

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Maternal NOEL = 10 mg/kg/day
Maternal LOEL = 30 mg/kg/day
Developmental Toxicity NOEL = 30 mg/kg/day
Developmental Toxicity LOEL = could not be determined

This study conforms to most of the guideline requirements for a developmental toxicity study according to guideline #83-3.

Classification: Core - supplementary and may be upgraded upon the clarification of said discrepancies.

A. Materials

Test Compound: RH-7592
Purity: 96.7%
Description: white solid
Lot No.: BPP-3-1786R

Vehicle(s): 0.5% aqueous methylcellulose

Test Animal(s): Species: rabbit
Strain: New Zealand White
Source: Hazleton Research Animals, Denver, PA
Age: 5 months
Weight: 2.5-4.5 kg

Statistical analysis

Included from pages 14 and 15 of the study.

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.
A signed Flagging statement was provided.

B. Study Design

This study was designed to assess the developmental toxicity potential of RH-7592 when administered by oral gavage to rabbits on gestation days 0 through 29, inclusive.

1. Mating Semen was collected from proven fertile bucks. After each ejaculate was evaluated for sperm motility, viability and concentration, the semen was artificially inseminated into does that had been induced to ovulate.

2. Group Arrangement: Females were randomly allocated to the following dose groups (levels were selected on the basis of data from a range-finding study in the same species) on Day

0 of gestation.

Test Group	Dose Level (mg/kg)	Number Assigned
Vehicle Control	0	21
Low Dose	10	21
Mid Dose	30	21
High Dose	60	21

3. Dosing: RH-7592 was administered as an active ingredient/kg bw/day on Days 7-19 of gestation at a constant volume of 5 ml/kg in 0.5% methylcellulose.

4. Observations: Females were weighed on Days 0, 7, 9, 11, 14, 17, 20 and 29 of gestation. Feed consumption was recorded daily during gestation.
Test animals were checked twice a day for any signs of toxicity due to treatment.

On day 29 all adult females were sacrificed and necropsied. They were examined grossly for evidence of pregnancy and gross lesions of the abdominal and thoracic cavity.

Results - There were no treatment-related deaths occurring in the 10 or 30 mg/kg groups. In the 60 mg/kg dose group 1/21 (5%) died and 1/21 (5%) was sacrificed in extremis. One doe in the control group and one in the 60 mg/kg dose group died due to intubation errors. There were treatment related clinical signs in the doe that died in the high dose (60 mg/kg/day) group that were evident from Day 1 to Day 18 of dosing. After being gavaged on Day 18, on Day 19 this doe showed signs of labored breathing and died due to a perforated lung before being gavaged.

The mid- (30 mg/kg/day) and high-dose (60 mg/kg/day) group animals had an increased incidence of anorexia accompanied by [illegible] during the [illegible] treatment period. There was also an increase in the incidence of red discharge and no feces observed during the treatment period. These same clinical signs continued among does in the high-dose group during the post-treatment period (Days 20-29 G).

6. Terminal C-Section:

After being euthanatized on Day 29 of the study, the thoracic and abdominal cavities were examined and the gravid uteri were weighed before being opened. The number and position of live and dead fetuses and resorptions were recorded. Corpora lutea were counted from each ovary of all does. Those does that appeared "nonpregnant" had their uterus stained with ammonium sulfide to detect very early resorptions. Data from these animals were used to determine

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the incidence of pregnancy and the number of litters with total resorptions.

Live fetuses were weighed and examined for external alterations. After the live fetuses were sacrificed, all fetuses were examined for soft tissue alterations and the sex of each was identified. The brain and eyes were examined. After being fixed and stained, all fetuses were examined for skeletal alterations.

Maternal Survival and Pregnancy Status+

	Control	LDT	MDT	HDT
#Females Assigned	21	21	21	21
#Females Mated	21	21	21	21
Pregnancy Rate (%)	20(95)	19(90)	18(86)	19(90)
Died or sacrificed moribund	1	0	0	3
Pregnant	1	0	0	3
Non pregnant	0	0	0	0
Aborted	0	0	0	6ab
Premature Delivery	0	1	0	0
Examined on Scheduled Necropsy (%)	20(95)	20(95)	21(100)	18(86)
Non Pregnant	1	2	3	2
Pregnant	19	18	18	16c
all Dead/Resorb.	0	0	0	15ac
Viable litters	19	18	18	1a

a = significantly different from control at p<0.05
b = includes 1 doe that aborted and was sacrificed moribund
c = includes 5 does that aborted and survived to necropsy
+ = Table excerpted from page 22 of the study

Summary of Reproduction Data+

	Control	LDT	MDT	HDT
#Females Mated	21	21	21	21
Pregnancy Rate (%)	20(95)	19(90)	18(86)	19(90)
Died or sacrificed moribund	1*	0	0	3*
Aborted	0	0	0	6ab
Premature Delivery	0	1	0	0
Viable litters at sacrifice (%)	19(90)	18(86)	18(86)	1a(4.8)
All dead/Resorbed	0	0	0	10a
Corpora Lutea(mean)	9.1	9.4	10.0	8.0
Implantation(mean)	7.3	7.2	7.3	9.0
Preimplantation Loss(%)	21.3	22.1	26.1	-12.5
Resorptions(mean)	0.4	0.2	0.2	4.4
Early	0.3	0.2	0.1	4.4
Late	0.1	0.0	0.1	0.0
Live Fetuses(mean)	6.9	6.9	7.2	8.0
Total Dead Fetuses	0	0	0	0
Fetal body weight (g)	45.3	48.2	45.3	48.5
Sex Ratio (% Male)	46:44	48:47	45:45	46:50

a = Significantly different from control at $p<0.05$
b = Includes 1 doe that aborted and was later sacrificed moribund.
* = 1 doe died due to an intubation error.
+ = Data extracted from table 9 (p. 49) and Appen. II (p. 136) of the study.

This reviewer noted that there were more implantation sites (9) observed than corpora lutea (8) in the high-dose group. The resulting preimplantation loss percentage rate (-12.5%) of this dose group was also significantly different from the control (21.3%). This data can not be properly assessed at this time.

7. Maternal body weight and body weight change

When compared to controls, there were no treatment-related

effects on body weight or body weight change in low- or high-dose animals. Because there was only one surviving dam in the high-dose group, accurate comparisons of body weignt and body weight gain between it and the control group were unable to be made.

This reviewer found some discrepancies in the calculations of mean corrected body weights and net weight changes from Day 0 in the control and high dose animals that need to be addressed. The difference with regard to net weight change in the Authors reported value and that calculated by this reviewer is significant. Therefore, the true value of the calculations for each tested group of females can not be properly assessed.

Summary of Uterine and Net Body Weight (grams)

Dosage	0 mg/kg/d	10 mg/kg/d	30 mg/kg/d	60 mg/kg/d
Gravid uterus				
mean	446.1	489.8	469.7	617.72
S.E.	37.02	35.84	37.56	0.00
N	17	18	18	1
Corr. body weight				
mean	3545.9a	3674.2	3570.6	3294.0*b
S.E.	51.8	52.46	59.50	78.59
N	17	18	18	16
Net wt. change from Day 0				
mean	-102.4c	15.5	-81.1	-368.0*d
S.E.	43.35	26.43	30.97	74.78
N	17	18	18	16

Table excerpted from page 26 of the study.
* = significantly different from control group at p<0.05
a = 3531.3 mg/kg/d as calculated by this reviewer
b = 3658.1 mg/kg/d as calculated by this reviewer
c = 96.7 mg/kg/d as calculated by this reviewer
d = -162.9 mg/kg/d as calculated by this reviewer

8. Maternal feed consumption

Because there was only one surviving doe in the high-dose group, an accurate comparison of maternal feed consumption between it and the control group could not be made. During the last 8 days of treatment at 60 mg/kg, 19/21 (90%) stopped eating. Most started to eat again during the post-treatment period.

9. Maternal necropsy

There were no gross abnormalities determined to be related

to treatment with RH-7592.

10. Reproductive outcome

In the high-dose group, treatment-related effects included the occurrence of only 1/19 (5%) pregnant does having produced a viable litter, while 6/19 (32%) of this group aborted. There appeared to be an excessive number of litters (10) that were totally resorbed (8 determined by visual observation and 2 by stain). In the low- and mid-dose groups there were no treatment-related changes evident.

Fetal body weight

Mean fetal body weights of male and female fetuses, both individually and combined, were comparable to controls. Because there were only 8 fetuses alive at C-section in the high-dose group, mean fetal body weights were not truly able to be evaluated.

Fetal malformations and variations

There were no treatment-related findings in any treated group when compared to controls. Those malformations or variations that were present were considered to be incidental findings.

Historical control data were not provided.

D. Discussion/Conclusions

The results of this study indicate that oral administration of RH-7592, by gavage, during gestation days 7-19 at dose levels of 10, 30 and 60 mg/kg of body weight had a maternal no observable effect level (NOEL) of 10 mg/kg. The no observable effect level for embryo-fetotoxicity and maternal reproductive effect was 30 mg/kg. Those malformations or variations demonstrated at dose levels up to 30 mg/kg were characterized as incidental. Fetal evaluations were not meaningful in the 60 mg/kg group because only 1/19 (5%) of the pregnant does produced a viable fetus.

This reviewer found some discrepancies in the calculations of mean corrected body weights and net weight changes from Day 0 in the control and high dose animals that need to be addressed. The difference with regard to net weight change in the Authors reported value and that calculated by this reviewer is significant. Therefore, the true value of the calculations for each tested group of females can not be properly assessed.

Maternal NOEL = 10 mg/kg/day
Maternal LOEL = 30 mg/kg/day
Developmental Toxicity NOEL = 30 mg/kg/day
Developmental Toxicity LOEL = could not be determined

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This study conforms to most of the guideline requirements for a developmental toxicity study according to guideline #83-3. It is classified core - supplementary and may be upgraded upon the clarification of said discrepancies.

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Page_____ is not included in this copy.

Pages 64 through 65 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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Reviewed by: SanYvette Williams, D.V.M. 3/23/93
Section IV, Toxicology Branch II
Secondary Reviewer: Elizabeth Doyle, Ph.D. E.A. Doyle 3/23/93
Section IV, Toxicology Branch II

DATA EVALUATION REPORT

STUDY TYPE: Multigeneration Reproduction - Rat (Guideline 83-4)

MRID NUMBER: 418750-15

TEST MATERIAL: RH-7592

REPORT NUMBER: 88R-241

SPONSOR: Rohm and Haas

TESTING FACILITY: Rohm and Haas Co. Toxicology Dept; 727 Norristown Rd., Spring House, PA 19477

TITLE OF REPORT: RH-7592: Two-Generation Reproduction Study in Rats

AUTHOR(S): H.M. Solomon and B.A. Kulwich

REPORT FINALIZED: August 16, 1990

CONCLUSIONS: Administration of RH-7592 to rats for two generations had a no-observable-effect-level for reproductive structure or function of 80 ppm. There were many adverse reproductive effects seen among females treated at 800 ppm in the P1 and P2 generations. These included increases in maternal death during delivery, increases in the number of dams not delivering viable or delivering nonviable offspring, increases in thyroid/parathyroid and adrenal weights, decreases in body weight and food consumption. Systemic toxicity was observed at 80 and 800 ppm.

Parental No-Observed-Effect Level (NOEL) = 4 mg/kg/day (80 ppm)
Parental Lowest-Observed-Effect Level (LOEL) = 40 mg/kg/day (800 ppm), based on decreased body weight and food consumption, increased number of dams not delivering viable or delivering nonviable offspring, and increases in adrenal and thyroid/parathyroid weights
Reproductive No-Observed-Effect-Level (NOEL) > 40 mg/kg/day (800ppm)

Core Classification: This study conforms to guidelines for a multigeneration reproduction study (#83-4) and is classified core - Guideline.

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I. PROTOCOL

A. Materials

1. Test material: RH-7592 Tech. Lot No.: BPP-3-1786R; Purity: 96.7% a.i. in the form of a white solid.

1. Test species: 21-day old male and female Crl:CD BR strain rats were obtained from Charles River Breeding Labs, Inc., Kingston Facility, Stone Ridge, NY. They were given physical examinations and were acclimated for a period of 14 days before being placed into the study. They were individually housed and maintained under adequate environmental conditions.

2. Diet preparation:

Test diets were analyzed for stability, homogeneity of mixtures and chemical stability in prepared diets. During Weeks 1, 3 and 5, diet samples were analyzed for concentration of the active ingredient and homogeneity. Every other week (i.e., Weeks 2, 4, 6 to termination), retention samples were analyzed for concentration of the active ingredient and stability.

Fresh diets were prepared every other week and fed ad libitum. P1 male and female rats were offered the test diet continuously from 6 weeks of age for a minimum of 10 weeks prior to mating, and throughout mating, gestation, lactation until euthanization. P2 test animals were offered the test diets from weaning for a minimum of 10 weeks, throughout mating, gestation and lactation until they were euthanatized.

Results - Analyses performed on test samples collected weekly indicated that the concentrations of RH-7592 Tech in all test diets were distributed uniformly and were stable for at least 28 days at room temperature. The nominal concentrations for the test were 0, 8, 80 and 800 ppm versus the actual concentrations of 0, 7, 82, and 771 ppm.

B. Procedures and Study Design

1. Mating: Adult female rats (P_1 and P_2) were cohabitated with an assigned male from the same test group until positive signs of mating (sperm plug) were observed. Females that were presumed pregnant were removed and housed individually throughout the gestation and lactation periods. If no evidence of mating was found in females after 10 days observation, the female was cohabitated with a different male from the same treatment group until mating was confirmed. If pregnancy was confirmed, these females were also housed individually through gestation and lactation periods. If after 21 days, mating was not confirmed,

then the remaining females were housed in the same manner as the presumed-pregnant females.

To determine whether RH-7592 had an adverse effect on the reproductive function in male rats, an additional mating was conducted for the P1 males in the control and high-dose (800 ppm) groups only. The male rats were cohabitated with untreated females until 20 males/group copulated.

During gestation, females were weighed and given physical examinations on Days 0, 7, 14 and 21 G. During lactation, females and offspring were weighed and examined on days 0 and 4 post-partum. Necropsies were performed on females not delivering by Day 25 G to determine pregnancy status. At necropsy, corpora lutea and implantation sites were counted, and if indicated the uterus was stained to determine early resorptions. Females and offspring were sacrificed on Day 4 post-partum without pathological examination. P1 males were necropsied after the lactation period.

2. **Observation Schedule**

a. Parental animals:
Mortality checks were performed twice a day on all (P1 and P2) test animals. Detailed clinical examinations were performed once a week throughout the study on all (P1 and P2) test animals.

Results - No treatment-related deaths or clinical signs were present in any of the P1 males. Death occurred in one control male at Week 19 and one low-dose (8 ppm) male at Week 21. The only treatment-related effects observed in the P1 females occurred at parturition. Four P1 females, that eventually died, in the high-dose (800 ppm) group became moribund and appeared to have difficulty in delivering. Another finding, in a low-dose female, was a subcutaneous tumor.

No treatment-related deaths or clinical signs were present in any of the P2 males or females during the premating period. There were no treatment-related findings in females treated with 8 or 80 ppm during gestation or lactation. There were, however, 3 deaths of female in the 800 ppm dose group during parturition. One died before delivering any offspring (Day 23G) and the other 2 during delivery of their litters. These deaths were considered to be related to treatment.

Body weights - Body weights were taken on all animals at the beginning of the study until cohabitation. Maternal body weights were taken on Days 0, 7, 14, and 21 of gestation and Days 0, 7, 14, and 21 of lactation.

Results - (P1 animals) - Treatment-related decreases in body weights were noted among females at 800 ppm during the pre-breeding period, and throughout gestation and lactation. No treatment-related changes were observed in any other dose groups.

(P2 animals) - At 800 ppm, decreases in female body weights were noted throughout the pre-breeding treatment period, gestation and lactation periods.

Feed consumption - Feed consumption values was recorded on Days 0, 7, 14, and 21 of lactation.

Results - (P1 animals) - There were slight decreases in feed consumption in females in the 800 ppm (statistically significant at Week 4) dose group during the pre-breeding period. During gestation,a statistically significant decrease in feed consumption was recorded during the 14-21 day interval. During lactation, feed consumption was significantly less than that of controls throughout this interval. These were the only treatment-related findings of significance in all treated groups (male and female). This reviewer noted that the Author stated that "The large increases in daily feed consumption for all P1 dams between Days 7-14 and 14-21 of lactation were a result of both dams and their offspring consuming feed." Summary data on page 66 of the study show values in the high-dose to be significantly lower than those of controls.

(P2 animals) - Males (high-dose) between Weeks 0-9 showed a statistically significant reduction in mean feed consumption. The P2 females (high-dose) also showed significantly reduced mean feed consumption during Weeks 0, 2, 3, 4, 7, 8 and 9 pre-breeding, during Days 7-14 and Days 14-21 of gestation and Days 7-14 of lactation. These were the only treatment-related findings.

Compound Intake
Summary data show that from premating through gestation, up to the first week of lactation (P1 and P2 females) a decrease in daily compound intake was observed. During the last 2 week of lactation in both P1 and P2 females, large increases in compound consumption were recorded. The Author attributes this to both the dam and offspring consuming treated feed.

c. Offspring:
On Day 0 post-partum, the number of live and dead offspring, and sex were determined. Each pup was examined for external alterations and clinical signs. Cage-site observations were made 2 times a day to detect dead or moribund animals and for animals exhibiting signs of abnormal behavior or appearance.

On Days 4, 7, 14 and 21 post-partum the offspring were individually weighed and examined.

On Day 4 post-partum, litters were randomly culled to 8 (4/sex) when possible and then were weaned on Day 21 post-partum. Individual litters (F1) were housed by sex and fed appropriate test diets. At 25 days of age, 1 male and 1 female from each litter was selected to serve as parents P2 for the F2 generation. The week in which the P2 generation was selected was designated Week 0 for purposes of measuring body weight, feed consumption and clinical signs. During the post-weaning period, cage-site observations were made 2 times a day.

3. **Pathology**

All organs, tissue and body cavities were examined in P1 and P2 animals found dead or scheduled for sacrifice. All offspring found dead before weaning were examined to determine the cause of death. Those culled on Day 4 post-partum, all F1 (except those selected for mating), and F2 offspring were sacrificed, necropsied and examined for malformations.

Gross Pathology - All P1 and P2 rats had the liver (weighed), thyroid/parathyroid (weighed), adrenals (weighed), pituitary, testes (weighed), epididymides, prostate, seminal vesicles, coagulating gland, ovaries (weighed), uterus, vagina, cervix and gross lesions collected from them at necropsy.

Results - There were no significant treatment-related findings among any of the test animals or offspring.

c. Microscopic - Tissues collected from the control and 800 ppm dose group animals and any gross lesions from the 8 or 80 ppm group animals of P1 and P2 rats were prepared for histopathology. Also, the liver and thyroids from the 80 ppm dose groups of males from the P1 group were prepared. The liver, adrenal glands and thyroids of the P1 females and the P2 male and females were examined.

Results - Treatment-related findings (at 800 ppm) in the P1 males included: 1) increase in follicular cell hypertrophy of the thyroid gland; 2) hypertrophy and vacuolation of the centrilobular hepatocytes.

P1 females (at (800 ppm) exhibited a significant amount of hypertrophy and vacuolation of the centrilobular hepatocytes and centrilobular necrosis. Hypertrophy of zona glomerulosa cells of the adrenal gland and thyroid follicular cell hypertrophy were also observed and indicative of a treatment-related effect.

Treatment-related findings in P2 (800 ppm) males consisted of hypertrophy and vacuolation of the centrilobular hepatic cells, and an increase in follicular cell hypertrophy. The adrenal gland also showed and increase in cortical vacuolation and hypertrophy of the zona glomerulosa.

Treatment-related findings in P2 (800 ppm) females consisted of hypertrophy and vacuolation of centrilobular hepatocytes and a slight increase in centrilobular necrosis. The adrenal gland showed signs of hypertrophy in the zona glomerulosa region. There was also an increased incidence in follicular hypertrophy of the thyroid gland. Other findings were the slight distension of the uterus, vagina and cervix. There were no treatment-related findings at the low- or mid-dose level.

4. Statistical Evaluation: Appended from pages 26 and 27 of the study.

5. **Reproductive Outcome and Litter Data**

a. P1 adult/F1 offspring

There were significant decreases in the live offspring/litter on Days 0 and 4 Post Partum at 8 ppm and 800 ppm, but not at 80 ppm. A treatment-related decrease in the number of females with liveborn offspring was noted at 800 ppm. At 800 ppm, 10/25 (40%) presumed-pregnant produced liveborn while 21/25 (84%) control females produced liveborn. At 800 ppm 4 females died while delivering their litters, 3 females produced litters with no viable offspring and 8 females did not deliver. Gestation seemed to last longer among pregnant females at 800 ppm.

Treatment-related effects were observed among offspring of dams at 800 ppm included an increase in the number of stillborn offspring, a decrease in the total number of offspring [illegible] decrease in the viability index (no. offspring that survived to Day 4 PP). There was a decrease in mean body weight at birth among offspring at 800 ppm which was considered to be biologically significant. Three of the 11 litters at 800 ppm were well below group mean (5.7g while in the three litters the means were 4.[illegible]g, 4.7g and 4.8g, respectively). Also, the control value appeared low when compared to the large litter sizes produced by control females. Offspring body weight at 800 ppm was decreased for all remaining periods during lactation.

Results for the parental animals are summarized from the report as follows:

Table 1

Observation	Control	Low	Mid	High
		(ppm)		
P_1 Generation	0	8	80	800
Females				
Number mated	25	25	25	25
Number fertile/pregnant	25	25	25	25
Female with liveborn	21	22	22	10(a)
Gestation index (1/1)	84	88	88	40
Females surviving Delivery	21	22	22	13(a)
Duration gestation interval (days)	22	22.2	22	22.8(a)
Number of litters with liveborn pups	21	22	22	10
Number of pups (Total)	313	279	296	10(a)
liveborn	311	279	294	84
stillborn	2	0	1	12(a)
uncertain	0	0	1	5
Mean pup weight/litter (g) (Day 0)	6.1	6.6	6.5	5.7
Mean pup weight/litter (g) (Day 21)	49.0	50.5	51.6	40.3a

a Statistically significantly different from control, $p<0.05$.
b 3 females delivered litters with no viable offspring (stillborn + uncertain delivery status)
Excerpted from pages 85-87

b. P1 Adult Males/F1b Offspring (Re-mating)
There were no treatment-related effects on male reproductive performance after the re-mating of control males and males at 800 ppm to untreated females at 800 ppm. No significant findings between untreated females mated to control males and untreated females mated to treated males (800 ppm) were observed.

c. P2 Adults/F2a Offspring
Treatment-related effects among P2 females at 800 ppm were similar to those observed for P1 females at 800 ppm. At this dose, only 4/21 (19%) presumed pregnant treated animals produced liveborn compared to 22/25 (88%) control females. At 800 ppm, 3 females died during parturition, 1 female produced

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a litter with no viable offspring and 13 females did not deliver. According to the Author no meaningful results could be obtained from offspring at 800 ppm since only 35 were liveborn compared to 279 liveborn controls.

Table 2

Observation	Control	Low	Mid	High
		(ppm)		
P_2 Generation	0	8	80	800
Females				
Number mated	25	25	25	21
Number fertile/pregnant	25	25	25	21
Females with liveborn	22	19	21	4(a)
Gestation Index 1/1	88	76	84	19
Females surviving delivery	22	19	21	5(a)
Duration gestation interval (days)				
Number of litters with live pups	22	19	21	4
Number of pups (Total)	279	263	294	38
liveborn	278	259	288	35
stillborn	1	4	6	3(a)
uncertain	0	0	0	0
Mean pup weight/litter (g) (Day 0)	6.4	6.8	6.5	6.1
Mean pup weight/litter (g) (Day 21)	51.9	52.9	53.6	46.0

(a) Statistically significantly different from control, p<0.05.
** Statistically significantly different from control, p<0.01.
Excerpted from pages 88-90

6. **Absolute and Relative Organ Weights**

P1 animals At 800 ppm, treatment-related increases were noted in relative and absolute liver weights for both males and females, absolute and relative thyroid/parathyroid weights for males and relative adrenal gland weights for females.

Table 3

GROUP MEAN ABSOLUTE AND RELATIVE ORGAN WEIGHTS

P1 Generation Males

Dose	Terminal Body Weight (g)	Liver Absolute	Liver Relative	Thyroid/ Parathyroid Absolute	Thyroid/ Parathyroid Relative
0 ppm	675.2	19.93	29.50	0.032	0.047
8 ppm	677.0	21.60	31.89*	0.031	0.046
80 ppm	705.6	22.05*	31.24	0.031	0.045
800 ppm	669.4	25.74*	38.49*	0.037*	0.055*

* = Significant difference from control group 1 ($p<0.05$)
Table excerpted from page 93 of the study
(Absolute organ weight expressed in grams)
(Relative organ wt. = Absolute organ wt.(g)X1000/Term. Body wt.

Table 4

GROUP MEAN ABSOLUTE AND RELATIVE ORGAN WEIGHTS

P1 Generation Females

Dose	Terminal Body Weight (g)	Liver Absolute	Liver Relative	Adrenal Absolute	Adrenal Relative
0 ppm	331.5	11.21	33.89	0.069	0.210
8 ppm	336.4	11.61	34.54	0.069	0.205
80 ppm	335.9	12.22	36.33	0.070	0.210
800 ppm	311.9	13.53*	43.40*	0.076*	0.245*

* = Significant difference from control group 1 ($p<0.05$)
Table excerpted from page 94 of the study
(Absolute organ weight expressed in grams)
(Relative organ wt. = Absolute organ wt.(g)X1000/Term. Body wt.)

P2 animals Treatment-related increases were observed in relative liver weight among males at 800 ppm and relative and absolute liver weights among females at 80 and 800 ppm. Treatment-related increases were also evident in the relative thyroid/parathyroid weight among males at 800 ppm and in the relative adrenal gland weight among females at 800 ppm. Increased relative gonad weights of both male and females at 800 ppm were not considered treatment-related but rather a result of the decreased terminal body weights of these animals. No histological changes were present in the testes or ovaries of treated animals at any diet concentration.

Table 5

GROUP MEAN ABSOLUTE AND RELATIVE ORGAN WEIGHTS

P1 Generation Males

Dose	Terminal Body Weight (g)	Liver Absolute	Liver Relative	Gonads Absolute	Gonads Relative	Thyroid/ Parathyroid Absolute	Thyroid/ Parathyroid Relative
0 ppm	675.2	19.93	29.50	3.75	5.99	0.032	0.047
8 ppm	677.0	21.60	31.89*	3.68	5.73	0.031	0.046
80 ppm	705.6	22.05*	31.24	3.74	5.69	0.031	0.045
800 ppm	669.4	25.74*	38.49*	3.47	6.92*	0.037*	0.055*

* = Significant difference from control group 1 ($p<0.05$)
Table excerpted from page 9[illegible] of the study
(Absolute organ weight expressed in grams)
(Relative organ wt. = Absolute organ wt.(g)X1000/Term. Body wt.

Table 6

GROUP MEAN ABSOLUTE AND RELATIVE ORGAN WEIGHTS

P1 Generation Males

Dose	Terminal Body Weight (g)	Liver Absolute	Liver Relative	Adrenals Absolute	Adrenals Relative	Gonads Absolute	Gonads Relative
0 ppm	349.8	11.18	31.98	0.065	0.186	0.140	0.390
8 ppm	352.6	11.18	31.70*	0.061	0.173	0.140	0.390
80 ppm	365.8	12.63*	34.51	0.068	0.188	0.140	0.390
800 ppm	309.7	13.10*	42.29*	0.068	0.221*	0.150	0.490*

* = Significant difference from control group 1 ($p<0.05$)
Table excerpted from page 9[illegible] of the study
(Absolute organ weight expressed in grams)
(Relative organ wt. = Absolute organ wt.(g)X1000/Term. Body wt.)

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2. Parental Histopathology: P1 and P2 Generation

Liver - Treatment-related microscopic changes were observed in the liver and thyroid of males and females and the adrenal glands of females only (P1 and P2 females). A decrease in the number of female rats from the 800 ppm dose group of the P2 generation with pigmented macrophages/sclerosis in the uterus was noted by the Author. The NOEL for histopathology findings in the P1 and P2 generations is considered to be 80 ppm.

Other changes in the liver (i.e., characterized by hypertrophy of centrilobular hepatocytes and an increased incidence and severity of vacuolation of hepatocytes in the centrilobular to midzonal regions) were attributed to treatment. These changes correlated with the increased liver weights observed for both sexes at 800 ppm. Minimal to moderate hypertrophy of centrilobular hepatocytes occurred in all males from Group 4 of both generations and in 20/25 (80%) and 18/21 (86%) females from the P1 and P2 generations, respectively. Hypertrophy of centrilobular hepatocytes (moderately severe) also occurred in 1 control male from the P2 generation.

Hepatocyte vacuolation in centrilobular to midzonal regions of the liver occurred in 8/25, 6/25, 7/25 and 22/25 P1 males and 2/25, 1/24, 4/25 and 18/21 P2 males from the control group, Group 2 (8 ppm), Group 3 (80 ppm) and Group 4 (800 ppm), respectively. This change was observed in 3/25, 0/25, 0/25 and 17/25 P1 rats and 0/25, 1/25, 1/25 and 19/21 P2 rats from the respective dose groups. Responses were minimal to moderately severe in males and minimal to slight in females at 800 ppm. In rats from the lower dose groups and controls, this change was minimal except for the P2 control male with hypertrophy of centrilobular hepatocytes in which data show that it was moderately severe.

Thyroid - There was an increased incidence of thyroid follicular cell hypertrophy in 800 ppm dose groups of males and females from the P1 and P2 generations compared to controls. This tended to correlated with the increase in organ weight observed for 800 ppm males in which this finding occurred most frequently. This change was generally minimal to slight and occurred in 4/25, 4/25, 5/25 and 17/25 males from the P1 generation and 6/25, 9/25, 4/25 and 14/21 males from the P2 generation of control, 8 ppm, 80 ppm and 800 ppm treated rats, respectively. In females 1/25, 3/25, 4/25 and 10/25 rats from the P2 generation and 2/25, 1/25, 0/25 and 2/25 rats from the P2 generation were affected from the respective groups. This change in the thyroid was considered to be secondary to hypertrophy of centrilobular hepatocytes in the liver.

Adrenal gland - Hypertrophy of the zona glomerulosa was increased in incidence in high-dose female rats from both the P1 and P2 generations compared to controls and correlated with the increase in organ weight observed in that group. This change was generally minimal to slight and occurred in 7/25, 6/25, 7/25 and 19/25 rats from the control, Group 2, Group 3 and Group 4 females, respectively. In P2 females, this change was observed in 7/25, 9/25, 7/25 and 15/21 rats from the respective diet concentration. In P1 males, hypertrophy of the zona glomerulosa was comparable between controls and high-dose rats (3/25 vs 2/25). This change was observed in 0/25, 2/25, 1/25 and 6/21 rats from the control, 8 ppm, 80 ppm and 800 ppm, respectively.

Centrilobular necrosis of the liver was cited as contributing to the cause of death in P1 and P2 females at 800 ppm. There were no other findings that were considered to be treatment-related.

c. CONCLUSIONS

--There were no treatment-related findings in males (P1 or P2) or females (P1 or P2) or offspring (P1 or P2) at 8 ppm.

--At 80 ppm, only the P2 females showed a treatment-related effect of an increase in absolute and relative liver weights.

--Treatment-related effects at 800 ppm in P1 males included: increased absolute and relative liver weights and thyroid/parathyroid weights and substantiating histopathological changes in each organ. The P2 males showed the same effects as P1 males in addition to decreased pre-mating body weights and feed consumption.

--Treatment-related effects at 800 ppm in P1 and P2 females included: decreased body weight throughout the study, increased maternal deaths during delivery, an increase in the number of dams not delivering or delivering dead offspring, a decrease in the number of dams delivering viable litters, an increase in absolute and relative adrenal and thyroid/parathyroid weights with histopathological findings to support the treatment effect.

--Treatment-related effects on P1 offspring included: an increase in the number of stillborn, and decreases in the total number of offspring delivered, live offspring/litter, viability index, and body weight at birth.

-- Treatment-related effects on P2 offspring included a decrease in the total number of offspring delivered.

Administration of RH-7592 to rats for two generations had a no-observable-effect-level for reproductive structure or function of 80 ppm. There were many adverse reproductive effects seen among females treated at 800 ppm in the P1 and P2 generations. These included increases in maternal death during delivery, increases in the number of dams not delivering viable or delivering nonviable offspring, decreases in body weight and food consumption. Systemic toxicity was observed at 80 and 800 ppm.

Parental No-Observed-Effect Level (NOEL) = 4 mg/kg/day (80 ppm)

Parental Lowest-Observed-Effect Level (LOEL) = 40 mg/kg/day (800 ppm), based on decreased body weight and food consumption, increased number of dams not delivering viable or delivering nonviable offspring, and increased in adrenal and thyroid/parathyroid weights

Reproductive No-Observed-Effect-Level (NOEL) > 40 mg/kg/day (800ppm)

Core Classification: This study conforms to guidelines for a multigeneration reproduction study (#83-4) and is classified core - Guideline.

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Reviewed by Steven L. Malish, Ph.D. S.L. Malish 4/17/92
Tox Branch II, Section IV (7509C) 4/13/92
Secondary Reviewer: R. Zenzdian, Ph.D., S. Williams, D.V.M. 4/20/92
Tox. Branch II, Section IV (H7509C)

DATA EVALUATION REPORT

STUDY TITLE: Dermal Absorption Study (85-3)

MRID NO.: 418750-19

TEST MATERIAL: RH-7592

SYNONYM: Fenbuconazole

SPONSOR: Rohm and Haas Co.
Independence Mall West
Phila.PA 19105

LABORATORY: Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, WI 53704

REPORT NO.: HLA 6228-110

REPORT TITLE: RH-7592: Dermal Absorption in Male Rats
(Preliminary and Definitive Phases)

AUTHORS: Theresa Cheng, Ph.D.

REPORT ISSUED: August 31, 1990

CONCLUSIONS:

The rate and extent of dermal absorption of ^{14}C-RH-7592 was studied in 78 male rats divided into a control and 4 treated groups. One group of four (4) male rats was dosed with ^{14}C-RH-7592 at 0.125 mg/kg [illegible] washing were analyzed for total radioactivity. Urine and feces were collected in 24 hour increments up to 168 hours postdose and also analyzed for total radioactivity.

Three (3) additional groups of 24 animals each were given, respectively, 0.125 mg/kg, 1.25 mg/kg and 125 mg/kg of the test compound. Urine, feces, carcass, skin site and skin washings from 4 animals at each time period were collected at 0.5, 1, 2, 4, 10 and 24 hours postdose and analyzed for total radioactivity.

The majority of ^{14}C-RH-7592 was not systemically absorbed and could be recovered in the skin wash (77.2 to 110%). The highest dermal absorption was found in animals that had the longest exposure to the dose. The mean percent of the dose absorbed (sum of the percent of dose in the urine, feces, carcass and skin of test site) was 12.35%, 5.24% and 1.59% of the total dose, respectively, at 0.125, 1.25 and 125 mg/kg. The amount left on or in the skin site ranged from 0.13% to 4.27%. Nondetectable to 8.08% of the total dose was eliminated in the excreta, primarily the feces over 160 hours, and retained in the carcass.

CLASSIFICATION: Core - acceptable

This study meets the criteria for a Dermal Absorption Study (85-3).

QUALITY ASSURANCE: - A quality assurance statement was issued.

FLAGGING CRITERIA: - No flagging criteria citation was included in the study. The flagging criteria does not apply to this study.

A. MATERIALS:

Test Material:

Chemical:	RH-7592 (non-labeled) (Ring-UL-^{14}C)-RH-7592, carbon atoms on phenyl ring were labeled
Lot (radiolabeled)	595.0113
Lot (nonlabeled)	BPP-3-1786R
Lot 2F (blank)	CDP-1065
Radiopurity:	95.75%
Purity:	technical (% purity not stated)
Spec. Activity:	20.83 Mci/g
Stability:	stable for duration of test
Physical state:	solid (RH-7592/^{14}C-RH-7592) liquid (formulation blank)
Storage:	room temperature

Dosing Solution:

Preliminary Phase (Groups A and B)

Radiolabeled dosing solutions were prepared by mixing known amounts of non-radiolabeled RH-7592 with ^{14}C-RH-7592 (dissolved in acetone). For group A, the appropriate amounts of deionized water:acetone (11:5) was added. For Group B, the appropriate amount of RH-7592-2F (formulation blank) was added.

Definitive Phase (Groups 2, 3, 4 and 5)

Group 2, 3, 4 and 5 were prepared by mixing known amounts of non-radiolabeled RH-7592 with ^{14}C-RH-7592 (dissolved in acetone). Carboxymethylcellulose 0.5% (CMC) solution was used as the suspending agent. The dose solutions prepared the day before dosing ere stirred at room temperature overnight. The appropriate amount of the blank formulation was added to Group 5.

Radioactivity Verification

The concentrations of the radiolabeled dosing solutions were verified by radioanalyses of predose and postdose aliquot of each solution for Groups A and B and predose, dosing and postdose aliquot of each solution for Groups 2 through 5.

Test Animals:

Species:	rat
Strain:	Sprague-Dawley Crl:CD^{R}BR
Sex:	male
Groups:	
Preliminary	2 groups of 4 animals each
Definitive	1 vehicle control group of 2 animals
	1 treated group of 4 animals
	3 treated groups of 24 animals
Age:	7 weeks old upon arrival
Weight:	200 - 250 gms at initiation
Source:	Portage MI facility of Charles River Labs, Inc. Wilmington, MA.

Housing and Maintenance

During acclimation, rats were housed individually. [The reviewer notes that the length of acclimation time was not given]. During testing, the rats were housed individually in metabolism cages designed for separation and collection of the urine and feces.

Certified Rodent Chow #5002 (Purina Mills, Inc.) and water was provided ad libitum. Animals in each dose group were assigned by random numbers.

METHODS:

STUDY DESIGN:

Animal Assignments

Eighty-six (86) male rats on test were assigned to control or treatment groups (Table 1).

Table 1

Animal Assignments and Group Treatments[1]

Group	No.	Dil.	Dose ^{14}C-RH-7592[e] ug/cm^2	mg/kg	ug/rat	Sacrifice[d] Hour Postdose
			Preliminary Phase			
A	4	1:1000	2	0.125	25	4
B[a]	4	0	2,000	125	25,000	4
			Definitive Phase			
1[b]	2	-	0	0	0	24
2[c]	4	1:1000	2	0.125	25	160
3[c]	24	1:1000	2	0.125	25	0.5,1,2,4,10,24
4[c]	24	1:100	20	1.25	250	"
5[a]	24	0	2000	125	25,000	"

[1]Adapted from the original report, p. 16.
[a]Animals in Group B and 5 received a dermal dose of 14C-RH-7592 dissolved in a 2F blank solvent (250 mg a.i. RH-7592/ml).
[b]Animals in Group 1 received 2F formulation blank solvent only.
[c]Animals received a dermal dose of [illegible] carboxymethylcellulose aqueous solution.
[d]Animals were washed (the skin of the test site) at presacrifice. Group 2 animals had an additional wash at approximately 10 hours postdose.
[e]Animals received approximately 100 ul of the dose on the skin area of 2.56 x 5 cm.
No. = number of animals, Dil. = dilution

Test Material Administration

Two days before dosing (preliminary) and on the day before dosing (definitive), the back and shoulders of each animal were shaved and washed with acetone. The application site for the test material was defined by a plastic enclosure (approximately 12.5 sq cm), which was affixed to the back with glue and sealed with adhesive. Care was taken not to abrade the skin. [The reviewer notes that the type and quantity of adhesive and glue were not specified].

At dosing, approximately 100 ul of the test material preparation was applied within the enclosure along the midline of the skin site. The weight of the dosing syringe was recorded before and after dosing to obtain the weight of the dosing solution. The test material was spread along the surface of the skin site using a glass stirring rod as a spreader. The spreader was then rinsed with about 4 ml of acetone and the rinse collected for analysis. The spreader rod was then wiped (dose wipe) and the wipe saved for analysis.

After administration of the test material, the application site was covered with a non-occlusive (filter paper) cover.

Antemortem Observations

All animals were observed at least twice daily for moribundity or mortality and once daily for signs of toxicity.

Body Weights

Individual body weights were recorded at the time of randomization and used for dose calculation.

Statistical Analyses

Means and standard deviation were calculated.

Sample Collection

Preliminary Phase (Groups A and B)

Urine and feces were collected from 0 thru 4 hours postdose.

Skin Washing Procedures

The radioactivity of 2 skin washing procedures, noted below, were compared to assess the best method to be used in the sample collection procedure.

Procedure 1 (wash before and after sacrifice)

Prior to the 4-hour postdose sacrifice, the non-occlusive cover was removed from each of 2 of the 4 animals in Group A and B and saved, the dose application site was washed with a 2% Ivory(tm) soap solution and the wash was collected and saved.

All animals were then anesthetized with Halothane(tm). The skin from the application site and the enclosure site was excised, and the skin washed a second time with the soap solution. The animals were then exsanguinated by cardiac puncture.

Procedure 2 (wash after sacrifice)

The remaining 2 animal each from Groups A and B were sacrificed at approximately 4 hours postdose. All animals were anesthetized with Halothane(tm) and exsanguinated by cardiac puncture. The skin from the application site was then excised, with the enclosure still affixed, and washed with the soap solution.

For procedures 1 and 2, all skin samples, skin washings etc. and residual carcass were saved for analyses. Cages were washed with acetone, water and wiped; these samples were also saved for analyses.

The washing Procedure 2 (without a second wash after the animal was sacrificed) was used in the Definitive Phase of the study (see below). This procedure gave less variability in removing the radioactive dose from the skin than Procedure 1.

Definitive Phase (Groups 1, 2, 3, 4 and 5)

Group 1 (Control, 0 mg/kg) - 24 hour postdose sacrifice.
Samples in this group were used for validation and background checks.

Urine:	0 thru 24 hours postdose collection period.
Feces:	0 thru 24 hour collection period postdose.
Site:	skin of dose application washed with gauze pads and cotton swabs using 2% Ivory soap solution approximately 10 hours postdose. Skin of the dose site with enclosure attached was excised and saved under Halothane anesthesia and the animal exsanguinated.
Cover:	saved before sacrifice.
Carcass:	saved at sacrifice.
Cage:	cages washed with acetone and water, wiped with gauze pad and saved.

Group 2 (0.125 mg/kg) - 168 hour postdose sacrifice

Sample	Comments
Urine:	0-24, 24-48, 48-72, 72-96, 96-120, 121-145, 145-168 hrs postdose collection periods.
Feces:	As above.
Site:	Washed at 10 hours postdose. Excised and saved before sacrifice (as in procedure above) at 160 hours postdose.
Cover:	Removed and saved before sacrifice.
Carcass:	Saved.
Cages:	Washed as above.

Group 3 (0.125 mg/kg) - 0.5, 1, 2, 4, 10 and 24 hrs postdose sacrifice.
Group 4 (1.25 mg/kg) same as above
Group 5 (125 mg/kg) same as above

Sample	Comments
Urine:	0-0.5, 0-1, 0-2, 0-4, 0-10 and 0-24 hours collection periods.
Feces:	same as above collection periods.
Site:	Washed, excised and saved before sacrifice at each time period (as in procedure above in Group 1).
Cover:	Removed and saved before sacrifice.
Carcass:	Saved at sacrifice.
Cages:	Washed as above.

Sample Storage

All samples were stored in the freezer before and after the analysis.

RESULTS and DISCUSSION:

Preliminary Phase

Skin Washing Studies 1 and 2

The radioactivity from the skin washings and test site were compared for the washing procedure 1 (performed before and after) sacrifice versus the washing procedure 2 performed after sacrifice As noted in Table 2, more consistent results were obtained from animals washed before sacrifice than for those washed after sacrifice (Table 2). This procedure, therefore, was used in the definitive study, but the skin wash after the sacrifice was deleted for very little of the radioactive dose was recovered (Table 2 .

Table 2

Amounts of Radioactive Dose in Skin Wash at 4 hours Postdose for Male Rats Administered a Dermal Dose of ^{14}C-RH-7592[1]

Animal No.	Percent (%) Radioactive Dose Individual	Mean	SD
(Preliminary Phase (Group A, 0.125 mg/kg)			
1	86.92[a]		
2	87.44[a]	87.2	0.37
1	1.60[b]		
2	1.03[b]	1.32	0.403
3	75.41[b]		
4	69.09[b]	72.3	4.47
(Preliminary Phase (Group B, 125 mg/kg)			
1	79.22[a]		
2	85.31[a]	82.3	4.31
1	0.12[b]		
2	1.30[b]	0.71	0.834
3	82.52[b]		
4	91.06[b]	86.8	6.04

[1]Adapted from the original report, p. 32.
[a]skin washed before animal was sacrificed (Procedure 1).
[b]skin washed after animal was sacrificed (Procedure 2).

The majority of the radioactivity was found in or on the skin with mean values of 80.4% (low dose) and 84.9% (high dose) of the total dose. Small amounts of radioactivity were retained in or on the skin of the test site (6.78% and 1.61%) and in the carcass (2.10% and 0.30%). The amount of radioactivity eliminated in the excreta was less than 0.1% of the total dose. The mean total recovery was 91.0% and 89.6% for Groups A and B, respectively.

Total radioactivity of 0.15% to 1.5% was detected in the skin of the test site from animals that had a skin washed before sacrifice, compared to 1.0% to 13% from animals that had the skin washed after sacrifice. This fact is reflected in the radioactivity contained in the skin washings (Table 3).

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Table 3

Preliminary Phase

Mean Percent (%) of Radioactivity In Samples at 4 Hours Postdose for Male Rats Administered a Dermal Dose of ^{14}C-RH-7592[1]

Percent of Radioactive Dose

(Group A - 0.125 mg/kg)

Animal No.	Enc (%)	TS (%)	Wash (%)	Feces (%)	Car (%)	Urine[2] (%)	Total (%)
1	0.35	1.49	88.52[3]	ND	2.28	0.12	92.79
2	0.71	1.51	88.47[3]	"	1.99	ND	92.70
3	0.55	10.74	78.41[4]	"	1.73	0.03	88.53
4	5.09	13.39	69.09[4]	"	2.41	ND	89.99
Mean	1.68	6.78	80.4	ND	2.10	0.04	91.0

(Group B - 125 mg/kg)

1	0.26	0.15	79.34[3]	ND	0.09	0.01	79.85
2	4.25	0.29	86.61[3]	"	0.45	0.02	91.62
3	0.97	1.03	82.52[4]	"	0.13	ND	85.07
4	5.04	4.98	91.06[4]	"	0.54	ND	101.9
Mean	1.83	1.61	84.9	ND	0.30	<0.01	89.6

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[1]Adapted from the original report, p. 30 thru 31.
[2]Includes cage wash and cage wipe.
[3]Percent mean radioactivity in washings when skin washed before and after sacrifice.
[4]Percent mean radioactivity in washings when skin washed after sacrifice.

Non-excretive cover mean radioactivity = 0.03% (Group A); 0.19% (Group B) of total dose. Enclosure mean radioactivity = 1.68% (Group A), 1.83% (Group B) of the total dose.
Enc=enclosure, TS=test site, Wash=skin washings, Car=carcass, ND=not detectable.

Definitive Phase (Table 4)

At all time periods among the 3 tested groups, the majority of the radioactivity was not absorbed and was detected in the skin wash, and ranged from 77.2% to 110% of the total dose in Groups 3, 4 and 5 respectively.

The amount of radioactive material in or on the skin site ranged from 0.13% to 4.27% of the total dose in Groups 3, 4 and 5.

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The mean percent of the dose absorbed (sum of percent of dose in urine, feces, carcass and skin of the test site) ranged from 0.13% to 12.35% and increased with time being the highest at 24 hours postdose. The mean percent of the absorbed dose was 12.35%, 5.24% and 1.59%, respectively in Groups 3, 4 and 5. Dermal absorption, was not linear when the longest exposure times were compared (168 hours in Group 2 and 24 hours in Groups 3, 4, and 5).

The data from Group 2 showed that the absorbed test material was excreted, primarily in the feces over a 7 day period and resulted in non-detectable residues in the carcass. Small amounts of the dose were eliminated in the urine over the time period studied.

The mean material balance (average of the means at all time periods) was 92.1%, 100.8% and 104.8% of the total dose at 0.125, 1.25 and 125 mg/kg, respectively.

Table 4

Definitive Phase

Mean Percent of Radioactivity In Samples at Various Time Periods Postdose for Male Rats Administered a Dermal Dose of ^{14}C-RH-7592[1]

Percent of Radioactive Dose

G	Dose (mg/kg)	Time (hr)	Enc.[2]	TS (%)	Wash. (%)	Feces (%)	Car. (%)	Urine[3] (%)	T.A.[5] (%)	Total[6] (%)
2	0.125	10	NA	NA	67.8[4]	NA	NA	NA	NA	NA
		160	0.51	0.43	68.8[4]	3.18	ND	0.19	3.80	73.1
3	0.125	0.5	0.31	0.72	77.2	ND	ND	ND	0.72	78.3
		1	0.27	0.99	81.9	ND	ND	ND	0.99	83.2
		2	0.39	1.17	96.0	ND	ND	ND	1.17	97.6
		4	1.43	2.40	89.4	ND	1.37	0.03	3.80	94.7
		10	6.85	1.69	77.8	0.09	2.42	0.05	4.25	88.8
		24	0.73	4.27	96.6	2.96	4.80	0.32	12.35	110
4	1.25	0.5	0.19	0.49	97.2	ND	0.39	ND	0.88	98.9
		1	0.34	0.55	99.7	ND	0.23	<0.01	0.78	101
		2	0.36	0.37	99.6	ND	0.44	<0.01	0.81	101
		4	0.24	0.56	100.0	ND	0.57	0.05	1.18	102
		10	0.22	0.91	100.0	0.02	1.11	0.04	2.08	102
		24	1.06	1.99	94.6	1.12	1.84	0.29	5.24	101
5	125.0	0.5	0.16	0.27	98.9	ND	0.86	<0.01	1.13	100
		1	0.37	0.22	101	<0.01	0.24	0.01	0.47	101
		2	0.17	0.13	102	ND	ND	<0.01	0.13	103
		4	0.27	0.20	107	ND	0.07	<0.01	0.27	107
		10	0.29	0.25	110	ND	0.17	0.03	0.45	111
		24	0.63	0.71	104	0.03	0.62	0.23	1.33	106

[1]Adapted from the original report, pp. 34 (Group 2), 35 Group 2, 40 thru 45 (Groups 3,4 and 5).
[2]The non-occlusive cover mean radioactivity = <0.01 to 0.06% of the total radioactivity.
[3]Includes cage wash and cage wipe.
[4]At 10 hours, 67.8% of total radioactivity recovered in the skin wash, at 168 hours an additional 1.05% was recovered for a total amount of 68.8%.
[5]Total amount of dose absorbed (skin test site, feces, carcass and urine).
[6]Total amount of dose recovered.
G= Group, Enc.= enclosure, TS=test site, Wash=skin washings, Car.=carcass, ND=not detectable, NA=not available (animals not sacrificed at this time period).

CONCLUSIONS:

The rate and extent of dermal absorption of ^{14}C-RH-7592 was studied in 78 male rats divided into a control and 4 treated groups. One group of four (4) male rats was dosed with ^{14}C-RH-7592 at 0.125 mg/kg followed by a postdose site wash at 10 and 168 hours. The washing were analyzed for total radioactivity. Urine and feces were collected in 24 hour increments up to 168 hours postdose and also analyzed for total radioactivity.

Three (3) additional groups of 24 animals each were given, respectively, 0.125 mg/kg, 1.25 mg/kg and 125 mg/kg of the test compound. Urine, feces, carcass, skin site and skin washings from 4 animals at each time period were collected at 0.5, 1, 2, 4, 10 and 24 hours postdose and analyzed for total radioactivity.

The majority of ^{14}C-RH-7592 was not systemically absorbed and could be recovered in the skin wash (77.2 to 110%). The highest dermal absorption was found in animals that had the longest exposure to the dose. The mean percent of the dose absorbed (sum of the percent of dose in the urine, feces, carcass and skin of test site) was 12.35%, 5.24% and 1.59% of the total dose, respectively, at 0.125, 1.25 and 125 mg/kg. The amount left on or in the skin site ranged from 0.13% to 4.27%. Nondetectable to 8.08% of the total dose was eliminated in the excreta, primarily the feces over 160 hours, and retained in the carcass.

DOC930063

FINAL

DATA EVALUATION REPORT

RH-7592

Study Type: Metabolism

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

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Principal Author Karen Gan, M.S. Date 7/23/92

Reviewer Sanju Diwan, Ph.D. Date 7/23/92

QA/QC Manager Sharon Segal, Ph.D. Date 7/23/92

Contract Number: 68D10075
Work Assignment Number: 1-75
Clement Number: 91-237, 91-238
Project Officer: James Scott

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GUIDELINE SERIES 85-1: Metabolism

EPA Reviewer: SanYvette Williams, D.V.M. Signature: [signature]
Review Section IV, Toxicology Branch II, Date: 8-11-92
Health Effects Division

EPA Section Head: Elizabeth Doyle, Ph.D. Signature: [signature]
Review Section IV, Toxicology Branch II, Date: 8/13/92
Health Effects Division

DATA EVALUATION REPORT

STUDY TYPE: Metabolism in Rats

EPA IDENTIFICATION NUMBERS:

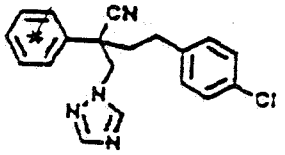
Tox. Chem. Number: 723-Q

HED Number: 1-2499

MRID Number: 418750-17; 418750-18

TEST MATERIAL: RH-7592 (CASRN 114369-43-6)

SYNONYM: [2-(4-Chlorophenyl)ethyl]-phenyl-1H-1,2,4-triazole-1-propanenitrile



The [^{14}C]-label (*) was uniformly distributed in the unsubstituted phenyl ring.

SPONSOR: Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA 19477

TESTING FACILITY: Hazleton Laboratories America, Inc., 3301 Kinsman Boulevard, Madison, WI 53704 (Report 1). Rohm and Haas Co., 727 Norristown Rd., Spring House, PA 19477 (Report 2).

AUTHORS: Leon LeVan (Report 1) and Richard Hanauer (Report 2)

REPORTS: 1. ^{14}C-RH-7592: Pharmacokinetic Study in Rats. Rohm and Haas Report No. 88RC-0071. 221 pp. Lab Project ID No.: HLA 6228-102.

2. ^{14}C-RH-7592: Range-Finding Kinetic and Metabolite Identification Study in Rats. Rohm and Haas Report No. 34-90-74. 327 pp.

DATE: August 3, 1990 (Report 1) and March 8, 1991 (Report 2)

9.

CONCLUSIONS: The absorption, distribution, metabolism, and excretion of RH-7592 were studied in groups of male and female Sprague-Dawley rats administered a single oral gavage dose of 1 or 100 mg/kg [^{14}C]RH-7592, or 1 mg/kg unlabeled RH-7592 in the diet for 14 days followed by a single gavage dose of 1 mg/kg [^{14}C]RH-7592 on day 15. An additional group of rats were administered a single intravenous injection of 1 mg/kg [^{14}C]RH-7592.

[^{14}C]RH-7592 was rapidly absorbed, distributed, metabolized, and excreted in rats for all dosing regimens. The 4-day recoveries were at least 82.6% of the administered dose for all dosing groups. The elimination of radioactivity in the feces (75.6-83.7% of administered dose) and urine (5.46-12.60%) were almost comparable for all oral-dose groups, with slightly higher radioactivity in the feces of the repeated oral-dosed group than the single-dose groups. The radioactivity in the blood peaked at 3 hours for the low-dose group and 3-6 hours in the high-dose group, indicating biphasic elimination. In the intravenous group, most of the recovery was in the feces (77.2-91.40% of administered dose). Therefore, the elimination and pharmacokinetic data suggest that absorption of RH-7592 is rapid, bioaccumulation is low, and excretion is primarily in the feces due to biliary excretion. The study also indicates that PH-7592 and/or its metabolites do not bioaccumulate to an appreciable extent following oral or intravenous exposure since all the tissues contained negligible levels (<1%) of radioactivity at 4 days postexposure.

The metabolism of RH-7592 appears to be extensive because the unmetabolized parent compound represented a minor amount of the recovered radioactivity in the excreta. Thirteen metabolites of RH-7592 and their conjugates were identified in the high-dose group. The highest radioactivities in the urine was represented by ketoacid, 3- and 4-phenol conjugates, and sulfate metabolites at 7 days postexposure. Lactone A and sulfates represented the highest radioactivities in the fecal extract. Sex-related differences were found for ketoacid and sulfate metabolites in the urine and feces. However, approximately 50% and 20% of the total radioactivity in the feces and urine, respectively, were not identified in the study, suggesting the lack of sensitivity of the analytical method used for metabolite analyses. Furthermore, dose-related differences of metabolism could not be determined since the metabolite pattern for the low-dose oral groups was not evaluated.

[illegible] other study results, [illegible] absorption and fecal elimination of RH-7592 were not sex or dose related. There was a sex-related difference in the metabolism of a single oral dose of 100-mg/kg [^{14}C]RH-7592 in rats. A dose-related effect for metabolic pathway could not be determined. The study also showed that administration of 1 and 100 mg/kg RH-7592 did not induce any apparent treatment-related clinical effects.

STUDY CLASSIFICATION: The study is classified as Supplementary. This study may be upgraded if additional data are provided regarding metabolite analysis for the low-dose oral groups. Thus, a dose-related difference in metabolism could be evaluated. There were no other major deficiencies in this study that would affect the overall study results and conclusions.

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A. MATERIALS

1. Test Substance

The unlabeled test material (Rohm and Haas, lot number EG 1442) administered in the diet for the repeated oral study was 96.4% active ingredient.

Radiolabeled RH-7592 (lot numbers 595.0106, 595.0107, 595.0108) was labeled with a ^{14}C-label that was uniformly distributed in the phenyl ring. The labeled preparations had specific activities of 20.83, 10.42, and 2.09 mCi/g. The mean radiochemical purity was 99.4%.

2. Test Animals

Male and female Sprague-Dawley Crl:CD BR rats were obtained from Charles River Laboratories, Portage, MI. A single oral gavage dose of 1 or 100 mg/kg labeled RH-7592 was administered to groups of 4 males and 4 females that were sacrificed at day 4 postexposure. The single high-dose group also had additional sacrifices with 3/sex at the following intervals: 1 and [illegible]rs and 1 and 2 days. For the repeated oral-dose study, rats rece[illegible] unlabeled RH-7592 in the diet for 2 weeks then received a single gavag[illegible]abeled dose. Another group of 4 male and 4 female rats received 1 mg/kg [illegible]abeled RH-7592 intravenously, however, the authors reported that a considerable amount of the test material remained at the injection site on the surface of the tail. Therefore, these animals were replaced with another group that was tested at a later date. The mean weights of rats in all groups ranged from 185 to 225 kg at the beginning of the study.

In a range-finding study, 3 groups of animals (4/sex/group) were administered a single oral dose of 100 mg/kg of labeled RH-7592 for the following purposes: (1) to evaluate metabolites in the urine and feces, (2) to measure the amount of radioactivity expired as CO_2, or (3) to examine elimination and tissue distribution at 7 days postexposure. The Crl:CD BR rats were obtained from Charles River Kingston, Kingston, NY.

B. METHODS

1. Acclimation

In the range-finding (CBI report 2) and primary study (CBI report 1), animals were acclimatized for 7, 10, 11, 14 or 21 days prior to administration of the test material. Rats were placed individually in Nalgene® metabolism chambers 3 days prior to exposure. If excreta were not being collected, the animals were housed individually in stainless steel cages before and during the study. Animals were provided a diet of Rodent Chow® 5002 (Purina Mills, Inc., St. Louis, MO) and water ad libitum throughout the study. No contaminants in the food and water were known to interfere with the study.

2. Dosing Solutions

The radiolabeled 1- and 100-mg/kg stock solutions for the single and repeated oral-dose studies were suspended in 0.5% aqueous carboxymethylcellulose (CMC). The solutions were evaporated, reconstituted in acetone, mixed, and resuspended in CMC, then sonicated, homogenized, and resonicated. The specific activities of the low-dose and high-dose solutions were 23,100 and 4,640 dpm/µg. In the repeated dose study, animals received ad libitum a diet premixed with nonradiolabeled RH-7592 in acetone for 14 days prior to the gavage dose to yield a treated diet of 10 ppm (1 mg/kg) RH-7592. The specific activity of the labeled solution was 23,100 dpm/µg. The oral gavage dose was administered at a volume of 5 mL/kg body weight. The intravenous solution was prepared in a similar manner as the other solutions except that it was dissolved in DMSO instead of CMC. The specific activity was 46,200 dpm/µg. The intravenous dose was administered at a volume of 0.25 mL/kg. Although the stability of the solutions were not reported, the test material is not volatile and probably is relatively stable.

Groups of rats (3-4/sex/group) were given single oral doses of 1 or 100 mg/kg [^{14}C]RH-7592, or were given a diet containing 1 mg/kg/day unlabeled RH-7592 for 2 weeks, followed by a single gavage administration of 1 mg/kg [^{14}C]RH-7592 on day 15. Animals were sacrificed at 4 days postexposure for all dosing groups, as well as at 1 and 6 hours, 1, 2, and 7 days postexposure for the 100-mg/kg group. There was no control group for the experiment. In the metabolism study, 100-mg/kg dosed rats were sacrificed at 7 days postexposure. The study doses were chosen based on a no-observed effect level of 10 ppm and an observed-effect level of 1000 ppm in a 2-week diet study ("86P-248" no other information provided).

3. Sample Collection

The urine and feces were collected, over dry ice, from animals at the following intervals: 0 hour and 1, 2, 3, and 4 days (also 7 days for high-dose group in the range-finding study) after exposure to the [^{14}C]-labeled dose of RH-7592. The primary study collected excreta over a 4-day period because results from the range-finding study indicated that nearly all of the radioactivity at 100-mg/kg dose was eliminated in rats within 4 days. Feces were homogenized in water and combusted in a Packard Model 306 Automatic Oxidizer (Packard Instrument Company). The metabolism cages were rinsed with distilled water and methanol, and the washing residues were collected. Urine funnels were also washed and residues were collected. Following ether anesthesia and exsanguination of rats on day 4 postexposure, major tissues (including the carcass) were weighed, homogenized, if necessary, and combusted. Analysis of the radioactivity in feces, urine, urine funnel washes, cage washings, tissues, and blood were performed by liquid scintillation counting (LSC) in duplicate. In the single-dosed groups, blood was collected at 0.5, 1, 3, and 6 hours and 1, 2, 3, and 4 days, then analyzed by LSC. Methods for statistical analyses were limited to means and standard deviations.

In the range-finding study, expired air as $^{14}CO_2$ was collected in the 100-mg/kg dosed animals. Expiration as CO_2 was 0.05% of the administered radioactivity after 7 days postexposure in the 100-mg/kg dosed rats. However, CO_2 was not collected in the primary study because of the low recovery by expiration.

4. Metabolite Analysis

In the metabolite analysis (report 2), animals were given an oral dose of 100-mg/kg [^{14}C]RH-7592. On day 7 postexposure, unextracted urine and extracted fecal samples were analyzed by high-performance liquid chromatography (HPLC) (Hewlett Packard HP 1090) and were then assayed by thin-layer chromatography (TLC) on silica gel plates (Merck Silica Gel 60-F254; 0.25 mm, no. 5715) or Whatman KC18F reverse phase plates (0.2 mm, No. 4803 800). There were seven different solvent development systems (CBI p. 19) used for elutions, followed by mass spectral analyses. The radioactivity on the TLC plates was scanned with the AMBIS Radioanalytic Imaging System Version 1.81, 2.03 (Automated Microbiology Systems, Inc., San Diego, CA) and identified with metabolite standards (CBI p. 20). Radioactive bands were quantitated by liquid scintillation spectrometry.

5. Protocols

The methods followed the study protocol.

C. REPORTED RESULTS

1. Elimination and Recovery

There were no major sex- or dose-related differences in the elimination of RH-7592 following oral dosing. In the single and repeated oral dose studies, the mean total recoveries of radioactivity ranged from 82.6 to 93.0% of the administered dose at 4 days postexposure (Table 1). In the single low-dose group, 79.00-79.20% and 6.67-7.88% of the administered dose was recovered in the feces and urine (including residues from urine funnel washes), respectively, and in the high-dose group, 75.60-76.70% and 5.46-12.60% [illegible]. In the repeated-dose [illegible] in the feces and urine was 82.3-83.7% and 7.63-9.98, respectively. Within 24 hours, 63.5-65.3% and 28.7-47.4% of the administered dose was recovered in the feces of the single low- and high-dose groups, respectively, and 59.3-61.8% in the feces of repeated low-dose group. The 7-day recoveries of the 100-mg/kg dose rats from the separate experiment indicated a total recovery of 98.6-99.3% of which 91.1-92.7% and 6.6-13.0% were contained in the feces and urine, respectively.

In the intravenous group, urine and feces contained 91.40% and 7.31% in males, respectively, and 77.20% and 10.22% in females, respectively. At 24 hours, 64.7-79.3% was recovered in the feces of the male and female rats.

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2. Tissue Distribution

The mean radioactivities in the tissues were low in the oral dosing groups. At 4 days postexposure, 0.2-0.7% of the administered dose was detected in the liver and <0.01-0.03% in the kidneys (CBI report 1, appendix I). The levels in the liver ranged from approximately 0.1 µg equiv/g in the low-dose groups to 4-5 µg equiv/g in the high-dose group. The levels in the kidney were approximately 0.01 and 0.7-1.2 µg equiv/g in the low- and high-dose groups, respectively. For the 100-mg/kg dose male rats, the kidneys contained 0.13% of the administered dose 7 days postexposure. In these high-dose animals, the tissue levels peaked at approximately 6 hours postexposure with radioactivity ranging from 3.23% to 3.82% in every tissue. Radioactivity in the other tissues were not detectable or ≤0.01% at 4 days postexposure. The carcass contained 0.3% of the administered dose in the high-dose group and was not detectable in the low-dose groups at 4 days postexposure.

Following intravenous dosing, the liver and kidney contained the highest radioactivity (0.03-0.68% of administered dose) while the other tissues had <0.01% or undetectable levels.

3. Pharmacokinetics

The radioactivity in the blood peaked at 3 hours and 3-6 hours for the single 1-mg/kg and 100-mg/kg doses, respectively. The data also appear to indicate that there is a biphasic elimination in which radioactivity is eliminated from the blood rapidly (24-48 hours postexposure), followed by a slower decline (48-96 hours). This is clearly evident in the the high-dose group (CBI report 1, figure 13 and 14).

4. Metabolism

There were several radioactive bands detected in the 7-day urine and feces of the 100-mg/kg dose rats (Table 2). The major classes of RH-7592 metabolites identified were: lactones, iminolactone, alpha alcohol, phenols, phenol lactones, ketoacids, phenol ketoacids, and sulfates. Trace amounts of RH-7592 and [illegible] were identified. The iminolactone and lactone metabolites were found in their diasteromeric forms (i.e., A and B). In the feces extract, lactone A, 4-phenol, 4-phenol lactone, and alpha alcohol represented 7.8-11.2%, 4.1-5.7%, 3.2-3.8%, and 3.9-7.6% of the total fecal radioactivity. In the urine, 3- and 4-phenol conjugates represented 17.7-19.4% and 19.1-25.9%, respectively, 3- and 4-phenol lactone conjugates represented 5.4-8.6% and 4.3-8.5%, respectively, and ketoacid represented 6.9-17.7% of the total urinary radioactivity.

Sex-related differences in fecal metabolites included 4-phenol ketoacid (5.5% in males vs. 0% in females) and sulfates (4.0% in males vs. 10.1% in females). Sex-related differences in urinary metabolites included ketoacid (17.7% in males vs. 6.9% in females) and sulfates (4.9% in males vs. 17.6% in females) Approximately 43.7-46.4% and 17.8-23% of the radioactivity in the feces and urine, respectively, represented unknown bands, smears, baselines, or nonextractable solids.

The author also used a "cold" method to look for triazole since evidence from other animal studies (not specified) suggests that this is a metabolite. Triazole was found in the urine of males (1.9% of the administered dose) and females (1.5%) by this method. The [^{14}C]-label study did not detect free triazole. In addition, the author identified less than 1% of RS-5922, a triazole cleavage product, in the urine and fecal samples by TLC. It was determined by comparison to an isolated sample from a goat metabolism study (reportedly in progress). Therefore, a minor metabolic pathway is the cleavage of RH-7592 to triazole and RS-5922.

The proposed metabolic pathway of RH-7592 is shown in Figure 1.

D. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES

The authors concluded that RH-7592 is eliminated primarily in the feces of rats. Most of the recovery of the radioactivity in the excreta occurred within 24 hours postexposure, which indicates the relatively rapid oral absorption of this chemical. There were no major sex- or dose-related differences in the rate and route of elimination of RH-7592. Biliary excretion appears to be a major route of excretion as indicated by the high fecal elimination following intravenous dosing. Four days following single and repeated oral dosing of RH-7592, the distribution of radioactivity in the rat tissues was highest in the liver and kidney compared to other tissues. Peak tissue concentration occurred at 6 hours postexposure for the high-dose group. No major sex- or dose-related differences in the tissue distribution pattern were evident. The radioactivity measurements in the blood suggest that there is dose proportionality since blood levels peak later in the high-dose group compared to the single low-dose group.

The pattern of metabolite radioactivity in the feces and urine was relatively similar for both sexes of the high-dose group. The majority of the activity is associated with known metabolites of RH-7592. The metabolites were from enzymatic oxidations at the chlorophenyl ring and at the 4- or 3-position of the phenyl ring of RH-7592, as well as subsequent nonenzymatic cylization, hydrolysis, and second oxidations. [illegible] triazole and RS-5922 is considered the minor metabolic pathway. The authors concluded that sex-related differences existed in RH-7592 metabolic pathway. The females had a higher amount of sulfate metabolites in the excreta, especially in the urine, probably due to greater ability to attach a sulfate moiety to the hydroxyl group. The ketoacid metabolite was higher in the urine of males than females, probably due to more availability of the enzyme for the second oxidation.

Quality assurance statements and statements of compliance with Good Laboratory Practices for the study were signed on August 3, 1990 (Report 1) and March 12, 1991 (Report 2). No flagging statements were listed for Report 1 or Report 2.

E. CONCLUSIONS BASED ON REVIEWERS' DISCUSSION AND INTERPRETATION OF DATA

The study adequately described the absorption, distribution, and excretion of [^{14}C]RH-7592 in rats following oral and intravenous exposure. The data indicate that labeled RH-7592 is rapidly and extensively absorbed from the gastrointestinal tract and eliminated primarily in the feces for all dosing groups. As the authors concluded, biliary excretion appears to be a major excretion route as indicated by high radioactivity in the feces of intravenous group. This suggestion is also supported by the high amount of metabolites detected in the feces rather than unabsorbed parent compound. However, it is possible that some metabolites were converted in the intestinal tract. The radioactivity in the blood of the high-dose group also suggests that biphasic elimination of radioactivity in the blood is occurring. The slightly increased elimination of radioactivity in the feces of the repeated low-dose group is probably due to saturation of the metabolic pathways. A more precise assessment of this hypothesis is not possible because of a lack of data regarding the metabolites in the single and repeated low-dose groups. Furthermore, radioactivity in the excreta should have been measured up until day 7 postexposure for higher recoveries, even though the authors stated that the range-finding study indicated recovery was practically complete within 4 days postexposure.

The low tissue levels of radioactivity, as well as the rapid elimination, at 4 days postexposure, demonstrate that bioaccumulation and retention of RH-7592 and/or its metabolites are low in rats. The metabolism of RH-7592 appears to be extensive following oral dosing since the unmetabolized parent compound accounted for a 8.5-14.8% of the recovered radioactivity in the feces and 0-2.7% in the urine. The major urinary metabolites of RH-7592 appeared to have been identified. However, the method used to analyze the radioactive components was not sensitive because approximately 50% and 20% of the total fecal and urinary activities, respectively, were not identified. As the authors concluded, major sex-related differences in metabolism of RH-7592 resulted in the formation of sulfate and ketoacid metabolites, possibly due to differences in metabolizing enzymes. The possibility of dose-related differences in the RH-7592 metabolite pattern of rats can not be assessed because metabolites were not identified in the single and repeated low-dose groups.

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FINAL

DATA EVALUATION REPORT

RH-7592

Thyroid Function and Hepatic Clearance of Thyroxine in Male Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

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Contract Number: 68D10075
Work Assignment Number: 1-75
Clement Number: 91-240
Project Officer: James E. Scott

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EPA Reviewer: S. Williams, D.V.M. Date: 7-10-92
SanYvette Williams, D.V.M.
Review Section IV, Toxicology Branch II

EPA Section Head: E. A. Doyle Date: 9/15/92
Elizabeth Doyle
Review Section IV, Toxicology Branch II

DATA EVALUATION REPORT

STUDY TYPE: Thyroid function STUDY COMPLETED: 03/04/91

EPA IDENTIFICATION NUMBERS:

Tox. Chem. Number: 723Q MRID Number: 418750-20 PC Number

TEST MATERIAL: RH-7592 SYNONYMS: None

SPONSOR: Rohm and Haas Co., Philadelphia, PA STUDY NUMBER: 90R-071

TESTING FACILITY: Rohm and Haas Co., Toxicology Dept., Spring House, PA

TITLE: Thyroid Function and Hepatic Clearance of Thyroxine in Male Rats

AUTHORS: Hazleton G. A., DiDonato L. J., Quinn D. L., Shade W. D., and Frantz J. D.

CONCLUSIONS: Male rats (10-20/group) were given diets containing 0, 8, 800, 1600, or 3200 ppm of test substance for 4 or 13 weeks. Additional groups received 1600 or 3200 ppm for 4 weeks followed by 9 weeks of normal diet. The control group received normal rat chow. The study authors calculated the mean daily intake to be 1, 57, 116, and 231 mg/kg/day for the 8-, 800-, 1600- and 3200-ppm dose groups, respectively. These calculations appear to overestimate the intake by about 10% since intake estimates were not used for weeks 6, 7, 9, 10, 11, or 12.

Thyroid function was affected and had the following NOEL and LOEL:
NOEL = 8 ppm no treatment-related effects
LOEL = 800 ppm (based on increased liver and thyroid weights, diffuse thyroid hyperplasia and increased TSH levels).

The increased incidence and severity of effects beginning at 800 ppm were dose-related. Body weights were decreased at 1600 and 3200 ppm throughout the duration of the study, and serum levels of T_4 were decreased at 1600 and 3200 ppm at week 13. Hepatic clearance of T_4 was tested in the control and 3200-ppm groups. The 3200-ppm group had increased biliary excretion of T_4, primarily as the glucuronide, and hepatic UDP glucuronosyltransferase activity with T_4 was increased.

CORE CLASSIFICATION: Supplementary based on design and intent.

A. MATERIALS AND METHODS

1. Test Material RH-7592 (purity 97.1%; lot number BPP3-1786R) was dissolved in acetone and mixed in food every 2 weeks. The animals were administered dose levels of 8, 800, 1600, or 3200 ppm for 4 and 13 weeks. The recovery groups were fed 1600 or 3200 ppm for 4 weeks followed by 9 weeks of basic diet.

2. Animals Male Crl:CDBR rats were individually housed in a room with controlled humidity, temperature and lighting. See Table 1 for the numbers of animals used. Animals were randomly assigned to groups so that the body weights were statistically equivalent. Animals were 6 weeks old and weighed 209-261 grams at the initiation of study. Animals were provided with the test substance mixed in Purina® Certified (#5002) rat chow, and filtered tap water was provided ad libitum. The control group consisted of 40 male rats (20 necropsied after 4 weeks, 20 necropsied after 13 weeks) that received 0 ppm of the test substance in the diet that was prepared with the same amounts of acetone (vehicle) as used in the high-dose group.

3. Diet Analysis

The diets were prepared by weighing the appropriate amounts of test substance, dissolving it in acetone and blending with untreated feed in an open Hobart mixer to evaporate the acetone. The final weights were achieved by adding the appropriate weights of untreated feed to the premix and blending for 15 minutes in a cross-flow blender. Control diets were prepared the same way, using only acetone. Fresh diets were prepared every 2 weeks.

Samples from the top, middle, and bottom of each dose level were submitted for analysis of active ingredient the first time they were prepared. The feed mixing procedures were determined to be satisfactory (variation between top, middle, and bottom layers was ≤10% at all dietary levels except 8 ppm; subsequent analysis for stability indicated that the 8-ppm level provided satisfactory concentrations of the test material). Analysis of the diet stored in an open container at room temperature for 7-8 or 17 days indicated that the product was stable for at least 17 days. Stability analyses were conducted on weeks 1, 3, 7 and 13 [illegible] material obtained from the stability analysis, expressed as a percent of target, ranged from 92 to 110%. Individual concentration determinations ranged from 77.6% to 122% of target. Quantification was by gas chromatography.

4. Statistics

An ANOVA model was used to test differences in body weight, food consumption, liver and thyroid organ weight, and serum thyroid hormone levels among the groups. If differences were significant, a Dunnet's t-test was used to compare the mean differences between the treated and control groups. The recovery groups (treated for 4 weeks and given normal food for 9 weeks) were compared to the treated groups that received the same doses for the full 13 weeks. No statistical analysis was used to compare recovery groups to controls. Histopathological data were not analyzed statistically.

For biliary excretion parameters, Student's t-tests were used to compare the values from the 3200-ppm group to the control group at each time period. For hepatic UDP-glucuronosyltransferase activity with L-thyroxine, values measured in animals fed 3200 ppm (13 weeks) were compared to controls. To determine the effects of the recovery period, Student's t-test was used to compare values from animals fed 3200 ppm (4 weeks with 9-week recovery period) to values from animals fed 3200 ppm for the full 13 weeks.

5. Data Reporting

Tables of individual data are provided for body weight, food consumption, and compound intake, measured weekly for the first 4-5 weeks, and on weeks 8 and 13. Means and standard deviations, and statistical analyses are provided in the summary tables. Tables of individual data and summary tables are also provided for liver and thyroid organ weights, thyroid histopathology, thyroid hormone concentrations, bile:plasma concentrations, bile flow, bile clearance, glucuronide excretion, and hepatic UDP-glucuronosyltransferase activity with L-thyroxine activity. The appendices also contained individual data for serum GOT and GPT levels, and summary tables for gross pathology and clinical observations.

B. METHODS AND RESULTS

1. Clinical Observations

No treatment-related mortalities occurred. The rats were observed daily for overt signs of toxicity and were examined more closely at weeks 1, 5, 8, and 13. The only consistent, abnormal clinical sign was "squinting of the eyes" observed in 48/60 animals in the 3200-ppm exposure groups. This effect was not observed among control animals or among animals receiving other dietary concentrations.

2. Body Weight

Body weights did not differ from controls in the groups that received doses of 8 or 800 ppm for 4 or 13 weeks. Body weights at the end of the 13-week period were decreased by 7% in the 1600-ppm group and by 22% in the 3200-ppm group. Statistically significant decreased body weights occurred at week 1 for these 2 groups. After 13 weeks, the differences between the control and recovery groups were only 2% in the 1600-ppm group and 10% in the 3200-ppm group. See Table 2.

Body weight gains were not reported.

3. Food Consumption and Compound Intake

Food consumption data were comparable to controls for the 8- or 800-ppm groups. There was a statistically significant decrease in food consumption, relative to controls, for the 1600-ppm group (17, 6, 7, and 6% for the first 4 weeks, respectively); and for the 3200-ppm group for the first 5 weeks (56, 36, 27, 24, and 16%, respectively), and week 8 (13%). All groups had normal food consumption on week 13.

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Compound intake (measured as mg compound/kg body weight/day) decreased throughout the study for the three lowest dose groups, and varied for the high-dose group. Authors state that the compound intake (time-weighted average) was calculated to be 1, 57, 116, and 231 mg/kg/day, respectively, for the four treated groups. This was calculated by averaging data from weeks 1, 2, 3, 4, 5, 8, and 13 only.

Since the compound intake decreased over the study, not using the intake from weeks 6, 7, 9, 10, 11, or 12 would lead to an overestimation of the mean intake for all 13 weeks. Including estimates of mean intakes for these weeks, the average intake for the full duration of the study was estimated by the reviewer to be about 10% less than those provided by the study authors.

Food consumption in the 3200-ppm recovery group was significantly greater than the 3200-ppm test group during the recovery period (at weeks 5, 8, and 13) and appeared to be comparable to controls.

4. Thyroid Function Tests

Blood samples were collected at necropsy for 10 animals/group. Serum samples were evaluated by radioimmunoassay for T_4 (L-thyroxine), T_3 (L-triiodothyronine, rT_3 (reverse triiodothyronine), and TSH (thyroid-stimulating hormone). Table 3 shows the serum thyroid hormone concentrations.

As compared to controls, after 4 weeks of treatment, TSH levels were statistically significantly increased in the groups treated with 800 ppm and above. T_4 levels were lower than those of controls in the 800- and 1600-ppm groups, but a significant decrease only occurred in the 3200-ppm group. T_3 had no significant changes, and rT_3 was significantly decreased in the 3200-ppm group.

After 13 weeks, TSH increased significantly in the 3200-ppm group; T_4 significantly decreased in the 1600 and 3200-ppm groups, and T_3 and rT_3 were not affected by treatment. In the recovery groups (fed 1600 or 3200 ppm for 4 weeks, and normal diet for 9 weeks), T_4 and T_3 levels increased to levels that were higher than the group fed 1600 or 3200 ppm for the full 13 weeks and appeared to be close to control levels. In the recovery group receiving 1600 ppm, TSH was reduced as compared to the group that were fed 1600 ppm for the full 13 weeks, but still higher than controls. The 3200-ppm recovery group was lower than the controls as well as the 3200-ppm (13-week) group.

5. Gross and Histopathology

(a) Thyroid Organ Weights

Organ weights were measured in 10 animals/group. After 4 weeks of treatment, there was a statistically significant increase in relative thyroid weights in the 1600-ppm group and 3200-ppm group, and the absolute thyroid weights were increased in the group receiving 1600 ppm but not in the 3200-ppm group. After 13 weeks, absolute and relative thyroid weights were increased at treatment doses of 800, 1600, and 3200 ppm. Table 4 shows the thyroid weights

and percent change from controls.

(b) Thyroid Histology

Tissues from 10 animals/group were studied. Photomicrographs showing representative histopathology from each of the groups were provided. The severity was categorized into four groups: minimal, slight, moderate, and moderately severe. Table 5 shows the incidence of hypertrophy.

For the animals treated for 4 weeks, there was dose-related increase in the incidence and severity of diffuse thyroid hypertrophy/hyperplasia. The changes were minimal in the 0- and 8-ppm groups, and slight to moderate in the 3200 ppm group. One animal in each of the top two doses had focal hyperplasia. Focal hyperplasia was not observed in the controls.

After 13 weeks of treatment, diffuse hypertrophy/hyperplasia was noted in all groups, including controls (6/10 affected); however, the severity increased with the dose. Changes were minimal to moderate in the 0- and 8-ppm groups and moderate to moderately severe in the 3200-ppm group. Focal hyperplasia occurred in one animal in the 3200-ppm group and in one animal in the 1600-ppm recovery group.

(c) Liver Organ Weights

After 4 and 13 weeks of treatment, there were statistically significant increases in absolute and relative liver weights at doses of 800 ppm and above. The groups that were treated for 4 weeks with a 9-week recovery period had significantly decreased liver weights as compared to the test animals that were treated for 13 weeks without a recovery period. The weights in the recovery groups were similar to those of the control animals. See Table 6.

6. Liver Toxicity as Indicated by Serum Enzymes

Activities of serum enzymes indicative of liver toxicity (serum glutamic pyruvic transaminase and serum oxaloacetic transaminase) were evaluated in the control and in animals treated with [illegible] for 13 weeks. Individual data and means and standard deviations are shown in Appendix 12 of the study. No other clinical chemistry parameters were measured. Values in the treated groups were similar to those in controls.

7. Biliary Excretion of T_4

Biliary excretion parameters were monitored in controls and 3200-ppm groups after 4-7 and 13-15 weeks of treatment. The anesthetized animals were cannulated in the bile duct. ^{125}I-T_4 was injected into the jugular vein. Bile samples were collected in 30-minute segments for the first two hours and then in 1-hour segments for the final 2 hours. Plasma samples were obtained via the orbital sinus. The recovered radioactivity was measured in a gamma counter and the percent of labeled T_4 excreted as the glucuronide conjugate was determined by thin-layer chromatography.

The bile:plasma ratio and total bile flow was increased in the 3200-ppm group

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at all time periods measured. Bile clearance (bile:plasma ratio multiplied by bile flow) increased for all time periods at both 4 and 13 weeks As compared to controls, all increases were found to be statistically significant. The recovery group (exposed to 3200 ppm for 4 weeks, followed by 9 weeks on a normal diet) had bile:plasma ratios, bile flow, and bile clearance rates that were less (statistically significant) than the 3200-ppm (13-week) group and appeared to be close to control levels.

The percent of the radiolabeled dose of ^{125}I which was excreted as the radiolabeled T_4-glucuronide was increased ($p < 0.05$) in the treated animals (3200 ppm for 4 or 13 weeks) as compared to the controls. See Table 7. The 3200-ppm recovery group was similar to controls.

Note: Two control animals died as the result of surgery at the 4-week test, and were replaced with controls from another study. This does not appear to affect the results of the study.

8. In vitro UDP Glucuronosyltransferase Activity

Hepatic UDP-glucuronosyltransferase activity with L-thyroxine was measured in the controls, 3200-ppm and 3200-ppm recovery groups from 6 animals/group. After 4 or 13 weeks, hepatic UDP-glucuronosyltransferase activity with L-thyroxine was increased ($p < 0.05$) when measured as activity/mg protein (54% at 4 weeks, 25% at 13 weeks); activity/gram liver (187% at 4 weeks, 144% at 13 weeks); or activity/whole liver (336% at 4 weeks, 300% at 13 weeks). The 3200-ppm recovery group had values similar to controls. See Table 8.

C. CONCLUSIONS

Study authors conclude that the thyroid follicular cell hypertrophy/hyperplasia (noted in the 1600- and 3200-ppm groups after 4 and 13 weeks) may have been due to over stimulation of the thyroid gland. The overstimulation could have been caused by increased levels of TSH (noted in the 3200-ppm group after 13 weeks), which could be due to rapid removal of T_4. T_4 would have been removed by increased hepatic metabolism (glucuronidation as indicated by the excretion of radiolabeled T_4-glucuronide, rapidly metabolized hepatic UDP-glucuronosyltransferase activity with L-thyroxine) and increased biliary clearance (220-336% of control levels for animals treated for 13 weeks). The data appear to support these conclusions.

D. COMPLIANCE

A signed and dated Quality Assurance Statement was presented.
A signed GLP Statement was included.
A signed and dated No Data Confidentiality Claim was included.
A No Flagging Statement was included.

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Table 1. Number of male rats exposed to RH-7592 in the diet

	Dietary level (ppm)						
	0	8	800	1600	3200	1600R	3200R
4 Weeks	20	10	10	20	20	20	20
13 Weeks	20	10	10	20	20	-	-

R = Rats in the recovery groups that were exposed for 4 weeks, received a normal diet for another 9 weeks, and were sacrificed at end of the total 13-week period.

Table 2. Mean body weights at 13 weeks in rats[a] exposed to RH-7592

	Dietary level (ppm)						
	0	8	800	1600	3200	1600R	3200R
Weight (grams)	575.3	568.9	561.8	537.5*	447.7*	564.5@	518.7@
% decrease[b]	-	1	1	7	22	2	10

R = Rats in the recovery groups that were exposed for 4 weeks, received a normal diet for another 9 weeks, and were sacrificed at end of the total 13-week period.

[a] 10 rats/group at 0, 8, and 800 ppm; 20 rats/group at 1600 and 3200 ppm.
[b] Percent decrease relative to controls as calculated by reviewer.
* Statistically significantly different from control group ($p < 0.0142$)
@ Statistically significant recovery ($p < 0.05$) as compared to animals that received the same doses for 13 weeks.

Table 3. Serum thyroid hormone concentrations in male rats exposed to RH-7592**

	Dietary level (ppm)						
	0	8	800	1600	3200	1600R	3200R
4 Weeks							
TSH (μU/ml)	212	202	380*	389*	436*		
T_4 (μg/ml)	7.21	6.12	5.71	6.19	3.39*		
T_3 (ng/dl)	104	93	104	102	96		
rT_3 (pg/ml)	148	111	145	146	65*		
13 Weeks							
TSH (μU/ml)	102	88	115	161	167*	149@	81@
T_4 (μg/ml)	4.87	4.40	4.47	3.19*	2.38*	5.01@	5.16@
T_3 (ng/dl)	68	74	70	75	72	84@	86@
rT_3 (pg/ml)	73	61	74	67	38[a]	71	23[a]

[a] This value appears to be significantly decreased from the control value; however, it was not indicated as such in the study report.
* Statistically significant from control group ($p < 0.01$).
@ Statistically significant recovery ($p < 0.05$) as compared to animals that received the same doses for 13 weeks.
** Table excerpted from page 39 of the study.

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Table 4. Thyroid weights in male rats (10/group) exposed to RH-7592**

	Dietary Level (ppm)						
	0	8	800	1600	3200	1600R	3200R
4 Weeks							
Absolute (mg)	25	23	27	34*	27		
% Increase[a]	-	-	8	36	8		
Relative (mg/kg)	62	53	62	83*	91*		
% Increase	-	-	0	34	47		
13 Weeks							
Absolute (mg)	32	34	42*	45*	43*	38@	31@
% Increase	-	6	31	41	34	19	-3
Relative (mg/kg)	57	61	74*	84*	95*	65@	60@
% Increase	-	6	30	47	67	14	5

* Significantly different from control group ($p < 0.0149$)
@ Statistically significant recovery ($p < 0.05$)
[a]Percent increase relative to controls as calculated by reviewer.
** Table excerpted from pages 34 and 35 of the study.

Table 5. Incidence of thyroid hyperplasia in male rats (10/group) exposed to RH-7592*

	Dietary Level (ppm)						
	0	8	800	1600	3200	1600R	3200R
4 Weeks							
Diffuse	1	2	4	9	10		
Focal	0	0	0	1	1		
13 Weeks							
Diffuse	6	5	9	10	10	6	8
Focal	0	0	0	0	1	1	0

*Table excerpted from pages 36 and 37 of the study.

Table 6. Liver weights in male rats (10/group) exposed to RH-7592**

	Dietary Level (ppm)						
	0	8	800	1600	3200	1600R	3200R
4 Weeks							
Absolute (mg)	18.34	18.38	22.21*	24.36*	24.89*		
% Increase[a]		-	21	33	36		
Relative (mg/kg)	44.65	44.27	51.84*	60.12*	82.51*		
% Increase[a]		-	16	33	85		
13 Weeks							
Absolute (mg)	20.42	19.30	24.63*	29.65*	31.19*	21.08@	19.20@
% Increase[a]		-	21	53	53	3	-6
Relative (mg/kg)	35.94	33.95	43.52*	54.25*	69.13*	36.52@	36.36@
% Increase[a]		-	21	51	92	2	1

* Significantly different from control group ($p < 0.0149$)
@ Statistically significant recovery ($p < 0.05$)
[a]Percent increase relative to controls as calculated by reviewer.
** Table excerpted from pages 34 and 35 of the study.

Table 7. Biliary Excretion of T_4 in RH-7592 Treated Rats (3200 ppm, 6-10/group)*

	Time Period in Minutes					
	0-30	30-60	60-90	90-120	120-180	180-240
			Bile:Plasma Ratios			
4 Weeks						
Control	0.41	0.84	1.05	1.28	1.29	1.17
RH-7592	0.61	1.79	2.31	2.61	2.64	2.44
% increase	49%	113%	120%	104%	105%	109%
13 Weeks						
Control	0.28	0.70	0.92	1.00	1.01	1.00
RH-7592	0.50	1.65	2.16	2.48	2.44	2.53
% increase	79%	136%	135%	148%	142%	153%
			Bile Flow (ml/hr/kg)			
4 Weeks						
Control	2.46	2.56	2.75	2.53	2.58	2.34
RH-7592	4.36	4.27	4.43	4.68	4.20	3.27
% increase	77%	67%	61%	85%	63%	40%
13 Weeks						
Control	1.83	2.04	2.24	2.19	2.11	2.17
RH-7592	3.20	3.61	3.35	3.62	3.15	3.52
% increase	75%	77%	50%	65%	50%	62%
			Biliary Clearance (ml/hr/kg)			
4 Weeks						
Control	1.01	2.18	2.69	3.27	3.32	2.74
RH-7592	2.68	7.77	10.13	12.45	11.33	8.36
% increase	165%	256%	250%	281%	241%	205%
13 Weeks						
Control	0.51	1.38	2.00	2.07	2.04	2.10
RH-7952	1.63	6.02	7.27	8.94	7.61	8.58
% increase	220%	336%	264%	332%	273%	308%

* Table excerpted from pages 40-45 of the study.

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Table 8. Hepatic UDP-Glucuronosyltransferase Activity with L-Thyroxine in Control and RH-7592 Treated Rats (3200 ppm, 6 rats/group)**

Treatment Group	Liver Weight (g)	Activity (nmol/min) per mg Protein	Activity (nmol/min) per gram Liver	Activity (nmol/min) per Whole Liver
4 Weeks				
Control	17.1±0.8	0.013±0.001	0.30±0.02	5.2±0.5
RH-7582	26.8±1.4*	0.020±0.003*	0.86±0.11*	22.7±2.5*
% Increase	57%	54%	187%	336%
13 Weeks				
Control	19.5±0.9	0.012±0.001	0.27±0.19	5.2±0.4
RH-7592	31.9±1.2*	0.015±0.001*	0.66±0.04*	20.8±1.1*
% Increase	64%	25%	144%	300%

* Significant difference from control group ($p < 0.05$)
** Table excerpted from pages 48-49 of the study.