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FINAL REPORT

Reproduction Study for Safety Evaluation of Rotenone using Rats

Contract No. 14-16-009-79-097

Study No. 81077 (3 Volumes)

Volume 1 of 3

for

Fish and Wildlife Service
LaCrosse, Wisconsin

by

Hazleton Raltech, Inc.
A Subsidiary of Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704

February 11, 1983

Protocol

Reproduction Study for Safety Evaluation of Rotenone using Rats

Contract No. 14-16-009-79-097

Study Number

81077

Test Material

Rotenone

Project Director

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Study Timetable

Starting Date	May 27, 1981
Completion Date (In-life)	June 25, 1982
Completion Date (Required Procedures)	August 30, 1982

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QUALITY ASSURANCE FINAL REPORT STATEMENT

Reproduction Study for Safety Evaluation
of Rotenone Using Rats

Study #81077

The final report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Raltech, Inc. in accordance with the Good Laboratory Practice Regulations as set forth in 21 CFR 58.35 (b) (6) (7). It has been found to accurately identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study and that the reported data accurately reflect the raw data of the laboratory study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Susan Glad Anderson

DATE 2/15/83

Susan Glad Anderson
Manager, Quality Assurance Unit

QUALITY ASSURANCE INSPECTIONS

Study #81077

Date of Inspection	Type of Inspection	Date Issued to Management
5/29/81	Protocol Review	5/21/81
8/05/81	Animal/Cage Identification Animal Care Animal Observations Body Weights/Feed Consumption	8/05/81
8/26/81	Diet Preparation	8/26/81
9/23/81	Reproduction	9/23/81
9/24/81	Diet Preparation	9/24/81
11/09/81	Pathology	11/09/81
12/08/81	Body Weights/Feed Consumption Animal Observations Diet Preparation	12/08/81
1/15/82	Diet Preparation	1/15/82
1/19/82	Body Weights/Feed Consumption Animal Observations	1/19/82
6/23/82	Pathology	6/23/82
1/28-2/03/83	Final Report Review	2/03/83

OBJECTIVE

The purpose of this study was to determine the effects of rotenone on reproductive function and development in two successive generations of rats that had been exposed continuously to the compound. All aspects of this study were conducted in compliance with the Good Laboratory Practice Regulations.[1]

A study (Study No. 79030) was initiated February 4, 1981 with animals from the Portage, Michigan facility of Charles River Breeding Laboratories, Wilmington, Massachusetts. The study was terminated May 13, 1981 because of the presence of Kilham Rat Virus (KRV) in the female rats. The study was reinitiated as Study No. 81077 using KRV-free animals obtained from the Kingston, New York facility of Charles River Breeding Laboratories (Protocol, Appendix M).

This Final Report presents data collected from a total of two litters (F_{1a} and F_{2a}) produced by two generations of rats (F_0 and F_{1a}) through weaning of F_{2a} litters and terminal necropsy of F_{1a} adult animals.

TEST MATERIAL

The test material in this study was rotenone (Aldrich Chemical Company, Catalog No. R200-1, Lot No. 091947 and 100287) and was furnished by the Sponsor. Lot No. 091947 was used for Weeks 1-52 of the study; Lot No. 100287 was used for Weeks 53-55 of the study. These samples of rotenone were analyzed at Hazleton Raltech, Inc. (HRI) prior to being used for this study and were determined to be 97-98% pure (Analytical Method, Appendix N).

TEST SYSTEM

Test Animal

Immature Charles River CD(SD)BR rats (non-littermates) were received from the Kingston, New York facility of Charles River Breeding Laboratories, Wilmington, Massachusetts on May 27, 1981 at 4 weeks of age to be used as the F_0 generation animals. The rat was selected as the test animal because it is the preferred species for reproduction studies.[2] The rats were acclimated in the test facility for 13 days prior to initiation of test material administration.

Identification

Each animal selected for the reproduction study or for necropsy was assigned a unique identification number and was permanently identified with a numbered metal tag. All data collected from an animal were recorded and filed under its identification number.

Group	Number of Animals	Sex	Diet Treatment ppm Rotenone	Animal Numbers
1	15	M	0	63105901-63105915
2	15	M	7.5	63105916-63105930
3	15	M	37.5	63105931-63105945
4	15	M	75	63105946-63105960
5	25	F	0	63105961-63105985
6	25	F	7.5	63105986-63106010
7	25	F	37.5	63106011-63106035
8	25	F	75	63106036-63106060

Fifteen males and 25 females from the first litters of the F_0 generation (F_{1a}; see Reproductive Procedures and Figure 1) were selected at random from each treatment group and were continued on test diets. The study design for the F_{1a} generation is presented below.

Group	Number of Animals	Sex	Diet Treatment ppm Rotenone	Animal Numbers
1	15	M	0	63117121-63117135
2	15	M	7.5	63117136-63117150
3	15	M	37.5	63117151-63117165
4	15	M	75	63117166-63117180, 63118924-63118925[a]
5	25	F	0	63117181-63117205
6	25	F	7.5	63117206-63117229[b]
7	25	F	37.5	63117231-63117255
8	25	F	75	63117256-63117280, 63118929-63118932[c]

[a]Animal Nos. 63117171 and 63117172 were replaced by 63118924 and 63118925, respectively.
[b]Animal No. 63117230 was not assigned to an animal - technical error.
[c]Animal Nos. 63117262, 63117264, 63117267, and 63117268 were replaced by 63118929, 63118930, 63118931, and 63118932, respectively.

Treatment Duration

F_0 generation animals received the test material at the appropriate dose levels in the diet continuously from 6 weeks of age (June 10, 1981) through weaning of F_{1a} litters, and until they were necropsied during the period of January 22-25, 1982 (i.e., during Week 33 on test).

F_{1a} generation animals selected to continue on the reproduction study were exposed to the test material *in utero*, through weaning, and in the diet continuously through termination of the study during the period of June 23-25, 1982 (i.e., during Week 32 on test).

Breeding. In each parent generation, breeding was initiated by selecting one male and two females at random from animals on the same treatment and placing them in a double-sized, screen-bottom cage. Sibling and half-sibling matings were avoided. Vaginal smears were taken daily, and the presence of a copulatory plug or sperm in the vaginal smear was considered evidence of positive mating. The day on which such evidence was found was considered Day 0 of gestation, and the female was removed and placed in a single-sized cage. If no positive mating was observed after 7 days, the female(s) were removed and placed with a different male from the same treatment group. Females were considered barren if no positive mating was observed after 14 days.

Breeding of the F_0 generation for F_{1a} litters was initiated on September 23, 1981, after the animals had received test material for 105 days. Breeding of the F_{1a} generation for F_{2a} litters was initiated on March 17, 1982, after test diets had been fed to the animals for a minimum of 120 days (post-weaning).

Data from the F_{1a} litters were evaluated to determine effects on the survival and body weight of the offspring. Based on these data, a decision was made not to produce an F_{1b} generation to be used for teratologic evaluation, and the F_0 animals were therefore sacrificed.

Gestation and Parturition. Mated females were observed daily during gestation, and body weights were recorded on Days 0, 7, 14, and 20 of gestation. Approximately 1 week prior to parturition, females were placed in individual plastic shoe box cages supplied with bedding material, a feed jar, and a water bottle. Females were allowed to deliver and care for their own young with a minimum of disturbance. The first day an entire litter was observed was considered Day 0 of lactation for that litter.

Lactation and Weaning. Litters were examined as soon as possible after delivery (Day 0 of lactation). Individual pup weights, the number of live pups and stillborns, and sex ratios, as well as any gross abnormalities, were recorded. Four days after birth (Day 4 of lactation), the number and sex of pups and individual pup weights were recorded. In addition, pups were culled from the litters at random to achieve a maximum litter size of 10, with five males and five females per litter if possible. None of the culled pups exhibited grossly abnormal appearance or behavior; therefore, they were subjected to an external examination and discarded.

The number and sex of pups and individual pup weights also were recorded on Days 7, 14, and 21 of lactation.

Females that had litters were weighed on Days 0, 7, 14, and 21 of lactation. Dams and pups were observed daily for survival and abnormal behavior. Pups found dead were examined for gross abnormalities and discarded. No pups exhibited grossly abnormal appearance or behavior.

Pups were allowed to nurse for 21 days prior to weaning. At weaning, animals were selected at random from the available F_{1a} litters so that each litter was represented during the continuation of the reproduction study (a maximum of one male per litter and at least one female but not more than two females per litter when possible) and 15 males and 25 females per treatment group were continued on the same diet level as that of their parents.

In addition, five male and five female F_{1a} and F_{2a} weanlings were selected at random from each treatment group (a maximum of one per sex per litter when possible) for gross necropsy and tissue collection. Specified tissues (see Pathology Data), lesions, and tissue masses were preserved in 10% phosphate-buffered formalin. Weanlings not selected for continuation on the reproduction study or for necropsy were subjected to a gross external examination, sacrificed, and discarded.

General Observations of Parental Generations

All animals were observed daily for any overt changes in appearance or activity, or any indication of toxicity, including death. Specific notations were made regarding appearance and behavior of dams during parturition (if observed) and lactation. Moribund animals were sacrificed if they were not expected to survive until the next day's observation. These animals, in addition to any animal that died on test, were subjected to a gross necropsy, and all lesions and tissue masses were preserved in 10% phosphate-buffered formalin and processed for histopathologic examination.

Body weight and feed consumption were recorded weekly during growth phases. Following initiation of breeding procedures, weekly body weights were recorded for males. Mated females were weighed on Days 0, 7, 14, and 20 of gestation and Days 0, 7, 14, and 21 of lactation; after all litters had been weaned, weekly body weights were recorded for all females.

F_0 generation adult animals (including barren males and females) were subjected to gross necropsy after F_{1a} litters had been weaned. Gonads were weighed and along with all lesions were preserved in 10% phosphate-buffered formalin and processed for histopathologic examination. A gross necropsy including tissue collection (see Pathology Data) was conducted on all F_{1a} adult animals approximately 30 days after weaning of F_{2a} litters; tissues and lesions were preserved in 10% phosphate-buffered formalin and processed for histopathologic examination.

OBSERVATIONS AND DATA COLLECTION

Male Data

The following data were recorded for each male in the F_0 and F_{1a} generations.

- Identification number
- Weekly body weight

- Any indication of abortion or premature delivery
- Any abnormalities in appearance or behavior, including those observed during gestation and lactation
- Age at death and reason for death (sacrificed or died on test, scheduled necropsy)

The following cumulative data were calculated for each dose level and generation.

- Mean weekly body weight from initiation of the study (F_0) or weaning (F_{1a}) until initiation of reproductive procedures, and after F_{1a} and F_{2a} litters, respectively, were weaned
- Mean weekly body weight gain from initiation of the study (F_0) or weaning (F_{1a}) until initiation of reproductive procedures
- Mean weekly feed consumption from initiation of the study (F_0) or weaning (F_{1a}) until initiation of reproductive procedures
- Mean daily compound consumption from initiation of the study (F_0) or weaning (F_{1a}) until initiation of reproductive procedures
- Mean body weight for pregnant females during gestation and lactation
- Reproductive indices
    - Mating index [(number of females with vaginal sperm or plug or that gave birth to a litter/number of females placed with males) x 100]
    - Gestation index [(number of litters with viable pups/number of females with vaginal sperm or plug or that gave birth to a litter) x 100]
- Number and percent of females with behavioral abnormalities during gestation or lactation

Litter Data

The following data were recorded for all litters.

- Identification numbers of parents
- Litter size: number born alive, number born dead

When a necropsy was performed with tissue collection, the tissues and organs listed below were systematically collected and trimmed of fat and extraneous tissue and placed in 10% phosphate-buffered formalin.

Tissues specified below were weighed before being placed in formalin. Gonads and lesions from F_0 adult animals as well as tissues and lesions collected from F_{1a} adult animals and F_{1a} and F_{2a} weanlings were examined histologically.

*Adrenals
Bone (tibia) with marrow
*Brain (3 levels)
Esophagus
Eyes
*Gonads
*Heart
*Kidneys
Large intestine
Lesions
*Liver
Lungs with mainstem bronchi
Lymph nodes (mesenteric and cervical)
Pancreas
Peripheral nerve (sciatic)
Pituitary
Prostate
Salivary gland (mandibular)
Seminal vesicles
Skeletal muscle
Skin (with mammary gland)
Small intestine (duodenum, ileum, jejunum)
Spinal cord (2 levels)
Spleen
Stomach
Thymus
*Thyroid (with parathyroids)
Trachea
Urinary bladder
Uterus

*Weighed

In addition to absolute organ weights for organs specified above, organ weight to body weight ratios were calculated.

All gross and histopatholgic observations were recorded for each animal on individual pathology data sheets.

Data Analysis

Analysis of variance techniques were used to examine differences in body weight, body weight gain, feed consumption, and organ weight. Where significant differences were indicated, Dunnett's test was performed to compare treatment to control means.[4] The Kruskal-Wallis test was used to compare the number of pups born, percent born alive, percent survival, and sex ratios.[5] Where significant differences were indicated, Dunn's test was used to compare treatment to control means.[6] Reproductive indices were analyzed using contingency table analysis, testing whether probability of successful mating or gestation was independent of treatment group.

Maintenance of Raw Data, Records, and Specimens

All raw data, records, and specimens generated by this study are the property of the Sponsor, but will be retained in the archives at Hazleton Raltech, Inc., 3301 Kinsman Boulevard, Madison, Wisconsin.

Body weight gain for males, when compared with that of controls, was significantly lower in the mid level group during Week 14, and in the high level group during Weeks 13, 14, and 15 (Table 5). Body weight gain for females in both the mid and high level groups was significantly lower than that of control animals during Weeks 8, 13, 14, and 15. In addition to these differences, body weight gain for females in the mid level group was significantly greater during Week 9 (Table 6).

Feed consumption for males in the high level group (75 ppm rotenone) was significantly lower than that of control animals for Week 15 (Table 7), but there were no significant differences in feed consumption for females (Table 8).

Compound consumption (expressed as mg rotenone/kg body weight/day) for males and females is summarized in Tables 9 and 10, respectively. Since, by design, different dose levels of test material were administered, these data were not analyzed statistically. However, they do indicate that compound consumption was proportional to the administered doses.

Body Weight Data for F_0 Generation Males and Females during the Reproductive Phase (Weeks 16 through 32)

With the exception of Weeks 22 and 32, body weights for males in the high level group (75 ppm rotenone) were significantly lower than those of control males throughout the entire reproductive phase (i.e., Weeks 16 through 32) (Table 11).

Body weight for females was recorded weekly from weaning of the last F_{1a} litter until the terminal necropsy (i.e., Weeks 23 through 32). Significantly lower body weights were detected in the mid level group (37.5 ppm rotenone) for Weeks 24, and 26 through 32, as well as in the high level group (75 ppm rotenone) for Weeks 23 through 32 (Table 12).

Reproduction Data for F_{1a} Litters

There were no significant differences between the control and treated groups in male fertility indices during breeding for F_{1a} litters (Table 13). Fertility in males was defined as a demonstrated ability to sire at least one litter.

In females, when compared with those of controls, body weights throughout gestation were significantly lower in the mid and high level groups (37.5 and 75 ppm rotenone, respectively); overall body weight change during gestation was significantly lower in the high level group; body weights during lactation were significantly lower in the mid and high level groups on Days 0, 7, and 14, as well as in the high level group on Day 21; and overall body weight change was significantly different in the mid and high level groups (Table 14). There were no significant differences between treated and control groups in mating or gestation indices, days required to mate, or the length of gestation (Table 15). No females displayed physical or behavioral abnormalities during F_{1a} gestation or lactation.

A common occurrence in rodents maintained on ground feed is malocclusion. One of the symptoms of this condition is the presence of a brownish nasal and/or ocular discharge which was observed in three males [animal No. 63105925, 63105930 (7.5 ppm rotenone), and 63105939 (37.5 ppm rotenone)] beginning at approximately 16 weeks of age. When necessary, the incisors of affected animals were clipped to alleviate the condition.

All F_0 animals were sacrificed during Week 33 on test (i.e., from January 22-25, 1982). These animals were subjected to a gross necropsy; gonads were weighed and along with lesions were preserved in 10% phosphate-buffered formalin and processed for histopathologic examination. A detailed pathology report with the data presented in tabular format is included on pages 89 through 254.

Macroscopic and Histopathologic Observations of the F_0 Generation

The most common macroscopic and histopathologic observations for the F_0 generation animals related to the kidneys. All observations are considered to be incidental findings and not treatment related. A detailed pathology report with the data presented in tabular format is included on pages 89 through 254. Gonad weights are summarized in Tables 20 and 21. Compared with that of control animals, terminal body weight for males was significantly lower in the high level group (75 ppm rotenone); terminal body weight for females was significantly lower in the mid and high level groups (37.5 and 75 ppm rotenone, respectively). Although no significant differences were indicated in gonad weights for males or females when analyzed as absolute values or after having been corrected for terminal body weight, analysis of weights relative to terminal body weight revealed a significantly greater gonad weight for males in the mid level group and females in the high level group when compared with those of their respective control group.

Body Weight, Feed Consumption, and Compound Consumption Data for F_{1a} Generation Males and Females during the Growth Phase (Weeks 0 through 17)

When compared with that of control animals, body weight for males was significantly lower in the mid level group (37.5 ppm rotenone) for Weeks 0 through 10, and in the high level group (75 ppm rotenone) for Weeks 0 through 17 (Table 22). Body weight for females in the mid and high level groups was significantly lower than that of controls for Weeks 0 through 17 (Table 23).

Body weight gain for treated males compared with that of controls was significantly lower in the mid level group for Week 1, and in the high level group for Weeks 1, 3, 4, and 10 (Table 24). Body weight gain for females was significantly less in the mid level group for Weeks 7 and 17, and in the high level group for Weeks 1, 4, 5, 7, 9, and 17 when compared with that of control animals (Table 25).

Analysis of litter data revealed significantly lower mean pup weights in the high level group compared with that of controls at Day 4 of lactation, and in both the mid and high level groups at Days 7, 14, and 21. Mean pup weight gain was significantly lower than that of the controls at Days 4, 7, 14, and 21 of lactation in the mid and high level groups. The mean number of pups born, as well as the mean number of survivors (i.e., mean litter size) at 0, 4, and 7 days of age, was significantly lower in the high level group compared with those of controls (Table 34). There were no significant differences between the control and treated groups in the mean percent of live offspring born per litter, sex ratios, or survivability through 21 days of age. No pups from the F_{2a} litters had physical or behavioral abnormalities.

Macroscopic and Histopathologic Observations of F_{2a} Weanlings

The most common macroscopic and histopathologic observations for F_{2a} weanlings pertained to the liver. All observations are considered to be incidental findings and not treatment related. A detailed pathology report with data presented in a tabular format is included on pages 89 through 254.

Although analysis of absolute organ weights (using data from controls for comparison) revealed significantly lower terminal body weight and kidney weight for males in the mid level group (37.5 ppm rotenone), as well as terminal body weight, brain, heart, liver, adrenal, kidney, and gonad weights for males in the high level group (75 ppm rotenone) (Table 35), no significant differences were detected in absolute values for females (Table 36).

For organ weights relative to terminal body weight, statistical analysis revealed significantly greater values in the high level group for heart weight of males, and brain weight for males and females compared with those of control animals (Tables 37 and 38, respectively).

No significant differences were indicated by analysis of organ weights which were corrected for terminal body weight.

General Study Observations of the F_{1a} Generation

Individual study observations for F_{1a} males and females are presented in Appendix K. Some F_{1a} males and females died on test. A detailed pathology report with data presented in tabular format is included on pages 89 through 254.

Several F_{1a} weanlings in the high level group (75 ppm rotenone) that were selected to continue on the reproduction study died before weaning of the last F_{1a} litter; with one exception, these animals were replaced with weanlings from the same treatment group. These animals and their replacements are listed on the following page.

Organ weights for males and females are summarized in Tables 39 through 42. Analysis of data for males as absolute values compared with those of control animal data revealed significantly lower liver and kidney weights in the mid level group (37.5 ppm rotenone). In the high level group (75 ppm rotenone), significantly lower terminal body weight, brain, heart, liver, gonad, and kidney weights were detected when compared with those of controls (Table 39). Significantly lower terminal body weight and kidney weight were detected for females in the mid and high level groups, as well as heart, liver, and gonad weights in the high level group compared with those of controls (Table 40).

Brain, heart, liver, adrenals, and kidney weights relative to terminal body weight were significantly greater than those of controls for males in the high level group (Table 41). Analysis of organ weights relative to terminal body weight for females revealed significantly greater brain and heart weights in the mid and high level groups; liver weight was significantly greater in the mid level group although it was significantly lower in the low level group compared with that of controls (Table 42).

For male data in the high level group, brain weight and gonad weight were significantly lower than those of controls when corrected for terminal body weight; liver weight was significantly greater than that of controls (Table 39). In females, analysis of organ weights corrected for terminal body weight revealed significantly lower liver weight in the low level group, and significantly greater liver weight in the mid level group compared to controls (Table 40).

APPROVAL

Daniel F. Kehoe
Study Supervisor
Reproductive Toxicology

Feb. 15, 1983
Date

Karen M. MacKenzie, PhD
Study Director
Assistant Director, Toxicology

2-15-83
Date

by and for Hazleton Raltech, Inc.