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

006537

DEC 22 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Studies Submitted to Support Registration of Rotenone (Reg. No. 6704-Q) Tox. Chem. No. 725: Tox. Project Nos. 1582 and 7-0865.

TO: William Miller
Product Manager (16)
Registration Division (TS-767C)

THRU: Judith Hauswirth, Ph. D., Section Head Judith W. Hauswirth 12/10/87
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: Roger Gardner, Toxicologist
Review Section 6 Roger Gardner 12-9-87
Toxicology Branch
Hazard Evaluation Division (TS-769) 12/23/87

Actions Requested

Review of the following studies:

A chronic feeding study in rats
An oncogenicity study in rats
An oncogenicity study in mice
Mutagenicity assays

Recommendations and Conclusions

1. Rotenone is under review as a Registration Standard, and it has been referred to the Toxicology Branch Peer Review Committee for consideration of oncogenicity data (see Points 6. and 7. below). Final conclusions regardng rotenone's oncogenic potential, data gaps (Point 13. below) and status of the Acceptable Daily Intake (Point 14. below) will not be made until a Peer Review determination has been made.

2. Rotenone has higher acute oral toxicity in female rats (Toxicity Category I) than it does in male rats (Toxicity Category II) (see Section I. C. 1. below).

3. The acute toxicity of a rotenone formulation that also contains related Cube resins and Pyrethrum (see Appendix I) places the formulation into Toxicity Category II with respect to oral toxicity and eye and skin irritation and Toxicity Category III for acute dermal toxicity (see Section I. C. 1. below).

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4. A NOEL of 0.4 mg/kg/day was established in a six-month dog study. The lowest-effect level (LEL) was 2 mg/kg/day, and dose-related effects included emesis and decreased body weight. The highest dose tested was associated with decreased hematocrit and hemoglobin, serum glucose, cholesterol, and total lipid levels (see Section I. C. 2. below).

5. In a two-year feeding study with rats, effects in females included significantly reduced food consumption, decreased body weight, decreased total protein and albumin levels in the blood, and increased serum urea nitrogen levels. At necropsy, female rats with these effects appeared thin, and organ weights and weight ratios reflected the generally reduced body weights. In male and female rats the incidence of angiectasis and hemorrhaging in the adrenals was increased. A NOEL for those effects could not be established because no histological examination was made of low and mid dose group rats. (see Section II. A. below).

6. An increase in the incidence of parathyroid adenomas in male rats given a 75 ppm diet was noted in an oncogenicity study. However, the study may not have evaluated a Maximum Tolerated Dose (MTD) (see Section II. B. 1. below).

7. There was no evidence that rotenone was carcinogenic in male or female mice, but an MTD may not have been achieved in the study (see Section II. B. 2. below).

8. In a rat teratology study, the NOEL for maternal toxicity (decreased body weight and body weight gain) was 3 mg/kg/day, and the LEL was 6 mg/kg (highest dose tested). The 6 mg/kg dose also caused a reduction in mean fetal weight and an increased incidence of unossified sternebrae and urinary tract abnormalities. The NOEL for fetotoxicity was 3 mg/kg/day, and the LEL was 6 mg/kg/day. The Adult-toDevelopmental toxicity ratio (A:D ratio) is equal to one (see Section I. C. 3. a. below).

9. In teratology studies with mice, the NOEL for maternal effects (weight loss and mortality) and fetal effects (decreased litter size) is 15 mg/kg/day. The lowest effect level (LEL) is 24 mg/kg/day. No fetoxocity or terata were found, and the ratio of adult-to-developmental toxicity NOEL's (A:D ratio) is equal to one (see Section I. C. 3. b. below).

10. The NOEL for effects observed in a two-generation reproduction study in rats was 7.5 ppm (approximately 0.375 mg/kg/day). The LEL for maternal toxicity (decreased body weight gain in dams) and for reproductive effects (decreased pup weights during lactation) was 37.5 ppm. Decreased litter sizes were also observed in rats given the 75-ppm diet (highest dose tested) (see Section I. C. 4. below).

11a. Rotenone did not induce gene mutations in bacteria with or without metabolic activation or in yeast. However, concentrations of 0.25 to 4.0 ug/ml without metabolic activation increased the frequency of mutations in mouse lymphoma cells in vitro. The pesticide failed to induce somatic mutations in a mouse spot test at doses of 0.05, 0.17, 0.5, or 1.0 mg/kg/day (see Sections I. C. 5. a. and II. C. 1. below).

11b. Rotenone did not increase the incidence of chromosomal aberrations or decrease the mitotic index in bone marrow cells of treated rats or the incidence of polychromatic erythrocytes with micronuclei in bone marrow of treated mice (see Sections I. C. 5. b. and II. C. 2. below).

11c. No mitotic recombination or mitotic gene conversion was observed in yeast exposed to rotenone (see Section I. C. 5. c. and II. C. 3. below).

12. Limited metabolism studies in rats indicated that the major route of excretion was in the feces, and females excreted administered radioactivity at a slower rate than males. Intravenous dosing results indicated that enterohepatic circulation is possible in treated rats, and the route of administration, dose level, and number of doses had no apparent effect on the excretion pattern. Most of the metabolites in the feces could not be identified (see Section I. C. 6. below).

13. Data gaps for technical grade rotenone include acute dermal and inhalation toxicity, primary eye and skin irritation studies, and a dermal sensitization study. Subchronic oral toxicity studies should be waived based on acceptable chronic studies, and a chronic toxicity study in a rodent species may be required unless additional histopathology for low and mid dose group rats is provided for the feeding study described in Section II. A. below. Oncogenicity studies in two species may not be required if Peer Review determines that available mouse and rat studies are acceptable. In addition, reports on sister chromatid exchange and chromosomal aberration assays summarized in the report on rat and mouse oncogenicity studies are needed before an assessment of the mutagenicity data can be completed (see Section III. B. below).

14. The Provisional Acceptable Daily Intake (PADI) is based on the two-generation reproduction study in rats (NOEL of 0.375 mg/kg body weight/day), and a 100-fold safety factor. It is calculated to be 0.00375 mg/kg/day.

I. Background

In May, 1980, the Agency prepared a support document entitled Rotenone: Pre-RPAR Review in which rotenone was considered as a potential candidate for a Rebuttable Presumption Against Registration (RPAR). The document concluded that the aquatic uses of rotenone should be regarded as food uses because residues might occur in edible fish. On that basis, establishment of tolerances or an exemption from tolerances were recommended, and Toxicology data requirements were determined to support those recommendations. Data submitted to meet the Toxicology data requirements are discussed in Sections I. C. and II. below.

A. Chemical Nature and Uses

Rotenone is a plant root extract (derris or cube roots) which is used as an insecticide or piscicide. Its chemical name is [2R-(2a, 6a, 12a)]-1, 2, 12, 12a-tetrahydro-8, 9-dimethoxy-2-(1-methylethenyl)[1]benzopyrano-(3,4-yl-furo(2, 3-dibenzopyran-6,(6aH)-one. The principal active ingredient is associated with derris or cube resins (depending upon the source of the rotenone extract) which are also classified as active ingredients. Formulations contain rotenone and associated resins in a ratio of 1:2 (see Environmental Protection Agency unpu-

blished report dated May. 1980. Rotenone PreRPAR Review. Office of Pesticides and Toxic Substances.). Rotenone can be separated from the resins to a purity of 99.5%.

B. Regulatory Considerations

1. Oncogenicity

On July 15, 1981 the Agency published a notice (Federal Register Vol. 46, No. 135, page 36745) that stated:

> The Agency placed rotenone on the RPAR review list because of evidence that rotenone posed the potential of meeting or exceeding certain of the 40 CFR 162.11 risk criteria. Specifically, with regard to oncogenicity, a 1973 study that indicated potential oncogenicity has protocol deficiencies, and attempts to duplicate its results have failed. More recent testing and scientific review of rotenone do not suggest the likelihood of oncogenicity or any other significant adverse effect of concern. Therefore, on the basis of available data, the Agency has concluded that rotenone has not met or exceeded the RPAR risk criteria, and that the issuance of a Rebuttable Presumption Against Registration is not warranted.

The Agency discussed three oncogenicity studies in the PreRPAR support document as follows:

> ..., male and female hamsters fed diets containing 0, 125, 250, 500, or 1000 ppm rotenone (0, 6, 12.5, 25, or 50 mg/kg) showed no significantly increased incidence of tumors after 18 months of treatment...
>
> ..., groups of 25 male and 25 female Sprague-Dawley rats were dosed by intraperitoneal injection or oral gavage at 1.7 or 3.0 mg/kg of rotenone for 42 consecutive days. Groups of 25 male and 25 female Wistar rats were given the same dosages orally by intubation for the same length of time. Control groups (15 of each sex) were dosed with corn oil only. The Sprague-Dawley rats were observed for 17 months, at which time survivors were sacrificed. Wistar rats were observed for 12 months prior to sacrifice.

The Agency concluded:

> ...the presently available data do not show that the criterion for oncogenicity (40 CFR [162.11(a) (3)(ii)(A)] has been met or exceeded for rotenone and its related compounds.

However, additional data were requested by the Agency.

2. Mutagenicity

The Agency's Pre-RPAR support document stated:

> Several studies have shown that rotenone is effective in arresting normal cell division, or mitosis. Such arrest can lead to chromo-

somal aberrations such as aneuploidy and polyploidy if the chromosomes fail to separate at anaphase...

According to the support document, several experiments showed that rotenone is capable of causing mitotic arrest, but no chromosomal aberrations were observed. Other reports that rotenone did not induce unscheduled DNA synthesis, but did cause DNA damage, were mentioned in the Pre-RPAR document. On the basis of Agency reviews of these studies, the following conclusion was reached:

> ...presently available data are not sufficient to show that the criterion for mutagenicity stated in 40 CFR 162.11(a)(3)(ii)(A) has been met or exceeded for rotenone and its related compounds...there is sufficient cause for concern with rotenone's mutagenic potential to support a requirement for additional mutagenicity testing.

3. Teratology

The Pre-RPAR support document described a rat teratology study as follows:

> ...doses of 1.5, 3.0, and 6.0 mg/kg body weight were administered to pregnant rats on days 5 through 13 of gestation. The dams in the high dose group...had reduced weight gain and activity level... the (fetuses) in the high dose group appeared to have decreased weights,...Skeletal abnormalities were observed in all dosed groups...
>
> ...The dose range was too narrow to determine a no effect level...

Based on these considerations, the Agency stated that additional teratology studies in two species were needed.

C. Summary of Previously Submitted Data

Appendix II contains the Toxicology Branch "One-Liners" for Rotenone.

1. Acute Toxicity

The acute toxicity of a rotenone formulation that also contains Cube resins and Pyrethrum (see Appendix III) places the formulation into Toxicity Category II with respect to oral toxicity and eye and skin irritation and Toxicity Category III for acute dermal toxicity.

A study with purified rotenone indicated that the pesticide should be classified into Toxicity Category I because of its high toxicity to female rats (LD_{50} = 39.5 $\pm$ 2.21 mg/kg for female rats and 102 $\pm$ 12.6 mg/kg for male rats).

2. Chronic Toxicity - Dogs

Groups of six male and six female dogs were given 0, 0.4, 2, or 10 mg/kg/day by capsule for six months. A NOEL of 0.4 mg/kg/day was established in the six-month study. The LEL was 2 mg/kg/day, and dose-related effects included emesis and decreased body weight. The highest dose tested was 10 mg/kg/day and was associated with decreased hematocrit and hemoglobin, serum glucose, cholesterol, and total lipid levels.

3. Teratology Studies

a. Rats

Groups of 25 pregnant rats were given doses of 0, 0.75, 1.5, 3, or 6 mg/kg/day on gestation days 6 through 19. Decreased body weight and body weight gain were observed in those rats given daily doses of 6 mg/kg/day. The NOEL for these effects was 3 mg/kg, and there were no effects observed at the 1.5 or 0.75 mg/kg dose levels. The 6 mg/kg dose also caused a reduction in mean fetal weight below that of controls, and an increased incidence of unossified sternabrae, renal cavitation, and distended ureters. The adult-to-developmental toxicity (A:D) ratio (ratio of the NOEL for adult toxicity and the NOEL for developmental toxicity) is equal to one.

b. Mice

Based on results of a range-finding study, the highest dose most likely to cause slight toxicity in a teratology study with mice was indicated to be between 12 and 24 mg/kg/day. The doses finally selected for the main study were 3, 9, and 15 mg/kg/day, and they were administered to groups of 30 females on days 5 through 17 of gestation. Results of the main study suggested a NOEL for maternal and fetal effects that may be higher than 15 mg/kg/day. Since there were no dose-related effects observed in the main study, its results were interpreted along with those from the preliminary study. On that basis, the NOEL for maternal effects (weight loss and mortality) and fetal effects (decreased litter size) in mice is 15 mg/kg/day. The lowest effect level (LEL) is 24 mg/kg /day. The adult-to-developmental toxicity ratio (A:D ratio) is equal to one.

4. Reproduction Toxicity

Rotenone had no effects in groups of 15 male and 25 female rats given dietary levels of 0, 7.5, 37.5, or 75 ppm. The lowest-effect level (LEL) with respect to decreased body weight gain in dams and in pups during lactation was 37.5 ppm. Litter sizes in the F_0 and F_{1A} generations were significantly reduced at 75 ppm, and the reproductive NOEL was 7.5 ppm. The NOEL for maternal toxicity was 7.5 ppm (approximately 0.375 mg/kg/day).

5. Mutagenicity

Most of the investigators reported limited solubility of rotenone in aqueous media (20 ug/ml).

a. Gene Mutation

Rotenone did not induce gene mutations in yeast with or without metabolic activation.

Mice given 0.05, 0.17, 0.5, or 1.0 mg rotenone per kg body weight on days 8 through 11 of gestation did not show signs of toxicity or somatic mutations in embryonic melanocytes. A dose of 1000 mg/kg administered to pregnant mice under the same conditions caused melanocyte toxicity, but did not cause somatic mutations.

b. Structural Chromosomal Aberration

Single oral doses of 0.7, 2.5, or 7 mg/kg did not increase the incidence of chromosomal aberrations or decrease the mitotic index in bone marrow cells of treated rats. No increase in the incidence of polychromatic erythrocytes with micronuclei were observed in bone marrow of mice 6 hours after the last of 2 consecutive daily doses of 0, 10, or 80 mg/kg.

c. Other Genotoxicity

No mitotic recombination or mitotic gene conversion was observed in yeast at dose levels up to 10,000 ug/ml.

6. Metabolism of Rotenone

A series of limited experiments generally characterized the excretion pattern of single or repeated (14 consecutive days) low oral doses (0.01 mg/kg) and single high oral doses (5 mg/kg) as well as a single low intravenous dose (0.01 mg/kg). Almost all of the administered rotenone was recovered from the feces, and most of the administered radioactivity (approximately 80 to 90%) was recovered within 48 hours after dosing. Female rats excreted administered radioactivity at a slower rate than males.

Intravenous dosing results indicated that enterohepatic circulation is possible in treated rats.

The route of administration (at the low dose), dose level, and number of doses had no apparent effect on the excretion pattern, and most of the metabolites in the feces could not be identified. They were characterized as polar compounds which were unaffected by glucuronidase or aryl sulfatase. No radioactivity was associated with unchanged rotenone.

II. New Data

Data Evaluation Records for the studies discussed in this section are included in Appendix II below.

A. Chronic Feeding Study in Rats

In a supplementary study, diets containing 0, 7.5, 37.5, or 75 ppm rotenone were fed to groups of 40 male and 40 female Fischer 344 strain rats for two years.

Those rats given the mid and high dose levels had significantly reduced terminal body weights, and food consumption for the mid and high dose group females was significantly reduced in comparison to that for the control group females.

Lower total protein and albumin levels in females given the 75 ppm diet and higher serum urea nitrogen levels in 37.5- and 75-ppm dose group females were observed. These changes were small and probably associated with reduced food consumption.

The only treatment-related macroscopic finding was thin female rats in the 37.5- and 75-ppm dose groups. The 75 ppm diet was associated with an increased

incidence of angiectasis and hemorrhaging in the adrenals of males and females, and reduced incidences of chronic progressive nephropathy (females only), hepatocellular degeneration, pituitary adenomas, and mononuclear cell leukemia.

The lowest-effect level for decreased body weight and food consumption was 37.5 ppm, and the no-observed effect level was 7.5 ppm.

Since no microscopic examinations were performed on low and mid dose group rats, a no-effect level for the adrenal gland lesions could not be established.

The incidence of pituitary adenomas and mononuclear cell leukemias was decreased in the supplementary chronic feeding study with Fischer 344 rats given dietary levels of 0, 7.5, 37.5, or 75 ppm rotenone for two years. Increased incidence of other tumors was not observed in treated rats.

At this time the study remains supplementary pending receipt and review of histopathology for the low and mid dose group male and female rats.

B. Oncogenicity

1. Rats

Rotenone was given to groups of 50 male and 50 female F344/N (Fischer 344) strain rats at dietary concentrations of 0, 38, or 75 ppm for two years. An increase in the incidence of parathyroid adenomas in male rats given the 75 ppm diet was noted (4/44 in the high dose group compared with 1/41 in the control group). The report noted that the incidence of these tumors in the study was not statistically significantly increased, but it was greater than historical control incidence (4/1,314).

Reported body weight decreases were not sufficient by themselves to indicate that an MTD was achieved in male rats, and adequate doses may not have been tested in females based on the marginally significant decrease in body weight gain reported for high dose group rats.

A Peer Review evaluation will determine if an additional oncogenicity study that evaluates an MTD is required.

2. Mice

Rotenone was given to groups of 50 male and 50 female $B6C3F_1$ strain mice at dietary concentrations of 0, 600 or 1200 ppm for two years in another supplementary study. A dose-related decrease in mortality for treated males was noted, and no significant effect on mortality in treated female mice was observed. No toxicologically significant changes in body weight gain or histopathological findings were noted that suggested a Maximum Tolerated Dose was achieved in the study. There was no evidence that rotenone was carcinogenic in male or female $B6C3F_1$ mice at doses tested.

Peer Review will determine if an additional study in mice that evaluates an MTD is required.

C. Mutagenicity

1. Gene Mutations

In two acceptable studies, rotenone did not induce gene mutations in Salmonella typhimurium with or without metabolic activation. In another acceptable study, concentrations of 0.25 to 4.0 ug/ml without metabolic activation increased the frequency of forward mutations at the Tk locus of L5178Y mouse lymphoma cells in vitro.

2. Structural Chromosomal Damage

Groups of male mice were given two consecutive daily doses of 0, 0.56, 1.13, or 2.75 mg rotenone per kg body weight by oral intubation in DMSO. The investigators concluded that rotenone did not induce micronucle under the test conditions, but there were inadequate data to support the conclusion. There was no information included on preliminary studies to select doses used, and the reported weight losses were similar for all groups except the positive control group. There was also insufficient information on the composition of the impurities that made up approximately 60% of the test substance. Therefore, the study is unacceptable as reported.

3. Other Genetic Effects

Concentrations of 0, 1, 100, or 1000 ug rotenone per ml medium with or without metabolic activation by liver microsomal fractions did not increase unscheduled DNA synthesis in human fibroblasts in vitro. Because there were no results presented in readable form (see Data Evaluation Record in Appendix II), no preliminary toxicity or solubility studies for rotenone in the test system, no positive control substances used, and the report was often illegible, the study is unacceptable.

Fresh and degraded rotenone at concentrations from 1 to 750 ng/ml was evaluated in mixed cultures of wild type/mutant V-79 Chinese hamster lung fibroblasts. The pesticide did not affect the survival of mutant cells in a manner similar to known tumor promoters. However, this study is unacceptable because the nature of the test substances was not characterized in the report.

III. Discussion

A. Oncogenicity

Data from one of the two rat long-term studies suggests that rotenone has oncogenic potential in laboratory animals. This conclusion is supported by the observation that parathyroid adenomas in male Fischer 344 rats are uncommon, and the incidence in rats given the highest test dose was outside the historically observed range for untreated control rats of the same strain. The conclusion is limited because the studies in rats and mice may not have tested Maximum Tolerated Doses (MTD).

This information will be presented to the Toxicology Branch Peer Review Committee for evaluation. The results of the evaluation will follow.

B. Mutagenicity

In addition to the mutagenicity studies described in Sections I. C. 5. and II. C. above, the National Toxicology Program (NTP) summarized results from four mutagenicity assays including an Ames assay with Salmonella typhimurium and the mouse lymphoma cell assay described below. The NTP report also summarized results from a sister chromatid exchange (SCE) assay in Chinese hamster ovary (CHO) cells and a chromosomal aberration assay in CHO cells. Reports on the CHO cell assys were not available for review.

Results of the SCE assay were characterized in the NTP report as equivocal since rotenone was associated with an increase in SCE's in the presence of liver microsomal activation and since those results could not be repeated. The NTP report stated that the cytogenetics assay was negative.

The reports for the CHO sister chromatid exchange and chromosomal aberration assays are needed before an assessment of the mutagenicity data can be completed.

C. Data Gaps

As mentioned in Section I. above, the Agency concluded that the aquatic uses of rotenone should be regarded as food uses because residues might occur in edible fish. Based on the assessment of Toxicology data in the previous sections, acute dermal and inhalation studies, primary eye and dermal irritation studies, and a dermal sensitization are required for technical grade rotenone. Subchronic oral toxicity studies should be waived based on acceptable chronic studies, and a chronic toxicity study in a rodent species may be required unless additional histopathology for low and mid dose group rats is provided for the feeding study described in Section II. A. above. Oncogenicity studies in two species may not be required if Peer Review determines that available mouse and rat studies are acceptable. Reports on the sister chromatid exchange and chromosomal aberration assays are needed before an assessment of rotenone's mutagenic potential can be completed.

D. Acceptable Daily Intake

In view of the above mentioned data gaps, a Provisional Acceptable Daily Intake (PADI) can be established from the following no-effect levels:

Study (species)	No-effect levels ppm	(mg/kg/day)
6-month feeding (dogs)	---*	0.4
Teratology (mouse)	---*	>15**
Reproduction (rat)	7.5†	0.375†

*Doses were administered by gavage rather than dietary.
**For maternal and developmental toxicity.
†For reproductive and maternal toxicity

Data from the rat oncogenicity study suggest that rotenone may have oncogenic potential in that species although the Maximum Tolerated Dose (MTD) may not have been tested (see above).

The PADI is based on the two-generation reproduction study in rats (NOEL of 0.375 mg/kg body weight/day), a 100-fold safety factor. It is calculated to be 0.00375 mg/kg/day.

This PADI has been approved by the Toxicology Branch ADI Committee.

APPENDIX I

Toxicology Branch "One-Liners" for
Rotenone (Tox. Chem. No. 725)

File Last Updated _____ Current Date 11/23/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Registration Standard					006420
Acute oral LD_{50} - rat; Hazleton Labs., Inc.; Proj. No. 419-137; Oct. 16, 1984	99.23% a. i.	MRID No. 00145490 Acc. Nos 253333 258849	LD_{50} for males = 102 ± 12.6 mg/kg LD_{50} for females = 39.5 ± 2.21 mg/kg Doses tested = 10, 25, 50, 75, and 150 mg/kg (10 per sex/dose)	I	Minimum 004652 004653
Chronic feeding - rat; Hazleton Labs America, Inc.; Report no. 6115-100: 12/30/85	95% rotenone 5% other cube resins	MRID No. 00156739	Doses tested = 0, 7.5, 37.5, and 75 ppm LEL was not be established in this study. Effects at highest dose tested = reduced body weight in males and females, decreased food consumption in females, lower total protein and albumin levels and higher blood urea nitrogen in females; the only histopathology observed was increased incidence of angiectasis and hemorrhaging in adrenals of males and females in the high dose group; no increase in tumor incidence was observed) NOEL can not be established until histopathology for low and mid dose groups is made available.		Supplementary 006420
6 Month feeding - dog; Midwest Research Institute; Report no. 4853-B; 1980	99% a. i.	MRID No. 00141406	Doses tested = 0, 0.4, 2, and 10 mg/kg/day LEL = 2 mg/kg/day (decreased body weights; hematocrit, hemoglobin, cholesterol, total lipids, and glucose levels were decreased in the blood; increased incidences of emesis and diarrhea) NOEL = 0.4 mg/kg/day (LDT)		Guideline 004816

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Oncogenic - rat; Battelle Columbus Labs; NIH Publication No. 86-2576; August, 1986	>98% a. i.	MRID No. 40179801	Doses tested = 0, 38, and 75 ppm LEL > 75 ppm (HDT) (decreased body weight and food consumption in females; increased incidence of hyperplasia in the anterior pituitary in males, and increased incidence of parathyroid adenomas in males (4/44 compared with 1/41: not statistically significant, tumors are rare in F344 strain rats) No MTD was achieved in the study.		Supplementary 006420
Oncogenic - rat; Battelle; Report No. EPA-600/1-79-040b; January, 1979	Rotenone (95+% a. i.)	Acc. No. 255278 MRID No. 00143257	Test material was administered by i. p. injection or gavage for 42 days, animals were maintained for 14 or 18 months before sacrifice. No treatment related tumors were observed. Doses tested = 0, 1, or 3 mg/kg/day		Supplementary 004610
Oncogenic - mice; Battelle Columbus Labs; NIH Publication No. 86-2576; August, 1986	>98% a. i.	MRID No. 40179801	Doses tested = 0, 600, and 1200 ppm LEL > 1200 ppm (HDT) (decreased body weight gain in males and females without histopathological effects, tumor incidence was not increased) No MTD was achieved in the study.		Supplementary 006420
18-Month oncogenic - hamster; Battelle; Report #EPA-600/1-79-040a; January, 1979	Rotenone (95+% a. i.)	Acc. No. 255278 MRID No. 00143256	96% mortality at 18 months in the control group females. Two mid-dose groups not evaluated microscopically. No dose-related tumor incidences were reported. Dietary levels tested = 0, 125, 250, 500, and 1000 ppm		Supplementary 004610

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - rat; Hazleton Raltech, Inc; Report no. 81178; June 17, 1982	Rotenone	Acc. No. 254725 MRID No. 00144294	Levels tested = 0, 0.75, 1.5, 3, and 6 mg/kg/day given on gestation days 6 through 19 by gavage. Teratogenicity NOEL > 6 mg/kg/day (HDT) Maternal NOEL = 3 mg/kg/day Maternal LOEL = 6 mg/kg/day (HDT) (decreased body weight) Fetotoxic NOEL = 3 mg/kg/day Fetotoxic LOEL = 6 mg/kg/day (renal pelvic cavitation and distended ureters, delayed ossification, and decreased fetal weights)		Minimum 004816 006420
Teratology - mice; Hazleton Raltech, Inc; Report no. 80049; Nov. 14, 1981	Rotenone Lot #100287 (94% a. i.)	Acc. No. 254722 MRID No. 00103047	Range-finding study Levels tested by gavage in CD-1 strain = 0, 0.75, 1.5, 3, 6, 12, and 24 mg/kg/day. Maternal NOEL = 12 mg/kg/day Maternal LOEL = 24 mg/kg/day (decreased gravid uterine weight and mortality) Fetotoxic NOEL = 12 mg/kg/day Fetotoxic LOEL = 24 mg/kg/day (decreased litter size and increased resorptions)		Supplementary 004816

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - mice; Hazleton Raltech, Inc; Report no. 80050; Nov. 24, 1981	Rotenone	Acc. No. 254724 MRID No. 00141407	Levels tested by gavage in CD-1 strain = 0, 3, 9, and 15 mg/kg/day (on gestation days 6 through 17) Teratogenic NOEL > 15 mg/kg/day(HDT) Maternal NOEL > 15 mg/kg/day (HDT) Maternal LOEL = 24 mg/kg/day (Hazleton Studies #80049 and #80050 were combined to obtain this LOEL) Fetotoxic NOEL > 15 mg/kg/day (HDT) Fetotoxic LOEL = 24 mg/kg/day (Hazleton Studies #80049 and #80050 were combined to obtain this LOEL)		Minimum when considered with Study 80049 004816
Reproduction- hamster; Battelle ;#EPA 600/1-79-040a; 1/79	Rotenone (95+% a.i.)	255278	500 ppm caused mortality in pups during lactation. No concurrent control group. Only one generation At 1000 ppm no litters were produced 3 males and 12 females died during treatment period before mating.		Supplementary 004610
2 Generation Reproduction - rat; Hazleton Raltech, Inc; Report no. 81077: Feb. 11, 1983	Rotenone (97.9% a. i.)	Acc. Nos. 254726 254727 254728 MRID No. 00141406	Levels tested = 0, 7.5, 37.5, and 75 ppm in the diet Reproductive NOEL = 7.5 ppm (decreased pup weight during lactation) Parental NOEL = 7.5 ppm Parental LOEL = 37.5 ppm (decreased body weight; highest dose tested also increased the incidence of gastric gland dilation in the stomach		Minimum 004816
Mutagenic - Salmonella typhimurium (Ames assay); EG&G Mason Research; Report no. 019-563-165-1; 11/3/78	>98% a. i.	MRID No. 40170502	Doses tested = 30 to 10,000 ug per plate with and without metabolic activation Not mutagenic		Acceptable 006420

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic - Salmonella typhimurium (Ames assay); SRI International; undated	>98% a. i.	MRID No. 40170506	Doses tested = 100 to 10,000 ug per plate with and without metabolic activation Not mutagenic		Acceptable 006420
Mutagenic - yeast - reverse mutation; Litton Bionetics; Report No. 22063; June 24, 1981	Rotenone (>97%)	Acc. No. 254720 MRID No. 00144292	Negative at dose levels up to 10,000 ug/ml		Acceptable 004816
Mutagenic - mouse L5178Y cells (point mutation assay); IRI; 11/27/84	>98% a. i.	MRID No. 40170505	Doses tested = 0 to 4 ug/ml without metabolic activation Mutagenic at doses from 0.25 (LDT) to 4 ug/ml without metabolic activation		Acceptable 006420
Mutagenic - mice - spot test; Litton Bionetics; Report No. 22063; June 24, 1981	Rotenone (>97%)	Acc. No. 254720 MRID No. 00144292	At dose levels of 0.05, 0.17, 0.5, or 1.0 mg/kg there were no mutations or cytotoxicity noted. A dose of 1000 mg/kg caused cytotoxicity. (Doses were administered on days 8 - 11 of gestation by gavage.)		Acceptable 004816
Mutagenicity - in vivo cytogenetics -rat; Biotech Research Labs., Inc.; January 10, 1992	Rotenone (>97% a. i.)	Acc. No. 254721 MRID No. 00093702	Doses tested = 0, 0.7, 2.5, or 7.0 mg/kg No increase in incidence of chromosomal aberrations, and no decrease in mitotic index of bone marrow cells in treated rats.		Acceptable 004816
Mutagenicity - micronucleus test - mice; Biotech Research Labs., Inc.; January 10, 1992	Rotenone (>97% a. i)	Acc. No. 254721 MRID No. 00093702	Doses tested = 0, 10, or 80 mg/kg (administered on two consecutive days) No increase in mono- or polychromatic erythrocytes with micronuclei.		Acceptable 004816

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic - yeast - mitotic recombination; Litton Bionetics; Report No. 22063; June 24, 1981	Rotenone (>97%)	Acc. No. 254720 MRID No. 00144292	Negative at dose levels up to 10,000 ug/ml		Acceptable 004816
Mutagenic - yeast - mitotic gene conversion; Litton Bionetics; Report No. 22063; June 24, 1981	Rotenone (>97%)	Acc. No. 254720 MRID No. 00144292	Negative at dose levels up to 10,000 ug/ml		Acceptable 004816
Metabolism - rat; Hazleton Labs., Inc.; Proj. No. 419-137; Oct. 16, 1984	94.64% a. i. spec. activ. 32.8479 uCi/mg	Acc. Nos. 258849 253333 MRID No. 00145496	Single oral dose = 0.01 mg/kg, 14 consecutive daily oral doses of 0.01 mg/kg/day, single i. v. dose of 0.01 mg/kg, and single oral dose of 5 mg/kg. Approximately 75% of all doses recovered within 72 hours following dosing in feces. 95 to 97% recovered during 144 hours after dosing. Unidentified metabolites were polar but were not specifically identified.		Acceptable 004652 004653

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral LD50 - rat; MB Research Lab; MB #82-6509A; 3/16/83	Rotenone . 1.1% Other Cube Extractives ..... 2.2% Pyrethrins ..... 0.8% Petroleum Distillate .. 3.2% Aromatic Petroleum Solvent ..... 84.7% Inerts .. 8.0% EPA Reg. No. 432-664)	250015	Oral LD50 (M) = 0.64 (0.42-0.97)g/kg Oral LD50 (F) = 0.21 (0.15-0.29)g/kg Combined oral LD50 = 0.34 (0.24-0.49) g/kg (frequent finding was hemorrhagic or congested lungs)	II	Minimum 003117
Acute dermal LD_{50} rabbit ; MB Research Labs;#MB 82-6509B;3/2/83	Rotenone . 1.1% Other Cube Extractives ..... 2.2% Pyrethrins ..... 0.8% Petroleum Distillate .. 3.2% Aromatic Petroleum Solvent ..... 84.7% Inerts .. 8.0% EPA Reg. No. 432-664)	250015	No mortalities at 2 g/kg LD_{50} > 2 g/kg	III	Minimum 003117

Tox Chem No. 725 - Rotenone File Last Updated Current Date 11/23/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary eye irritation - rabbit; MB Research Labs;#MB 82-6509D;3/2/83	Rotenone . 1.1% Other Cube Extractives ..... 2.2% Pyrethrins ..... 0.8% Petroleum Distillate .. 3.2% Aromatic Petroleum Solvent ..... 84.7% Inerts .. 8.0% EPA Reg. No. 432-664)	250015	One rabbit showed corneal involvement on day 21, others were clear on day 21 or with very slight conjunctival irritation. Corneal opacity in 4/6 rabbits.	II	Minimum 003117
Primary dermal irritation - rabbit;#MB Res. Labs;#MB 82-6509C;3/2/83	Rotenone . 1.1% Other Cube Extractives ..... 2.2% Pyrethrins ..... 0.9% Petroleum Distillate .. 3.2% Aromatic Petroleum Solvent ..... 84.7% Inerts .. 8.0% EPA Reg. No. 432-664)	250015	Severe erythema/eschar at 72 hours, slight edema (4 hr exposure). Irritation had healed at 14 day.	II	Minimum 003117

APPENDIX II

Data Evaluation Records for the Following Studies

Tisdel, M. (1985) Chronic Toxicity Study of Rotenone in Rats: Final Report: Study No. 6115-100. Unpublished study prepared by Hazleton Laboratories. Americas, Inc. 1222 p. MRID No. 00156739

Abdo, K. (1983) NTP Technical Report on the Toxicology and Carcinogenesis Studies on Rotenone in F344/N Rats and B6C3F1 Mice (Feed Studies) Draft. Prepared by Battelle Columbus Laboratories for the National Toxicology Program, NIH Publication No. 86-2576. 186 p. MRID No. 40179801 (Separate DER's for rat and mouse studies).

Haworth, S. (1978) Salmonella/mammalian-microsome Plate Incorporation Mutagenesis Assay (Rotenone): Laboratory Project ID: #019-563-165-1. Unpublished study prepared by EG&G Mason Research Institute. 26 p. MRID No. 40170502.

SRI International. (1987??) The Salmonella/microsome Mutagenicity Test System (Rotenone). Unpublished compilation. 7 p. MRID No. 40170506

IRI. (1984) Mouse Lymphoma Protocol (Rotenone): Laboratory Project ID: 28037. Unpublished compila tion. 8 p. MRID No. 40170505

Jones, D. C. L., V. F. Simmon, K. E. Mortelmans, A. D. Mitchel, E. L. Evans, M. M. Jotz, E. S. Riccio, D. E. Robinson, and B. A. Kirkhart September, 1984 In Vitro and In Vivo Mutagenicity Studies of Environmental Chemicals: Micronucleus Test (includes Rotenone). Unpublished report prepared by SRI International for U. S. Environmental Protection Agency. Submitted by U. S. Fish and Wildlife Service. MRID No. 401705-01

Ahmed, F. E.; Hart, R. W.; Lewis, N. J. July, 20, 1976. Pesticide Induced DNA Damage and Its Repair in Cultured Human Cells (Includes Rotenone). Mutation Research 42:161-174. MRID No. 401705-03

Maltese, L. J.; Hartman, T. July 8, 1985. Stillwell and Gladding Study on Relative Safety of Rotenone: Cytotoxicity and Tumor Promoting Activity of Fresh and Degraded Rotenone Resins. Unpublished report prepared by Stillwell and Gladding Laboratories. Submitted by U. S. Fish and Wildlife Service. MRID No. 401705-04

006537

Reviewed by: Roger Gardner R.G. 11-19-87
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 11/19/87
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic feeding - rat (Guideline §83-1)

MRID NUMBER: 00156739

TEST MATERIAL: Technical grade rotenone (Lot No. 215-LCD-1 L19123; the stated purity was 95% rotenone, 5% other cube resins) was used in the study. The test substance was described as a white powder and was obtained from Penick Corporation. According to the report, analysis of the test substance as received indicated a purity of 89.6% a. i., and the material was recrystalized to a purity of 96.4%.

SYNONYMS: 1,2,12,12a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl-[1]benzopyrano-[3,4-b]furo[2,3-h][1]benzopyran-6[6H]-one; CAS No. 83-79-4

STUDY NUMBER(S): 6115-100

SPONSOR: U. S. Fish and Wildlife Service

TESTING FACILITY: Hazleton Laboratories America, Inc., Madison, WI

TITLE OF REPORT: Final Report: Chronic Toxicity Study with Rotenone in Rats.

AUTHOR(S): Tisdel, M.

REPORT ISSUED: December 30, 1985

CONCLUSION: Diets containing 0, 7.5, 37.5, or 75 ppm rotenone were fed to male and female Fischer 344 rats for two years. Male and female rats given the mid and high dose levels had significantly reduced terminal body weights when compared to control group rats. Food consumption for the mid and high dose group females was also significantly reduced in comparison to that for the control group females. Lower total protein and albumin levels in females given the 75 ppm diet and higher serum urea nitrogen levels in 37.5- and 75-ppm dose group females were observed. These changes were small and probably associated with reduced food consumption. The only treatment-related macroscopic finding was thin female rats in the 37.5and 75-ppm dose groups. The 75 ppm diet was associated with an increased incidence of angiectasis and hemorrhaging in the adrenals of males and females, and reduced incidences of chronic progressive nephropathy (females only), hepatocellular degeneration, pituitary adenomas, and mononuclear cell leukemia.

The lowest-effect level for decreased body weight and food consumption was 37.5 ppm, and the no-observed effect level was 7.5 ppm.

Core classification: Supplementary. Since no microscopic examinations were performed on low and mid dose group rats, a no-effect level for the adrenal gland lesions could not be established, and a dose-response relationship can

Core classification (continued):

not be established for the decreased incidences of chronic progressive nephropathy, hepatocellular degeneration, pituitary adenomas, and mononuclear cell leukemia. The classification could be upgraded when histopathology for the low and mid dose groups is available for review.

I. PROTOCOL

A. MATERIALS

1. Test species: Male and female weanling Charles River Fischer 344 [(CDG® F-344)/CrlBR)] strain rats were used. The animals were approximately 6 weeks of age when placed on test diets.

2. Diet preparation: Basal diet consisted of NIH07 rodent diet in meal form. Test diets were prepared weekly and stored under refrigeration. Samples of test diets were analyzed for stability, homogeneity and accuracy of test concentration before the study began. Diets were analyzed for concentration of test substance during weeks 1, 2, 3, and 4, and one test diet was randomly chosen for analysis each week through termination of the study.

B. STUDY DESIGN

1. Animal assignment: Animals were randomly assigned to test groups as follows:

Test groups No.	Designation	Dose (ppm)	Animals per sex: Main study	Pre-test*	Interim Sacrifice
1	Control	0.0	40	10	--
2	Low (LDT)	7.5	40	0	--
3	Mid	37.5	40	0	--
4	High (HDT)	75.0	40	0	--

*In addition to animals assigned to test groups.

2. Observations schedule

Type of observation	Number of animals per sex per group	Frequency
Mortality	All	Twice a day*
Signs of toxicity	All	Twice a day*
Body weight	All	On day of arrival at laboratory, at pre-test during randomized group assignment, at weekly intervals through the first 12 weeks, every fourth week thereafter, and on the day of necropsy.

2. Observations schedule (continued)

Type of observation	Number of animals per sex per group	Frequency
Food consumption	All	Weekly for the first 12 weeks, and during weeks 26, 39, 52, 65, 78, 92, and 104.
Blood samples	10	At 3, 6, 12, 18, and 24 months.
Urine samples	10	At 3, 6, 12, 18, and 24 months.
Necropsy	Animals found dead or moribund	When found.
	Survivors	At 24 months

*The report stated that at least once a week each animal was removed from its cage and examined carefully. The report also indicated that the incidence, size, and location of all tissue masses were determined by palpation.

3. Observation of blood samples: Blood was collected from the retro-orbital sinus, and the animals were fasted overnight prior to sampling.

a. Hematology

- X Hematocrit
- X Hemoglobin
- X Red cell count
- X Platelet count
- X Total white cell count
- X Differential white cell counts
- X Mean corpuscular hemoglobin concentration
- X Mean cell volume
- X Mean corpuscular hemoglobin

Additional Hematology

Nucleated red blood cell count

b. Blood chemistry

- X Alanine aminotransferase (ALT)
- X Albumin
- X Albumin/globulin ratio
- X Alkaline phosphatase (AP)
- X Aspartate aminotransferase (AST)
- ___ Creatinine
- ___ Electrolytes
- X Gamma glutamyl transferase
- X Glucose
- ___ Lactate dehydrogenase (LDH)
- ___ Total bilirubin
- ___ Total cholesterol
- ___ Total globulins
- X Total protein
- ___ Triglycerides
- X Urea nitrogen
- X Uric acid

4. Urine observations

X Volume	X glucose	X occult blood	X specific gravity
X pH	X ketones	X urobilinogen	X microscopic examination
X protein	X bilirubin	X Appearance	of centrifuged deposits

5. Necropsy Gross examinations were conducted on all animals regardless of fate, and macroscopic lesions were noted.

a. Weighed organs

X Liver	X Spleen	X Adrenals	X Brain
X Kidneys	Heart	X Gonads	Pituitary

Organ to body weight ratios were calculated.

b. Tissues preserved for microscopic examination:

X Adrenals	X Kidneys	X Skin and subcutis
Aorta	X Liver	X Spinal cord
X Bone and marrow	X Lungs	X Spleen
X Brain	X Lymph nodes	X Stomach
X Cecum	X Mammary glands	X Testes
X Colon	X Ovaries	X Thymus
X Duodenum	X Pancreas	X Thyroid with para-
Ears	X Pituitary	thyroid
X Epididymides	X Prostate	X Tongue
X Esophagus	X Rectum	X Trachea
X Eyes with	X Salivary gland	X Urinary bladder
Harderian gland	X Seminal vessicles	X Uterus with cervix
X Heart	X Sciatic nerve	X Vagina
X Ileum	X Skeletal muscle	X All macroscopic
X Jejunum		abnormalities

Tissues from all animals in the control and high dose groups were examined microscopically.

6. Statistical analysis:

a. Continuous variables: (body weight, hematology, clinical chemistry, organ and weights.

Statistical procedure	Purpose
Analysis of variance	Determine significance of differences between group means.**
Dunnett's "t" test	Determine significance of differences between the control and each treatment group.

b. Frequency data (urine protein, glucose, ketones, bilirubin, blood, and urobilinogen)

Statistical procedure	Purpose
Contingency table techniques	Determine significance of differences between individual groups, overall variations, or trends

II. REPORTED RESULTS

A. Mortality and Signs of Toxicity: Mortality during the study was summarized in the report as follows:

Dose (ppm)	Number of Animals: Alive at initiation	Number of Animals: Accidental deaths	Adjusted number alive	Number died or sacrificed moribund	Number of survivors at week 104
			Males		
0.0	40	1	39	14	25
7.5	40	2	38	14	24
37.5	40	0	40	6	34
75.0	40	1	39	8	31
			Females		
0.0	40	0	40	11	29
7.5	40	0	40	8	32
37.5	40	2	38	11	27
75.0	40	1	39	4	35

No significant compound-related effect on survival of treated rats was noted.

The investigators noted that during weeks 20 and 21 several animals exhibited swelling in the neck or submandibular area. The numbers of males with the swelling were 4, 5, 10, and 5 in the control, low, mid, and high dose groups, respectively. The respective number of females exhibiting the swelling were 4, 3, 9, and 1 in the control, low, mid, and high dose groups. These observations were attributed by the investigators to a transient sialodacryoadenitis (SDA) virus infection.

According to the report, there were no dose-related effects on the incidence of palpable masses in male or female rats. There were 19, 16, 14, and 17 males in the control, low, mid, and high dose groups reported to have palpable masses during the feeding period, while 25, 15, 16, and 6 females were observed to have palpable masses in the control, low, mid, and high dose groups, respectively.

B. Body Weight and Food Consumption: Group mean body weights for mid and high dose group rats were statistically significantly lower than those for the control group. For males the differences were significant from week 68 of

B. Body Weight and Food Consumption (continued)

the study, and the differences for females were significant throughout the experiment. The investigators noted that by the end of the study mid dose group males had a group mean body weight that was 8% less than that for the controls, and the male high dose group mean weight was 15% less than the control mean. Female group mean weights for the mid and high dose groups were 22 and 55% less than their control group mean at the end of the study, according to the report.

Group mean body weight gains during the first 12 weeks of the study are summarized as follows:

Observation	Dose level (ppm) 0	7.5	37.5	75.0
Males				
Body weight (g) at week 0	118.3	117.8	115.0*	115.9
Body weight (g) at week 12	320.5	314.3	312.7	312.9
Cumulative weight gain (weeks 0-12)	+202.2	+196.5	+197.7	+197.0
Body weight (g) at termination	358.8	359.7	333.5*	305.4*
Females				
Body weight (g) at week 0	90.1	90.5	89.1	89.8
Body weight (g) at week 12	193.7	196.4	186.2*	186.4*
Cumulative weight gain (weeks 0-12)	+103.6	+ 95.9	+ 97.8	+ 96.6
Body weight (g) at termination	288.9	287.7	220.0*	168.5*

*Statistically significantly different from controls ($p<0.05$).

The report noted a significant dose-related decrease in food consumption for female rats given the mid and high dose diets. Selected group mean food consumption for the female rats were summarized as follows:

Week of study	Dose level (ppm) 0	7.5	37.5	75.0
1	81.3	87.9*	85.1*	83.8*
2	90.0	92.7*	86.1*	84.5*
3	94.2	93.3	85.1*	75.8*
4	97.8	98.4	86.7*	78.7*
8	90.8	95.7	80.7*	67.0*
12	86.7	86.0	83.9	76.0*
26	83.8	81.5	80.4*	64.7*
52	93.7	95.2	82.0*	75.2*
78	102.8	98.7	83.6*	69.2*
104	102.1	107.6	95.4	76.5*

*Statistically significantly different from controls ($p<0.05$).

B. Body Weight and Food Consumption (continued)

The investigators noted that the food consumption for mid dose group females averaged 8% less than that for controls, and the average food consumption for the high dose group females was 20% less than that for the control group during the study.

C. Clinical Pathology

1. Hematology: Although the authors noted statistically significant differences for some hematological observations, none were dose-related or consistently observed throughout the two-year study.

2. Clinical chemistry: Total protein, albumin and urea nitrogen were affected in treated female rats. Reported mean values for both sexes are summarized as follows:

Month of observation	Dose level (ppm) Males 0	7.5	37.5	75.0	Females 0	7.5	37.5	75.0
				Total protein (g/dl)				
3	6.62	6.62	6.42	6.39	6.66	6.44	6.47	6.04
6	6.93	7.04	7.02	6.92	7.29	7.54	7.30	6.65*
12	7.16	6.97	7.13	7.09	7.76	7.86	7.62	6.95*
18	6.59	6.81	6.71	6.39	7.03	6.99	6.77	6.63*
24	6.8	6.8	6.5	6.3*	7.3	7.4	7.2	7.1
				Albumin (g/dl)				
3	3.24	3.21	3.17	3.25	3.45	3.30	3.33	2.94*
6	2.91	2.94	2.98	2.94	3.21	3.24	3.23	2.98*
12	3.39	3.41	3.41	3.43	4.06	4.15	3.93	3.50*
18	3.17	3.27	3.45*	3.32	3.84	3.80	3.62	3.65
24	2.9	3.0	3.0	3.2	3.4	3.5	3.8	3.5
				Urea nitrogen (mg/dl)				
3	13.9	15.6*	15.7*	15.9*	14.5	15.6	16.1	17.1*
6	14.9	15.5	16.3	16.3	17.2	17.5	17.[illegible]	18.6
12	15.8	14.5	16.7	16.2	16.7	16.6	16.4	17.4
18	15.5	14.1	15.7	16.3	15.4	16.6	17.7*	20.6*
24	17.9	16.5	18.2	18.2	14.2	15.3	18.6*	20.2*

*The group mean was statistically significantly different from the control group mean ($p<0.05$).

3. Urine analysis: There were no significant changes noted in the report for these parameters.

D. Necropsy

1. Organ weights: According to the report, decreased weights were observed for the spleen, kidney, liver, and right adrenal in male and female rats given the 75-ppm diet. Organ-to-body weight ratios were increased for brain, kidney, ovaries, and right adrenal in high dose group females. The investigators attributed these changes to the decreases observed in terminal body weights. Organ weights and weight ratios are presented for the kidneys as an example of the changes observed as follows:

Observation	Dose level (ppm) 0	7.5	37.5	75.0
	Males			
Terminal body weight (g)	358.8	359.7	333.5*	305.4*
Absolute weight (g)				
Left kidney	1.49	1.47	1.43	1.37
Right kidney	1.47	1.47	1.41	1.36*
Organ-to-body weight ratio				
Left kidney	0.417	0.410	0.430	0.450*
Right kidney	0.411	0.413	0.425	0.446*
	Females			
Terminal body weight (g)	288.9	287.7	220.0*	168.5*
Absolute weight (g)				
Left kidney	1.16	1.13	1.00*	0.84*
Right kidney	1.17	1.14	0.99*	0.83*
Organ-to-body weight ratio				
Left kidney	0.406	0.393	0.460*	0.500*
Right kidney	0.407	0.388	0.453*	0.493*

*Statistically significantly different from control ($p<0.05$).

2. Macroscopic observations: The only macroscopic observation attributed to treatment by the investigators was an increased incidence of thin females. There were 1, 3, 10, and 25 of the 40 females in the control, low, mid, and high dose groups, respectively.

3. Microscopic observations

a. Non-neoplastic findings: The only lesions with increased incidence in high dose group males and females were angiectasis and hemorrhage in the adrenals. Female rats in the high dose group also had an increased incidence of mineralization in the kidneys. The incidence of these lesions is summarized as follows:

3. Microscopic observations (continued)

a. Non-neoplastic findings (continued)

Observations	Dose level (ppm) Males 0	Males 75	Females 0	Females 75
Adrenals				
Angiectasis	1/40	13/40	6/40	13/40
Hemorrhage	3/40	14/40	7/40	14/40
Kidneys				
Mineralization	0/40	0/40	0/40	5/40

The investigators noted that other non-neoplastic lesions were decreased at the high dose. The incidence of these lesions is summarized as follows:

Observations	Males 0	Males 75	Females 0	Females 75
Chronic progressive nephropathy	39/40	40/40	40/40	10/40
Hepatocellular degeneration	12/40	18/40	18/40	1/40

b. Neoplastic lesions: No tumors had a significantly increased incidence in the high dose group in comparison to the control group. However, the authors noted that the incidence of pituitary adenomas and monuclear cell leukemia were decreased. These results are summarized as follows:

Observations	Males 0	Males 75	Females 0	Females 75
Pituitary adenomas	26/40	13/40	35/40	19/40
Mononuclear cell leukemia	5/40	2/40	9/40	2/40

III. DISCUSSION

A. Authors' conclusions:

In the discussion section of the report's chapter entitled "Pathology Report" the following conclusions were presented:

> No toxicologically important changes were observed in this study except for lower terminal body weights in both sexes treated with 37.5 and 75 ppm of Rotenone. Statistically significant changes in organ weights were incidental, and were due to the lower terminal body weights of the treated rats. Lower total protein and albumin levels in 75 ppm dose group females and higher serum urea nitrogen levels in 37.5- and 75-ppm dose group females were treatment related. However, the magnitude of the changes was small and was not considered biologically important. The only treatment-related macroscopic finding was thin female rats in the 37.5- and 75-ppm dose groups.

A. Authors' conclusions (continued)

This finding correlated with the lower terminal body weights of these rats. No treatment-related changes were observed in microscopic findings.

B. Reviewer's discussion: The conclusions made in the report did not consider the body weight, or total protein, albumin, and serum urea nitrogen results in the context of the decreased food consumption reported for the mid and high dose group females. Cummulative body weight gains during the first 12 weeks of the study were approximately 7% less in the mid and high dose group females than that for the control group (see page 6 above), and group mean body weights at week 12 were 3 to 3.5% less for mid and high dose group female rats than that for the control group. Group mean body weights for the mid and high dose groups were not toxicologically significantly different from control values until late in the study (see page 8 above). By the end of the study group mean body weights for the mid and high dose group females were 24 and 42% less than that for the control group females. This pattern of weight changes, the absence of clinical signs of toxicity or histopathology, and the presence of changes in total protein, albumin and urea nitrogen are more likely to be a reflection of the chronically reduced food consumption noted in the report. Therefore, the lowest-effect level is more appropriately based on reduced palatability of diets containing 37.5 ppm rotenone or more.

The reduced organ weights and changes in organ-to-body weight ratios noted by the investigators were consistent with reductions in body weights observed in treated rats.

The investigators did not discuss the significance of the increased incidences of angiectasis and hemorrhaging in the adrenals which was observed in high dose group rats (see page 8 above). Since the protocol did not include microscopic examination of the adrenals in low and mid dose group rats, a full assessment of the significance of these lesions can not be made.

006537

Reviewed by: Roger Gardner R.G. 11-14-87
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 11/19/87
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity - Rat (Guideline §83-2)

MRID NUMBER: 401798-01

TEST MATERIAL: Technical grade Rotenone (Lot no. 735-RAP-1502) purity >98%) was used. It was described as a white crystaline powder.

SYNONYMS: 1,2,12,12a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl-[1]benzopyrano-[3,4-b]furo[2,3-h][1]benzopyran-6[6H]-one; CAS No. 83-79-4

STUDY NUMBER(S): NIH Publication No. 86-2576

SPONSOR: National Toxicology Program (Submitted by the U. S. Department of the Interior, Fish and Wildlife Service, National Fisheries Research Laboratory)

TESTING FACILITY: Battelle Columbus Laboratories

TITLE OF REPORT: NTP Technical Report on the Toxicology and Carcinogenesis Studies of Rotenone (CAS No. 83-79-4) in F344/N Rats and B6C3F1 Mice. (Feed Studies)

AUTHOR(S): K. Abdo, Chemical Manager

REPORT ISSUED: August, 1986

CONCLUSIONS: Rotenone was given to male and female F344/N strain rats at dietary concentrations of 0, 38, or 75 ppm for two years. An increase in the incidence of parathyroid adenomas in male rats given the 75 ppm diet was noted (4/44 in the high dose group compared with 1/41 in the control group). The report noted that the incidence of these tumors in the study was not statistically significantly increased, but it was greater than historical control incidence (4/1,314). The increased incidence of subcutaneous tissue tumors in female rats was considered to be equivocal because there was no statistically significant dose-related trend, and the statistically significant increase observed in the low dose group was attained only by combining morphologically different tumors.

Although group mean body weights for treated rats of both sexes at 3 months were comparable to appropriate control groups, weight gain in males given the 38-ppm and 75-ppm diets were 10 and 7.6% greater than that for the controls. Weight gains for the low and high dose group females were 19 and 9% less than that of the control group, respectively. In the absence of other effects, these body weight changes suggest that a Maximum Tolerated Dose (MTD) may not have been achieved.

Core classification: Supplementary. An MTD may not have been tested.

I. PROTOCOL

A. MATERIALS

1. Test compound: Technical grade Rotenone (Lot no. 735-RAP-1502) purity >98%) was used. It was described as a white crystaline powder.

2. Test species: Male and female 57-day old F344/N strain rats were used. Breeding stock for these animals originated at the National Institutes of Health Repository, and those animals used in the study were provided by the Frederick Cancer Research Laboratory.

3. Diet preparation: Test diets were prepared biweekly and stored at 4° C. The test substance was added to Purina Lab Chow®, and samples of test diets were analyzed for stability, homogeneity and accuracy of test concentration before the study was begun. Diets were analyzed for concentration of test substance at approximately 8-week intervals during the two year feeding period.

4. Preliminary studies: See the Addendum below for a discussion of the feeding studies conducted to characterize the toxicity of rotenone and determine the highest dose to be tested.

B. STUDY DESIGN

1. Animal assignment: Animals were randomly assigned to test groups as follows:

Test groups		Dose	Animals per sex		
No.	Designation	(ppm)	Main study*	Pre-test	Interim Sacrifice
1	Control	0	50	5	0
2	Low (LDT)	38	50	0	0
3	High (HDT)	75	50	0	0

*24 months.
**These were examined to determine health status of the animals used in the assay.

B. STUDY DESIGN (continued)

2. Observations schedule

Type of observation	Number of animals per sex per group	Frequency
Mortality	All	Twice a day*
Signs of toxicity	All	Twice a day*
Body weight	All**	Once per week during the first 8 weeks of the study, and once a month thereafter, and on the day of sacrifice.
Necropsy	Animals found dead or moribund	When found.
	Survivors	At 24 months

*The report stated that observations were made only once each month after the first 5 months.
**For each cage of 5 animals.

3. Necropsy Gross lesions were noted.

a. Tissues examined microscopically

Adrenals	Lymph nodes	Stomach
Bone and marrow	Mammary glands	Testes/ovaries
Brain	Pancreas	Thymus
Colon	Pituitary	Thyroid with parathyroid
Esophagus	Prostate	Trachea
Eyes	Salivary gland	Urinary bladder
Heart	Skin	Uterus
Kidneys	Small intestine	All macroscopic abnormalities
Liver	Spleen	
Lungs		

b. Animals examined: The protocol stated that complete histological examinations were conducted on all animals in the control and high dose groups as well as all animals in any test group that died during the course of the study. When lesions observed in the high dose group were interpreted as chemically ralated, the appropriate tissues from animals in the low dose group were also examined. In this study those organs were the thyroid and parathyroid in low dose males.

C. STATISTICAL ANALYSIS

The probability of survival was determined by a product-limit method. According to the report, animals were censored from this analysis at the time they were found dead of other than natural causes or were missing. The report further stated that those animals dying of natural causes were not censored from the analysis. A method of testing two groups for

B. STATISTICAL ANALYSIS (continued)

equality and a life-table method of testing for a dose-related trend were used to analyze the possible dose-related effects on survival. If an effect was detected the report stated that additional analyses were conducted to detect the time point at which survival differences became significant. Reported p values in these analyses were described as two-sided.

Three statistical procedures were used for analysis of tumor incidence data. The first was the Mantel-Haenszel method, and the second was a pairwise comparison of the high dose group and the low dose group with the control group for significant differences. The third test was one for significant trends. The report described reported p values for these analyses as one-sided.

II. REPORTED RESULTS

A. Mortality and toxic signs:

1. Body weight: There were no apparent effects on body weight in treated male rats (see Table 1 below), and during weeks 58 through 88 the high dose group females had mean body weights that were 5 to 9% less than group means for the control group during that time. After the first three months, group mean body weights were similar in treated and control rats of both sexes. Low and high dose group males had greater weight gains than the control group (3 and 6%, respectively). In the females given the low dose, body weight gains during the first three months were the same as that for the cntrol group (53 g). High dose females showed a weight gain that was approximately 2% less than that for the control group during the first three months.

Table 1

Selected group mean body weights (g) in rats given Rotenone in their diets for two years.*

	Dose (ppm)					
	Males			Females		
Week	0	38	75	0	38	75
0	204	199	197	143	142	146
12	348	347	350	196	195	194
26	412	414	416	223	220	217
54	463	465	459	280	275	270
79	464	473	460	331	318	304
104	432	440	439	343	321	332

*No statistically significant differences were noted in the report.

2. Food consumption: There were no statistically significant differences noted with respect to daily food consumption during the study. The average daily consumption for the control, low, and high dose group males

A. Mortality and toxic signs (continued)

was reported to be 17.9, 18.0, and 18.2 g/day, respectively. The respective values for control, low, and high dose group females were 12.5, 11.8, and 12.0 g/day.

Based on these results and body weight observations, the average daily dose for males and females in the low dose group were 1.7 and 1.8 mg/kg/day, respectively. High dose group males received 3.4 mg/kg/day, and high dose group females received 3.6 mg/kg/day.

3. Mortality: Table 2 summarizes mortality during the in-life phase of the study, and Table 3 shows the results of the survival analysis as reported.

Table 2

Mortality in rats given rotenone in their diets for two years.

Dose (ppm)	Mortalities* during weeks: Males 1-26	27-54	55-79	80-103	Females 1-26	27-54	55-79	80-103
0	0	1	3	24	0	3	4	16
38	0	0	3	16	0	0	3	14
75	0	1	2	17	0	0	3	16

Table 3

Results of the survival analysis (reproduced as reported)

Observation	Dose (ppm): Males 0	38	75	Females 0	38	75
No. animals/ group	50	50	50	50	50	50
No. died during study	28	19	20	23	18	19
No. sacrificed at termination	22	31	30	27	32	31
Survival P values*	0.109	0.076	0.142	0.438	0.367	0.505

*Results of the life table trend test is in the 0 ppm column, and results of pairwise comparisons of each dose group with the control group are in the appropriate column.

B. Necropsy

1. Non-neoplastic observations: The only non-neoplastic lesion found to be significantly increased by rotenone administration was focal hyperplasia in the anterior pituitary glands of high dose male rats. The report stated that the observed incidences of that lesion were 7 of 49 (14%), 2 of 15 (13%), and 13 of 50 (26%) in the control, low, and high dose groups, respectively.

2. Neoplastic observations (continued)

2. Neoplastic observations

a. Males

Table 4 summarizes the results for tumors considered to be related to test substance administration.

Table 4

Summary of the incidences for tumors reported to be associated with administration of rotenone.*

Observation	Dose (ppm) 0	38	75
Parathyroid Adenoma			
Intercurrent deaths	0/23	0/17	2/18
Terminal sacrifice	1/18	0/27	2/26
Overall	1/41	0/44	4/44
Week of first observation	104	---	91

*No statistically significant trends or differences were noted in the report.

The report stated that the adenomas were not observed grossly, and microscopically they appeared as spherical masses with enlarged cells with vesicular nuclei. These tumors also slightly compressed adjacent normal tissue according to the report. Although no statistical significance was associated with the incidence of the tumor, the investigators cited historical control data for the Fisher 344 strain used in this study which indicated that the parathyroid adenoma is uncommon in the test strain. The historical incidence of the tumor was reported to be 4 in 1,314 male F344 rats, and no more than one parathyroid adenoma was observed in any single control group. The reported range was from 0 of 70 to 1 of 38.

b. Females

Table 5 summarizes the statistically significantly increased incidences of subcutaneous tissue tumors which the report noted.

2. Neoplastic observations (continued)

Table 4

Summary of the incidences for tumors reported to be statistically significantly increased in a treatment group.

Observation	Dose (ppm) 0	38	75
Fibromas			
Overall incidence	0/50	1/50	0/50
Neurofibroma			
Overall incidence	0/50	1/50	0/50
Sarcoma			
Overall incidence	0/50	1/50	1/50
Fibrosarcoma			
Overall incidence	0/50	1/50	2/50
Myxosarcoma			
Overall incidence	0/50	1/50	0/50
Combined incidence			
Intercurrent deaths	0/23	3/18	3/19
Terminal sacrifice	0/27	2/32	0/31
Overall	0/50	5/50*	3/50
Week of first observation	---	64	77

*Statistically significantly different from controls (p=0.049 in life table tests; p=0.013 by Fisher Exact Test).

The historical incidence of the tumor was reported to be 50 in 2,021 female F344N rats, and the reported range was from 0 of 70 to 5 of 49.

III. DISCUSSION

A. Conclusions of the Investigators: The report noted no dose-related effects on mortality in the study, and body weight decreases indicated that the doses tested were reasonable.

The incidence of parathyroid tumors was described as follows:

> Adenomas of the parathyroid gland occurred in 1/41 control, 0/44 low dose, and 4/44 high dose male rats. Although not statistically significant, the incidence in the high dose group greatly exceeds the historical incidence in untreated control male rats (4/1,314, 0.3%). The biologic behavior of this proliferative lesion is unknown. Carcinomas of the parathyroid have not occurred in NTP untreated control male F344/N rats, nor does morphologic evidence exist for progression from adenoma to carcinoma. Parathyroid adenoma is distinguished from hyperplasia by its focal nature and compression of adjacent

A. Conclusion of the Investigators (continued):

> normal tissue. Parathyroid hyperplasia is relatively much more common in male rats and generally occurs secondary to severe renal disease (spontaneous progressive nephropathy); whether parathyroid adenoma is related to this process is unknown. The unusually high incidence of parathyroid adenoma in high dose male rats may be related to the administration of rotenone. However, the severity of renal disease in the four high dose male rats was not marked.
>
> ..., focal hyperplasia in the anterior pituitary occurred with an increased incidence in the high dose males (control, 4/49, 8%; low dose, 2/15, 13%; and high dose, 13/50, 26%), but the incidence of tumors of the anterior pituitary gland did not increase.
>
> ...Because of a lack of a significant dose-related trend...and because statistical significance was attained by combining tumors of differing morphology, the evidence for an association between rotenone administration and subcutaneous tissue tumors in female rats is equivocal.

B. Reviewer's Discussion: Reported body weight decreases were not sufficient to indicate toxicity in male or female rats at the highest dose level (see page 4 and Table 1 above), and results from the thirteen-week study (see Addendum below) suggest that the highest dose probably could have been increased. Body weight gain for males given the 75, 150, and 300 ppm diets during the thirteen-week study were decreased by 7.2, 11.5, and 34.7%, respectively, and the respective body weight gains for females at those dose levels were 12.7, 32.3, and 53.9% less than that for the control.

Indep[illegible] analyses of the apparently dose-related increased incidence of hyperplasia in the anterior pituitary gland showed no significant linear trend ($p>0.05$, Cochran-Armitage trend test), and there were no significant difference between the control and high dose group ($p = 0.115$ by the Fisher Exact test).

These results suggest that a Maximum Tolerated Dose (MTD) may not have been achieved in the study.

ADDENDUM

Review of Preliminary Feeding Studies
for the Two-Year Feeding Study of Rotenone
in Fisher 344 Strain Rats

MRID No. 40179801

I. First 14-Day Feeding Study

A. Materials and Methods: Groups containing 5 male and 5 female seven week old F344/N strain rats were given diets containing 0, 50, 100, 200, 400, or 600 ppm test substance for fourteen days.

According to the report, the animals were observed twice daily, and moribund animals were sacrificed. Feed consumption was determined for each cage of five animals, and individual body weights were measured weekly. At the end of the 14-day feeding period, surviving rats were sacrificed and subjected to necropsy. Those animals dying during the study were necropsied when possible.

The tissues examined microscopically were not listed in the report, but the protocol stated that the animals given the highest dose level and those in the control group were subjected to histological examination.

B. Reported Results: The group mean body weights for treated males at the end of the 14-day feeding period were 8 to 13% lower than that for the control group, and the final group mean body weights for the female rats receiving the 200, 400, and 600 ppm diets were 4, 8, and 13% less than that for the control group female rats.

There were no mortalities, clinical signs, macroscopic observations, or microscopic findings that could be associated with treatment.

C. Discussion and Conclusions: Based on the results of the first 14-day study the investigators initiated a second study at higher doses to characterize the potential toxicity of rotenone in rats.

II. Second 14-Day Feeding Study

A. Materials and Methods: Groups containing 5 male and 5 female nine-week old F344/N strain rats were given diets containing 0, 300, 600, 1200, 2400, or 4800 ppm test substance for fourteen days.

According to the report, the animals were observed twice daily, and moribund animals were sacrificed. Feed consumption was determined for each cage of five animals and individual body weights were measured weekly. At the end of the 14-day feeding period, surviving rats were sacrificed and subjected to necropsy. Those animals dying during the study were necropsied when possible.

The tissues examined microscopically were not listed in the report, but the protocol stated that the animals given the highest dose level and those in the control group were subjected to histological examination.

B. Reported Results: Three males in the 2400 ppm dose group, one male in the 4800 ppm dose group, and four female rats in the 2400 ppm dose group died. Body weight and feed consumption were also reduced by treatment (see Table 1 below).

II. B. Reported Results (continued)

Table II-1

Summary of group mean body weights and feed consumption in the second fourteen day feeding study of rotenone in rats.*

Dose (ppm)	Mean Body Weight (g) Week 0	Week 2	Food consumption (g/day) Week 1	Week 2
		Males		
0	130	189	25.2	25.2
300	128	176	21.6	23.4
600	131	167	21.6	23.3
1200	128	108	16.2	16.3
2400	131	79	11.1	11.3
4800	131	90	12.2	11.9
		Females		
0	105	138	18.8	17.3
300	106	134	17.2	18.7
600	102	102	16.7	16.5
1200	105	83	16.0	16.3
2400	103	69	12.0	11.7
4800	105	71	11.8	11.8

*No statistical analysis was reported for these results.

According to the report, all male and female rats given the 1200, 2400, and 4800 ppm diets had rough hair coats. Those rats given the 2400 and 4800 ppm diets also had hard feces and hunched posture. No treatment related gross or microscopic lesions were observed at necropsy.

C. Discussion and Conclusions: The results as presented supported the conclusions of the ivestigators that the 1200 ppm diet should be the highest dose in a thirteen-week feeding study (see Section III. below). The conclusion was based on the absence of compound-related lesions and deaths at the 1200 ppm dose level.

III. Thirteen-Week Feeding Study

A. Materials and Methods: Groups containing 10 male and 10 female four to five week old Fischer 344/N strain rats were given diets containing 0, 75, 150, 300, 600, or 1200 ppm test substance. for thirteen weeks.

According to the report, the animals were observed twice daily, and moribund animals were sacrificed. Feed consumption was determined for each cage of five animals, and individual body weights were measured weekly. At the end of the 13-week feeding period, surviving rats were sacrificed and

III. A. Materials and Methods (continued)

subjected to necropsy. Those animals dying during the study were necropsied when possible.

The following tissues were examined microscopically:

Adrenals	Heart	Ovaries/uterus	Stomach
Brain	Kidneys	Pancreas	Thymus
Colon	Liver	Pituitary	Thyroid with parathyroid
Esophagus	Lungs	Salivary gland	
Eyes	Lymph nodes	Small intestine	Trachea
Femur	Mammary glands	Skin	Urinary bladder
		Spleen	

The report noted that these tissues from the control, 300, 600, and 1200 ppm dose groups were the only ones examined. The bone marrow and stomach of 75 ppm female rats, liver, bone marrow and stomach for 150 ppm male rats, and bone marrow and stomach for 150 ppm female rats were also examined microscopically.

B. Reported Results

1. Mortality, body weight and feed consumption: All 10 of the males and 6 of the 10 females given the 1200 ppm diet died before the end of the 13-week feeding period. In the 600-ppm dose group 3 of 10 males and 4 of 10 females died.

The group mean body weights for the 300 and 600-ppm dose group males were 21 and 52% below that for the control group at the end of the 13-week feeding period, respectively. Female rats given the 150 and 300 ppm doses had group mean body weights that were 16 and 26% less than control group mean body weight at 13 weeks, while the respective weight decreases for the 600 and 1200 ppm dose groups were 54 and 58% less than the control group mean weight (see Table III-1 below).

Reported mean body weight gains for the 75 ppm dose group males and females were approximately 8% and 13% less than appropriate control group mean body weight gains, respectively.

There were no effects noted on feed consumption (see Table III-2 below).

2. Organ weights: Table III-3 shows the results for liver weight data. The report noted that the increases in liver-to-body weight ratios were the result of decreased body weights in treated animals because there were no statistically significant dose-related differences with respect to absolute liver weight.

B. Reported Results (continued)

Table III-1

Group mean body weight (g) for rats given diets containing rotenone for 13 weeks†

Dose (ppm)	Males		Females	
	Week 0	Week 13	Week 0	Week 13
0	122	357	101	203
75	126	344	103	192
150	132	340	101	170
300	128	282	103	150
600	122	170	104	93
1200	133	---*	100	84

†No statistical analyses for their results were reported for these data.
*All animals in the group died before determination.

Table III-2

Group mean feed consumption (g/day) for rats given diets containing rotenone for 13 weeks

Dose (ppm)	Males		Females	
	Week 4	Week 12	Week 4	Week 12
0	19.3	15.8	13.6	11.6
75	17.9	14.7	12.8	10.0
150	17.5	16.7	12.0	11.4
300	17.8	15.6	16.0	9.8
600	16.7	16.3	15.3	15.6
1200	18.5	---*	15.8	11.5

*All animals in the group died before determination.

Reported Results (continued)

Table III-3

Group mean liver weight (mg) and liver-to-body weight ratios (mg/g) for rats given diets containing rotenone for 13 weeks

	Males		Females	
Dose (ppm)	Weight	Ratio	Weight	Ratio
0	13,850	37.9	7,382	36.2
75	15,744†	44.2	8,533††	41.5
150	14,234	39.7	7,262	37.2
300	13,232	45.0†	7,522	42.2
600	8,780††	44.5	7,540	60.2††
1200	---*	---*	6,812	64.0††

*All animals in the group died before determination.
†Statistically significantly different from controls ($p<0.05$)
††Statistically significantly different from controls ($p<0.01$)

Clinical observations: The authors stated that rough hair coats and arched backs were exhibited by 600- and 1200-ppm dose group animals, and males in the 600 ppm dose group also exhibited generalized weakness.

Necropsy findings: Lesions observed to be dose-related included bone marrow atrophy and inflammation and hyperplasia in the forestomach. The incidences of these lesions are summarized in Table III-4 below.

B. Reported Results (continued)

Table III-4

Incidences of dose-related lesions in rats given diets containing rotenone for 13 weeks.

Observation	0	75	150	300	600	1200
			Males			
Bone marrow atrophy	0/10	---	0/10	10/10	10/10	9/10
(severity)*	---	---	---	2.2	2.8	3.9
Forestomach						
Inflammation	0/10	---	0/10	3/10	4/8	---**
(severity)*	---	---	---	2.3	3.8	---**
Hyperplasia	0/10	---	0/10	3/10	4/8	---**
(severity)*	---	---	---	2.3	2.8	---***
			Females			
Bone marrow atrophy	0/10	1/10	6/10	9/10	9/10	9/10
(severity)*	---	1.0	1.0	1.8	3.2	3.8
Forestomach						
Inflammation	0/10	0/10	4/10	7/9	3/7	---**
(severity)*	---	---	2.3	2.3	3.0	---**
Hyperplasia	0/10	0/10	5/10	6/9	4/7	---**
(severity)*	---	---	2.8	2.5	2.0	---***

*Severity is determined according to the following scale: 1=minimal, 2=mild, 3=moderate, 4=severe. The values entered above represent means of the animals examined and having the lesion in each group.

C. Discussion and Conclusions: Based on the 8 to 13% decrease in body weight gain observed in the 75 ppm dose group, mortality in groups given doses of 600 or 1200 ppm, and the incidence of bone marrow atrophy in the 150, 300, and 600 ppm dosed groups, the highest dose selected for the two-year oncogenicity assay was 75 ppm.

The data as presented supported the conclusion of the investigators regarding dose selection for the long-term feeding study.

006537

Reviewed by: Roger Gardner R.G. 11-19-87
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 11/19/87
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity - Mouse (Guideline §83-2)

MRID NUMBER: 401798-01

TEST MATERIAL: Technical grade Rotenone (Lot no. 735-RAP-1502; purity >98%) was used. It was described as a white crystaline powder.

SYNONYMS: 1,2,12,12a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl-[1]benzopyrano-[3,4-b]furo[2,3-h][1]benzopyran-6[6H]-one; CAS No. 83-79-4

STUDY NUMBER(S): NIH Publication No. 86-2576

SPONSOR: National Toxicology Program (Submitted by the U. S. Department of the Interior, Fish and Wildlife Service, National Fisheries Research Laboratory)

TESTING FACILITY: Battelle Columbus Laboratories

TITLE OF REPORT: NTP Technical Report on the Toxicology and Carcinogenesis Studies of Rotenone (CAS No. 83-79-4) in F344/N Rats and B6C3F1 Mice. (Feed Studies)

AUTHOR(S): K. Abdo, Chemical Manager

REPORT ISSUED: August, 1986

CONCLUSIONS: Rotenone was given to male and female B6C3F$_1$ strain mice at dietary concentrations of 0, 600 or 1200 ppm for two years. A dose-related decrease in mortality for treated males was noted, and no significant effect on mortality in treated female mice was observed. Group mean body weights at the end of the study for low and high dose group males were 92 and 87% of that for the control group, respectively. Final group mean body weight for the low dose group females was 83% of the control group value and the high dose group females had a mean body weight that was 76% of the control group at the end of the study.

During the first three months of the study body weight gain for the low dose group males was increased by 5% above the control group weight gain, and that for high dose group males was decreased by 8% in comparison to controls. Weight gains for low and high dose group female mice during the first three months were decreased by 15 and 9% below that of the controls, respectively. These weight changes are of questionable toxicological significance in the absence of histopathological effects, and in the case of female mice the decreased weight gain is not dose-related.

In the absence of other effects, these body weight changes suggest that a Maximum Tolerated Dose (MTD) may not have been achieved.

CONCLUSION (continued)

The report concluded that there was no evidence that, under the conditions of the study, rotenone was carcinogenic in male or female B6C3F$_1$ mice.

Core classification: Supplementary. An MTD may not have been tested.

I. PROTOCOL

A. MATERIALS

1. Test species: Male and female 56-day old B6C3F$_1$ strain mice were used. Breeding stock for these animals originated at the National Institutes of Health Repository, and those animals used in the study were provided by the Frederick Cancer Research Laboratory.

2. Diet preparation: Test diets were prepared biweekly and stored at 4° C. The test substance was added to Purina Lab Chow®, and samples of test diets were analyzed for stability, homogeneity and accuracy of test concentration before the study was begun. Diets were analyzed for concentration of test substance at approximately 8-week intervals during the two year feeding period.

3. Preliminary studies: See the Addendum below for a discussion of the feeding studies conducted to characterize the toxicity of rotenone and determine the highest dose to be tested.

B. STUDY DESIGN

1. Animal assignment: Animals were randomly assigned to test groups as follows:

Test groups		Dose	Animals per sex		
No.	Designation	(ppm)	Main study*	Pre-test	Interim Sacrifice
1	Control	0	50	5	0
2	Low (LDT)	600	50	0	0
3	High (HDT)	1200	50	0	0

*24 months.
**These were examined to determine health status of the animals used in the assay.

B. STUDY DESIGN (continued)

2. Observations schedule

Type of observation	Number of animals per sex per group	Frequency
Mortality	All	Twice a day*
Signs of toxicity	All	Twice a day*
Body weight	All**	Once per week during the first 8 weeks of the study, and once a month thereafter, and on the day of sacrifice.
Necropsy	Animals found dead or moribund	When found.
	Survivors	At 24 months

*The report stated that observations were made only once each month after the first 5 months.
**For each cage of 5 animals.

3. Necropsy Gross lesions were noted.

a. Tissues examined microscopically

Adrenals	Lungs	Stomach
Bone and marrow	Lymph nodes	Testes/ovaries
Brain	Mammary glands	Thymus
Colon	Pancreas	Thyroid with parathyroid
Esophagus	Pituitary	Trachea
Eyes	Prostate	Urinary bladder
Gall bladder	Salivary gland	Uterus
Heart	Skin	All macroscopic abnormalities
Kidneys	Small intestine	
Liver	Spleen	

b. Animals examined: The protocol stated that complete histological examinations were conducted on all animals in the control and high dose groups as well as all animals in any test group that died during the course of the study. When lesions observed in the high dose group were interpreted as chemically ralated, the appropriate tissues from animals in the low dose group were also examined. In this study those organs were the liver and lung in low dose males.

C. STATISTICAL ANALYSIS

The probability of survival was determined by a product-limit method. According to the report, animals were censored from this analysis at the time they were found dead of other than natural causes or were missing. The report further stated that those animals dying of natural causes were not censored from the analysis. A method of testing two groups for

B. STATISTICAL ANALYSIS (continued)

equality and a life-table method of testing for a dose-related trend were used to analyze the possible dose-related effects on survival. If an effect was detected the report stated that additional analyses were conducted to detect the time point at which survival differences became significant. Reported p values in these analyses were described as two-sided.

Three statistical procedures were used for analysis of tumor incidence data. The first was the Mantel-Haenszel method, and the second was a pairwise comparison of the high dose group and the low dose group with the control group for significant differences. The third test was one for significant trends. The report described reported p values for these analyses as one-sided.

II. REPORTED RESULTS

A. Toxic signs and mortality:

1. Body weight: According to the report, group mean body weights for the high dose group males were 5 to 10% lower than those for the control group during weeks 4 through 33 of the study. During weeks 37 through 103 group mean weights for the high dose males were 10 to 19% lower than controls (see Table 1 below). Mean body weight for the low dose group males were also 5 to 13% lower than controls from week 29 to the end of the study.

During weeks 15 through 103 the treated group female mean body weights were 7 to 30% less than group means for the control group (see Table 1).

Table 1

Selected group mean body weights (g) in mice given Rotenone in their diets for two years.*

	Dose (ppm)					
	Males			Females		
Week	0	600	1200	0	38	75
0	23.3	22.8	22.9	17.8	18.0	17.4
12	31.8	31.7	30.7	24.4	23.6	23.4
24	34.9	34.1	32.1	30.0	27.0	27.0
51	40.7	38.0	35.7	36.5	31.8	29.1
77	44.0	38.1	35.8	41.6	34.4	31.0
103	38.9	36.6	34.0	42.6	35.5	32.3

*No statistically significant differences were noted in the report.

A. Toxic signs and mortality (continued)

2. Food consumption: The reported noted that food consumption for low and high dose group male mice was 103 and 106% that of the control group, respectively. Those respective values for low and high dose group female mice were 113 and 115%.

Based on these results and body weight observations, the average daily dose for males and females in the low dose group were 111 and 124 mg/kg/day, respectively. High dose group males received 242 mg/kg/day, and high dose group females received 265 mg/kg/day.

3. Mortality: Table 2 summarizes mortality during the in-life phase of the study, and Table 3 shows the results of the survival analysis as reported.

Table 2

Mortality in mice given rotenone in their diets for two years.
(Derived from Table III-14 of the original report.)

Dose (ppm)	Mortalities* during weeks							
	Males				Females			
	1-24	25-51	52-77	78-103	1-24	25-51	52-77	78-103
0	6	6	2	7	0	2	3	8
600	1	2	3	7	1	0	5	3
1200	0	1	0	2	0	0	1	4

Table 3

Results of the survival analysis
(Reproduced from Table III-15 of the original report.)

Observation	Dose (ppm)					
	Males			Females		
	0	600	1200	0	600	75
No. animals/ group	50	50	50	50	50	50
No. died during study	21	14**	3	12**	7**	5
No. sacrificed at termination	29	36**	47	38	43	45
Survival P values*	<0.001	0.143	<0.001	0.071	0.351	0.093

*Results of the life table trend test is in the 0 ppm column, and results of pairwise comparisons of each dose group with the control group are in the appropriate column.

**This number inidicated one more death during the study than was shown in Table 3 above.

These results suggested that rotenone significantly increases survival of male mice (see Table 3 above).

B. Necropsy

1. Non-neoplastic observations: No non-neoplastic lesions were reported to be significantly increased or decreased by rotenone in the diets of mice after two years.

2. Neoplastic observations

a. Males

Tables 4 and 5 summarize the results for tumors considered to be related to test substance administration.

Table 4

Summary of the incidences of liver tumors reported to be associated with administration of rotenone.*

Observation	Dose (ppm) 0	600	1200
Hepatocellular Adenomas			
Intercurrent deaths	2/13	2/13	0/3
Terminal sacrifice	5/29	7/36	1/47
Overall	7/47†	9/49	1/50††
Week of first observation	89	88	104
Hepatocellular Carcinoma			
Intercurrent deaths	2/13	1/13	0/3
Terminal sacrifice	4/29	2/36	0/47
Overall	6/47*	3/49	0/50**
Week of first observation	76	87	---
Hepatocellular Adenomas and Carcinoma			
Intercurrent deaths	4/13	3/13	0/3
Terminal sacrifice	8/29	9/36	1/47
Overall	12/47†	12/49	1/50††
Week of first observation	76	87	---

†Statistically significant negative trend ($p<0.001$; life table analysis)
††Statistically significantly decreased incidence ($p<0.001$; pairwise comparison, life table analysis)
*Statistically significant negative trend ($p=0.002$; life table analysis)
**Statistically significantly decreased incidence ($p<0.002$; pairwise comparison, life table analysis)

2. Neoplastic observations (continued)

Table 5

Summary of the incidences of subcutaneous tissue tumors reported to be associated with administration of rotenone to male mice.*

Observation	Dose (ppm) 0	600	1200
Subcutaneous Tissue Fibrosarcomas			
Intercurrent deaths	4/20	1/14	0/3
Terminal sacrifice	2/29	1/36	0/47
Overall	6/49	2/50	0/50
Week of first observation	83	88	---
Life Table Tests*	P=0.002	P=0.087	P=0.004
Incidental Tumor Tests*	P=0.008	P=0.086	P=0.017
Cochran-Armitage Trend Test*	P=0.007	---	---
Fisher Exact Test*	---	P=0.128	P=0.012
Subcutaneous Tissue Sarcomas, Fibrosarcomas, or Neurofibrosarcomas			
Intercurrent deaths	5/20	1/14	0/3
Terminal sacrifice	3/29	2/36	2/47
Overall	8/49	3/50	2/50
Week of first observation	72	88	104
Life Table Tests*	P=0.008	P=0.059	P=0.010
Incidental Tumor Tests*	P=0.028	P=0.054	P=0.070
Cochran-Armitage Trend Test*	P=0.023	---	---
Fisher Exact Test*	---	P=0.094	P=0.043
Subcutaneous Tissue Fibroma or Fibrosarcomas			
Intercurrent deaths	4/20	1/14	0/3
Terminal sacrifice	2/29	2/36	0/47
Overall	6/49	3/50	0/50
Week of first observation	83	88	---
Life Table Tests*	P=0.002	P=0.162	P=0.004
Incidental Tumor Tests*	P=0.011	P=0.171	P=0.017
Cochran-Armitage Trend Test*	P=0.019	---	---
Fisher Exact Test*	---	P=0.233	P=0.012
Subcutaneous Fibromas, Sarcomas, Fibrosarcomas, or Neurofibromas			
Intercurrent deaths	5/20	1/14	0/3
Terminal sacrifice	3/29	3/36	2/47
Overall	8/49	4/50	2/50
Week of first observation	72	88	104
Life Table Tests*	P=0.006	P=0.107	P=0.010
Incidental Tumor Tests*	P=0.030	P=0.104	P=0.070
Cochran-Armitage Trend Test*	P=0.027	---	---
Fisher Exact Test*	---	P=0.168	P=0.043

*P values listed under the control group column indicate the significance of trends, and those under dose group columns indicate significance of pairwise comparisons for that dose group and the control group.

2. Neoplastic observations (continued)

b. Females

There were no significant increases or decreases in the incidences of tumors in female mice given rotenone in the diet according to the report.

III. DISCUSSION

A. Conclusions of the Investigators: The report noted a dose-related decrease in mortality of the high dose group males, and no significant effect on mortality in treated female mice. Group mean body weights at the end of the study for low and high dose group males were 92 and 87% of that for the control group, respectively. Final group mean body weight for the low dose group females was 83% of the control group value and the high dose group females had a mean body weight that was 76% of the control group at the end of the study.

The report concluded that there was no evidence under the conditions of the study of carcinogenic activity for rotenone in male or female $B6C3F_1$ mice.

B. Reviewer's Discussion: As indicated in Table 1 (page 4 above), group mean terminal body weights were reduced in the high dose group males by approximately 12.6% and in the high dose group females by about 24.2%. During the first three months of the study body weight gain for the low dose group males was increased by 5% above the control group weight gain, and that for high dose group males was decreased by 8% in comparison to controls. Weight gains for low and high dose group female mice during the first three months were decreased by 15 and 9% below that of the controls, respectively. These weight changes are of questionable toxicological significance in the absence of histopathological effects, and in the case of female mice the decreased weight gain is not dose-related.

In the preliminary thirteen-week feeding study body weight gains for male mice given diets containing 600, 1900, and 5000 ppm were 17.6, 23.5, and 49% less than that for controls. Respective group mean body weights for the 600, 1900, and 5000-ppm dose group males were 8.6, 5.0, and 13.6% below control mean weights at the end of the study. Respective weight gains for female mice given 600, 1900, or 5000-ppm diets in the preliminary study were 19.3, 6.8, and 51.1% less than controls, and respective mean body weights for those animals at the end of the study were 6.8, 5.7, and 22.1% less than that for the control group. Although liver weights and weight ratios were increased in both sexes (see Addendum Table III-3 below), there were no indications of histopathology observed at necropsy.

The results of the preliminary and main experiments suggest that a Maximum Tolerated Dose (MTD) may not have been achieved in the two-year study with mice.

ADDENDUM

Review of Preliminary Feeding Studies
for the Two-Year Feeding Study of Rotenone
in $B6C3F_1$ Strain Mice .

MRID No. 40179801

I. First 14-Day Feeding Study

A. Materials and Methods: Groups containing 5 male and 5 female eight-week old $B6C3F_1$ strain mice were given diets containing 0, 50, 100, 200, 400, or 600 ppm test substance for fourteen days.

According to the report, the animals were observed twice daily, and moribund animals were sacrificed. Feed consumption was determined for each cage of five animals and individual body weights were measured weekly. At the end of the 14-day feeding period, surviving mice were sacrificed and subjected to necropsy. Those animals dying during the study were necropsied when possible.

The tissues examined microscopically were not listed in the report, but the protocol stated that the animals given the highest dose level and those in the control group were subjected to histological examination.

B. Reported Results: According to the report, there were no effects observed on group mean body weights, mortality, clinical signs, macroscopic observations, or microscopic findings that could be associated with treatment.

C. Discussion and Conclusions: Based on the results of the first 14-day study the investigators initiated a second study at higher doses to characterize the potential toxicity of rotenone in mice.

II. Second 14-Day Feeding Study

A. Materials and Methods: Groups containing 5 male and 5 female eight-week old $B6C3F_1$ strain mice were given diets containing 0, 300, 600, 1200, 2400, or 4800 ppm test substance for fourteen days.

According to the report, the animals were observed twice daily, and moribund animals were sacrificed. Feed consumption was determined for each cage of five animals and individual body weights were measured weekly. At the end of the 14-day feeding period, surviving mice were sacrificed and subjected to necropsy. Those animals dying during the study were necropsied when possible.

The tissues examined microscopically were not listed in the report, but the protocol stated that the animals given the highest dose level and those in the control group were subjected to histological examination.

B. Reported Results: According to the report, there were no effects observed on group mean body weights, mortality, clinical signs, macroscopic observations, or microscopic findings that could be associated with treatment.

III. Thirteen-Week Feeding Study

A. Materials and Methods: Groups containing 10 male and 10 female five to six week old $B6C3F_1$ strain mice were given diets containing 0, 600, 1200, 5000, 16,000, or 50,000 ppm test substance for thirteen weeks.

According to the report, the animals were observed twice daily, and moribund animals were sacrificed. Feed consumption was determined for each

III. A. Materials and Methods (continued)

cage of five animals and individual body weights were measured weekly. At the end of the 13-week feeding period, surviving mice were sacrificed and subjected to necropsy. Those animals dying during the study were necropsied when possible.

The following tissues were examined microscopically:

Adrenals	Heart	Ovaries/uterus	Stomach
Brain	Kidneys	Pancreas	Thymus
Colon	Liver	Pituitary	Thyroid with para-thyroid
Esophagus	Lungs	Salivary gland	
Eyes	Lymph nodes	Small intestine	Trachea
Femur	Mammary glands	Skin	Urinary bladder
		Spleen	

The report noted that these tissues from the control, 5000, 16.000, and 50,000 ppm dose groups were the only ones examined. The liver, spleen, and testes of 1900 ppm male mice, and the livers of 1900 ppm female mice were also examined microscopically.

B. Reported Results

1. Mortality, body weight and feed consumption: All 10 of the males and females given the 50,000 ppm diet died before the end of the 13-week feeding period. In the 16,000-ppm dose group 9 of 10 males and 8 of 10 females died.

The group mean body weights for the 5000 and 16,000-ppm dose group males were 14 and 26% below that for the control group at the end of the 13-week feeding period, respectively. Female mice given the 5000 and 16,000 ppm diets had group mean body weights that were 22 and 12% less than control group mean body weight at 13 weeks (see Table III-1).

There were no effects noted on feed consumption (see Table III-2 below).

Table III-1

Group mean body weight (g) for mice given diets containing rotenone for 13 weeks†

	Males		Females	
Dose (ppm)	Week 0	Week 13	Week 0	Week 13
0	23.6	33.8	19.2	28.0
600	22.6	31.0	19.0	26.1
1,900	24.3	32.1	18.2	26.4
5,000	24.0	29.2	17.5	21.8
16,000	24.5	25.0	19.5	24.5
50,000	24.4	---*	19.3	---*

†No statistical analyses or their results were reported for these data.
*All animals in the group died before determination.

B. Reported Results (continued)

Table III-2

Group mean feed consumption (g/day) for mice given diets containing rotenone for 13 weeks

Dose (ppm)	Males		Females	
	Week 4	Week 12	Week 4	Week 12
0	7.3	6.9	6.8	10.4
600	7.3	8.0	7.3	7.7
1,900	7.6	8.1	8.2	7.5
5,000	8.3	10.9	7.2	9.3
16,000	---	---	---	---
50,000	---*	---*	---*	---

*All animals in the group died before determination.

2. Organ weights: Table III-3 shows the results for liver weight data.

Table III-3

Group mean liver weight (mg) and liver-to-body weight ratios (mg/g) for mice given diets containing rotenone for 13 weeks

Dose (ppm)	Males		Females	
	Weight	Ratio	Weight	Ratio
0	1.401	45.6	1,183	45.6
600	1.915††	62.5††	1,410	54.4†
1,900	1.903††	59.2††	1.516††	58.2††
5,000	1.877††	62.8††	1,612††	62.4††
16,000	---*	---*	1,555	51.9

*All animals in the group died before determination.
†Statistically significantly different from controls ($p<0.05$)
††Statistically significantly different from controls ($p<0.01$)

3. Clinical observations: The authors noted no effects.

4. Necropsy findings: No findings were reported.

C. Conclusions: Based on the decrease in body weight gain and mortality, the investigators selected dietary levels of 600 or 1200 ppm for the two-year feeding study.

006537

Reviewed by: Roger Gardner R. G. 11-19-87
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 11/19/87
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Ames Assay (Guideline §84-2)

MRID NUMBER: 40170502

TEST MATERIAL: Rotenone (Lot no. 735-RAP-1502; purity unspecified) was used. It was described as a white crystaline solid.

SYNONYMS: 1,2,12,12a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl-[1]benzopyrano-[3,4-b]furo[2,3-h][1]benzopyran-6[6H]-one; CAS No. 83-79-4

STUDY NUMBER(S): Study No. 019-563-165-1

SPONSOR: U. S. Fish and Wildlife Service

TESTING FACILITY: EG&G Mason Research Institute

TITLE OF REPORT: Salmonella/Mammalian Microsome Plate Incorporation Mutagenesis Assay (Rotenone)

AUTHOR(S): Haworth, S. R.

REPORT ISSUED: November 3, 1978

DISCUSSION AND CONCLUSIONS: There were adequate data presented to support the conclusions of the investigators. They concluded that rotenone did not induce mutations in five strains of Salmonella typhimurium in the presence or absence of metabolic activation. The concentrations tested were from 30 to 10,000 ug/plate, and preliminary studies indicated that concentrations of 48 ug/plate or more precipitated suggesting that several test doses exceeded the limit of solubility for the test substance.

Core classification: Acceptable

I. PROTOCOL

A. Materials

1. Reference mutagens: 1,3-propane sulfone, 2-nitrofluorene, and 2-aminoanthracene were used as positive controls.

2. Vehicle: Dimethyl sulfoxide (DMSO) was used as the vehicle for the test substance.

3. Test species: The bacterial strains used were TA98, TA100, TA1535, TA1537, and TA1538 of Salmonella typhimurium.

4. Bacterial culture media: Top agar for selection of histidine revertants contained 8 g/l agar, and 5 g/l NaCl, and the medium also contained 0.05 mM L-histidine and 0.05 mM biotin. Minimal bottom agar with salts and glucose (Vogel-Bonner Medium E) was used for plating of test strains with and without metabolic activation mixture.

5. Microsomal enzyme (S-9) preparation: Liver microsomal preparations were obtained from Aroclor 1254 induced rats. Each ml sample of the S-9 mix contained the following:

S-9	0.05 ml
0.4M $MgCl_2$	0.02 ml
1.65M KCl	0.02 ml
0.04M NADP	0.10 ml
0.05M glucose-6-phosphate	0.10 ml
1.0M sodium phosphate buffer (pH 7.4)	0.10 ml
Water	0.61 ml

B. Methods

1. Toxicity testing and dose-selection procedures: Concentrations of 2, 5, 15, 48, 153, 489, 1563, 5000, and 10,000 ug test substance per ml medium were tested with and without metabolic activation. The remainder of the preliminary assay protocol is similar to that described below for the main experiment.

2. Mutagenicity assay procedure: The test substance was dissolved in DMSO and added to test plates at concentrations of 0, 30, 100, 330, 1000, 3300, and 10,000 ug/plate. Each dose, vehicle and positive control was tested in triplicate and two separate assays were conducted. For tests without metabolic activation, 50 ul of each tester strain culture and 50 ul test or control solution were added to 2.5 ml top agar. In tests with metabolic activation, 0.5 ml of the activation mixture were added to 2.0 ml top agar. These solutions were overlaid on minimal bottom agar, and the plates were then incubated under unspecified conditions. After incubation the revertant colonies on each plate were counted, and the arithmetic mean of plate counts at each test concentration was calculated. The authors stated that the results were considered to be positive if the colony count at any test concentration was at least double that of the vehicle control, and a dose-related increase in the number of revertants/plate was observed.

II. REPORTED RESULTS

Table 1 summarizes the results of the preliminary toxicity assay with strain TA100.

Table 1

Summary of results for the preliminary toxicity assay with strain TA100

Dose (ug per plate)	Count per plate	Reveratnts per plate
50 ul DMSO	353	103
100 ul DMSO	186	104
2	355	106
5	371	93
15	333	102
48*	317	93
153*	243	95
489*	291	104
1,563*	339	81
5,000**	348	97
10,000**	246	85

*Precipitation of test substance noted.
**Heavy precipitation of test substance noted.

No significant differences were noted at any concentration of the test substance with respect to the number of revertant colonies per plate when compared with that for the vehicle control (see Tables 2 and 3).

Table 2

Summary of revertants per plate for the first trial

Dose (ug per plate	TA98	TA100	TA1535	TA1537	TA1538
		Without activation			
0	21	151	9	8	14
30	17	140	3	8	19
100	16	153	8	8	14
330	15	148	6	9	15
1,000	16	152	1	8	17
3,300	16	130	7	7	10
10,000	8	115	2	5	11
2-Nitrofluoone					
10	1066	---	---	---	1039
1,3-propane sultone					
0.04 ul	--	1100	1005	---	---
9-Aminoacridine					
75	--	---	---	722	---
		With activation			
0	24	119	12	6	24
30	28	131	9	7	21
100	30	120	12	9	23
330	24	135	11	10	23
1,000	26	119	10	8	25
3,300	24	125	9	7	17
10,000	23	122	9	5	17
2-Aminoanthracene					
1.0	1480	2517	---	---	---

Table 3

Summary of revertants per plate for the second trial

Dose (ug per plate	TA98	TA100	TA1535	TA1537	TA1538
		Without activation			
0	19	109	23	6	15
30	11	121	20	5	15
100	19	116	18	6	13
330	21	116	20	7	17
1,000	21	129	21	6	16
3,300	18	107	21	5	17
10,000	16	107	13	6	15
2-Nitrofluoone					
10	1351	---	---	---	---
10	---	---	---	---	931
1,3-propane sultone					
0.04 ul	--	1247	1349	---	---
9-Aminoacridine					
75	--	---	---	1543	---
		With activation			
0	23	106	11	8	22
30	22	99	11	8	21
100	22	93	11	6	27
330	21	111	13	6	25
1,000	25	106	9	6	20
3,300	23	103	8	7	27
10,000	13	83	7	4	16
2-Aminoanthracene					
1.0	1509	1300	---	---	---
1.0	—	3793	---	---	---

Reviewed by: Roger Gardner R.G. 11-19-87
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D.
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Ames assays (Guideline §84-2)

MRID NUMBER: 40170506

TEST MATERIAL: Technical grade Rotenone (Lot no. 735-RAP-1502) purity >98%) was used. It was described as a white crystaline powder.

SYNONYMS: 1,2,12,12a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl-[1]benzopyrano-[3,4-b]furo[2,3-h][1]benzopyran-6[6H]-one; CAS No. 83-79-4

STUDY NUMBER(S):

SPONSOR: National Toxicology Program (Submitted by the U. S. Department of the Interior, Fish and Wildlife Service, National Fisheries Research Laboratory)

TESTING FACILITY: SRI International

TITLE OF REPORT: The Salmonella/Microsome Mutagenicity Test System (Rotenone

AUTHOR(S): Anon.

REPORT ISSUED: Undated

CONCLUSIONS: Rotenone was not mutagenic in Salmonella typhimurium at concentrations of 100 to 10,000 ug/plate with or without metabolic activation by rat or hamster liver microsomal enzymes.

Core classification: Acceptable

I. PROTOCOL

A. Materials

1. Reference mutagens: 2-Aminoanthracene, 9-aminoacridine, sodium azide, and 4-nitro-o-phenylenedidiamine were used as positive controls.

2. Vehicle: Ethanol (95%) was used as the vehicle for the test substance.

3. Test species: The bacterial strains used were TA98, TA100, TA1535, and TA1537 of Salmonella typhimurium were used in the assays.

4. Bacterial culture media: Media used in the assay were not described in detail.

5. Microsomal enzyme (S-9) preparation: Liver microsomal preparations were obtained from Aroclor 1254-induced male Sprague-Dawley rats or Syrian hamsters. No further details were included in the report.

B. Methods

1. Toxicity testing and dose-selection procedures: The report stated that selection of the high dose was limited by toxicity or solubility of the test substance, but it was not to exceed 10.0 mg/plate. No indication of a toxicity study or determination of rotenone's solubility was included in the report. The doses selected for the assay were 100, 333, 1000, 3333, and 10,000 ug/plate. A solvent control and positive control were also tested.

2. Mutagenicity assay procedure: The test substance was dissolved in DMSO, and each dose, vehicle, and positive control was tested in triplicate. The report stated that the test chemical was incubated with the test strain in rat or hamster liver microsomal fraction plus cofactor mix or a buffer for 20 minutes at 37°C. Then top agar was added to these solutions, and the resulting mixtures were overlaid on minimal bottom agar. No further information regarding subsequent procedures was included in the report.

   The protocol also stated that the entire assay or those portions which resulted in a positive response were repeated at least one week after the first assay was conducted.

   The revertant colonies on each plate were counted, and the arithmetic mean of plate counts at each test concentration was calculated. The authors stated that the results were considered to be positive if a reproducible dose response was observed in the number of revertants per plate. Results were to be described as equivocal if either a non-dose related increase or a non-reproducible response was observed.

II. REPORTED RESULTS

No significant differences were noted at any concentration of the test substance with respect to the number of revertant colonies per plate when compared with that for the vehicle control (see Addndum below). According to the report the two highest concentrations of the test substance (3333 and 10,000 ug/ml) formed a precipitate.

III. DISCUSSION

Although individual plate counts for each series of assays were not included in the report, there are adequate data presented to support the conclusions of the investigators.

ADDENDUM

Results of a Salmonella Mutation Assay
with Rotenone

(Reproduced from MRID No. 40170506)

CELLULAR AND GENETIC TOXICOLOGY BRANCH, NTP
SALMONELLA TESTING RESULTS

CAS #:
ALIQUOT: 687373 LAB: SRI INTERNATIONAL ← (Chemical Abstract Registry Number and Chemical Name)
MUTAGENICITY CONCLUSION: - ← (Overall Program Call) ← ('In-House' Code Number and Testing Laboratory)

(Strain) → TA100 SOLVENT: DMSO PROTOCOL: PREINCUBATION

(Trial Number) →

DOSE	1 NA (-) MEAN	SE	2 NA (-) MEAN	SE	1 10% HLI (-) MEAN	SE	2 10% HLI (-) MEAN	SE	1 10% RLI (-) MEAN	SE	2 10% RLI (-) MEAN	SE
ug/PLATE												
0.000	70	5.4	92	5.9	85	7.5	91	6.0	100	4.4	75	7.4
100.000	119	3.6	92	6.7	94	2.1	95	6.8	111	6.9	108	13.4
333.300	125	6.6	103	11.0	86	12.1	94	4.7	109	6.6	104	4.5
1000.000	106	10.1	84	3.3	89	7.1	89	7.8	100	2.2	94	5.9
3333.300	116	8.0	89	6.2	91	6.4	92	8.2	100	5.5	94	5.0
10000.000	114	1.2	94	3.3	85	7.8	85	7.2	97	3.1	96	5.4
POS	318	21.4	394	5.7	428	22.9	1492	34.2	234	10.5	684	44.0

TA1535 SOLVENT: DMSO PROTOCOL: PREINCUBATION

(Activation System) → 1 NA (-) 2 NA (-) 1 10% HLI (-) 2 10% HLI (-) 1 10% RLI (-) 2 10% RLI (-)

DOSE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
ug/PLATE												
0.000	19	2.3	8	0.6	5	0.6	6	0.7	12	1.8	7	0.9
100.000	24	3.8	10	2.1	11	3.2	9	2.3	13	1.8	7	1.0
333.300	20	5.2	7	0.9	10	0.3	11	2.6	12	2.1	7	1.5
1000.000	21	3.5	9	2.3	11	1.7	8	3.2	9	1.3	8	0.0
3333.300	24	2.6	8	2.8	9	1.7	8	0.9	12	2.1	7	1.0
10000.000	24	0.0	8	1.3	7	0.7	4	0.7	12	3.2	10	2.7
POS	215	8.2	244	5.8	293	11.9	274	6.9	155	7.6	139	7.5

→ NA = No Activation
+ = positive; - = negative, +w = weak positive, ? = equivocal HLI = Hamster Liver Induced RLI = Rat Liver Induced

137 SOLVENT: ET95 PROTOCOL: PREINCUBATION

SE	1 NA (-)		2 NA (-)		1 10% HLI (-)		2 10% HLI (-)		1 10% RLI (-)		2 10% RLI (-)	
LATE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
.000	9	2.3	10	1.9	6	1.8	8	2.1	3	1.2	10	2.3
.000	10	1.8	5	1.8	5	1.7	7	1.2	10	1.8	11	1.5
.000	5	0.3	10	1.2	8	1.9	5	1.5	11	2.7	9	2.0
.000	9	3.5	7p	1.5	13	1.5	4	3.0	14	0.9	6	3.3
.000	6n	0.9	4n	0.6	6n	2.0	7n	1.0	5p	0.9	6p	3.7
.000	9p	1.8	8p	2.0	7p	0.7	7p	1.0	9p	2.7	6p	1.0
	226	29.6	667	33.4	546	9.3	537	13.5	184	20.9	178	5.8

SOLVENT: ET95 PROTOCOL: PREINCUBATION

E	1 NA (-)		2 NA (-)		1 10% HLI (-)		2 10% HLI (-)		1 10% RLI (-)		2 10% RLI (-)	
AT_	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
000	28	1.2	28	1.5	40	1.8	41	4.3	37	4.1	38	3.8
000	23	3.5	24	5.4	34	3.5	34	3.7	37	7.2	38	3.5
000	17	3.3	25	4.1	36	2.4	31	3.7	47	5.2	31	1.3
000	19	0.9	35p	2.5	38	7.9	29	1.0	39	7.6	27	3.8
000	20p	4.6	24p	2.0	40p	0.6	29p	2.3	29p	3.5	35p	3.1
000	20n	1.2	15n	2.5	40n	3.4	33n	1.7	32n	6.2	32p	3.2
	493	35.3	952	8.2	1211	47.4	1738	38.5	456	22.4	560	23.4

TOUCT

CELLULAR AND GENETIC TOXICOLOGY BRANCH, NTP
SALMONELLA TESTING RESULTS

S #: A3-79-4 ROTENONE
EQUOT: 535953 LAB: SRI INTERNATIONAL
MUTAGENICITY CONCLUSION: -

TA100 SOLVENT: ET95 PROTOCOL: PREINCUBATION

DOSE	1 NA (-)		2 NA (-)		1 10% HLI (-)		2 10% HLI (-)		1 10% RLI (-)		2 10% RLI (-)	
MG/PLATE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
0.000	145	13.5	159	7.5	179	10.1	134	1.9	165	9.4	127	5.7
100.000	139	1.5	116	9.7	175	1.3	103	1.7	140	13.0	120	5.2
333.000	134	17.0	122	2.8	156	1.5	101	3.8	149	9.4	123	4.1
1000.000	146	17.8	110p	15.8	181	11.1	114	13.6	135	9.2	128	2.3
3333.000	124p	9.2	118s	5.7	169p	5.0	115p	3.3	157p	11.1	131p	5.3
10000.000	119p	5.2	124s	5.9	150p	12.4	122p	1.2	160p	16.8	130p	5.8
POS	301	3.1	416	15.2	1405	54.5	1781	11.9	553	26.8	712	22.3

TA1535 SOLVENT: ET95 PROTOCOL: PREINCUBATION

DOSE	1 NA (-)		2 NA (-)		1 10% HLI (-)		2 10% HLI (-)		1 10% RLI (-)		2 10% RLI (-)	
MG/PLATE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
0.000	25	2.9	41	2.1	35	3.3	31	2.9	34	2.3	41	1.8
100.000	22	2.5	34	1.3	27	1.7	33	3.4	45	3.8	29	1.3
333.000	25	3.8	34	2.9	24	2.7	31	3.1	27	5.5	35	1.5
1000.000	29	7.5	27p	1.5	27	1.3	31	3.6	38	3.2	26	2.6
3333.000	28p	1.7	29p	4.3	23p	5.2	33p	4.9	32s	3.3	23p	3.3
10000.000	25p	2.6	25p	1.7	19p	0.3	25p	1.3	32s	3.7	19p	2.1
POS	299	4.3	536	4.4	361	14.7	486	4.3	167	7.9	273	5.9

Reviewed by: Roger Gardner R.G. 11-4-87
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W. Hauswirth 11/19/87
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Mouse Lymphoma cells (Guideline §[illegible]-2)

MRID NUMBER: 40170505

TEST MATERIAL: Technical grade Rotenone (Lot no. 735-RAP-1502) purity >98%) was used. It was described as a white crystaline powder.

SYNONYMS: 1,2,12,12a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl-[1]benzopyrano-[3,4-b]furo[2,3-h][1]benzopyran-6[6H]-one; CAS No. 83-79-4

STUDY NUMBER(S):

SPONSOR: National Toxicology Program (Submitted by the U. S. Department of the Interior, Fish and Wildlife Service, National Fisheries Research Laboratory)

TESTING FACILITY: IRI

TITLE OF REPORT: Mouse Lymphoma Protocol (Rotenone)

AUTHOR(S):

REPORT ISSUED: November 27, 1984

CONCLUSION: Concentrations of 0.25 to 4.0 ug rotenone per ml of medium without metabolic activation increased the frequency of forward mutations at the Tk locus of L5178Y mouse lymphoma cells under the test conditions.

Core classification: Acceptable

I. PROTOCOL

A. Materials

1. Reference mutagens: Methylmethane sulfonate (MMS) was used as positive control substances.

2. Vehicle: Acetone was used as the vehicle for the test substance.

3. Test species: L5178Y mouse lymphoma cells ($Tk^{+/-}$) were used.

4. Cell culture conditions: Cultures were grown in unspecified medium. Cloning and selective media were also unspecified, and the report indicated that cultures were incubated at 37°C in 5% CO_2.

5. Microsomal enzyme (S-9) preparation: Aroclor 1254-induced S9 from an unspecified source was used when the assays without metabolic activation resulted in a negative response according to the report. No further details were provided.

B. Methods

1. Experimental procedures---Preliminary toxicity assay: The report stated that the highest dose tested was determined by solubility or toxicity, and was not to exceed 5 mg/ml. No further details were included in the report.

2. Experimental procedure---Main study: Cultures (6×10^6 cells) were incubated with test substance for 4 hours. The five concentrations used in the assay without metabolic activation were 0.5, 1, 2, 4, and 8 mg/ml in the first trial and 0.125, 0.5, 1, 2, and 4 in the second trial. The cells were then washed and resuspended in medium for 2 days to allow expression of mutants. Cultures were kept in the log phase of growth during the expression period.

At the end of the 2-day expression period, samples of each culture were plated to determine cloning efficiency (600 cells) and mutant counts (3×10^6 cells). The report stated that cells plated for counting of mutants were treated with trifluorothymidine. All plates were incubated for 10 to 12 days before they were counted.

The criteria for a positive result included: a statistically significant increase ($p<0.05$) in the mutation frequency at any of the 3 highest concentration in comparison to the solvent control value and a significant positive trend ($p<0.05$). In addition, a positive response must be confirmed with a second assay. The results are considered questionable if there is significance for only one of the two statistical tests. Results are considered negative if there is no significance for either statistical test in assays with and without metabolic activation.

II. REPORTED RESULTS

The results are summarized in Table 1 below. According to the protocol, there were no assays conducted with metabolic activation because those without the microsomal fraction indicated a positive response.

III. DISCUSSION

The report was limited with respect to descriptions of the methods used in the assay. The details of the protocol that were included in the report are described in Section I. above. The report also cited Myhr et al. 1985, as the basis of the procedures used.

There were adequate data presented to indicate that rotenone induced forward mutations in mouse L5178Y lymphoma cells in vitro at concentrations ranging from 0.25 to 4 ug/ml without metabolic activation.

Table 1

Summary of reported results from the mouse lymphoma assay with rotenone.

Compound	Concentration (ug/ml)	Cloning efficiency (%)	Relative total growth (%)	Mutation frequency (per 10^6 cells)
		First trial		
Acetone	---	77.7	100.0	59
Rotenone	0.5	60.0	21.0	208*
	1.0	55.5	14.5	322*
	2.0	40.0	6.5	1,310*
	4.0	15.0	2.5	3,142*
	8.0	Lethal	--	---
MMS	15.0	38.0	31.5	236*
		Second trial		
Acetone	---	77.8	100.0	71
Rotenone	0.25	64.0	25.0	116*
	0.5	58.0	32.5	119*
	1.0	65.0	20.0	238*
	2.0	54.0	17.0	268*
	4.0	Lethal	--	---
MMS	15.0	35.0	28.0	191*

*Statistically significantly increased ($p<0.05$) when compared with the control group mutation frequency (Statistical test was not specified in the report).

IV. REFERENCE

Myhr, B.; Bowers, L.; Caspary, W. (1985) Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture. Prog. Mutat. Res. 5:555-568.

Reviewed by: Roger Gardner R. G. 12-9-87
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith W Hauswirth 12/10/87
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Micronucleus Test (Guideline §84-2)

MRID NUMBER: 401705-01

TEST MATERIAL: Technical grade Rotenone (Lot no. IN#-772-262; purity 41.59%) was used.

SYNONYMS: 1,2,12,12a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl-[1]benzopyrano-[3,4-b]furo[2,3-h][1]benzopyran-6[6H]-one; CAS No. 83-79-4

STUDY NUMBER(S): NIH Publication 86-2576

SPONSOR: National Toxicology Program

TESTING FACILITY: SRI International

TITLE OF REPORT: In Vitro and In Vivo Mutagenicity Studies of Environmental Chemicals: Micronucleus Test (includes Rotenone)

AUTHOR(S): D. C. L. Jones, V. F. Simmon, K. E. Mortelmans, A. D. Mitchel, E. L. Evans, M. M. Jotz, E. S. Riccio, D. E. Robinson, and B. A. Kirkhart

REPORT ISSUED: September, 1984

CONCLUSIONS: Groups of male mice were given two consecutive daily doses of 0, 0.56, 1.13, or 2.75 mg rotenone per kg body weight by oral intubation in DMSO. The investigators concluded that rotenone did not induce micronuclei under the test conditions, but there were inadequate data to support the conclusion. There was no information included on preliminary studies to select doses used, and the reported weight losses were similar for all groups except the positive control group. There was also insufficient information on the composition of the impurities that made up approximately 60% of the test substance.

Core classification: Unacceptable

I. PROTOCOL

A. Materials

1. Test species: Male Swiss-Webster strain mice were used. They weighed from 20 to 30 g.

2. Positive control substance: Trimethyl phosphate was used as the positive control.

A. Materials (continued)

3. Preliminary considerations The investigators stated that dose selection was based on the acute oral LD_{50} for mice. No description of an acute oral LD_{50} study in mice was included in the report, but the authors stated that the doses were selected at 20, 40, and 80% of that value.

B. Methods

1. Experimental procedure: Groups containing 24 male mice were given two consecutive daily doses of 0.56, 1.13, or 2.75 mg/kg by oral intubation, and a group of 24 animals was given the DMSO vehicle without test substance. Another group containing 8 mice was given two consecutive daily doses of 1250 mg/kg TMP by intraperitoneal injection. The animals were weighed and observed daily for toxic signs during the study.

Subgroups of 8 animals of each treated group and vehicle control group were subsequently sacrificed 30, 48, and 72 hours after administration of the last dose. The 8 male mice in the positive control group were sacrificed 24 hours after the last treatment.

Two blood smears were made for each animal by mixing a drop of cardiac blood with a drop of fetal calf serum, and three bone marrow smears were made for each mouse. The report stated that the slides were stained with buffered Giemsa stain and air dried. They were then covered with coverslips for scoring. No other details of these procedures were included in the report.

Slides were scored by examining 500 polychromatophilic erythrocytes (PCE) and noting the micronucleated cells. The number of mature erythrocytes was also counted while examining the first 200 PCE's, and a ratio of mature erythrocytes to PCE's was calculated.

2. Analysis of data: The procedures used to analyze the results were described as follows:

> The method of Mackey and MacGregor (41, reference not included in the report) is used to determine whether a dose group is positive or negative in the micronucleus test. This method is based on the negative binomial distribution and uses a decision table constructed using the following parameters:

Parameter	Definition	Value in this study
$\bar{x}_1$	The mean number of micronucleated cells per 500 PCE's scored in the historical control animals.	0.9
$\bar{x}_2$	The true mean number of micronucleated cells per 1000 PCE's needed to declare a compound mutagenic.	3.0
k	The negative binomial constant, estimated from historical control data using a FORTRAN program supplied by Bruce Mackey.	6.9

2. Analysis of data (continued):

Parameter	Definition	Value in this study
alpha	The chance of declaring mutagenic a group whose true mean equals x_1 (false positive). The alpha error decreases as the true mean for a group increases.	(from table)
beta	The chance of declaring mutagenic a group whose true mean equals x_2 (false negative). The actual beta error decreases as the true mean for any particular group decreases.	(from table)

Using these values in the formulae of Mackey and MacGregor, the following decision table has been calculated:

No. of animals	Negative decision beta < 0.001	0.005	0.01	0.05	0.10	No decision alpha & beta >0.10	0.10	Positive decision alpha < 0.05	0.01	0.005	0.001
5	<1	2,3	---	4,5	6	7-10	11	12,13	--	14,15	16
6	<3	4	5	---	7	8-12	13	14	15	16	18
7	<4	6	7	8	9	10-14	--	15	17	18	20
8	<6	7,8	---	10	11	12-15	16	17,18	19	20	21

To use the table, the row for the number of animals in the group is read across until the value for the total number of micronucleated cells found in the group is located. The decision and level of significance of the decision are indicated at the top of the column in which the total is located.

The data generated are considered unacceptable if the positive or the negative control group does not fall within its range in the decision table. If either control group is unacceptable, the slides are recoded and evaluated by another observer. If the results remain outside the acceptable range for the group, the study is repeated.

According to the report, a compound is considered mutagenic when two of the dose-time observations fall within the positive range shown in the above decision table.

A test substance is characterized as negative if the highest dose is a maximum tolerated dose for the test animal, and the number of micronuclei observed falls into the negative range indicated in the decision table. The maximum tolerated dose was defined in the report as the dose that causes significant weight loss in treated animals or some animals die before they are scheduled for sacrifice.

Results are classified as inconclusive when the number of micronuclei in all groups falls within the "no decision" range shown in the table above.

2. Analysis of data (continued):

Results can also be called inconclusive if extreme bone marrow depression is indicated (mean ratio of PCE's to red blood cells is less than 0.1 to 0.15) in all dose groups in a negative study.

II. REPORTED RESULTS

The animals in each test group (including the vehicle control group) lost weight during the study. The vehicle and positive control groups lost 1.2 and 2.4 g, respectively. Reported weight losses for the low, mid, and high dose groups were 1.3, 1.0, and 1.0 g, respectively. No deaths were reported.

Rotenone was described as negative at all doses with a beta error less than 0.005 (see Addendum for results). The PCE to mature erythrocyte ratios were characterized as within normal ranges (1.0 to 1.5) with the exception of the low dose 96-hour group. The investigators attributed the increase in the PCE to RBC ratio for that group to one of the eight animals in the group (see Addendum).

III. DISCUSSION

The investigators concluded that rotenone met all the criteria for classification as non-mutagenic under the conditions of the test.

There were inadequate data to support the conclusions of the investigators since there was no information included on preliminary studies to select doses used, and since the reported weight losses were similar for all groups except the positive control group. There was also insufficient information on the composition of the impurities that made up approximately 60% of the test substance.

Based on these considerations the assay as reported is unacceptable.

IV. REFERENCES

References were not included with the report. Only the citations were included.

ADDENDUM

Reported Results of a Micronucleus
Test in Male Mice with Rotenone

MRID No. 40170501

Table 294

INCIDENCE OF MICRONUCLEATED POLYCHROMATOPHILIC ERYTHROCYTES*

(500 PCE scored per animal)

Group	48 Hours Animal No.	PCE with MN	PCE/RBC	72 Hours Animal No.	PCE with MN	PCE/RBC	96 Hours Animal No.	PCE with MN	PCE/RBC
DMSO 2 x 5 ml/kg Vehicle Control	101♂	1	1.30	103♂	1	1.98	102♂	0	0.70
	101r	0	1.49	103r	1	1.83	102r	1	1.00
	101ℓ	2	1.09	103ℓ	0	1.61	102ℓ	0	1.09
	101b	0	0.88	103b	2	0.59	102b	0	0.81
	104♂	1	0.81	106r	1	1.28	105♂	1	0.98
	104r	1	0.75	106ℓ	1	1.47	105r	1	1.56
	104ℓ	1	0.92	106b	1	1.23	105ℓ	1	1.24
	104b	0	1.47				105b	0	1.67
Total		6†			7§			4†	
Mean			1.09			1.43			1.13
Rotenone 2 x 0.56 mg/kg	112♂	0	1.44	111♂	0	0.93	110♂	0	1.45
	112r	1	1.79	111r	0	1.08	110r	1	1.62
	112ℓ	0	0.73	111ℓ	0	1.26	110ℓ	1	1.74
	115♂	0	1.17	111b	0	1.45	110b	0	0.93
	115r	0	1.18	114♂	0	1.01	113♂	0	2.94
	115ℓ	0	1.18	114r	0	1.76	113r	1	1.77
	115b	0	1.09	114ℓ	0	0.92	113ℓ	0	1.28
				114b	1	0.73	113b	0	1.44
Total		1†			1†			3†	
Mean			1.23			1.14			1.65
Rotenone 2 x 1.13 mg/kg	117♂	0	1.14	116♂	1	0.98	118♂	0	1.26
	117r	0	1.09	116r	0	1.26	118r	0	0.87
	117ℓ	0	1.05	116ℓ	1	1.30	118ℓ	1	1.58
	117b	1	0.66	116b	1	1.27	118b	0	0.74
	119♂	0	0.92	120♂	0	0.10	121♂	1	0.84
	119r	0	1.80	120r	0	0.81	121r	1	2.27
	119ℓ	1	0.99	120ℓ	0	2.51	121ℓ	1	1.13
	119b	0	1.37	120b	1	0.60	121b	2	0.90
Total		2†			4†			6†	
Mean			1.13			1.10			1.20

* PCE = Polychromatophilic erythrocytes, RBC = mature erythrocytes, MN = micronucleus.
† ≤ 0.001.
§ ≤ 0.01.

Table 294 (Concluded)

Group	48 Hours Animal No.	PCE with MN	PCE RBC	72 Hours Animal No.	PCE with MN	PCE RBC	96 Hours Animal No.	PCE with MN	PCE RBC
Rotenone	125ø	0	1.40	122ø	0	1.35	124ø	0	1.00
2 x 2.25 g/kg	125r	1	1.28	122r	0	1.63	124	1	0.64
	125ℓ	1	1.90	122ℓ	0	1.26	124b	1	1.59
	125b	0	1.18	122b	1	1.08	126ø	0	1.64
	127ø	0	1.40	123ø	0	0.91	126r	2	1.59
	127r	2	1.34	123r	2	1.41	126ℓ	1	1.20
	127ℓ	1	1.30	123ℓ	0	1.11	126b	0	0.83
	127b	2	1.16	123b	0	0.78			
Total		7†			3†			5†	
Mean			1.37			1.19			1.21
TMP	107ø	4	0.92						
2 x 1 g/kg	107r	18	0.62						
Positive	107ℓ	2	0.92						
Control	107b	5	1.51						
	108ø	3	0.71						
	108r	4	1.32						
	108ℓ	0	1.01						
	108b	2	0.90						
Total		38**							
Mean			0.99						

† ≤ 0.001.
* ≤ 0.005.
** ≤ 0.001.

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Reviewed by: Roger Gardner R.G. 12-9-87
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Judith D Hauswirth 12/10/87
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Unscheduled DNA Synthesis (Guideline §84-2)

MRID NUMBER: 401705-03

TEST MATERIAL: Rotenone (Lot no. unspecified; purity unspecified) was used.

SYNONYMS: 1,2,12,12a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl-[1]benzopyrano-[3,4-b]furo[2,3-h][1]benzopyran-6[6H]-one; CAS No. 83-79-4

STUDY NUMBER(S): Mutation Research 42:161-174

SPONSOR:

TESTING FACILITY: Ohio State University, Columbus, OH

TITLE OF REPORT: Pesticide Induced DNA Damage and Its Repair in Cultured Human Cells (Includes Rotenone)

AUTHOR(S): Ahmed, F. E.; Hart, R. W.; Lewis, N. J.

REPORT ISSUED: July, 20, 1976

DISCUSSION AND CONCLUSIONS: Concentrations of 0, 1, 100, or 1000 ug rotenone per ml medium with or without metabolic activation by liver microsomal fractions did not increase unscheduled DNA synthesis in human fibroblasts. Because there were no results presented in readable form (see Addendum below), no preliminary toxicity or solubility studies for rotenone in the test system, no positive control substances used, and the report was often illegible, the study is unacceptable.

Core classification: Unacceptable

I. PROTOCOL

A. Materials

1. Reference mutagens: None were used.

2. Vehicle: Acetone was used as the vehicle for the test substance.

3. MATERIALS AND METHODS

4. Test species: SV 40 transformed human fibroblast line VA-1 cells were used.

B. Materials (continued)

5. Cell culture conditions: Cultures were grown in Minimum Essential Medium (MEM) supplemented with 5% fetal bovine serum and antibiotics (streptomycin, penicillin, and fungizone). They were incubated in a humidified 5% CO_2 atmosphere. Cultures were grown on 11 X 22 mm coverslips contained in 100 mm diameter Petri dishes. These coverslips were seeded with cells (amount was not legible) 24 hours before the assays were begun. In order to inhibit unscheduled DNA synthesis, hydroxyurea was added to a concentration of 2 mM five hours prior to addition of the test substance.

6. Metabolic activation (S-9): Microsomal preparations were obtained from livers perfused with cold KCl and 0.05 M Tris buffer (pH 7.4). The livers were excised from uninduced rats, and the tissue was homogenated and centrifuged at 9000 X g and 4°C for 20 minutes. The supernatant was separated and centrifuged again under the same conditions for 15 minutes. To each volume of the S-9 was added an equal volume of a cofactor solution containing NAD (0.1 mM), NADP (0.4 mM), glucose-6-phosphate (5 mM), and $MgCl_2$ (25 uM).

B. Methods

1. Experimental procedures---Preliminary toxicity assay: Concentrations of 0, 1, 10, and 1000 ug/ml were evaluated in the assay. Medium was removed from prepared cultures (see Section A. 5. above) and replaced with fresh medium containing the test substance, ^{3}H-thymidine (specific activity of 5 Ci/mM; at a concentration of 2 uCi/ml), and hydroxyurea at a concentration of 2 mM. The S-9 mix was also added to cultures in which metabolic activation of the test substance was required.

   At intervals (0.5, 3, 5, and 8 hours), coverslips were removed from the test medium. The cultures were fixed, mounted, and coated with an emulsion for radiography; details of these procedures were not legible in the report. The prepared slides were stored in the dark for 4 days before they were developed and scored for the number of granules per nucleus (corrected for background). One to fifty cells were counted according to the report.

2. Analysis of results: A t test was used to determine the significance of the differences between mean grain counts for each concentration of test substance at each time interval and the vehicle control group values. The report noted that the level of significance was 95%.

II. REPORTED RESULTS

According to the report, there was no induction of unscheduled DNA synthesis by rotenone with or without metabolic activation. Although the report indicated that grain counts for cultures exposed to rotenone without metabolic activation for 8 hours were reported, none were readable (see Addendum below).

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ADDENDUM

Results as Reported for an
Unscheduled DNA Synthesis Assay
with Rotenone in Human Fibroblasts

006537

TABLE I

THE INDUCTION OF UNSCHEDULED DNA SYNTHESIS IN HUMAN FIBROBLAST CULTURES (VA-4) BY VARIOUS PESTICIDES

Results shown represent those obtained following an 8 h exposure to the chemical agents, days of exposure to Ilford-4 photographic emulsion at 4°C and the dark silver grains over the nucleus in 30 cells per cover slip counted and corrected for background. Results were analyzed by subprogram *t*-test that compared the mean number of grains in controls and cells treated with pesticides at 95% confidence level.

Compound	Concentration (μM)	Unscheduled DNA synthesis [a]	
		Non-activated	Activated
Lindane	1000	[c]	
	1		
Rotenone	1000		
	10		
	1		
Chlordane	1000	[b]	
	100	•	
	10	•	
	1	•	
Heptachlor	1000		
	100		•
Heptachlor-epoxy	1000		
	100		•
	10		•
DDT	1000		
	100	-	
	10		
Aldrin	1000	•	•
	100	•	•
	10	•	•
	1	•	•
Dieldrin	100	•	•
	10	•	•
	1	•	•
Carbaryl	1000	•	•
	100	•	•
	10	•	
	1	•	-
Diquat	1000	•	•
	100	•	•
	10	•	•
	1	•	•
2,4-D fluid	1000		
	100		
	10	•	•
	1	•	•
Dimethoate	1000	-	•
	100	-	•
	10	-	-
Captan	1000	•	•
	100	•	•
	10	•	•
	1	•	•

[a] Measured as grains per nucleus.
[b] Indicates a significant difference from the control at $P < 0.05$.
[c] Indicates no significant difference from the control.

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Cell Transformation (Guideline §84-2)

MRID NUMBER: 401705-04

TEST MATERIAL: Commercially available (source unspecified) technical grade rotenone (purity unspecified) was used. The test also evaluated "degraded" rotenone resins. No further descriptions of the test substances were provided.

SYNONYMS: 1,2,12,12a-Tetrahydro-8,9-dimethoxy-2-(1-methylethenyl-[1]benzopyrano-[3,4-b]furo[2,3-h][1]benzopyran-6[6H]-one; CAS No. 83-79-4

STUDY NUMBER(S): None

SPONSOR: None

TESTING FACILITY: Stillwell and Gladding Laboratories

TITLE OF REPORT: Stillwell and Gladding Study on Relative Safety of Rotenone: Cytotoxicity and Tumor Promoting Activity of Fresh and Degraded Rotenone Resins.

AUTHOR(S): Maltese, L. J.; Hartman, T.

REPORT ISSUED: July 8, 1985

DISCUSSION AND CONCLUSIONS: Fresh and degraded rotenone at concentrations from 1 to 750 ng/ml was evaluated in mixed cultures of wild type/mutant V-79 Chinese hamster lung fibroblasts. The pesticide did not affect the survival of mutant cells in a manner similar to known tumor promoters.

Core classification: Unacceptable because the nature of the test substances was not characterized in the report.

I. PROTOCOL

A. Materials

1. Reference mutagens: 12-O Tetradecanoyl-phorbol-13-acetate (TPA) was used.

2. Vehicle: Dimethyl sulfoxice (DMSO) was used as the vehicle for the test substance.

3. Test species: Chinese hamster V-79 lung fibroblasts were used. Genotypes for the cells were wild type (HG-PRT +) and mutant (HG-PRT -). The wild type possesses the gene for hypoxanthine guanine phosphoribosyl transferase, an enzyme that phosphorylates guanine in a salvage pathway that recovers free guanine for DNA synthesis. The mutant lacks HG-PRT enzyme activity and can not recover free guanine; mutant cells must synthesize guanine de

3. Test species (continued)

novo. The HG-PRT enzyme is also capable of phosphorylating 6-thioguanine which is substituted for guanine in DNA synthesis. Such a substitution results in cytotoxicity in HG-PRT + cells. In mixed wild type and mutant monolayer cell cultures, metabolic cooperation takes place, and mutant cells become susceptible to 6-thioguanine toxicity also. Tumor promotors inhibit the processes making cooperative metabolism possible and can enhance survival of mutant cells in a mixed mutant/wild type monolayer culture.

4. Cell culture conditions: Cultures were grown in 5 ml Modified Eagles Medium (MEM, formula #78-5470) supplemented with 5% fetal calf serum in 60 X 15 mm tissue culture dishes. The report indicated that cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

B. Methods

1. Preliminary cytotoxicity assay: Concentrations of 0, 50, 100, 250, 500, 750, and 1000 ng/ml of fresh rotenone and 0, 250, 500, 750, 1000, 1250, or 1500 ng/ml degraded rotenone were evaluated with HG-PRT - cells. Approximately 100 cells were seeded in 5 ml of medium and 50 ul of a test solution, and the cultures were incubated for 3 days. At the end of incubation, medium was decanted and the cultures were stained with 1.0% crystal violet. The number of live cells was counted in each culture.

2. Transformation assay: Cultures seeded with 100 HG-PRT - and 4.5×10^5 HG-PRT + cells were incubated for 4 hours to allow attachment of the cells. Then test solutions were added so the final concentrations of fresh rotenone were 0, 1, 10, 50, 100, and 250 ng/ml, and final concentrations for the degraded rotenone were 0, 50, 100, 250, 500, and 750, ng/ml. 6-Thioguanine was also added to a final concentration of 10 ug/ml. The positive control substance was added to a final concentration of 100 ug/ml, and the vehicle control cultures contained 10 ul DMSO. The cloning efficiency control cultures were seeded with 100 HG-PRT - cells.

Ten plates were used at each concentration, and all cultures were incubated for 3 days. At the end of the incubation period the medium was replaced without addition of test substance, and incubation was continued for 3 more days. At the end of the second incubation the medium was decanted, and the cells were washed with phosphate buffered saline (pH 7.2) and stained with 1.0% crystal violet. The number of colonies on each plate was then counted.

II. REPORTED RESULTS

According to the report the cytotoxicity assays indicated LC_{50} values for fresh and degraded rotenone to be 691 and 1265 ng/ml, respectively. Table 1 summarizes the mean survival rates for HG-PRT - cells in the preliminary assays.

In the main assay fresh or degraded rotenone did not enhance the growth of HG-PRT - cells as tumor promotors do under the experimental conditions described above. The reduced survival (see Table 2) indicated that cooperative metabolism occurred between the HG-PRT + and HG-PRT - cells in the treated cultures resulting in cytotoxicity to the mutant cells.

II. REPORTED RESULTS (continued)

Table 1

Results of the Preliminary Cytotoxicity Assays with Fresh and Degraded Rotenone and V-79 Chinese Hamster Lung Cells

Test group or concentration (ng/ml)	% Survival (HG-PRT - cells) Fresh Rotenone	Degraded Rotenone
Plating efficiency controls	91.2	91.2
Solvent control	89.4	89.4
50	89.4	--**
100	90.2	--**
250	86.0	88.7
500	67.2	89.3
750	39.6	84.5
1000	18.4	61.6
1250	--*	36.8
1500	--*	32.2

*Concentrations not tested with fresh rotenone.
**Concentrations not tested with degraded rotenone.

Table 2

Promoting Activity of Fresh or Degraded Rotenone in V-79 Chinese Hamster Lung Cells

Test group or concentration (ng/ml)	% Survival (HG-PRT - cells) Fresh Rotenone	Degraded Rotenone
Plating efficiency controls	88.0	88.0
Positive control (TPA)	82.0	82.0
Solvent control	20.0	20.0
1	19.3	--**
10	20.0	--**
50	20.3	20.9
100	20.0	20.6
250	17.7	21.3
500	--*	21.3
750	--*	21.1

*Concentrations not tested with fresh rotenone.
**Concentrations not tested with degraded rotenone.