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

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FEB 10 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: **FENBUCONAZOLE:** Review of a Metabolism Study

FROM: SanYvette Williams-Foy, D.V.M. 2/8/94
Review Section IV, Toxicology Branch II (H7509C)

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

THRU: Jess Rowland, M.S., Acting Section Head 2/8/94
Section IV, Toxicology Branch II (H7509C)

and

Marcia van Gemert, Ph.D., Chief concur 2/10/94
Toxicology Branch II
Health Effects Division (H7509C)

EPA IDENTIFICATION NUMBERS:
PC Code: 129011
DP Barcode: D194780
MRID #: 429008-01
Submission #: S447326

Registrant: Rohm and Haas Company

[redacted] metabolism study in order to fulfill data requirements studies that had been classified as Core-supplementary.

Response: A copy of the Data Evaluation Report is attached. An Executive Summary is provided below:

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EXECUTIVE SUMMARY: The absorption, distribution, metabolism, and excretion of RH-7592 were studied in groups of Crl:CD BR rats (3-5/sex/group) administered a single oral gavage dose of 1 or 100 mg/kg ^{14}C-RH-7592 or administered 1 mg/kg/day unlabeled RH-7592 (in the diet) for 14 days followed by a single dose of 1 mg/kg ^{14}C-hydrogen cyanamide on day 15. [MRID #: 429008-01]

The study demonstrated that radiolabeled RH-7592 is rapidly absorbed, distributed, and excreted following oral administration in rats. Total 3- or 4-day recoveries of the radioactivity were high for all groups [90.40-104.49% of the administered dose]. Biliary excretion data indicated that systemic absorption of RH-7592 was high for all dosing groups. The feces was the major route of excretion [78.74-94.43% of administered dose] after 4 days post-dosing, while recovery in the urine was low [<1% of the administered dose]. Tissue distribution and bioaccumulation of RH-7592 appeared to be minimal since <1% of the administered dose was recovered in tissues 4 dose after oral administration for all dosing groups. No sex- or dose-related differences in absorption, distribution, or elimination were found. Metabolism of RH-7592 was extensive as shown by the numerous metabolites characterized and isolated in the feces, bile, and urine. Furthermore, a dose-related difference in metabolism was evident. The higher amount of unmetabolized parent compound in the feces of the high-dose group compared to the low-dose and repeated-dose groups suggested that saturation of the metabolic pathway may be occurring at the high dose.

This study is classified as Core-Guideline. This study alone satisfies the guideline requirement for a metabolism study [#85-1] in rats. It fulfills the guideline requirement for oral low-dose, high-dose, and repeated-dose studies.

The study was conducted to upgrade a previously submitted metabolism study [MRID #: 418750-17 and -18]. The results were similar for the two studies. In the previous metabolism study, metabolite analysis data for the low-dose and repeated-dose groups were not provided; however, this study has addressed and satisfied this deficiency.

FINAL

DATA EVALUATION REPORT

FENBUCONAZOLE
(RH-7592)

Study Title:
^{14}C-RH-7592: Disposition and Elimination Study in Rats

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

December 13, 1993

Principal Reviewer: Karen N. Gan, M.S. Date 1/25/94

Independent Reviewer: William McLellan, Ph.D. Date 1/25/94

QA Reviewer: John Liccione, Ph.D. Date 1/25/94

Contract Number: 68D10075
Work Assignment Number: 3-39
Clement Number: 172
Project Officer: Caroline Gordon

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RH-7592 Metabolism Study (85-1)

EPA Reviewer: SanYvette Williams-Foy, D.V.M. SW Foy D.V.M. Date 2/2/94
Review Section IV, Toxicology Branch II (7509C)
Acting EPA Section Head: Jess Rowland, M.S. Date 2/3/94
Review Section IV, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism - Rat (85-1)

TOX CHEM. NUMBER: 723Q

P.C. CODE: 129011

MRID NUMBER: 429008-01

TEST MATERIAL: RH-7592; Fenbuconazole

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

REPORT NUMBER: 92R-060

SPONSOR: Rohm and Haas Company, Toxicology Dept., Spring House, PA

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

TITLE OF REPORT: ^{14}C-RH-7592: Disposition and Elimination Study in Rats.

AUTHORS: L.J. DiDonato and G.A. Hazelton

REPORT ISSUED: August 6, 1993

EXECUTIVE SUMMARY: The absorption, distribution, metabolism, and excretion of RH-7592 were studied in groups of Crl:CD®BR rats (3-5/sex/group) administered a single oral gavage dose of 1 or 100 mg/kg ^{14}C-RH-7592 or administered 3 mg/kg/day unlabeled RH-7592 (in the diet) for 14 days followed by a single dose of 1 mg/kg ^{14}C-hydrogen cyanamide on day 15.

The study demonstrated that radiolabeled RH-7592 is rapidly absorbed, distributed, and excreted following oral administration in rats. Total 3- or 4-day recoveries of the radioactivity were high for all groups (90.40-104.49% of the administered dose). Biliary excretion data indicated that systemic absorption of RH-7592 was high for all dosing groups. The feces was the major route of excretion (78.74-94.43% of administered dose) after 4 days postdosing, while recovery in the urine was low (<1% of the administered dose). Tissue distribution and bioaccumulation of RH-7592 appeared to be minimal since <1% of the administered dose was recovered in tissues 4 days after oral administration for all dosing groups. No sex- or dose-related differences in absorption, distribution, or elimination were found. Metabolism of RH-7592 was extensive as shown by the numerous metabolites characterized and isolated in the feces, bile, and urine. Furthermore, a dose-related difference in metabolism was evident. The higher amount of unmetabolized parent compound in the feces of the high-dose group compared to

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the low-dose and repeated-dose groups suggested that saturation of the metabolic pathway may be occurring at the high dose.

This study is classifie as Core-guideline. This study alone satisfies the guideline requirement for a metabolism study (85-1) in rats. It fulfills the guideline requirement for oral low-dose, high-dose, and repeated-dose studies.

The study was conducted to upgrade a previously submitted metabolism study (MRID No. 418750-17, 418750-18; Tox. Chem. No. 723-Q; HED number 1-2499). The results were similar for the two studies. In the previous metabolism study, metabolite analysis data for the low-dose and repeated-dose groups were not provided; however, this study has addressed and satisfied this deficiency.

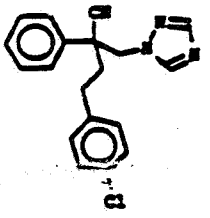
Special Review Criteria (40 CFR 154.7): None.

A. MATERIALS

1. Test compound:

Description	Unlabeled RH-7592	^{14}C-RH-7592
Purity	96.7 - 99.97%	98.7%
Specific Activity	Not applicable	25.6 mCi/g
Position of Radiolabel	Not applicable	phenyl ring

The structure and radiolabel position of RH-7592 are shown below:



uniformly labeled with ^{14}C on the phenyl ring

2. Vehicle: Aqueous 5% methylcellulose

3. Test animals:

Species: Rat
Strain: Charles River Crl:CD®BR
Age and weight at study initiation: Not reported
Source: Charles River Kingston, Stone Ridge, NY
Housing: Individually in Nalgene metabolism cages; rats in groups E and F were housed individually in metal hanging cages during the dietary pretreatment phase.
Environmental conditions:

Temperature: 73±2°F
Humidity: 40-60%
Air changes: Not reported
Photoperiod: 12 hour dark/light
Acclimation period: at least 3 days

4. Preparation of dosing solutions:

All ^{14}C-labeled dosing solutions were prepared on the day of dosing. The solutions contained nonlabeled and labeled RH-7592 suspended in 0.5% methylcellulose and then sonicated prior to use. The labeled dose solution was administered by gavage at 5 mL/kg to rats. Although the stability of the dosing solutions was not reported, the test material is not volatile and probably is relatively stable.

For the repeated-dosing group, unlabeled RH-7592 was dissolved in acetone, mixed with a small amount of untreated feed, and blended to evaporate the acetone. This mixture was weighed and then mixed with additional untreated feed and blended for 15 minutes.

B. STUDY DESIGN

A total of 52 rats was used in the study. The study was designed to determine the absorption, distribution, metabolism, and elimination of labeled RH-7592 after oral administration to rats. The test groups and dose levels used in the study are shown below.

Group	Dose level (mg/kg)	Route of Administration	Number of animals	Time of Sacrifice
A	1	single (low)	5M	4 days
B	1	single (low)	5F	4 days
C	100	single (high)	5M	4 days
D	100	single (high)	5F	4 days
E	1	repeated	5M	4 days
F[a]	1	repeated	5F	4 days
G	1	single (low)	3M	3 hours
H	1	single (low)	3F	3 hours
I	1	single (low)	3M	12 hours
J	1	single (low)	3F	12 hours
K[b]	1	single (bile)	5M	3 days
L[b]	1	single (bile)	5F	3 days

[a]Animals received unlabeled RH-7592 (10 ppm) in the diet for 14 days. On day 15 of the study, the animals were administered labeled test material as a single oral dose.
[b]Surgery was performed prior to dosing to insert 2 cannulas, one in the bile duct to collect bile and one in the duodenum for infusion of taurocholic acid to replace bile salts. Once a consistent bile flow was established, these animals each received a single oral dose of the labeled test material.

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C. TREATMENT OF ANIMALS AND ANALYSIS OF EXCRETA & TISSUES

1. Oral Administration

Urine and feces were collected, over dry ice, at 0 and 6 hours and 1, 2, 3, and 4 days after administration for ^{14}C-analysis; remaining excreta samples were stored frozen for ^{14}C-metabolite analysis. Urine and fecal samples were analyzed for radioactivity by combustion and liquid scintillation counting (LSC).

The animals in Groups G and H were sacrificed at 3 hours postdosing (when plasma ^{14}C-concentrations were at peak levels), and the animals in Groups I and J were killed at 12 hours postdosing (when plasma ^{14}C-concentrations were 1/2 of peak levels). Urine and feces in these animals were collected as described above.

Urine funnel washes also were collected at 3 hours (Groups G and H), 12 hours (Groups I and J), and 3 days (Groups K and L).

2. Biliary Excretion Study (Groups K and L)

Bile was collected at 0, 6, and 12 hours and 1, 2, and 3 days following administration of ^{14}C-RH-7592 and then analyzed for ^{14}C radioactivity by LSC.

3. Tissue Distribution Study

Four days after administration (Groups A-F) or three days after administration (Groups K and L), animals were sacrificed and the tissues were dissected out for radioanalysis. Animals also were sacrificed after 3 hours (Groups G and H) and 12 hours (Groups I and J) for tissue radioanalysis. Collected tissue samples included: blood, liver, fat, kidney, bone marrow, heart, lungs, brain, testes/ovaries, muscle, spleen, adrenals, and thyroids. In addition, the carcasses (including gastrointestinal tract contents were collected for radioanalysis. Radioactivity in samples was determined by tissue combustion and LSC.

4. Identification of Metabolites

Urine and feces samples from Groups A-F and bile samples from Groups K and L were analyzed for ^{14}C-metabolite analysis (Study Report, Appendix E, p. 149[1]). Feces samples were pooled, homogenized, and then extracted with methanol, ethyl acetate, and/or butanol. Post extraction solids (PES) were hydrolyzed with HCl. Similarly, urine samples also were extracted directly with ethyl acetate and butanol. Urine and bile samples were subjected to C18 solid phase extraction (preconditioned with methanol and water).

Concentrated extracts that contained high levels of radioactivity were subjected to reverse-phase high-performance liquid

[1]Liu, D.D.W. 1993. ^{14}C-RH-7592: Disposition and Elimination Study in Rats. Performed by XenoBiotic Laboratories, Inc., for Rohm and Haas Company. TR No. 34-93-52. XBL Report No. RPT00130. July 16, 1993. 263 pp.

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chromatography (HPLC). Thin-layer chromatography (TLC) (two solvent systems) was used to identify and characterize ^{14}C-RH-7592 and its metabolites. Reference standards were used for comparison with isolated radioactive components. Radioactivity in solid samples was determined by combustion, while radioactivity in liquid samples was determined by LSC.

Enzyme hydrolysis (with β-glucuronidase, aryl sulfatase) was performed on isolated metabolites and HPLC eluates. Some of the isolated metabolites also were derivatized with trimethylsilyl (TMS). Isolated metabolites were analyzed by gas chromatography (GC) with flame ionization detector and radioactivity monitor, GC/mass spectrometry (MS), direct insertion probe/MS, and/or fast atom bombardment/MS.

D. STATISTICS

Statistical analyses were limited to calculations of means, standard deviations, and pharmacokinetic calculations, as appropriate.

E. QUALITY ASSURANCE

A signed and dated quality assurance statement was present.
A signed and dated GLP statement was present.

QC dated August, 6, 1993; GLP dated 8/23/93

F. RESULTS

1. Urinary and fecal excretion

As shown in Table 1, absorption and elimination of ^{14}C-RH-7592 were rapid and occurred primarily in the feces. The 3- to 4-day recovery of radioactivity was 88.14–104.49% of the administered dose. In both the low and high dose groups, the ^{14}C label was found predominantly in the feces (78.74–94.43% of the administered dose). Most of the dose was recovered within the first 2 days after administration (>80% of the administered dose for Groups A–F). Recoveries at 3 and 12 hours postdosing was 71–103% of the administered dose following a single dose of 1 mg/kg (Groups G–J), with most of the radioactivity found in the tissues and carcass.

2. Biliary Excretion

Three days after administration of 1 mg/kg ^{14}C-RH-7592 to rats with cannulated bile ducts (Groups K and L), total recovery in the excreta and tissues was 95–98% of the administered dose (Table 1). Recovery of ^{14}C radioactivity in the bile was rapid; 79–87% of the administered dose in the bile by 3 days postdosing, with most of the radioactivity collected within 24 hours postdosing. The feces, urine, and carcass contained 6–7%, 3–7%, and 1–2% of the administered dose, respectively, at 3 days postdosing. Therefore, these data indicate that most of an oral dose of RH-5792 is absorbed systemically.

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3. Tissue Distribution

RH-7592 or its metabolites do not appear to bioaccumulate in blood or tissues of rats, and no sex- or dose-related differences were found in the tissue distribution of RH-7592. As shown in Table 1, the percentage of dose in all tissues 3 or 4 days after dosing was <0.01% for all dosing groups. At 4 days, radioactivity concentrations ranged from 0.001 ppm to 0.19 ppm in the low-dose groups and 0.1 ppm to 4.2 ppm in the high-dose groups; the highest tissue levels were found in the liver (0.08-4.217 ppm). In the low-dose animals, the amount of radioactivity in the tissues was higher at 3 hours (Groups G and H) and 12 hours postdosing (Groups I and J) than at 4 days postdosing. A large amount of the radioactivity in the carcass was represented by the gastrointestinal tract content, indicating that RH-7592 was rapidly eliminated in the bile.

4. Metabolites

A summary of the metabolites of RH-7592 in the urine and feces of rats following oral administration of RH-7592 is shown in the appendix of this report. Metabolites were identified in the ethyl acetate, butanol, aqueous, and PES fractions of extractions. RH-7592 and Phase I metabolites were detected in the ethyl acetate extracts, glucuronide and sulfate conjugates were found in the butanol extracts, and polar metabolites were detected in the aqueous fractions. The ethyl acetate fractions contained nonconjugated nonpolar metabolites, including lactone A, lactone B, unmetabolized RH-7592, iminolactone A, 3-phenol lactone, phenol lactone A, ketone, iminolactone B, m-chlorophenol, 4-phenol, and keto-m-chlorophenol. The butanol fractions contained conjugated polar metabolites, such as 3-phenol and/or m-chlorophenol derivatives, sulfate and/or glucuronide conjugate of benzylic alcohol, glucuronide conjugate of keto-m-chlorophenol, glucuronide conjugates of saturated and/or unsaturated keto-m-chlorophenol and of dihydrodiol, glucuronide conjugates of 4-phenol.

In the feces, 75.16-100.79% of the administered dose was characterized for Groups A-J. The ethyl acetate fractions contained 48.85-68.81% of the administered dose for all dosing groups. The major metabolites in this fraction included unmetabolized RH-7592, benzylic alcohol, 4-phenol, dihydrodiol, and an acid-related metabolite F16F. Higher levels of the parent compound in the high-dose group (20.58-36.68% of the administered dose) compared to the low-dose and repeated-dose groups (2.18-4.57% of the administered dose) suggest that saturation of metabolic enzymes is occurring at the high dose. Furthermore, the amounts of some major fecal metabolites in the high-dose group were slightly less than those in the low-dose and repeated-dose group. The butanol fractions contained 5.77-14.18% of the administered dose (mostly lactone A, lactone B, RH-7592, keto-phenol, iminolactone, phenol, and metabolite F16F). The aqueous fractions contained 0.92-2.57% of the administered dose, represented by polar metabolites that could not be analyzed due to low percentages of radioactivity present. The PES-1 fractions contained 9.92-22.85% of the administered dose, consisting mostly of lactone A, lactone B, keto-phenol, acid-related metabolite F16F, and unmetabolized RH-7592.

In the urine, 7.28-11.70% of the administered dose was characterized for Groups A-F. The ethyl acetate fractions contained 2.42-6.64% of the administered dose for all dosing groups; mostly hydroxy-dihydrodiol, dihydrodiol, and acid-related metabolite F16F, keto-m-chlorophenol. The butanol fractions contained 2.13-4.60% of the administered dose (mostly sulfate and glucuronide conjugates). Aqueous fractions contained 0.71-2.57% of the administered dose.

The bile contained mostly conjugated metabolites (in butanol extracts), with the majority being glucuronide conjugates. The majority of the metabolites identified were benzylic alcohol conjugates, 4-phenol, and m-chlorophenol.

The registrant-postulated metabolic pathways are shown in Figure 1. The metabolism of RH-7592 may include the following reactions:

Major reactions
1. Oxidation
2. Hydroxylation
3. Conjugation, primarily glucuronide

Minor reactions
1. Nonenzymatic cyclization

The reviewer concurs with the registrant's metabolic pathway of RH-7592.

G. DISCUSSION

The study demonstrated that radiolabeled RH-7592 is rapidly absorbed, distributed, and excreted following oral administration in rats. Total 3- or 4-day recoveries of the radioactivity were high for all groups (90.40-104.49% of the administered dose). The feces was the major route of excretion (78.74-94.43% of administered dose), while <1% of the administered dose was recovered in the urine at 4 days postdosing. Biliary excretion data indicated that systemic absorption of RH-7592 was high for all dosing groups. Tissue distribution and bioaccumulation of [illegible] were minimal since radioactivity was <1% in tissues 4 days after oral administration for all dosing groups. Based on the results, no sex- or dose-related differences in absorption, distribution, or elimination were found. Metabolism of RH-7592 was extensive as shown by the numerous metabolites characterized and isolated in the feces and urine. Furthermore, there appeared to be a dose-related difference in metabolism as indicated by the higher amount of unmetabolized parent compound in the feces in the high-dose group (~21-37% of the administered dose) compared to the low-dose and repeated-dose groups (~2-5% of the administered dose). This finding suggests that saturation of the metabolic pathway may be occurring at the high-dose group. The authors have made a thorough attempt at isolating or characterizing the metabolites in the excreta.

The findings were very similar to a previous metabolism study (MRID No. 418750-17, 418750-18; Tox. Chem. No. 723-Q; HED number 1-2499). The recoveries were higher in the present study, but both studies demonstrated that there were no major sex- or dose-related differences in the absorption, distribution, and elimination of RH-7592. This study

however, also demonstrated that there was a dose-related difference in metabolism of RH-7592, possibly due to saturation of the metabolic pathway at the high dose level. This metabolism study is Core-Guideline and has addressed the deficiency from the previous metabolism study (metabolite analysis data for the low-dose groups for comparison with high-dose group).

H. STUDY DEFICIENCIES:

No major deficiencies were found in the review of the study.

Table 1. Radioactivity in the Feces, Urine, Tissues, and Carcass as Percent (%) of Dose From Rats Following Oral Administration of ^{14}C-RH-7592[a]

Group	Sex	Dose level (mg/kg)[b]	Route of Administration	% Administered Dose (Mean)				
				Feces	Urine[c]	Bile	Tissue & Carcass	Total
A	M	1	oral (low)	85.31	9.92	-	0.78	96.01
B	F	1	oral (low)	92.15	11.53	-	1.06	103.51
C	M	100	oral (high)	90.19	7.00	-	0.39	97.58
D	F	100	oral (high)	78.74	11.23	-	0.43	90.40
E	M	1	oral (repeated)	94.43	8.92	-	0.93	104.28
F	F	1	oral (repeated)	89.36	11.44	-	2.69	104.49
G	M	1	oral (low)	0.00	1.67	-	101.14	102.81
H	F	1	oral (low)	0.00	2.67	-	99.28	101.94
I	M	1	oral (low)	13.37	4.67	-	70.09	88.14
J	F	1	oral (low)	4.32	7.33	-	59.07	70.64
K	M	1	oral (bile)	6.87	3.24	87.05	0.98	98.14
L	F	1	oral (bile)	6.33	7.70	79.05	1.65	94.73

[a]Data extracted from Report 92R-060, Table 1 and Table 6; radioactivities determined at 4 days postdosing (Groups A-F), 3 hours postdosing (Groups G and H), 12 hours postdosing (Groups I and J), or 3 days postdosing (Groups K and L)

[b]All animals (3 or 5/group) were dosed orally by gavage (5 mL/kg). Animals in Groups E and F received 10 ppm (a.i.) nonradiolabeled RH-7592 in the diet for 14 days prior to receiving a single gavage dose (5 mL/kg) of ^{14}C-RH-7592.

[c]Includes cage rinses and washes

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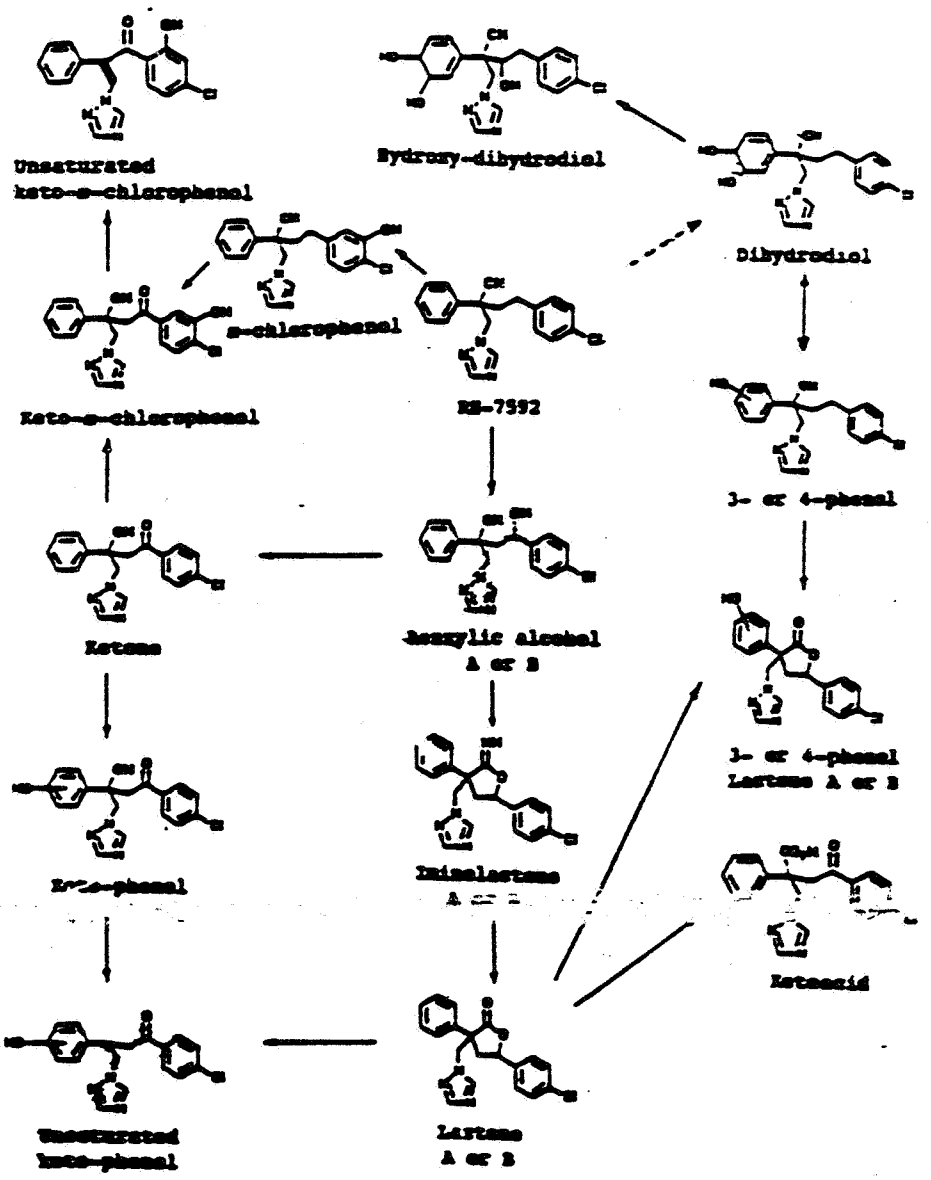

Figure 1. Postulated Metabolic Pathway of RH-7592 in Rats
(Report 92R-060, Figure 1, p. 17)

CBI Appendix
(Study Report No. 92R-060, p. 194, 195, 198-201)

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Page_____ is not included in this copy.

Pages 15 through 20 are not included.

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