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|-------------------------|----------|---------------|---|--------------|----------------|
| Document No. | | | OTS0509764 | | |
| New Doc ID. | | 89-8680144 | Old Doc ID. | | SEHQ-0296-0578 |
| Date Produced | 02/07/86 | Date Received | 02/14/86 | TSCA section | |
| | | | | 8E | |
| Submitting Organization | | | Syntex Inc | | |
| Contractor | | | Syntex Inst Tox Ser | | |
| Document Title | | | Eye irritation, Oral and Dermal Toxicity of Acetyl Ferrocene Final reports with cover letters | | |
| Chemical Category | | | Acetyl Ferrocene | | |

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CORPORATE FACILITIES ENGINEERING & SERVICES DIVISION

February 13, 1986

8E HQ-4286-0578 FLWP
89-8680144

Document Control Officer (WH-557)
Information Management Division
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

EPA-OTS
000411168N

Subject: TSCA Section 8 (e) Submission on Acetyl Ferrocene
EPA Document Control Number 8E HQ-1285-0578

Dear Sir:

In response to your request of January 16, 1986, we are hereby submitting complete copies of the final reports from all of the acute in vivo studies of acetyl ferrocene that formed the basis for the company's submission under Section 8 (e) of the Toxic Substances Control Act. Final reports on the acute dermal and oral toxicity of acetyl ferrocene, as well as eye irritation studies are being provided. These studies outline the experimental procedures and results of all Syntex testing of acetyl ferrocene. Where histopathological examinations were conducted, these results are also provided.

We have reviewed these reports and have concluded that we have no present interest in asserting a confidentiality claim as to these reports.

In our previous submission, we hypothesized that the absence of any reported adverse health effects among employees might suggest that there may be important differences in sensitivities between humans and animals. In the next few months, we are planning to initiate one additional acetyl ferrocene test in a higher animal species, such as a dog or monkey. Any additional pertinent test results will be transmitted to the Agency.

Should you have further questions on this subject, please do not hesitate to contact us. We will, of course, keep you informed if any additional developments arise.

Very truly yours,


Dennis Paustenbach
Chairperson
Toxic Risk Evaluation Committee

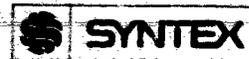
REPORT 841-R-85-96526-000-PO-TXE



DISTRIBUTION

Study File
Dr. H. Leung

RS-96526-000



REPORT 841-R-85-96526-000-PO-TXE

FINAL REPORT

**ACUTE ORAL TOXICITY OF RS-96526-000 (ACETYL FERROCENE)
WHEN TESTED ON THE RAT**

STUDY TEAM

Study Director:

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Diplomate, A.B.T.
Institute of Toxicologic Sciences
Department of Toxicology

Veterinary Pathologist:

Leonard D. Shott, D.V.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.
Institute of Toxicologic Sciences
Department of Pathology

Study Started:

30 October 1985

**In-Life
Completion Date:**

13 November 1985

Date of Report:

7 February 1986

**SYNTEX RESEARCH
Institute of Toxicologic Sciences
3401 Hillview Avenue, Palo Alto, California**

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REPORT TITLE: ACUTE ORAL TOXICITY OF RS-96526-000 (ACETYL FERROCENE) WHEN TESTED ON THE RAT

PURPOSE: In a previous study, all rats given single oral doses of 50 mg/kg or more of RS-96526-000, hereafter referred to as acetyl ferrocene, died. The present study is to extend the dose range downward to 5 mg/kg.

CHEMICAL NAME: RS-96526-000 = Acetyl Ferrocene

DATA RETENTION: The raw data and report for this study are maintained in Syntex Research Archives located at 3401 Hillview Avenue, Palo Alto, California.

PROCEDURE: All procedures were conducted according to the Standard Operating Procedures for the Institute of Toxicologic Sciences, Syntex Research.

SUMMARY

The acute oral toxicity of acetyl ferrocene (RS-96526-000) was studied using the rat as the test system. Three groups each composed of 5 male and 5 female rats were used. They were given doses of 0 (untreated control), 5, or 50 mg/kg of acetyl ferrocene. The rats were observed for signs of toxicosis for 2 weeks after dosing.

None of the rats in the control group died during the observation period and all animals in this group appeared normal. All of the female rats given 5 mg/kg of acetyl ferrocene were dead by the second day after dosing. None of the male rats given this dose died. The animals in these groups had non-specific signs of toxicosis. These included rough coat, decreased activity, increased and/or labored respiration and wasting. The males appeared to have recovered by 6 days after dosing and all appeared normal at the end of the 2-week observation period.

SUMMARY (continued)

All of the females and 4/5 of the males given the 50 mg/kg dose of acetyl ferrocene died. Signs of toxicosis for animals in these groups were similar to those for the animals in the low-dose group, but also included decreased body temperature, diarrhea, nasal discharge, kyphosis, salivation, and urogenital staining.

Gross pathological examinations were carried out on 2 male and 8 female rats that died. These animals were diagnosed as having pneumonopathy. Some of these animals also had dark discoloration of the liver.

Conclusions

The acutely toxic oral dose of acetyl ferrocene for female rats is less than 5 mg/kg. For the male rat the acutely toxic dose is between 5 and 50 mg/kg.



COMMENT

No unforeseen circumstances were noted which would have affected the quality or the integrity of the study.

APPROVED BY:


Duane W. Hallesy, Ph.D.
Director, Toxicology
Study Director
Diplomate, A.B.T.

6 Feb 86
Date


Leonard D. Shott, D.V.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.

7 Feb 86
Date


Irwin A. Heyman, Ph.D.
Vice President, Syntex Research
Director, ITS

7 Feb 86
Date

EXPERIMENTAL PROCEDURES AND RESULTSTest Article Formulations

The RS-96526 (Lot #187-120) was released for use in this study by the Institute of Organic Chemistry, Syntex Research, Palo Alto, California. 0.125 grams of RS-96526 was mixed with 24.9 ml of Mazola corn oil so that a concentration of 5.0 mg/ml was attained. Mixing was done with a Polytron homogenizer for approximately 30 seconds. A summary of analytical results will be maintained on file with the Environmental Health and Safety group rather than being presented in this report.

Animals

The rat was selected because of its small size and because it is one of the species recommended for the evaluation of acute toxicity. Sprague-Dawley derived rats (CD) purchased from Charles River Breeding Laboratories, Inc., Portage, Michigan were received on 23 October 1985. Age and weight were not specified but a random sample of rats weighed between 139 and 149 g upon receipt.

After receipt at Syntex, the rats were individually housed in stainless steel cages with wire-mesh fronts and bottoms. The rats were transferred into clean cages biweekly and maintained in an environmentally controlled room. Food (Purina Certified Rodent Chow®) and water were available to the animals ad libitum except for an overnight period of food deprivation before dosing. Food was withheld for a further 2 hours (approximately) postdosing.

The rats were acclimatized to laboratory conditions for 1 week before the start of the study. Only those animals considered to be in satisfactory health were used. Each animal was individually identified by using a standard ear punch code. The animal number and the study number were used to identify the card attached to each animal's cage.

EXPERIMENTAL PROCEDURES AND RESULTS (continued)Treatment

The rats were assigned to 3 groups composed of 5 males and 5 females. Randomization was not used since it was considered unnecessary. Treatment, based on predose individual animal body weights, was as follows:

| <u>Group/ Number Series</u> | <u>Dose of RS-96526 (mg/kg)</u> | <u>Dose Volume ml/100 g</u> | <u>Concentration of RS-96526 (mg/ml)</u> |
|---------------------------------|-------------------------------------|---------------------------------|--|
| 1 (100 series) | Untreated Control | -- | -- |
| 2 (200 series) | 5.0 | 0.1 | 5.0 |
| 3 (300 series) | 50.0 | 1.0 | 5.0 |

The test formulation was administered as a single dose using a metal rodent intubator. The day of administration of the test formulation was considered study day 1. The oral route was selected to evaluate effects which would occur if the test compound was accidentally ingested.

Observations

Each animal received a single dose and was observed for 14 days. Clinical observations were recorded daily. Body weights were recorded on study days 1 (day of dosing), 6, and 15 (last day of the study).

At the end of a 14-day postdose observation period, all surviving animals were euthanatized and discarded.

RESULTS

The nature and incidence of clinical observations, observations for individual animals, and body weights are presented in the In-Life Tables 1 through 3.

EXPERIMENTAL PROCEDURES AND RESULTS (continued)

All animals in group 1 remained normal throughout the study.

The females in group 2 had the following clinical observations: closed eyes, inactivity, increased and labored respiration, rough coat and emaciation. All group 2 females were found dead on day 3. Rough coat was the only clinical observation noted in group 2 males; all group 2 males survived to day 15.

The animals in group 3 had the following clinical observations. closed eyes, inactivity, increased and labored respiration, unthriftiness, rough coat, emaciation, hypothermia, urogenital stain, diarrhea, kyphosis, listlessness, brown, black and/or red nasal discharge and salivation. All females in group 3 were found dead by study day 4.

Four out of 5 males in group 3 were found dead by study day 8. Animal #307 exhibited urogenital staining on study day 2 and was normal the remainder of the study.

Due to the increased mortality in groups 2 and 3 body weights could not be compared.

Signs of toxicity were noted in all treated groups. Females were more sensitive to RS-96526.

SUBMITTED AND REPORTED BY:

Janice A. Mackay, ifa
Calvin Harris, B.S.
Toxicology Biologist

2-7-86
Date

TABLE 3

BODY WEIGHTS (G) FOR RATS GIVEN A
SINGLE ORAL DOSE OF ACETYL FERROCENE
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL ID | STUDY WEEK | | |
|--------------|------------------------|-----|-----|
| | 1 | 2 | 3 |
| GROUP | I M: UNTREATED CONTROL | | |
| 0101 | 189 | 200 | 215 |
| 0103 | 182 | 200 | 207 |
| 0105 | 178 | 200 | 204 |
| 0107 | 181 | 200 | 205 |
| 0109 | 182 | 201 | 205 |
| N | 5 | 5 | 5 |
| MEAN | 184 | 200 | 205 |
| STD | 6 | 6 | 7 |
| GROUP | I F: UNTREATED CONTROL | | |
| 0100 | 133 | 173 | 191 |
| 0102 | 160 | 200 | 213 |
| 0104 | 164 | 215 | 222 |
| 0106 | 161 | 216 | 233 |
| 0108 | 140 | 177 | 204 |
| N | 5 | 5 | 5 |
| MEAN | 152 | 197 | 213 |
| STD | 14 | 21 | 18 |

TABR101/TABR02

TABLE 3

BODY WEIGHTS (G) FOR RATS GIVEN A SINGLE ORAL DOSE OF ACETYL FERROCENE FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL ID | STUDY WEEK | | |
|-----------|------------|--------------------------|-----|
| | 1 | 2 | 3 |
| GROUP | 2 M | 5 MG/KG ACETYL FERROCENE | |
| 0201 | 202 | 276 | 310 |
| 0203 | 193 | 257 | 294 |
| 0205 | 192 | 280 | 310 |
| 0207 | 169 | 246 | 281 |
| 0209 | 182 | 248 | 278 |
| N | 5 | 5 | 5 |
| MEAN | 188 | 261 | 295 |
| STD | 13 | 16 | 15 |
| GROUP | 2 F | 5 MG/KG ACETYL FERROCENE | |
| 0200 | 162 | D | D |
| 0202 | 133 | D | D |
| 0204 | 152 | D | D |
| 0206 | 173 | D | D |
| 0208 | 142 | D | D |
| N | 5 | 0 | 0 |
| MEAN | 152 | NA | NA |
| STD | 16 | * | * |

D- ANIMAL DECEASED / REMOVED FROM STUDY
 *- SAMPLE SIZE TOO SMALL TO CALCULATE STD

TABR101/TABRB02

TABLE 3

BODY WEIGHTS (G) FOR RATS GIVEN A SINGLE ORAL DOSE OF ACETYL FERROCENE FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL ID | STUDY WEEK | | |
|--------------------------------|------------|-----|-----|
| GROUP | 1 | 2 | 3 |
| 3 M: 50 MG/KG ACETYL FERROCENE | | | |
| 0301 | 201 | D | D |
| 0303 | 178 | 158 | D |
| 0305 | 194 | D | D |
| 0307 | 188 | 245 | 278 |
| 0309 | 188 | D | D |
| N | 3 | 2 | 1 |
| MEAN | 181 | 188 | 278 |
| STD | 8 | * | * |
| 3 F: 50 MG/KG ACETYL FERROCENE | | | |
| 0300 | 148 | D | D |
| 0302 | 148 | D | D |
| 0304 | 148 | D | D |
| 0306 | 140 | D | D |
| 0308 | 147 | D | D |
| N | 5 | 0 | 0 |
| MEAN | 148 | NA | NA |
| STD | 3 | * | * |

D- ANIMAL DECEASED / REMOVED FROM STUDY
 *- SAMPLE SIZE TOO SMALL TO CALCULATE STD

TABR101/TABR02

REPORT 841-R-85-96526-000-PO-TXE

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PATHOLOGY TABLE

DEPARTMENT OF PATHOLOGY
INSTITUTE OF TOXICOLOGIC SCIENCES
SYNTEX RESEARCH
PALO ALTO, CA 94304

STUDY 8841-R-85
GROSS PATHOLOGY INCIDENCE TABLE
ACUTE ORAL TOXICITY OF RS-88528-000
(ACETYL FERROCENE) WHEN TESTED IN THE RAT
REPORT: 841-R-85-88528-PO-THE

TABLE PAGE 1
PATH/TON SYSTEM
PRINTED: 24-JAN-88

SPECIES: SPRAGUE DAWLEY RAT

ROUTE: ORAL-STOMACH TUBE

STUDY TYPE: ACUTE

TEST ARTICLE
DESCRIPTION:

THE ACETYL FERROCENE (RS-88528) WAS RELEASED FOR USE BY THE INSTITUTE
OF ORGANIC CHEMISTRY (IOC), SYNTEX RESEARCH.

TREATMENT SCHEDULE:

SINGLE DOSE FOLLOWED BY A 2 WEEK OBSERVATION PERIOD.

SACRIFICE INTERVAL:

TERMINAL SACRIFICE AFTER A 2 WEEK OBSERVATION PERIOD.

EXPERIMENTAL GROUPS:

SEE ITS PROTOCOL

COMMENTS:

GROSS PATHOLOGY BY:

C. BROWN, W. CRAWFORD
S. WINSLOW, D. SIDLACK, DVM

MICROSCOPIC PATHOLOGY BY: NOT APPLICABLE

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DEPARTMENT OF PATHOLOGY
INSTITUTE OF TOXICOLOGIC SCIENCES
SYNTEX RESEARCH
PALO ALTO, CA 94304

STUDY #841-R-85
GROSS PATHOLOGY INCIDENCE TABLE
ACUTE ORAL TOXICITY OF MS-8526-000
(ACETYL FERROCENE) WHEN TESTED IN THE RAT

TABLE PAGE: 112
PATH/TOX SYSTEM

PRINTED: 24-JUN-86

THIS SHEET PRESENTS THE SYMBOLS, ABBREVIATIONS AND CODES USED IN THE "MACROSCOPIC EVALUATION OF TISSUES" TABLES TO INDICATE WHICH ORGAN/TISSUE FINDINGS WERE OBSERVED AND TO ASSIST IN MORE FULLY DESCRIBING THEM.

***** GRADES FOR OBSERVATION SEVERITY *****

- " 1 " MINIMAL OR VERY SLIGHT DEGREE OR AMOUNT PRESENT
- " 2 " SLIGHT DEGREE OR AMOUNT PRESENT
- " 3 " MODERATE DEGREE OR AMOUNT PRESENT
- " 4 " MARKED DEGREE OR AMOUNT PRESENT
- " 5 " SEVERE DEGREE OR LARGE AMOUNT PRESENT

***** ANIMAL DEATH CODES *****

- " 1 " - " 6 " INTERIM SACRIFICES 1 THROUGH 6
- " T " TERMINAL SACRIFICE
- " P " POST-RECOVERY SACRIFICE #1
- " Q " POST-RECOVERY SACRIFICE #2
- " B " DIED FROM BLOOD COLLECTION
- " C " EUTHANATIZED FOR CAUSE
- " D " FOUND DEAD
- " O " OTHER (ACCIDENT, LACK OF FOOD OR WATER, ETC -
SEE CLINICAL OBSERVATIONS)

***** OTHER SYMBOLS AND NOTATIONS *****

- " G " THIS ANIMAL HAS GROSS OBSERVATIONS NOTED.
- " U " ORGAN/TISSUE NOT MACROSCOPICALLY REMARKABLE
- " - " KEYWORD/PHRASE NOT PRESENT OR OBSERVED
- " P " INDICATES PRESENCE (WHERE SEVERITY INAPPROPRIATE)
- " nC " FREE-TEXT COMMENT ("C") ACCOMPANIES THE OBSERVATION WHERE "n" INDICATES SEVERITY (1-5) OR PRESENCE (P).
- " * " INDICATES SIGNIFICANCE AT .05 LEVEL (DUNNETT'S).
- " ** " INDICATES SIGNIFICANCE AT .01 LEVEL (DUNNETT'S).
- " EX " INDICATES THAT ORGAN IS EXCLUDED FROM CALCULATIONS.

***** ORGAN WEIGHING STATUSES *****

- " NOT TAKEN " ORGAN WEIGHT NOT TAKEN; NO EXPLANATION GIVEN
- " MISSING " ORGAN MISSING OR LOST
- " UNSUITABLE " ORGAN TECHNICALLY UNSUITABLE FOR WEIGHING
- " AUTOLYTIC " ORGAN AUTOLYTIC AND COULD NOT BE WEIGHED
- " EXCLUDE " WEIGHT HAS BEEN TAKEN, BUT WILL BE EXCLUDED FROM ALL CALCULATIONS.

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DEPARTMENT OF PATHOLOGY
INSTITUTE OF TOXICOLOGIC SCIENCES
SYNTEX RESEARCH
PALO ALTO, CA 94304

STUDY 8841-R-85
GROSS PATHOLOGY INCIDENCE TABLE
ACUTE ORAL TOXICITY OF RS-86528-000
(ACETYL FERROSENE) WHEN TESTED IN THE RAT

TABLE PAGE 1-3
PATH/TOX SYSTEM

PRINTED: 24-JAN-86

DESCRIPTION OF THE "TABLE INCLUDES:" PORTION OF THE OUTPUT TABLE HEADING

- "SD" - THIS IS THE SEX/DOSAGE GROUP NUMBER. THE FIRST DIGIT INDICATES THE SEX OF THE GROUP. MALES ARE "1" AND FEMALES ARE "2". THE SECOND DIGIT IS THE DOSAGE GROUP WITHIN THE SEX. THE "SEX" AND "DOSAGE-GROUP" DESCRIPTIONS ARE THE EXPANDED FORMS OF THE "SD" NUMBER.
- "SEX" - INDICATES WHICH SEXES WERE SELECTED FOR THIS OUTPUT TABLE. IF BOTH MALE AND FEMALE WERE SELECTED OR THE SEX WAS NOT USED AS A SELECTION CRITERION THE SEX WILL BE "B" - BOTH. IF ONLY MALE OR ONLY FEMALE ANIMALS WERE SELECTED THE SEX WILL BE "M" OR "F", RESPECTIVELY (EG. "SEX=F").
- "GROUP" - INDICATES THE DOSAGE GROUP OR GROUPS SELECTED FOR THIS OUTPUT TABLE. IF ALL GROUPS WERE SELECTED OR THE DOSAGE GROUP WAS NOT USED AS A SELECTION CRITERION THE GROUP WILL BE "ALL". IF INDIVIDUAL GROUPS WERE SPECIFIED, THE GROUPS WILL BE DISPLAYED WITH COMMAS SEPARATING THEM (EG. "GROUP=1,3,4").
- "WEEKS" - INDICATES THE NUMBER OF WEEKS AN ANIMAL WAS ON THE STUDY AT THE TIME OF DEATH. IF ALL WEEKS WERE SPECIFIED OR EITHER A SINGLE WEEK OR THE LOWER AND UPPER BOUNDS OF A RANGE OF WEEKS ARE DISPLAYED (EG. "WEEKS=14", "WEEKS=4-15").
- "DEATH" - INDICATES THE DEATH CODES OF THE ANIMALS SELECTED FOR THE OUTPUT TABLE. IF ALL DEATH CODES WERE SUPPLIED OR THE DEATH CODE WAS NOT USED AS A SELECTION CRITERION, THE DEATH CODE WILL BE "ALL". IF INDIVIDUAL DEATH CODES WERE SUPPLIED, THE DEATH CODES WILL BE DISPLAYED WITH COMMAS SEPARATING THEM (EG. DEATH=1,2,3,D,T).
- "INTERIM" - INDICATES THAT THE OUTPUT TABLE HAS BEEN GENERATED BEFORE THE STUDY WAS SIGNED OFF. NORMALLY, "DRAFT" WILL BE DISPLAYED ON EITHER SIDE OF THE REPORT HEADING. IF THE OUTPUT TABLE IS GENERATED BEFORE THE STUDY IS SIGNED OFF, THE "DRAFT" NOTATION MAY BE CHANGED TO "INTERIM" BY ENTERING THE "INTERIM" KEYWORD.
- "SUBSET" - INDICATES WHETHER ALL OF THE TISSUES APPEAR ON THE REPORT OR JUST SOME TARGET TISSUES WERE SELECTED. IF ALL OF THE TISSUES WERE SELECTED OR TISSUES WERE NOT USED AS A SELECTION CRITERION, THE SUBSET WILL BE "ALL". IF A SUBSET OF THE TISSUE LIST WAS USED, THE SUBSET INDICATOR WILL BE "-1". THE USE OF BODY SYSTEM SUBSETS IS INDICATED BY A "B".

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DEPARTMENT OF PATHOLOGY
INSTITUTE OF TOXICOLOGIC SCIENCES
SYNTEX RESEARCH
PALO ALTO, CA 94304

STUDY 8841-R-85
GROSS PATHOLOGY INCIDENCE TABLE
ACUTE ORAL TOXICITY OF RS-98528-000
(ACETYL PERIOCENE) WHEN TESTED IN THE RAT

TABLE PAGE 17
PATH/TOR SYSTEM

PRINTED: 24-JAN-86

AN-NUM ORGAN KEYWORD OR PHRASE FREE-TEXT COMMENT

| | | | |
|------|------|------------|-------------|
| 0301 | LUNG | DISCOLORED | RED/BLACK |
| 0301 | SKIN | STAINED | FACE, BLACK |
| 0305 | LUNG | DISCOLORED | RED/BLACK |
| 0305 | SKIN | STAINED | FACE, BLACK |

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DEPARTMENT OF PATHOLOGY
 INSTITUTE OF TOXICOLOGIC SCIENCES
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STUDY 8841-R-85
 GROSS PATHOLOGY INCIDENCE TABLE
 ACUTE ORAL TOXICITY OF RS-88526-000
 (ACETYL FERROCENE) WHEN TESTED IN THE RAT

TABLE PAGE 1133
 PATH/ICR SYSTEM

PRINTED: 24-JAN-88

AN-NUM

ORGAN

KEYWORD OR PHRASE

FREE-TEXT COMMENT

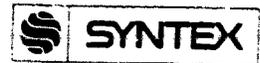
| | | | | |
|------|---------|--------------------|--------------------------------------|--|
| 0100 | LIVER | DISCOLORED | DARK | |
| 0200 | LUNG | DISCOLORED | RED/BLACK | |
| 0202 | LIVER | DISCOLORED | DARK | |
| 0202 | UTERUS | CONTAINED MATERIAL | CLEAR FLUID | |
| 0202 | LUNG | DISCOLORED | RED/BLACK, MOTTLED | |
| 0202 | TRACHEA | CONTAINED FROTH | WHITF | |
| 0204 | LIVER | DISCOLORED | DARK | |
| 0204 | LUNG | DISCOLORED | RED/BLACK, MOTTLED | |
| 0206 | LIVER | DISCOLORED | DARK | |
| 0206 | LUNG | DISCOLORED | RED/BLACK, MOTTLED | |
| 0206 | LIVER | DISCOLORED | DARK | |
| 0214 | EYE | CLOUDY | RIGHT | |
| 0208 | LUNG | DISCOLORED | RED/BLACK, MOTTLED | |
| 0300 | STOMACH | INGESTA DISCOLORED | ORANGE/BROWN, OILY | |
| 0300 | EYE | CLOUDY | RIGHT | |
| 0300 | LUNG | DISCOLORED | ALL LOBES, DIFFUSE, RED/PURPLE/BLACK | |
| 0302 | STOMACH | INGESTA DISCOLORED | ORANGE/BROWN, OILY | |
| 0302 | LUNG | DISCOLORED | ALL LOBES, RED/PURPLE/BLACK | |
| 0302 | SKIN | STAINED | FACE AND PERINEUM, YELLOW/BROWN | |
| 0308 | LIVER | DISCOLORED | DARK | |
| 0308 | LUNG | DISCOLORED | RED/BLACK, MOTTLED | |

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REPORT 841-R-85-96526-000-PO-TXE

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APPENDICES

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**SPONSOR AND TESTING FACILITY:**

SYNTEX RESEARCH
 Institute of Toxicologic Sciences
 Departments of Toxicology and Pathology
 3401 Hillview Avenue, Palo Alto, California

PROTOCOL NUMBER:

041-R-85-96256-PO-TXE

REVISED**PROTOCOL DATE:**

September 4, 1985

SEE REVISION**SEP 10 1985****PROTOCOL TITLE:**

ACUTE ORAL TOXICITY OF RS-96256-000 (ACETYL FERROCENE) WHEN TESTED ON THE RAT.

PROJECT NUMBER:

Z.O.18

PROTOCOL PURPOSE:

In a previous study, all rats given single oral doses of 50 mg/kg or more of RS-96256-000 hereafter referred to as acetyl ferrocene, died. The present study is to extend the dose range downward to 5 mg/kg.

PROCEDURE:

Unless otherwise stated, all procedures will be done according to Standard Operating Procedures for the Institute of Toxicologic Sciences.

A. Test Articles

1. The acetyl ferrocene will be released for use by the Institute of Organic Chemistry (IOC), Syntex Research. A summary of their analytical results will be included with the report of this study.
2. On receipt, the test article will be stored at 4 degrees C. The elapsed time between release of article by IOC and testing in ITS shall not exceed 90 days. If the elapsed time should exceed 90 days an aliquot of the test article will be returned to IOC for reanalysis.
3. The test article will be mixed with vegetable oil just before dosing. Suspend 0.125 grams of acetyl ferrocene in 24.9 ml of vegetable oil. Mix for at least 30 seconds using a Polytron homogenizer.

B. Animals

1. Healthy animals, not subject to any previous experimental procedures, will be used.
2. Obtain enough rats so that 15 males and 15 females will be available for this study.

Strain: (CD) Sprague Dawley Derived
 Source: Charles River, Kingston, NY, or Portage, MI
 Age: Not specified (Young Adult)
 Weight: Not specified (Young Adult)

B. Animals (continued)

3. Upon receipt the rats will be placed into individual cages and held for an acclimatization period of approximately 1 week.
4. Food (Purina Certified Rodent Chow) and water will be available ad lib except for an overnight period of food deprivation before dosing. No materials are anticipated to be present in the food or water which would interfere with the purpose or conduct of this study.
5. Following random assignment to the study each rat is to be individually identified using a standard ear punch code, or with indelible ink. The method used is to be documented.
6. The animals will be inspected during the acclimatization period. Any animal exhibiting unusual behavior, significant change in condition of the fur, color of the skin, scratching, loud or irregular breathing, unusual discharges from orifices, unusual condition of urine or stool, changes in food or water consumption, or wounds caused by fights will not be used for the study.

C. Test Procedures

1. Assign the rats to 3 groups as shown below.

| <u>Group*</u> | <u>Compound</u> | <u>(mg/kg)</u> | <u>Dose of Formulation</u> |
|----------------|-------------------|----------------|----------------------------|
| 1 (100 series) | untreated control | | |
| 2 (200 series) | acetyl ferrocene | 5.0 | 0.1 ml/100g |
| 3 (300 series) | acetyl ferrocene | 50.0 | 1.0 ml/100g |

* Groups composed of 5 males and 5 females

2. Remove food from animals overnight before dosing. Do not replace food until approximately 2 hours after dosing.
3. Route - single oral dose (stomach tube). The maximum volume of aqueous solution that can be given in one dose should not exceed 2 ml/100 g body weight. For nonaqueous liquids and suspensions, the volume should not exceed 1 ml/100 g body weight. When possible, variability in test volume should be minimized.
4. Duration - single dose followed by a 2 week observation period.

D. Observations

1. A careful clinical examination should be made at least once each day. Cageside observations should include changes in the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behavior pattern. Particular attention should be directed to observation for tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. All toxicological and pharmacological signs should be recorded including time of onset, intensity and duration. The time of death should be recorded as precisely as possible. Individual weights of animals should be determined shortly before the test substance is administered, weekly thereafter, and at study termination. At the end of the test, surviving animals are to be submitted for necropsy.
2. Animals found dead or killed for cause are to be submitted to the Department of Pathology. Attempt to obtain blood and urine samples from animals to be killed for cause.
3. If any deaths or if no deaths are observed, the need for additional testing will be discussed with Environmental Health and Safety.

E. Clinical Pathology Studies

Terminal blood samples for hematology and chemistry will be taken from the surviving animals in each group. Urine samples will be collected during the week prior to scheduled sacrifice.

Perform the following analyses:

1. Hematology

- a) hemoglobin
- b) hematocrit
- c) total leukocyte count
- d) differential leukocyte count
- e) total erythrocyte count
- f) mean corpuscular volume (MCV)
- g) mean corpuscular hemoglobin (MCH)
- h) mean corpuscular hemoglobin concentration (MCHC)
- i) platelet count

2. Plasma or Serum Chemistry (Perform in the following order as sample permits)

- a) total bilirubin
- b) urea nitrogen
- c) glutamic oxalacetic transaminase (GOT/AST)
- d) glutamic pyruvic transaminase (GPT/ALT)
- e) alkaline phosphatase

E. Clinical Pathology Studies (continued)

- f) chloride
- g) sodium
- h) potassium
- i) glucose
- j) triglycerides
- k) cholesterol
- l) total protein

3. Urinalysis - Perform in the following order as sample permits.

- a) color
- b) appearance
- c) glucose
- d) protein
- e) ketones
- f) blood
- g) bilirubin
- h) urobilinogen
- i) reaction (pH)
- j) specific gravity

F. Pathology

The procedures to be used will include but not necessarily be limited to the following.

1. Gross Pathology

- a. Necropsy - Conduct a complete examination on each animal that dies during or at termination of the study.
- b. Tissue Preservation and Organ Weights - The following tissues or organs from each animal are to be sampled and preserved in neutral buffered 10% formalin. Organs indicated with a (W) are to be weighed from animals necropsied at times of scheduled sacrifice:

Altered Tissues: From any tissue/organ

Cardiovascular System: Aorta
Heart (W)



F. Pathology (continued)

Digestive System:

Esophagus
Intestine - Large
Cecum
Colon
Rectum
Intestine - Small
Duodenum
Ileum
Jejunum
Liver (W)
Pancreas
Salivary Gland
Stomach
Tongue

Endocrine System:

Adrenal Glands (W)
Parathyroid Glands
Pituitary Gland
Thyroid Gland (W)

Hematopoietic System:

Bone Marrow, Sternum
Lymph Node, Mandibular
Lymph Node, Mesenteric
Spleen (W)
Thymus Gland

Integumentary System:

Auditory Sebaceous Gland
Lacrimal Gland
Skin, Abdomen

Musculoskeletal System:

Bone, Femur
Bone, Spinal Column
Bone, Sternum
Skeletal Muscle

Nervous System:

Brain (W)
Eyes
Nerve, Sciatic
Spinal Cord



F. Pathology (continued)

Reproductive System:

Female

Mammary Gland
Ovaries (W)
Uterus (W)
Vagina

Male

Mammary Gland
Epididymides
Prostate Gland/
Seminal Vesicles (W)
Testes (W)

Respiratory System:

Lung w/major bronchi
Trachea

Urinary System:

Kidneys (W)
Urinary Bladder

2. Microscopic Pathology

- a. Histological examination shall include evaluation of the above listed tissues from all males and all females from groups 1 and 3 that were necropsied at the end of the treatment period.
- b. The tissues to be examined histologically from the other groups and animals found dead or killed for cause will be determined after necropsy.
- c. Tissues found at necropsy to be altered will be examined histologically.

G. Results

Tabulation of data and individual results will accompany each report in sufficient detail to permit independent evaluation of results, including summaries and tables that show, as appropriate, the relationship of effects to time of dosing, sex etc.

H. Statistical Evaluation

No statistical evaluation will be required.



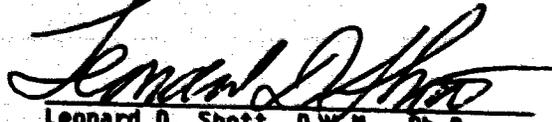
I. Data Retention

All raw data will be maintained in Syntex Research Archives managed from 3401 Hillview Avenue, Palo Alto, California.

APPROVED BY:


Duane W. Hallesy, Ph.D.
Director, Toxicology
Study Director
Diplomate, A.B.T.

9/5/85
Date


Leonard D. Shott, D.W.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.

5 Sept 85
Date


Irwin A. Heyman, Ph.D.
Vice President, Syntex Research
Director, ITS

6 Sept 85
Date

SPONSOR AND TESTING FACILITY:

SYNTEX RESEARCH
Institute of Toxicologic Sciences
Departments of Toxicology and Pathology
3401 Millview Avenue, Palo Alto, California

0044



PROTOCOL NUMBER:

841-R-85-96526-000-PO-TXE

REVISION NUMBER:

One

REVISION DATE:

September 10, 1985

REVISED
SEE REVISION ~~NOV - 1 1985~~

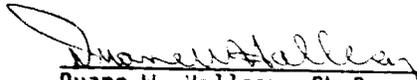
PURPOSE OF REVISION:

The purpose of this revision is to correct the compound number. It should be RS-96526-000.

REVISION:

Change 96256 to 96526 wherever it occurs.

APPROVED BY:


Duane W. Hallesy, Ph.D.
Director, Toxicology
Study Director
Diplomate, A.B.T.

10 Sept 85
Date


Leonard D. Shott, D.V.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.

10 Sept 85
Date

0045



SPONSOR AND TESTING FACILITY:

SYNTEX RESEARCH
Institute of Toxicologic Sciences
3401 Hillview Avenue, Palo Alto, California

PROTOCOL NUMBER:

96526 ~~96526~~
~~941-R-85-9838-000-PO-TXE~~

REVISION NUMBER:

Two

REVISION DATE:

November 1, 1985

PURPOSE OF REVISION:

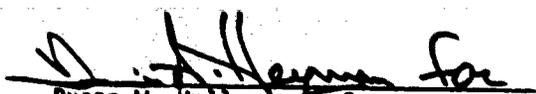
The purpose of this revision is to remove requirement for necropsy and clinical pathology examinations. Deaths in groups 2 and 3 have resulted in too small of group size for meaningful terminal procedures.

REVISION:

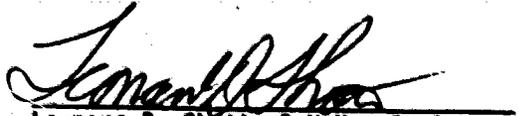
D. Observations

No animal beyond those already examined on and before 1 November 1985 will be submitted for clinical (Section E) or anatomic (Section F) examination. At the end of the observation period, all animals will be sacrificed and discarded.

APPROVED BY:


Duane W. Hallesy, Ph.D.
Director, Toxicology
Study Director
Diplomate, A.B.T.

5 Nov 85
Date


Leonard D. Shott, D.V.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.

4 Nov 85
Date

0046



STUDY SCHEDULING SHEET

PROTOCOL NUMBER: 841-R-85-96526-000-PO-TXE

STUDY DIRECTOR:

Duane W. Hallesy 10/25/85
Duane W. Hallesy, Ph.D.
Director, Toxicology

PROPOSED

STARTING DATE: 10/30/85

PROPOSED IN-LIFE

COMPLETION DATE: 11/13/85



APPENDIX B

ACKNOWLEDGMENTS

STUDY DIRECTOR, SCIENTISTS, AND SUPERVISORY
PERSONNEL INVOLVED IN TOXICITY STUDY

Institute of Toxicologic Sciences

Director: I. Heyman
Information Section: L. Thunen

Department of Toxicology

Director, Toxicology: D. Hallesy
Study Director: D. Hallesy
Animal Colony, Supervisor of Toxicology Biology: L. Machholz

Department of Pathology

Director, Pathology: L. Shott
Veterinary Pathologist: L. Shott

Institute of Pharmaceutical Sciences

Assistant Director: S. Shastri
Head, Department of Clinical Manufacturing: Z. Shaikh
Manager, Quality Assurance/Quality Control: G. Shah

Institute of Organic Chemistry

Director, Analytical Research: L. Throop

Environmental Health and Safety

Director, Environmental Health and Safety: F.J. Murray

RS-96526-000



REPORT 832-R-85-96526-000-SK-TXE

FINAL REPORT

ACUTE DERMAL TOXICITY OF RS-96526-000 WHEN TESTED ON THE RAT

STUDY TEAM

Study Director:

Duane W. Mallesy, Ph.D.
Director, Toxicology
Diplomate, A.B.T.
Institute of Toxicologic Sciences
Department of Toxicology

Veterinary Pathologist:

Leonard G. Shott, D.V.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.
Institute of Toxicologic Sciences
Department of Pathology

Study Started:

18 June 1985

In-Life
Completion Date:

02 July 1985

Date of Report:

26 November 1985

SYNTEX RESEARCH
Institute of Toxicologic Sciences
Departments of Toxicology and Pathology
3401 Hillview Avenue, Palo Alto, California

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| 3 | Body Weights (Group 2) | 12 |
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APPENDICES

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REPORT TITLE: ACUTE DERMAL TOXICITY OF RS-96526-000 WHEN TESTED ON THE RAT

PURPOSE: The Environmental Health and Safety Group has requested that we carry out safety evaluation studies on RS-96526-000 (acetyl ferrocene). This study was part of the safety evaluation package.

CHEMICAL NAME: RS-96526 = Acetyl Ferrocene

DATA RETENTION: The raw data and report for this study are maintained in Syntex Research Archives located at 3401 Hillview Avenue, Palo Alto, California.

PROCEDURE: All procedures were conducted according to the Standard Operating Procedures for the Institute of Toxicologic Sciences, Syntex Research.

SUMMARY

The acute dermal toxicity of RS-96526 (acetyl ferrocene) was investigated using the rat as the test system. An initial study was done in which rats were given single dermal doses of 2 g/kg applied under occlusion to abraded skin. The test sites were occluded for 24 hours after dosing. Three of the four animals given this dose of compound died before the end of the observation period. The first animal died 2 days after dosing. Signs of toxicosis were rather non-specific.

Because of the high mortality for the group given 2 g/kg, the study was repeated with 2 additional groups each composed of 2 male and 2 female rats. These animals were given single dermal doses of 0.05 or 0.50 g/kg. The experimental procedures were the same as those used for the 2 g/kg group. One male given 0.50 g/kg was found dead 3 days after dosing. Signs of toxicosis included rough coat, hypothermia and increased respiratory rate. One animal given 0.05 and one given 0.50 g/kg of RS-96526 had well defined and very slight erythema, respectively, on the day after dosing. All signs of irritation were absent by 2 days after dosing.

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REPORT 832-R-85-96526-000-SK-TXE

SUMMARY (continued)

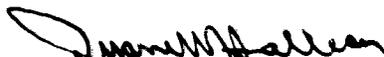
Conclusions

The acutely lethal dermal dose of RS-96526 (acetyl ferrocene) is between 50 and 500 mg/kg.

COMMENT

No unforeseen circumstances were noted which would have affected the quality or the integrity of the study.

APPROVED BY:



Duane W. Hallesy, Ph.D.
Director, Toxicology
Study Director
Diplomate, A.B.T.

21 Nov 85

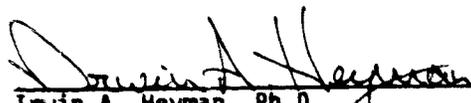
Date



Leonard D. Shott, D.V.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.

21 Nov 85

Date



Irwin A. Heyman, Ph.D.
Vice President, Syntex Research
Director, ITS

26 Nov 85

Date



EXPERIMENTAL PROCEDURES AND RESULTS

Test Article Formulations

RS-96526 (Lot No. 187-124) for group 2 and RS-96526 (Lot No. 187-120) for groups 3 and 4 were released for use in this study by the Institute of Organic Chemistry, Syntex Research, Palo Alto, California. To facilitate application to the test sites, the test article was mixed with water. Final use of RS-96526 (Lot No. 187-124) for this study was on 18 June 1985. The last date of analysis as noted on the test article container was 04 February 1985. Final use of Lot No. 187-120 was on 17 September 1985. Leftover compound will be returned to the Institute of Organic Chemistry to determine if reanalysis is necessary. A summary of analytical results will be maintained on file with the Environmental Health and Safety Group rather than being presented in this report.

Animals

The rat was selected for this study because of its small size and because it is one of the recommended rodent species for acute toxicity studies. The rats used ((CD)Sprague-Dawley-derived) were purchased from Charles River, Portage, MI and were specified to be young adults. The rats for group 2 were received at Syntex on 15 May 1985 and weighed between 307 g and 334 g for males and between 166 g and 178 g for females upon receipt. The rats for groups 3 and 4 were received at Syntex on 3 July 1985 and weighed between 184 g and 222 g for males and between 141 g and 161 g for females upon receipt.

The rats were individually housed in stainless steel cages with wire mesh fronts and bottoms. The trays under the cages were flushed with water daily. The rats were transferred into clean cages biweekly. Food (Purina Certified Rodent Chow®) and water were available ad libitum.



REPORT 832-R-85-96526-000-...-TXE

EXPERIMENTAL PROCEDURES AND RESULTS (continued)

The rats were acclimatized for 34 days (group 2) and 76 days (groups 3 and 4) before the start of the study. Following assignment to the study, each rat was individually identified using a standard ear punch code. The study number and the animal number were used to identify the cage in which the animal was housed.

Treatment

The rats were assigned to 3 groups each composed of 2 males and 2 females. Treatment employed was as follows:

| <u>Group/Animal ID</u> | <u>Compound</u> | <u>Dose (g/kg)</u> |
|------------------------|-----------------|--------------------|
| 2 (200 series) | RS-96526 | 2.00 |
| 3 (300 series) | RS-96526 | 0.50 |
| 4 (400 series) | RS-96526 | 0.05 |

The dermal route was selected to evaluate toxic changes from accidental skin exposure. The back of each animal was clipped free of hair the day before dosing. Just prior to applying the test substance, the skin was abraded by making four epidermal incisions with a clean needle. Each animal received a single dose of the test formulation. The day of application of the test material was considered to be the study day 1.

The test formulation was held in contact with the skin for a 24-hour exposure period using a porous gauze dressing for group 2 and Hilltop chambers for groups 3 and 4 which were secured in place with non-irritating tape. All animals were fitted with collars to prevent ingestion of test material during the exposure period.

Following the 24-hour exposure period the residual test substance was removed and the back of each animal was washed with water to remove any remaining test material.

EXPERIMENTAL PROCEDURES AND RESULTS (continued)Observations

Clinical observations on the day after dosing were made one-half hour after the residual test substance was washed from the test site. The rats were observed daily and clinical signs including observations on the dosed area were recorded daily for the remainder of the study. Body weights and food intakes were recorded weekly.

The animals for group 2 were dosed on 18 June 1985 and the final observations were made on 2 July 1985. The animals for groups 3 and 4 were dosed on 17 September 1985 and the final observations were made on 1 October 1985. At the end of the 14-day observation period, all surviving animals were euthanatized and discarded. No animals were necropsied.

Results

The clinical observations, body weight and food intake data are presented in In-Life Tables 1-4, respectively, for group 2 animals. ~~Body weight and food intake data are presented in in-Life Tables 3A and 4A, respectively,~~ for groups 3 and 4 animals.

Group 2

There were no signs of skin irritation on any animals. After unwrapping, all dosed areas of the back were stained bright orange-yellow (due to the drug), which disappeared over time. No clinical observations for redness and swelling were recorded on the computer if there were no signs of redness or swelling present on the original manual entry data sheets. Three animals were found dead: animals #200, #203, and #202 on study days 3, 4, and 11, respectively. Clinical observations noted for animal #202 prior to death were: unthriftiness, wasting, rough coat, pallor, inactivity, increased and labored respiration, cold body temperature, urogenital staining, dehydration, ataxia and kyphosis.



EXPERIMENTAL PROCEDURES AND RESULTS (continued)

Animal #201 appeared clinically normal throughout the 14-day observation period.

On study day 8, surviving animals had their backs clipped due to fast hair growth and the inability to see the skin.

Due to the large mortality, body weights and food intakes could not be compared.

Groups 3 and 4

The clinical observations for groups 3 and 4 animals are fully described below. Because most animals remained normal for the majority of the study, it was considered unnecessary to present clinical observation tables for these groups. Four of 8 animals appeared clinically normal throughout the 14-day postdose observation period. Following the 24-hour exposure period, the only signs of skin irritation observed were as follows: animals #302 and #401 were scored for very slight and well-defined erythema, respectively, on study day 2. These signs disappeared on study day 3, and these animals remained normal for the duration of the study. Animal #301 was found dead on study day 4, after exhibiting rough coat, hypothermia, and increased respiration on study day 3. Animal #400 exhibited alopecia on the fore and hind limbs but this was considered unrelated to drug toxicity.

0007



REPORT 832-R-85-98526-000-SK-TXE

EXPERIMENTAL PROCEDURES AND RESULTS (continued)

Body weight and food intake values were comparable between groups (In-Life Tables 3A and 4A).

SUBMITTED BY:

Rubi Leonardi
Rubi Leonardi
Laboratory Animal Specialist

11-25-85
Date

Calvin Harris
Calvin Harris, B.S.
Toxicology Biologist

11-26-85
Date

REPORTED BY:

Laura A. Machholz
Laura A. Machholz, B.S.
Supervisor of Toxicology Biology

11-21-85
Date

0008



REPORT 832-R-85-96526-000-SK-TXE

IN-LIFE TABLES

0013

PAGE 01.00

REPORT 0832-R-85-05520-000-SK-TXE

TABLE 3 A

BODY WEIGHTS (G) FOR RATS GIVEN
A SINGLE DERMAL APPLICATION OF RS-06520
FOLLOWED BY A TWO-WEEK OBSERVATION PERIOD

| ANIMAL ID | STUDY WEEK | | |
|-----------|-------------------------|-----|-----|
| | 14 | 15 | 16 |
| GROUP | 3 M: 0.50 G/KG RS-06520 | | |
| 0301 | 501 | D | D |
| 0303 | 508 | 500 | 480 |
| N | 2 | 1 | 1 |
| MEAN | 505 | 500 | 480 |
| STD | * | * | * |
| GROUP | 3 F: 0.50 G/KG RS-06520 | | |
| 0300 | 298 | 283 | 290 |
| 0302 | 328 | 318 | 315 |
| N | 2 | 2 | 2 |
| MEAN | 312 | 301 | 302 |
| STD | * | * | * |

D- ANIMAL DECEASED

*- SAMPLE SIZE TOO SMALL TO CALCULATE STD

TABR101/TABR02

0014

PAGE 02.00

REPORT 0832-R-85-96526-000-SK-TXE

TABLE 3 A

BODY WEIGHTS (G) FOR RATS GIVEN
A SINGLE DERMAL APPLICATION OF RS-96526
FOLLOWED BY A TWO-WEEK OBSERVATION PERIOD

| ANIMAL ID | STUDY WEEK | | |
|-----------|-------------------------|-----|-----|
| | 14 | 15 | 18 |
| GROUP | 4 M: 0.05 G/KG RS-96526 | | |
| 0401 | 484 | 495 | 483 |
| 0403 | 497 | 485 | 498 |
| N | 2 | 2 | 2 |
| MEAN | 490 | 490 | 490 |
| STD | * | * | * |
| GROUP | 4 F: 0.05 G/KG RS-96526 | | |
| 0400 | 322 | 308 | 312 |
| 0402 | 281 | 273 | 271 |
| N | 2 | 2 | 2 |
| MEAN | 302 | 290 | 292 |
| STD | * | * | * |

* - SAMPLE SIZE TOO SMALL TO CALCULATE STD

TABR101/TABR802

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REPORT 8832-R-85-88528-000-SK-TXE

TABLE 4

AVERAGE DAILY FOOD INTAKES (G) FOR
RATS GIVEN A SINGLE DERMAL APPLICATION OF
RS-88528 FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

ANIMAL STUDY WEEK

| ID | 1 | 2 | 3 |
|-------|------------------------|----|----|
| GROUP | 2 M: 2.0 G/KG RS-88528 | | |
| 201 | 28 | 32 | D |
| 203 | D | D | D |
| N | 1 | 1 | 0 |
| MEAN | 28 | 32 | NA |
| STD | * | * | * |
| GROUP | 2 F: 2.0 G/KG RS-88528 | | |
| 200 | D | D | D |
| 202 | 10 | D | D |
| N | 1 | 0 | 0 |
| MEAN | 10 | NA | NA |
| STD | * | * | * |

NA DATA NOT AVAILABLE
D ANIMAL DEAD, MISSING, OR REMOVED FROM STUDY
* SAMPLE SIZE TOO SMALL TO CALCULATE STD

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REPORT 0832-R-85-96526-000-SK-TXE

TABLE 4 A

AVERAGE DAILY FOOD INTAKES (G) FOR
RATS GIVEN A SINGLE DERMAL APPLICATION OF
RS-96526 FOLLOWED BY A TWO-WEEK OBSERVATION PERIOD

ANIMAL STUDY WEEK *

| ID | 1 | 2 | 3 |
|-------|------------------------|----|----|
| GROUP | 3 M:0 50 G/KG RS-96526 | | |
| 301 | D | D | D |
| 303 | 33 | 33 | D |
| N | 1 | 1 | 0 |
| MEAN | 33 | 33 | NA |
| STD | * | * | * |
| GROUP | 3 F:0 50 G/KG RS-96526 | | |
| 300 | 18 | 21 | D |
| 302 | 18 | 23 | D |
| N | 2 | 2 | 0 |
| MEAN | 18 | 22 | NA |
| STD | * | * | * |

*Study weeks 1, 2, and 3 on this table correspond to study weeks 14, 15, and 16, respectively. The study week numbers on this table were normalized to reflect the first food intake interval for these animals (called study week 1) and the second food intake interval for these animals (called study week 2). The study week numbers on this table do not reflect the chronological study week.

NA DATA NOT AVAILABLE
D ANIMAL DEAD, MISSING, OR REMOVED FROM STUDY
* SAMPLE SIZE TOO SMALL TO CALCULATE STD

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0017

PAGE 02.00

REPORT 8832-R-85-88520-000-SK-TXE

TABLE 4 A

AVERAGE DAILY FOOD INTAKES (G) FOR
RATS GIVEN A SINGLE DERMAL APPLICATION OF
RS-88520 FOLLOWED BY A TWO-WEEK OBSERVATION PERIOD

ANIMAL STUDY WEEK *

| ID | 1 | 2 | 3 |
|-------|------------------------|----|----|
| GROUP | 4 M:0.05 G/KG RS-88520 | | |
| 401 | 28 | 35 | D |
| 403 | 28 | 32 | D |
| N | 2 | 2 | 0 |
| MEAN | 27 | 34 | NA |
| STD | * | * | * |
| GROUP | 4 F:0.05 G/KG RS-88520 | | |
| 400 | 21 | 24 | D |
| 402 | 18 | 18 | D |
| N | 2 | 2 | 0 |
| MEAN | 20 | 22 | NA |
| STD | * | * | * |

* See comment, page 1 of this table.

NA DATA NOT AVAILABLE
D ANIMAL DEAD, MISSING, OR REMOVED FROM STUDY
* SAMPLE SIZE TOO SMALL TO CALCULATE STD

00071

REPORT 832-R-85-96526-000-SK-TXE

0018



APPENDICES

**SPONSOR AND TESTING FACILITY:**

SYNTEX RESEARCH
Institute of Toxicologic Sciences
Departments of Toxicology and Pathology
3401 Hillview Avenue, Palo Alto, California

PROTOCOL NUMBER:

832-R-85-96526-000-SK-TXE

PROTOCOL DATE:

May 2, 1985

PROTOCOL TITLE:

ACUTE DERMAL TOXICITY OF RS-96526-000 WHEN TESTED ON THE RAT

PROJECT NUMBER:

2.0.18

PROTOCOL PURPOSE:

The Environmental Health and Safety Group has requested that we carry out safety evaluation studies on RS-96526-000 (acetyl ferrocene). This study is part of the safety evaluation package.

SCHEDULING:

Starting and in-life completion dates will be added when the study is scheduled.

PROCEDURE:

Unless otherwise stated, all procedures will be done according to Standard Operating Procedures for the Institute of Toxicologic Sciences.

A. Test Articles

1. The RS-96526-000 will be released for use by the Institute of Organic Chemistry (IOC), Syntex Research. A summary of their analytical results will be included with the report of this study.
2. On receipt, the test article will be stored at 4 degrees C. The elapsed time between release of article by IOC and testing in ITS shall not exceed 90 days. If the elapsed time should exceed 90 days an aliquot of the test article will be returned to IOC for reanalysis.
3. The test article will be used as received.

B. Animals

1. Healthy animals, not subject to any previous experimental procedures, will be used.
2. Obtain enough rats so that 2 males and 2 females will be available for this study.

REVISED
SEE REVISION AUG 22 1985

PROTOCOL 832-R-85-96526-000-S, TXE

Page 2

B. Animals (continued)

Strain: (CD) Sprague Dawley Derived
 Source: Charles River, Kingston, NY, or Portage, MI
 Age: Not specified (Young adult)
 Weight: Not specified (Young adult)

3. Upon receipt the rats will be placed into individual cages and held for an acclimatization period of approximately 1 week.
4. Food (Purina Certified Rodent Chow[®]) and water will be available ad libitum. No materials are anticipated to be present in the food or water which would interfere with the purpose or conduct of this study.
5. Following assignment to the study each rat is to be individually identified using a standard ear punch code, or with indelible ink. The method used is to be documented.
6. The animals will be inspected during the acclimatization period. Any animal exhibiting unusual behavior, significant change in condition of the fur, color of the skin, scratching, loud or irregular breathing, unusual discharges from orifices, unusual condition of urine or stool, changes in food or water consumption, or wounds caused by fights will not be used for the study.

C. Test Procedures

| <u>Group*</u> | <u>Compound</u> | <u>Dose (ml/kg) or (g/kg)</u> |
|----------------|-----------------|-----------------------------------|
| 2 (200 series) | RS-96526-000 | 2.0 |

* Groups composed of 2 males and 2 females

Preparation of Animal

1. Route - dermal. The dermal route was selected to evaluate toxic changes that might occur in cases of accidental skin exposure.
2. The back of each animal should have an area of at least 15 square centimeters clipped free of hair.
3. The animals are to be fitted with collars to prevent ingestion of test material during the exposure period.

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Page 3

C. Test Procedures (continued)

4. Just prior to applying the test substance, abrade the skin by making four epidermal incisions with a clean needle through the stratum corneum (not deep enough to disturb the derma or produce bleeding).

Test Substance

1. Liquids should be tested directly; solid test substances should be moistened sufficiently with normal saline or tap water to make a paste that will insure good contact with the skin. For some test substances, it may be appropriate to use other vehicles. If a carrier or diluent is used, it should be non-irritating and of known low toxicity. When such vehicles are used, the vehicle should be reported and acknowledgment should be made of the effects of the vehicles on absorption of the test substance in reporting the results.
2. The test substance should be applied uniformly over the abraded area and should be held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24 hour exposure period. The day of dosing is considered to be day 1.
3. Following the 24 hour exposure period, residual test substance shall be removed using water to wash the test site.

D. Observations

1. Observation of the test site for skin irritancy and clinical observations (including changes in skin, fur, eyes, mucous membranes, respiratory, cardiovascular system, autonomic and central nervous system, motor activities, tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma) should be made about one-half hour after the residual test substance has been washed from the test site (day 2).
2. Clinical and skin irritancy observations should be recorded daily for a period of two weeks. Animals with visible signs of systemic toxicity should be held for longer than 2 weeks.
3. See Table 1 for criteria in grading skin reaction recommended by IRLG.
4. Mortality - Daily observations should be made.
5. Body weight and food intake should be measured weekly and at sacrifice.

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Page 4

D. Observations (continued)

6. At the end of the observation period, all surviving animals should be euthanatized and discarded.
7. No animal will be necropsied.
8. If any deaths occur, the test will be repeated at doses below 2 g/kg or 2 ml/kg.

E. Results

Tabulation of data and individual results will accompany each report in sufficient detail to permit independent evaluation of results, including summaries and tables that show, as appropriate, the relationship of effects to time of dosing, sex, etc.

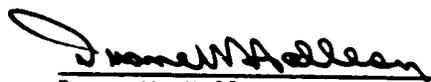
F. Statistical Evaluation

No statistical evaluation will be required.

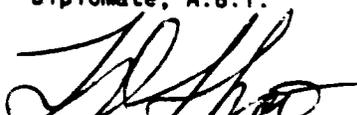
G. Data Retention

All raw data will be maintained in Syntex Research Archives managed from 3401 Millview Avenue, Palo Alto, California.

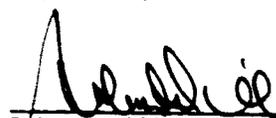
APPROVED BY:


 Duane W. Hallesy, Ph.D.
 Director, Toxicology
 Study Director
 Diplomate, A.B.T.

3 May 85
 Date


 Leonard D. Shott, D.V.M., Ph.D.
 Director, Pathology
 Diplomate, A.C.V.P.

6 May 85
 Date


 Robert Hill, Ph.D.
 Vice President, Syntex Research
 Director, ITS
 Diplomate, A.B.T.

6 May 85
 Date



TABLE 1

EVALUATION OF SKIN REACTIONErythema and Eschar Formation

| | |
|--|---|
| No erythema..... | 0 |
| Very slight erythema (barely perceptible)..... | 1 |
| Well-defined erythema..... | 2 |
| Moderate to severe erythema..... | 3 |
| Severe erythema (beet redness to slight eschar formation (injuries in depth)..... | 4 |

Edema Formation

| | |
|--|--------------------|
| No edema..... | 0 |
| Very slight edema (barely perceptible)..... | 1 |
| Slight edema (edges of area well defined by definite raising)..... | 2 |
| Moderate edema (raised approximately 1 mm)..... | 3 |
| Severe edema (raised more than 1 mm and extending beyond the area of exposure)..... | 4 |
| Severe eschar and/or corrosion..... | Note occurrence |

0024



SPONSOR AND
TESTING FACILITY:

SYNTEX RESEARCH
Institute of Toxicologic Sciences
Departments of Toxicology and Pathology
3401 Hillview Avenue, Palo Alto, California

PROTOCOL NUMBER: 832-R-85-96526-000-SK-TXE

REVISION NUMBER: One

REVISION DATE: August 22, 1985

PURPOSE OF REVISION: Because 3/4 animals dosed with 2 g/kg of acetyl ferrocene (RS-96526-000) died before the end of the 2 week postdose observation period, the provision for additional dose groups is being implemented.

REVISION:

Two additional groups of 2 male and 2 female rats will be added to the study. The dose group information is shown below. All other provisions of the study remain the same.

| <u>Group</u> | <u>Compound</u> | <u>Dose (g/kg)</u> |
|----------------|-----------------|--------------------|
| 3 (300 series) | RS-96526-000 | 0.50 |
| 4 (400 series) | RS-96526-000 | 0.05 |

APPROVED BY:


Duane W. Hallesy, Ph.D.
Director, Toxicology
Study Director
Diplomate, A.B.T.

26 Aug 85
Date


Leonard D. Shott, D.V.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.

27 Aug 85
Date

0025



STUDY SCHEDULING SHEET

PROTOCOL NUMBER: 832-R-85-96326-010-2K-TYE

STUDY DIRECTOR: Duane Halliday, Ph.D.

Duane Halliday
5 Jun 85

PROPOSED STARTING DATE: June 13, 1985

PROPOSED IN-LIFE COMPLETION DATE: July 2, 1985



APPENDIX B

ACKNOWLEDGMENTS

STUDY DIRECTOR, SCIENTISTS, AND SUPERVISORY
PERSONNEL INVOLVED IN TOXICITY STUDY

Institute of Toxicologic Sciences

Director: I. Heyman
Information Section: L. Thunen

Department of Toxicology

Director, Toxicology: D. Hallesy
Study Director: D. Hallesy
Animal Colony, Supervisor of Toxicology Biology: L. Machholz

Department of Pathology

Director, Pathology: L. Shott
Veterinary Pathologist: L. Shott

Institute of Organic Chemistry

Director, Analytical Research: L. Throop

Environmental Health and Safety

Director, Environmental Health and Safety: F.J. Murray



02-2624-000

REPORT 001-0-02-2624-000-00-THE

FINAL REPORT

ACUTE ORAL TOXICITY OF 02-2624-000 WHEN TESTED IN THE RAT

STUDY TEAM

Study Director:

**Osborne W. Mallesy, Ph.D.
Director, Toxicology
Diplomate, A.S.T.
Institute of Toxicologic Sciences
Department of Toxicology**

Interim Pathologist:

**Leonard G. Shett, D.V.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.
Institute of Toxicologic Sciences
Department of Pathology**

Study Started:

10 July 1985

**In-Life
Completion Date:**

25 July 1985

Date of Report:

25 October 1985

**SYNTEX RESEARCH
Institute of Toxicologic Sciences
Departments of Toxicology and Pathology
3001 Wilshire Avenue, Palo Alto, California**



REPORT 831-R-85-96526-000-PO-TXE

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| 2 Clinical Observations | 10 - 16 |
| 3 Body Weights | 17 - 19 |
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REPORT TITLE: ACUTE ORAL TOXICITY OF RS-96526-000 WHEN TESTED IN THE RAT

PURPOSE: The Environmental Health and Safety Group has requested that safety evaluation studies be performed on RS-96526-000 (acetyl ferrocene). This study was part of the safety evaluation package.

CHEMICAL NAME: RS-96526 - Acetyl Ferrocene

DATA RETENTION: The raw data and report for this study are maintained in Syntex Research Archives located at 3401 Hillview Avenue, Palo Alto, California.

PROCEDURE: All procedures were conducted according to the Standard Operating Procedures for the Institute of Toxicologic Sciences, Syntex Research.

SUMMARY

The acute oral toxicity of RS-96526 was evaluated using the rat as the test species. Three groups each composed of 2 males and 2 females were used for this study. The animals were given single oral doses of 0.05, 0.15, or 0.50 g/kg. The animals were observed for signs of toxicity for a 2-week period after dosing.

All rats given single oral doses of 0.05, 0.15, or 0.50 g/kg of RS-96526 died before the end of the 2-week post dosing observation period. The time to death was related to dose. All rats given 0.5 g/kg were dead by 2 days after dosing, those given 0.15 g/kg were dead by 6 days after dosing, and the last of the rats given 0.05 g/kg died 12 days after dosing. Signs of toxicosis included but were not limited to: kyphosis, ptosis, rough coat, unthrifty appearance, wasting, labored respiration, raling, tremors, decreased activity, and death. No animals were necropsied.

0002



REPORT 831-R-85-96526-000-PO-TXE

SUMMARY (continued)

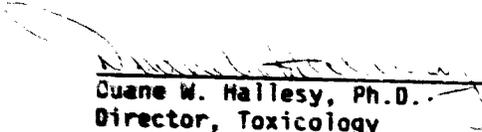
Conclusions

The acutely toxic oral dose of RS-96526 for rats is less than 0.05 g/kg.

COMMENT

No unforeseen circumstances were noted which would have affected the quality or the integrity of the study.

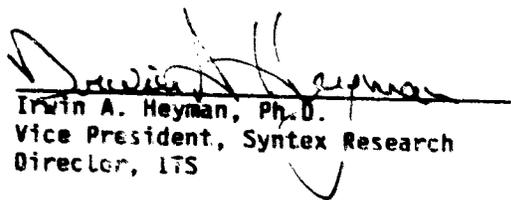
APPROVED BY:


Duane W. Hallesy, Ph.D.
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Date


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24 Oct 85
Date


Irwin A. Heyman, Ph.D.
Vice President, Syntex Research
Director, IIS

25 Oct 85
Date



EXPERIMENTAL PROCEDURES AND RESULTS

Test Article Formulations

The RS-96526 (Lot # 187-124) was released for use in this study by the Institute of Organic Chemistry, Syntex Research, Palo Alto, California. The RS-96526 was mixed with vegetable oil so that a concentration of 50 mg/ml was attained. Dosing was done within 15 minutes of mixing. The last date of analysis for this compound, as noted on the test article container was 4 February 1985. Leftover material will be returned to the Institute of Organic Chemistry to determine if reanalysis is necessary. A summary of analytical results will be maintained on file with the Environmental Health and Safety Group rather than being presented in this report.

Animals

The rat was selected because of its small size and because it is one of the species recommended for the evaluation of acute toxicity. Sprague-Dawley derived rats (CD) purchased from Charles River, Portage, MI, were received on 29 May 1985. The rats weighed between 164 and 321 g. They were young adults as specified.

After receipt at Syntex, the rats were individually housed in stainless steel cages with wire-mesh fronts and bottoms. The rats were transferred into clean cages biweekly and maintained in an environmentally controlled room. Food (Purina Certified Rodent Chow®) and water were available to the animals ad libitum except for an overnight period of food deprivation before dosing. Food was withheld for a further 2 hours (approximately) postdosing.

The rats were acclimatized to laboratory conditions for over 12 days before assignment to the study. Only those animals considered to be in satisfactory health were used. Each animal was individually identified by

EXPERIMENTAL PROCEDURES AND RESULTS (continued)

using an ear notch. The animal number and the study number were used to identify the card attached to each animal's cage.

Treatment

The rats were assigned to 3 groups each composed of 2 males and 2 females. Treatment, based on predose individual animal body weights, was as follows:

| <u>Group</u> | <u>(g/kg)</u> | <u>Dose Formulation</u> <u>ml/100 g</u> | <u>Concentration of</u> <u>RS-96526 (mq/ml)</u> |
|--------------|---------------|--|--|
| 2 | 0.05 | 0.1 | 52.63 |
| 3 | 0.15 | 0.3 | 52.63 |
| 4 | 0.50 | 1.0 | 52.63 |

The test formulations were administered as single doses using a metal rodent intubator. The day of administration of the test formulation was considered study day 1. The oral route was selected to evaluate effects which would occur if the test compound were accidentally ingested.

Observations

Each animal received a single dose and was observed for 14 days. Clinical observations were recorded daily. Body weights were recorded on study days 1 (day of dosing), 8, and 15 (last day of the study).

RESULTS

The nature and incidence of clinical observations, observations for individual animals, and body weights are presented in the In-Life Tables 1 through 3.

After dosing on study day 1, only the males in group 2 remained normal. The females in group 2 exhibited orange urine, slight kyphosis, and one

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REPORT R91-R-95-26526-000-PO-TXEEXPERIMENTAL PROCEDURES AND RESULTS (continued)

female exhibited slight ptosis. Following dosing on day 1, animals in group 3 exhibited slight to moderate orange urine, kyphosis, ptosis, salivation, and orange saliva. Animals in group 4 exhibited mild to severe signs of inactivity, kyphosis, rough coat, unthriftiness, salivation, orange urine, and ptosis.

On day 2 the males in group 2 exhibited slight rough coats and all the remaining animals exhibited some or all of the following: kyphosis, ptosis, salivation, unthrifty appearance, rough coat, and orange urine. On day 2, 3/4 of the animals in group 4 and one male in group 3 were found dead. By day 3 all the animals in group 4 were dead and 2/4 the animals in group 3 were scored for some or all of the following: unthrifty appearance, orange urine, rough coats, ptosis, kyphosis, labored respiration, and wasting. Animals in group 4 were consistently scored more severely than the animals in group 3 which were scored more severely than the animals in group 2. All the animals in group 3 were dead by day 7 and all the animals in group 2 were dead by day 13 of the study. No animals were submitted for necropsy.

SUBMITTED AND REPORTED BY:

David Schumacher
David Schumacher, B.S.
Toxicology Biologist

12/24/85
Date

0006



REPORT 831-R-85-96526-000-PO-TXE

IN-LIFE TABLES

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REPORT 831-R-85-80528-000-P0-TX
TABLE 1

INCIDENCE OF CLINICAL OBSERVATIONS FOR
RATS GIVEN A SINGLE ORAL DOSE OF RS-80528
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

GROUP 2 - 0.050 G/KG RS-80528

MALES

| ANIMAL CLINICAL DESCRIPTOR /LOCATION | STUDY DAY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------------------------------------|-----------|---|---|---|---|---|---|---|---|---|----|----|----|
| NUMBER OF ANIMALS | | 2 | 2 | 2 | 2 | 2 | 2 | 1 | | | | | |
| NORMAL | | | | | | | | | | | | | |
| KYPHOSIS | | | | | | | | | | | | | |
| LABORED RESPIR | | | | | | | | | | | | | |
| PTOSIS | | | | | | | | | | | | | |
| ROUGH COAT | | | | | | | | | | | | | |
| UNTIMELY | | | | | | | | | | | | | |
| WASTING | | | | | | | | | | | | | |
| DEAD/MISSING/REMOVED FROM STDY | | | | | | | | 1 | | | | | |

FEMALES

| ANIMAL CLINICAL DESCRIPTOR /LOCATION | STUDY DAY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------------------------------------|-----------|---|---|---|---|---|---|---|---|---|----|----|----|
| NUMBER OF ANIMALS | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 |
| NORMAL | | | | | | | | | | | | | |
| BLEEDING H FOOT LEFT DIGITA | | | | | | | | | | | | | |
| COLD | | | | | | | | | | | | | |
| COLLAPSED | | | | | | | | | | | | | |
| DISCOLORED BODY BLUE | | | | | | | | | | | | | |
| DISCOLORED P LIMS UPPER RED | | | | | | | | | | | | | |
| DISCOLORED URINE ORANGE | | | | | | | | | | | | | |
| GASPING | | | | | | | | | | | | | |
| INACTIVE | | | | | | | | | | | | | |
| KYPHOSIS | | | | | | | | | | | | | |
| LABORED RESPIR | | | | | | | | | | | | | |
| PTOSIS | | | | | | | | | | | | | |
| ROUGH COAT | | | | | | | | | | | | | |
| SWOLLEN H FOOT LEFT | | | | | | | | | | | | | |
| TREMORS | | | | | | | | | | | | | |
| UNTIMELY | | | | | | | | | | | | | |
| WASTING | | | | | | | | | | | | | |
| DEAD/MISSING/REMOVED FROM STDY | | | | | | | | | | | | | |

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REPORT 831-R-85-98526-000-PO-TX
TABLE 1

INCIDENCE OF CLINICAL OBSERVATIONS FOR
RATS GIVEN A SINGLE ORAL DOSE OF RS-98526
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

GROUP 3 - 0.150 G/KG RS-98526

MALES

| ANIMAL CLINICAL DESCRIPTOR /LOCATION | STUDY DAY | | | | | | | | | | | |
|---|-----------|---|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| NUMBER OF ANIMALS | 2 | 2 | 1 | - | - | - | - | - | - | - | - | - |
| NORMAL | - | - | - | - | - | - | - | - | - | - | - | - |
| KYPHOSIS | - | - | - | - | - | - | - | - | - | - | - | - |
| PTOSIS | - | - | - | - | - | - | - | - | - | - | - | - |
| SALIVATING | - | - | - | - | - | - | - | - | - | - | - | - |
| UNTHRIFTY | - | - | - | - | - | - | - | - | - | - | - | - |
| DEAD/MISSING/REMOVED FROM STDY | - | - | - | - | - | - | - | - | - | - | - | - |

FEMALES

| ANIMAL CLINICAL DESCRIPTOR /LOCATION | STUDY DAY | | | | | | | | | | | |
|---|-----------|---|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| NUMBER OF ANIMALS | 2 | 2 | 2 | 2 | 2 | 1 | 1 | - | - | - | - | - |
| NORMAL | - | - | - | - | - | - | - | - | - | - | - | - |
| DISCOLORED URINE ORANGE | - | - | - | - | - | - | - | - | - | - | - | - |
| KYPHOSIS | - | - | - | - | - | - | - | - | - | - | - | - |
| PTOSIS | - | - | - | - | - | - | - | - | - | - | - | - |
| ROUGH COAT | - | - | - | - | - | - | - | - | - | - | - | - |
| UNTHRIFTY | - | - | - | - | - | - | - | - | - | - | - | - |
| WASTING | - | - | - | - | - | - | - | - | - | - | - | - |
| DEAD/MISSING/REMOVED FROM STDY | - | - | - | - | - | - | - | - | - | - | - | - |

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REPORT 831-R-85-00520-000-PO-TX
TABLE 1

INCIDENCE OF CLINICAL OBSERVATIONS FOR
RATS GIVEN A SINGLE ORAL DOSE OF RS-00520
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

GROUP 4 - 0.500 G/NG RS-00520

MALES

| ANIMAL CLINICAL DESCRIPTOR /LOCATION | STUDY DAY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------------------------------------|-----------|---|---|---|---|---|---|---|---|---|----|----|----|
| NUMBER OF ANIMALS | | 2 | 2 | 1 | - | - | - | - | - | - | - | - | - |
| NORMAL | | - | - | - | - | - | - | - | - | - | - | - | - |
| INACTIVE | | 1 | 1 | - | - | - | - | - | - | - | - | - | - |
| KYPHOSIS | | 1 | 1 | - | - | - | - | - | - | - | - | - | - |
| PTOSIS | | - | - | - | - | - | - | - | - | - | - | - | - |
| ROUGH COAT | | 2 | 1 | - | - | - | - | - | - | - | - | - | - |
| SALIVATING | | 1 | 1 | - | - | - | - | - | - | - | - | - | - |
| UNTHIRTY | | 1 | 1 | - | - | - | - | - | - | - | - | - | - |
| DEAD/MISSING/REMOVED FROM STDY | | 1 | 1 | - | - | - | - | - | - | - | - | - | - |

FEMALES

| ANIMAL CLINICAL DESCRIPTOR /LOCATION | STUDY DAY | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------------------------------------|-----------|---|---|---|---|---|---|---|---|---|----|----|----|
| NUMBER OF ANIMALS | | 2 | 2 | - | - | - | - | - | - | - | - | - | - |
| NORMAL | | - | - | - | - | - | - | - | - | - | - | - | - |
| DISCOLORED URINE | | 1 | - | - | - | - | - | - | - | - | - | - | - |
| KYPHOSIS | | 2 | - | - | - | - | - | - | - | - | - | - | - |
| PTOSIS | | 2 | - | - | - | - | - | - | - | - | - | - | - |
| DEAD/MISSING/REMOVED FROM STDY | | 2 | 2 | - | - | - | - | - | - | - | - | - | - |

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REPORT 831-R-15-86528-000-PO-TXE
TABLE 2

CLINICAL OBSERVATIONS FOR RATS
GIVEN A SINGLE ORAL DOSE OF RS-86528
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL /LOCATION | CLINICAL DESCRIPTOR | MALES | | | | | | | | | | | | |
|---------------------|---|-----------|---|---|---|---|---|---|---|---|----|----|----|--|
| | | STUDY DAY | | | | | | | | | | | | |
| 0211 | NORMAL KYPHOSIS LABORED RESPIR PTOSIS ROUGH COAT UNTHRIFTY FOUND DEAD | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| | | * | | | | | | | | | | | | |
| | | | 2 | 2 | 3 | | | | | | | | | |
| | | | | 2 | 2 | 3 | | | | | | | | |
| | | | 1 | 1 | 2 | 3 | | | | | | | | |
| | | | 2 | 2 | 3 | | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 0213 | NORMAL KYPHOSIS LABORED RESPIR PTOSIS ROUGH COAT UNTHRIFTY WASTING FOUND DEAD | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| | | * | | | | | | | | | | | | |
| | | | 2 | 2 | 3 | 3 | | | | | | | | |
| | | | | 2 | 2 | 3 | 3 | | | | | | | |
| | | | 1 | 2 | 2 | 3 | 3 | | | | | | | |
| | | | 2 | 2 | 3 | 3 | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |

GROUP 2 - 0.050 G/KG RS-86528
SCORING : 0-NORMAL 1-SLIGHT 2-MODERATE 3-MARKED *-PRESENT D-DEAD M-MISSING R-REMOVED FROM STUDY
SIZE : S0-0MM S1=0-10MM S2=10-30MM S3=30-50MM S4=50-100MM S5=OVER 100MM

0010

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09/19/85
10:11:18

PAGE 2 00

REPORT 831-R-15-86528-000-PO-THE
TABLE 2

CLINICAL OBSERVATIONS FOR CATS
GIVEN A SINGLE ORAL DOSE OF RS-86528
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL CLINICAL DESCRIPTION / LOCATION | STUDY DAY | | | | | | | | | | | | |
|--|-----------|---|---|---|---|---|---|---|----|----|----|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | |
| 0210 BLEEDING | | | | | | | | | | | 1 | 1 | 0 |
| H FOOT LEFT DIGITS | | | | | | | | | | | | | |
| COLD | | | | | | | | | | | | | |
| DISCOLORED | | | | | | | | | | | | | |
| BODY BLUE | | | | | | | | | | | | | |
| DISCOLORED | | | | | | | | | | | | | |
| URINE ORANGE | 2 | 1 | 0 | | | | | | | | | | |
| INACTIVE | | | | | | | | | | | | | |
| KYPHOSIS | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| LABORED | | | | | | | | | | | | | |
| RESPIR | | | | | | | | | | | | | |
| PTOSIS | | | | | | | | | | | | | |
| RALING | 2 | 2 | 2 | 1 | 1 | 1 | 0 | | | | | | |
| ROUGH COAT | | | | | | | | | | | | | |
| SWOLLEN | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 1 |
| H FOOT LEFT | | | | | | | | | | | | | |
| TREMORS | | | | | | | | | | | | | |
| UNTHIRTY | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| WASTING | | | | | | | | | | | | | |
| 0212 COLD | | | | | | | | | | | | | |
| COLLAPSED | | | | | | | | | | | | | |
| DISCOLORED | | | | | | | | | | | | | |
| BODY BLUE | | | | | | | | | | | | | |
| DISCOLORED | | | | | | | | | | | | | |
| LIMB UPPER RED | | | | | | | | | | | | | |
| DISCOLORED | 1 | 1 | 0 | | | | | | | | | | |
| URINE ORANGE | | | | | | | | | | | | | |
| GASPING | | | | | | | | | | | | | |
| INACTIVE | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| KYPHOSIS | | | | | | | | | | | | | |
| LABORED | | | | | | | | | | | | | |
| RESPIR | | | | | | | | | | | | | |
| PTOSIS | | | | | | | | | | | | | |
| RALING | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 0 | | | | | |
| ROUGH COAT | | | | | | | | | | | | | |
| (THIS ANIMAL IS CONTINUED) | 1 | 2 | 1 | 1 | 1 | 2 | 2 | | | | | | |

GROUP 2 - 0.050 G/KG RS-86528
SCORING 0=NORMAL 1=SLIGHT 2=MODERATE 3=MARKED 4=PRESENT 5=DEAD 6=MISSING 7=REMOVED FROM STUDY
SIZE 50-60MM 51-60-10MM 52-10-30MM 53-20-50MM 54-50-100MM 55-50-100MM 56-50-100MM

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16:11:18

PAGE 3 00

REPORT 831-R-85 86526-000-PO-TXE
TABLL 2

CLINICAL OBSERVATIONS FOR RATS
GIVEN A SINGLE ORAL DOSE OF RS-98526
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL CLINICAL DESCRIPTOR /LOCATION | STUDY DAY | FEMALES |
|---|----------------------------|---------|
| 0212 | 1 2 3 4 5 6 7 8 9 10 11 12 | |
| TREMORS | | |
| UNTHRIFTY | | 1 0 |
| WASTING | 2 2 2 2 2 2 2 2 2 2 2 2 | 3 3 3 |
| FOUND DEAD | | 1 2 3 3 |
| DAY | 8 9 10 | 0 |

DISCOLORED RED REFERS TO THE FUR ON THE INSIDE OF BOTH FORE LIMBS

0012

GROUP 2 - 0.050 G/KG RS-98526

SCORING : 0=NORMAL 1=SLIGHT 2=MODERATE 3=MARKED * =PRESENT D=DEAD M=MISSING R=REMOVED FROM STUDY
SIZE : S0=0MM S1=0-10MM S2=10-30MM S3=30-50MM S4=50-100MM S5=OVER 100MM

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16:11:18

REPORT 831-R-85-88526-000-PC-THE
TABLE 2

CLINICAL OBSERVATIONS FOR RATS
GIVEN A SINGLE ORAL DOSE OF RS-85526
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL CLINICAL DESCRIPTION /LOCATION | MALES | | | | | | | | | | | |
|--|-------|---|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 0311 KYPHOSIS | | | | | | | | | | | | |
| PTOSIS | | | | | | | | | | | | |
| SALIVATING | | | 2 | | | | | | | | | |
| UNTHRIFTY | | | | | | | | | | | | |
| FOUND DEAD | | | | | | | | | | | | |
| DAY | 1 | | | | | | | | | | | |
| SALIVA ON FACE IS ORANGE | | | | | | | | | | | | |
| 0313 KYPHOSIS | | | | | | | | | | | | |
| PTOSIS | | | | | | | | | | | | |
| SALIVATING | | | 1 | | | | | | | | | |
| UNTHRIFTY | | | | | | | | | | | | |
| FOUND DEAD | | | | | | | | | | | | |
| DAY | 1 | | | | | | | | | | | |
| SALIVA ON FACE IS REDDISH BROWN | | | | | | | | | | | | |

GROUP 3 - 0.150 G/KG RS-88526

SCORING : 0-NORMAL 1-SLIGHT 2-MODERATE 3-MARKED 4-PRESENT 5-DEAD 6-DEAD
 SIZE : 50-0MM 51-0-10MM 52-10-20MM 53-10-50MM 54-50-100MM 55-OVER 100MM

0-MISSING R-REMOVED FROM STUDY

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PAGE 5.00

REPORT 831-R-85-98526-000-PO-TXE
TABLE 2

CLINICAL OBSERVATIONS FOR RATS
GIVEN A SINGLE ORAL DOSE OF RS-88526
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL CLINICAL DESCRIPTOR /LOCATION | FEMALES | | | | | | | | | | | |
|---|---------|---|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 0310 DISCOLORED URINE ORANGE | 1 | 1 | 1 | 0 | | | | | | | | |
| KYPHOSIS | 1 | 3 | 2 | 1 | | | | | | | | |
| PTOSIS | 2 | 2 | 1 | | | | | | | | | |
| ROUGH COAT | 1 | 1 | 1 | | | | | | | | | |
| UNTHRIFTY | 3 | 2 | 1 | | | | | | | | | |
| FOUND DEAD | | | | | | | | | | | | D |
| 0312 DISCOLORED URINE ORANGE | 1 | 1 | 1 | 0 | | | | | | | | |
| KYPHOSIS | 2 | 1 | 2 | 2 | 3 | 3 | 2 | 2 | | | | |
| PTOSIS | 1 | 1 | 2 | 2 | 3 | 2 | 2 | 2 | | | | |
| ROUGH COAT | | | 1 | 2 | 2 | 2 | 1 | | | | | |
| UNTHRIFTY | | | 2 | 2 | 2 | 2 | 3 | 2 | | | | |
| WASTING | | | | | | | 1 | 2 | | | | |
| FOUND DEAD | | | | | | | | | | | | D |

0014

GROUP 3 - 0.150 G/KG RS-88526

SCORING : 0=NORMAL 1=SLIGHT 2=MODERATE 3=MARKED * =PRESENT D=DEAD M=MISSING R=REMOVED FROM STUDY
SIZE : S0=0MM S1=0-10MM S2=10-30MM S3=30-50MM S4=50-100MM S5=OVER 100MM

09/18/85
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REPORT 831-R-85-06526-000-P0-THE
TABLE 2

CLINICAL OBSERVATIONS FOR RATS
GIVEN A SINGLE ORAL DOSE OF RS-86526
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL CLINICAL DESCRIPTOR /LOCATION | MALES | | | | | | | | | | | |
|---|-------|---|---|---|---|---|---|---|---|----|----|----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 0411 INACTIVE | | | | | | | | | | | | |
| KYPHOSIS | | | | | | | | | | | | |
| ROUGH COAT | | | | | | | | | | | | |
| UNTHRIFTY | | | | | | | | | | | | |
| FOUND DEAD | | | | | | | | | | | | |
| 0413 KYPHOSIS | | | | | | | | | | | | |
| PTOSIS | | | | | | | | | | | | |
| ROUGH COAT | | | | | | | | | | | | |
| SALIVATING | | | | | | | | | | | | |
| UNTHRIFTY | | | | | | | | | | | | |
| FOUND DEAD | | | | | | | | | | | | |

GROUP 4 - 0.500 G/KG RS-86526

SCORING : 0-NORMAL 1-SLIGHT 2-MODERATE 3-MARKED 4-PRESENT 5-DEAD 6-MISSING 7-REMOVED FROM STUDY

SIZE : S0-0MM S1=0-10MM S2=10-30MM S3=30-50MM S4=50-100MM S5=OVER 100MM

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PAGE 7 00

REPORT 831-R-85-96526-000-PO-TAE
TABLE 2

CLINICAL OBSERVATIONS FOR RATS
GIVEN A SINGLE ORAL DOSE OF RS-96526
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL CLINICAL DESCRIPTOR /LOCATION | STUDY DAY | FEMALES |
|---|----------------------------|---------|
| 0410 DISCOLORED | 1 2 3 4 5 6 7 8 9 10 11 12 | |
| URINE | 2 | |
| ORANGE | | |
| KYPHOSIS | 3 | |
| PTOSIS | 2 | |
| FOUND DEAD | D | |
| 0412 KYPHOSIS | 3 | |
| PTOSIS | 2 | |
| FOUND DEAD | D | |

GROUP 4 - 0.500 G/KG RS-6652F

SCORING : 0=NORMAL 1=SLIGHT 2=MODERATE 3=MARKED 4=PRESENT 5=DEAD M=MISSING R=REMOVED FROM STUDY
SIZE : S0=0MM S1=0-10MM S2=10-30MM S3=30-50MM S4=50-100MM S5=OVER 100MM

0016

REPORT 0031-R-85-00520-000-PO-TKE

TABLE 3

BODY WEIGHTS (G) FOR RATS
GIVEN A SINGLE ORAL DOSE OF RS-00520
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL ID | STUDY WEEK 1 | STUDY WEEK 2 |
|-----------|--------------|---------------------|
| GROUP | 2 M | 0.050 G/KG RS-00520 |
| 0211 | 432 | D |
| 0213 | 484 | D |
| N | 2 | 0 |
| MEAN | 458 | NA |
| STD | 8 | 8 |
| GROUP | 2 F | 0.050 G/KG RS-00520 |
| 0210 | 233 | 188 |
| 0212 | 212 | 170 |
| N | 2 | 2 |
| MEAN | 222 | 178 |
| STD | 8 | 8 |

D- ANIMAL DECEASED
S- SAMPLE SIZE TOO SMALL TO CALCULATE STD

TABR101/TABR02

REPORT 8831-R-85-96526-000-PO-TXE

TABLE 3

BODY WEIGHTS (G) FOR RATS
GIVEN A SINGLE ORAL DOSE OF RS-96526
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL ID | STUDY WEEK | | |
|-----------|------------|------------|----------|
| | 1 | 2 | |
| GROUP | 3 M | 0.150 G/KG | RS-96526 |
| 0311 | 499 | D | |
| 0313 | 499 | D | |
| N | 2 | 0 | |
| MEAN | 499 | NA | |
| STD | * | * | |
| GROUP | 3 F | 0.150 G/KG | RS-96526 |
| 0310 | 265 | D | |
| 0312 | 227 | D | |
| N | 2 | 0 | |
| MEAN | 2.6 | NA | |
| STD | * | * | |

D- ANIMAL DECEASED

*- SAMPLE SIZE TOO SMALL TO CALCULATE STD

TABR101/TABR802

0019

REPORT 8831-R-88-88528-000-PO-TXE

PAGE 02.00

TABLE 3

BODY WEIGHTS (G) FOR RATS
GIVEN A SINGLE ORAL DOSE OF RS-88528
FOLLOWED BY A TWO WEEK OBSERVATION PERIOD

| ANIMAL ID | STUDY WEEK | |
|-----------|------------|---------------------|
| | 1 | 2 |
| GROUP | 4 M | 0.500 G/KG RS-88528 |
| 0411 | 499 | D |
| 0413 | 487 | D |
| N | 2 | 0 |
| MEAN | 478 | NA |
| STD | * | * |
| GROUP | 4 F | 0.500 G/KG RS-88528 |
| 0410 | 231 | D |
| 0412 | 248 | D |
| N | 2 | 0 |
| MEAN | 240 | NA |
| STD | * | * |

D- ANIMAL DECEASED

* - SAMPLE SIZE TOO SMALL TO CALCULATE STD

TABR101/TABR802

00501

0020



REPORT 831-R-85-96526-000-PO-TXE

APPENDICES

**SPONSOR AND TESTING FACILITY:**

SYNTEX RESEARCH
Institute of Toxicologic Sciences
Departments of Toxicology and Pathology
3401 Hillview Avenue, Palo Alto, California

PROTOCOL NUMBER:

~~031-R-85-96526-000-PO-TXE~~

PROTOCOL DATE:

May 2, 1985

PROTOCOL TITLE:

ACUTE ORAL TOXICITY OF RS-96526-000 WHEN TESTED ON THE RAT

PROJECT NUMBER:

2.0.18

PROTOCOL PURPOSE:

The Environmental Health and Safety Group has requested that we carry out safety evaluation studies on RS-96526-000 (acetyl ferrocene). This study is part of the safety evaluation package.

SCHEDULING:

Starting and in-life completion dates will be added when the study is scheduled.

PROCEDURE:

Unless otherwise stated, all procedures will be done according to Standard Operating Procedures for the Institute of Toxicologic Sciences.

A. Test Articles

1. The RS-96526-000 will be released for use by the Institute of Organic Chemistry (IOC), Syntex Research. A summary of their analytical results will be included with the report of this study.
2. On receipt, the test article will be stored at 4 degrees C. The elapsed time between release of article by IOC and testing in ITS shall not exceed 90 days. If the elapsed time should exceed 90 days an aliquot of the test article will be returned to IOC for reanalysis.
3. The test articles will be mixed with vegetable oil just before dosing. Suspend 1.25 grams of RS-96526-000 in 23.75 ml of vegetable oil. Mix for at least 30 seconds using a Polytron homogenizer.

B. Animals

1. Healthy animals, not subject to any previous experimental procedures, will be used.



PROTOCOL 831-R-85-96526-000-PO-TXE
Page 2

B. Animals (continued)

2. Obtain enough rats so that 6 males and 6 females will be available for this study.

Strain: (CD) Sprague Dawley Derived
Source: Charles River, Kingston, NY, or Portage, MI
Age: Not specified (Young adult)
Weight: Not specified (Young adult)

3. Upon receipt the rats will be placed into individual cages and held for an acclimatization period of approximately 1 week.
4. Food (Purina Certified Rodent Chow®) and water will be available ad libitum except for an overnight period of food deprivation before dosing. No materials are anticipated to be present in the food or water which would interfere with the purpose or conduct of this study.
5. Following assignment to the study each rat is to be individually identified using a standard ear punch code, or with indelible ink. The method used is to be documented.
6. The animals will be inspected during the acclimatization period. Any animal exhibiting unusual behavior, significant change in condition of the fur, color of the skin, scratching, loud or irregular breathing, unusual discharges from orifices, unusual condition of urine or stool, changes in food or water consumption, or wounds caused by fights will not be used for the study.

C. Test Procedures

1. Assign the rats to 3 groups as shown below. Randomization is not necessary.

| <u>Group*</u> | <u>Compound</u> | <u>(mg/kg)</u> | <u>Dose of Formulation</u> |
|----------------|-----------------|----------------|----------------------------|
| 2 (200 series) | RS-96526-000 | 0.050 | 0.1 ml/100 g |
| 3 (300 series) | RS-96526-000 | 0.150 | 0.3 ml/100 g |
| 4 (400 series) | RS-96526-000 | 0.500 | 1.0 ml/100 g |

* Groups composed of 2 males and 2 females

Preparation of Animal

2. Remove food from animals overnight before dosing. Do not replace food until approximately 2 hours after dosing.



PROTOCOL 831-R-83-96526-000-PO-TAE
Page 3

C. Test Procedures (continued)

3. Route - Single oral dose (stomach tube). The maximum volume of aqueous solution that can be given in one dose should not exceed 2 ml/100 g body weight. For nonaqueous liquids and suspensions, the volume should not exceed 1 ml/100 g body weight. When possible, variability in test volume should be minimized.
4. Duration - single dose followed by a 2 week observation period. Animals exhibiting obvious signs of toxicity may be held for more than 2 weeks.

D. Observations

1. A careful clinical examination should be made at least once each day. Cageside observations should include changes in the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behavior pattern. Particular attention should be directed to observation for tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. All toxicological and pharmacological signs should be recorded including time of onset, intensity and duration. The time of death should be recorded as precisely as possible. Individual weights of animals should be determined shortly before the test substance is administered, weekly thereafter, and at study termination. Changes in weight should be calculated and recorded when survival exceeds one day. At the end of the test, surviving animals are to be euthanatized and discarded.

2. No animals will be submitted to necropsy.
3. If any deaths or if no deaths are observed, the need for additional testing will be discussed with Environmental Health and Safety.

E. Results

Tabulation of data and individual results will accompany each report in sufficient detail to permit independent evaluation of results, including summaries and tables that show, as appropriate, the relationship of effects to time of dosing, sex, etc.

F. Statistical Evaluation

No statistical evaluation will be required.

0024

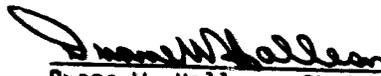


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Page 4

6. Data Retention

All raw data will be maintained in Syntex Research Archives managed from 3401 Hillview Avenue, Palo Alto, California.

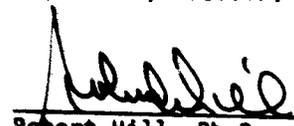
APPROVED BY:


Ruane W. Hallesy, Ph.D.
Director, Toxicology
Study Director
Diplomate, A.B.T.

3 May 85
Date


Leonard D. Snott, D.V.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.

6 May 85
Date


Robert Hill, Ph.D.
Vice President, Syntex Research
Director, ITS
Diplomate, A.B.T.

6 May 85
Date

0025

**SPONSOR AND TESTING FACILITY:**

SYNTEX RESEARCH
 Institute of Toxicologic Sciences
 Departments of Toxicology and Pathology
 3401 Hillview Avenue, Palo Alto, California

PROTOCOL NUMBER:

031-R-85-96526-000-PO-TXE

REVISION NUMBER:

One

REVISION DATE:

May 14, 1985

REVISEDSEE REVISION MAY 23 1985**PURPOSE OF REVISION:**

The purpose of this revision is to 1) Correct doses shown in section C.1. Doses are in g/kg, not mg/kg.
 2) Delete sentence in section D.1. concerning calculating body weights when survival exceeds 1 day since body weights of dead animals provide little useful information.

REVISION:**C. Test Procedures**

1. Assign the rats to 3 groups as shown below. Randomization is not necessary.

| <u>Group*</u> | <u>Compound</u> | <u>(g/kg)</u> | <u>Dose of Formulation</u> |
|----------------|-----------------|---------------|----------------------------|
| 2 (200 series) | RS-96784-000 | 0.050 | 0.1 ml/100 g |
| 3 (300 series) | RS-96784-000 | 0.150 | 0.3 ml/100 g |
| 4 (400 series) | RS-96784-000 | 0.500 | 1.0 ml/100 g |

* Groups composed of 2 males and 2 females

D. Observations

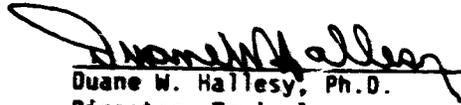
1. A careful clinical examination should be made at least once each day. Cageside observations should include changes in the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behavior pattern. Particular attention should be directed to observation for tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. All toxicological and pharmacological signs should be recorded including time of onset, intensity and duration. The time of death should be recorded as precisely as possible. Individual weights of animals should be determined shortly before the test substance is administered, weekly thereafter, and at study termination. At the end of the test, surviving animals are to be euthanatized and discarded.

0026



PROTOCOL 831-R-85-96526-000-PO-TXE
Rev. 1, Page 2

APPROVED BY:



Duane W. Hallesy, Ph.D.
Director, Toxicology
Study Director
Diplomate, A.S.T.

15 May 85
Date



Leonard D. Shott, D.V.M., Ph.D.
Director, Pathology
Diplomate, A.C.V.P.

15 May 85
Date

0027



SPONSOR AND TESTING FACILITY:

SYNTEX RESEARCH
Institute of Toxicologic Sciences
Departments of Toxicology and Pathology
3401 Hillview Avenue, Palo Alto, California

PROTOCOL NUMBER:

83-R-85-96526-000-PO-TXE

REVISION NUMBER:

Two

REVISION DATE:

May 23, 1985

PURPOSE OF REVISION:

The purpose of this revision is to correct compound numbers in revision one, section C-1.

REVISION:

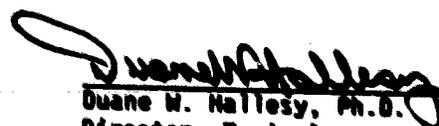
C. Test Procedures

1. Assign the rats to 3 groups as shown below. Randomization is not necessary.

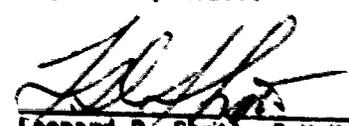
| <u>Group*</u> | <u>Compound</u> | <u>(g/kg)</u> | <u>Dose of Formulation</u> |
|----------------|-----------------|---------------|----------------------------|
| 2 (200 series) | RS-96526-000 | 0.050 | 0.1 ml/100 g |
| 3 (300 series) | RS-96526-000 | 0.150 | 0.3 ml/100 g |
| 4 (400 series) | RS-96526-000 | 0.500 | 1.0 ml/100 g |

* Groups composed of 2 males and 2 females

APPROVED BY:

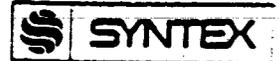

 Duane M. Mallesy, Ph.D.
 Director, Toxicology
 Study Director
 Diplomate, A.B.T.

23 May 85
Date


 Leonard D. Shott, D.V.M., Ph.D.
 Director, Pathology
 Diplomate, A.C.V.P.

23 May 85
Date

0028



STUDY SCHEDULING SHEET

PROTOCOL NUMBER: 831-R-85-96526-000-PO-TXE

STUDY DIRECTOR: Duane W. Hallesy, Ph.D.
Director, Toxicology

PROPOSED
STARTING DATE: 07/10/85

PROPOSED IN-LIFE
COMPLETION DATE: 07/24/85

*Penumathy, L. For
D. W. Hallesy
7/9/85*

00110



APPENDIX B

ACKNOWLEDGMENTS

STUDY DIRECTOR, SCIENTISTS, AND SUPERVISORY
PERSONNEL INVOLVED IN TOXICITY STUDY

Institute of Toxicologic Sciences

Director:
Information Section:

I.A. Heyman
L. Thunen

Department of Toxicology

Director, Toxicology:
Study Director:
Animal Colony, Supervisor of Toxicology Biology:

D. Hallesy
D. Hallesy
J. Hull

Department of Pathology

Director, Pathology:
Veterinary Pathologist:

L. Shott
L. Shott

Environmental Health and Safety

Director, Environmental Health and Safety:

F.J. Murray