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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Toxicology Studies with Rotenone and (Reg. No. 6704-Q) Tox. Chem. No. 725.

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

THRU: Jane Harris, Ph. D., Section Head
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769)
Jane E. Harris 11/26/85

FROM: Roger Gardner, Toxicologist
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Roger Gardner 11-26-85
11/26/85

Actions Requested

Review of the following toxicity studies:

1. Mutagenicity studies in Escherichia coli (Acc. No. 254719), yeast (reverse mutation, mitotic recombination, mitotic gene conversion) and mouse embryonic melanocytes (somatic cell point mutation) (Acc. No. 254720) rats (bone-marrow cytogenetics), mice (micronucleus test), and Drosophila (sex chromosome loss assay) (Acc. No. 254721)

2. Teratology studies in mice (Acc. No. 254722 and 254725) and rats (Acc. No. 254724)

3. Six-month feeding study in dogs (Acc. No. 254723)

4. Two-generation reproduction study in rats (Acc. Nos. 254726-254728)

Recommendations and Conclusions

1. The submitted studies satisfy the recommendations of the Agency's pre-RPAR review (see Section I. below) that mutagenicity, reproduction, and teratology studies should

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be submitted to support registration of proposed uses for rotenone.

2. According to the pre-RPAR review (see Section I. B. 2., below), studies indicate that rotenone has the potential of causing DNA damage and stopping cell division (mitotic arrest). A study with DNA repair deficient and proficient strains of bacteria (see Section II. C., and Appendix I) provided marginal results which were incompletely reported. More detailed data from that study are needed to support a more definitive interpretation of the results.

3. Rotenone did not cause chromosomal damage in rat bone marrow, nor did it increase the numbers of micronuclei in polychromatic or monochromatic erythrocytes of mice <u>in vivo</u>. The sensitivity of the Drosophila assay is in question because of the absence of a response to MMS (methyl methanesulfonate) which was used as the positive control. Reverse mutations, mitotic gene conversion, or recombination were also not seen in treated yeast, and rotenonone did not induce somatic cell mutations in mouse embryonic melanocytes <u>in vivo</u> (see Appendix I. and Section II. C. below).

4. Results from the range-finding and main teratology studies in mice suggest a NOEL of 15 mg/kg/day (highest dose tested in the main study) for fetal effects (reduced litter size and increased resorptions), maternal effects (increased mortality and decreased gravid uterine weight), and teratogenicity. An LEL of 24 mg/kg/day is suggested by the results from the range-finding study (see Section II. A. 1., and Appendix I below).

5. Results from the rat teratology study (see Appendix I and Section II. A. 2. below) suggest a NOEL of 3 mg/kg for maternal toxicity (decreased body weight gain) and fetal effects (decreased fetal weight and an increased incidence of fetuses with unossified sternabrae). An LEL of 6 mg/kg/day (highest dose tested) was indicated for maternal and fetal toxicity. No compound-related terata were observed.

6. The results from a multigeneration reproduction study (see Appendix I and Section II. B., below) suggested a NOEL of 7.5 ppm in adult and young rats. The LEL was 37.5 ppm (decreased body weight in pups during lactation and body weight gain of females before, during, and following pregnancy). Litter sizes in the group given the highest dietary level of rotenone were statistically significantly reduced in first and second generations of the study, but there were no treatment-related effects on reproductive indices observed in the study.

7. The six-month feeding study in dogs was reviewed previously (see Appendix II.). That study indicated that the NOEL was 0.4 mg/kg/day, and the LEL was 2 mg/kg/day (emesis and decreased body weight).

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. The support document further recommended that the Agency discuss with the U. S. Fish and Wildlife Service (FWS) and other interested parties the specific data required to support tolerances for rotenone.

The Toxicology data requirements were subsequently determined, and a list of studies needed by the Agency was included in a memorandum dated June 22, 1981 (From: Marcia Williams, Director, Special Pesticides Review Division. To: Douglas Campt, Director, Registration Division. Subject: Completion of Pre-RPAR Review of Rotenone.). Those requirements included the following:

1. A full complement of mutagenicity studies.
2. Teratology studies in two mammalian species.
3. A multigeneration reproduction study in rats.
4. Chronic feeding studies.

The FWS has resubmitted the mutagenicity studies and the six-month feeding study in dogs (Acc. Nos. 254720, 254721, and 254723). The mutagenicity studies are reconsidered along with the teratology and reproduction studies submitted with the current action (see Appendix I below). The previous Toxicology Branch review of the dog study is included in Appendix II.

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 unpublished 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%.

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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 Pre-RPAR 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 (Leber and Persing, 1978)...

> ..., 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 (Leber and Thake, 1978). 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.

Subsequently, a Toxicology Branch review (see memorandum dated August 15, 1985. From: R. Gardner. To: W. Miller,

Registration Division. Subject: Review of Oncogenicity Studies with Rotenone and Request for Data Waiver. (Reg. No. 16-58T) Tox. Chem. No. 725.) concluded that the results of the hamster study were not conclusive with respect to the oncogenic potential of rotenone. In addition, the review stated that the rat studies were designed to confirm results obtained in a specific protocol rather than to support registration of rotenone. Based on these considerations, additional oncogenicity studies with rotenone were recommended.

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 chromosomal 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

In addition to the data mentioned above (see Section I. B. for discussions of the oncogenicity, mutagenicity, and teratology data reviewed previously), Appendix III 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 (see Memorandum dated August 16, 1985. From: R. Gardner. To: W. Miller, Registration Division. Subject: Review of a Draft Final Report on Rat Metabolism Studies with Rotenone and Request for Comments. (Reg. No. 6704-Q) Tox. Chem. No. 725.) 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).

2. Subchronic and chronic toxicity

A NOEL of 0.4 mg/kg/day was established in a six-month dog 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 (see Appendix II below).

3. Metabolism

Results of a series of experiments generally characterized the excretion pattern associated with single or repeated (14 consecutive days) low 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). The major route of excretion (95 to 97% of the administered dose) is in the feces, and female rats excrete administered radioactivity at a slower rate than males. The route of administration, dose level, and number of doses had no apparent effect on the excretion pattern.

II. Summary of New Data

Data Evaluation Records for the studies described in this section are included in Appendix I.

A. Teratology Studies

1. Mice

Based on results of a range-finding study (1), the highest

dose most likely to cause slight toxicity in a teratology study with mice is between 12 and 24 mg/kg/day.

The doses finally selected for the main study (2) were 3, 9, and 15 mg/kg/day, and they were administered on days 5 through 17 of gestation. However, the results of the main study suggested a NOEL for maternal and fetal effects that may be higher. Since there were no dose-related effects observed in the main study, its results should be interpreted along with those from the preliminary study. On that basis, the NOEL for maternal effects (weight loss and mortality), fetal effects (decreased litter size) in mice is 15 mg/kg/day. The lowest effect level (LEL) is 24 mg/kg/day.

2. Rats

Decreased body weight and body weight gain were observed in pregnant rats given daily doses of 6 mg/kg/day on gestation Days 6 through 19. The NOEL for these effects was 3 mg/kg, and there were also 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 sternebrae. The NOEL for these effects was also 3 mg/kg/day. There were no dose-related terata observed in the study.

B. Reproduction Toxicity

Rotenone had no effect on reproduction in rats at dietary levels of 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 in both adult and young rats of both sexes. The NOEL was 7.5 ppm (approximately 0.375 mg/kg/day).

C. Mutagenicity

Nine assays were described in three reports (5-7), and a common observation was made; the solubility of rotenone in aqueous media is low (20 ug/ml, see 7). In some cases, investigators attributed the negative results they observed in assays to the low solubility of the test material.

In a report on differential toxicity assays in Escherichia coli (5), the investigators noted no zone of inhibition around filter paper disks saturated with rotenone which, they concluded, was the result of the low solubility of the test material in aqueous solutions. However, studies with liquid media indicated results classified by the authors as marginal. Without individual replicate data reports, the significance of their conclusion can not be determined, and the report is considered to be unacceptable.

Rotenone did not induce reverse mutations in yeast (Saccharomyces cereviciae strains S138 and S211) at dose levels up to 10,000 ug/ml. No mitotic recombination or mitotic gene conversion was observed in yeast (strains D4 and D5) at the same dose levels. Again, low solubility of the test substance may be a factor as well as other limitations of such assays (see the Data Evaluation Record in Appendix I and Reference 6).

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 induce 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 (6). The estimated acute oral LD_{50} in the preliminary range-finding study was near 3 mg/kg.

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 (7). 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 (7).

Reported results for the positive control group in a Drosophila sex chromosome loss assay (7) failed to indicated that the test procedure used for rotenone was sensitive to genotoxic effects. A pilot study with MMS (the positive control) showed that another procedure was sensitive to the genotoxicity of the reference mutagen, but the main study did not follow that procedure. In addition, the authors stated that a feeding method for rotenone has not been established. Under these conditions, the assay is unacceptable.

III. BIBLIOGRAPHY

1. Hazleton Raltech, Inc. November 24, 1981. Final Report: Range Finding Study with Rotenone in Pregnant Mice. Unpublished report No. 80049 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254722.

2. Hazleton Raltech, Inc. November 24, 1981. Final Report: Teratology Study with Rotenone in Mice. Unpublished report No. 80050 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254724.

3. Hazleton Raltech, Inc. June 17, 1982. Report: Teratology Study with Rotenone in Rats. Unpublished report No. 81178 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254725.

4. Hazleton Raltech, Inc. February 11, 1983. Final Report: Reproduction Study for Safety Evaluation of Rotenone Using Rats. Unpublished report No. 81077 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254726, 254727, and 254728.

5. Van Goethem, D., B. J. Barnhart, and S. S. Fotopoulos. April 1, 1981. Mutagenicity Studies on Rotenone: Final Report. Unpublished report no. 7029-E prepared by Midwest Research Institute, Kansas City, MO. Submitted by U. S. Fish and Wildlife Service, Department of the Interior. EPA Acc. No. 254719.

6. Litton Bionetics, Inc. June 24, 1981. Mutagenicity Studies on Rotenone: Final Report. Unpublished report no. 22063 prepared by Litton Bionetics, Inc., Kensington, MD. Submitted by the U. S. Fish and Wildlife Service, National Fishery Research Laboratory. EPA Acc. No.254720.

7. Biotech Research Laboratories, Inc. January 10, 1982. Analytical studies for the detection of chromasomal aberration in fruit flies, rats, mice, and horse beans. Unpublished report prepared by Biotech Research Laboratories, Inc. Submitted by the U. S. Fish and Wildlife Service, National Fishery Research Laboratory, La Crosse, Wisconsin. EPA Acc. No. 254721.

APPENDIX I

Data Evaluation Records for Teratology, Reproduction, and Mutagenicity Studies with Rotenone

NOTE: The Data Evaluation Records are included in the same order listed in Section III. above.

DATA EVALUATION RECORD

1. CHEMICAL: Rotenone

2. TEST MATERIAL: Rotenone of unspecified purity was used.

3. STUDY/ACTION TYPE: Teratology - rats

4. STUDY IDENTIFICATION: Hazleton Raltech, Inc. June 17, 1982. Report: Teratology Study with Rotenone in Rats. Unpublished report No. 81178 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254725.

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7. CONCLUSIONS: Decreased body weight gain were observed in pregnant rats given daily doses of 6 mg/kg on gestation Days 6 through 19. The NOEL for these effects was 3 mg/kg.

The 6 mg/kg dose also caused a reduction in mean fetal weight below that of controls, and an increased incidence of unossified sternebrae. The NOEL for these effects was also 3 mg/kg/day. There were no dose-related terata observed in the study.

Core Classification: Minimum

8. MATERIALS AND METHODS

Test species: Seven- to ten-week-old male and female COBS® CD® strain rats were used. Each female was mated with a male and was examined daily for a vaginal plug or the presence of sperm in vaginal smears. The day either were found was designated Day 0 of gestation.

Experimental procedures: The test substance was suspended in corn oil and administered by gavage on days 6 through 19 of gestation. Doses of 0, 0.75, 1.5, 3, or 6 mg test substance per kg body weight were given to groups of 25 mated dams.

8. MATERIALS AND METHODS (continued)

Each dam was observed at least once daily for occurrence of toxic signs and mortality. Body weight determinations were made on days 0, 6, 9, 12, 15, 18, and 20 of gestation.

The rats were weighed and sacrificed on day 20 of gestation. Gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Live fetuses were grossly examined and half of them were prepared for skeletal examination. The remainder were prepared for soft tissue examination, and all abnormalities and variations were noted. Apparently non-gravid uteri were placed in 10% ammonium sulfide and examined.

Statistical procedures are discussed below as appropriate. The report noted that animals dying during gestation, aborted, or not pregnant were not included in the analysis of results.

9. REPORTED RESULTS

Maternal observations: The most frequently observed clinical signs, according to the investigators, were excessive salivation and rubbing of the face and paws on the bottom of their cages. These signs occurred only in the treated groups, and they were observed throughout the study. The report also noted that rough coats, a reddish-brown tinge on the fur, lethargy, poor muscle tone, nasal exudate, and a wet urogenital area were frequently seen. None of the control group animals were found to exhibit these signs.

At necropsy eight of the dams in the 6 mg/kg/day group had distended stomachs because of the presence of excess amounts of food. The control, 0.75 and 3 mg/kg/day dose groups each contained one dam with renal pelvic cavitation, and the 6 mg/kg/day dose group had one animal with fused fetal placentae.

Table 1 summarizes reported maternal body weight results.

As shown in Table 2, there were 4 deaths reported. The animal from the 1.5 mg/kg/day dose group exhibited tonic convulsions shortly before death. One of the three 6 mg/kg/day dose group animals was sacrificed in moribund condition on Day 18 of gestation while the others were found dead on Days 10 and 17 of gestation. All three 6 mg/kg dose group animals exhibited the clinical signs most commonly reported among treated animals.

Litter observations: The report stated that pregnancy rates ranged from 96% to 100% (see Table 2), and only one dead fetus was found (in a dam from the 1.5 mg/kg/day dose group). There were no significant differences noted with respect to

8. REPORTED RESULTS (continued)

Table 1

Group Mean Maternal Body Weight Data (g)

Weight parameter	Dose (mg/kg/day) 0	0.75	1.5	3	6
Body weight at					
Day 0	249	249	241	234*	240
Day 20	379	381	365	376	316
Gravid uterus wt.	69.9	72.7	70.1	72.2	57.6
Body wt. excl. uterus at Day 20	309	307	295**	284**	258**
Body wt. gain					
Incl. uterus	130	132	124*	122**	76**
Excl. uterus	60	59	54**	50**	24**

*Statistically significantly different from controls; p<0.05.
**Statistically significantly different from controls; p<0.01.

the group mean number of corpora lutea, implantation sites, live fetuses, or resorptions. These results are reflected in the parameters summarized in Table 2 below.

Mean fetal weight in the 6 mg/kg/day dose group was statistically significantly decreased below that for the controls.

Fetal observations: The only grossly observed lesion noted by the investigators was described as a 1 mm clear dermal cyst found extending from the head to the mid abdominal region. One fetus in the 0.75 mg/kg/day dose group and two from the same litter in the 1.5 mg/kg dose group were observed to have the lesion.

Soft tissue abnormalities did not occur frequently according to the report. There was one fetus from each of the control, 0.75, and 3 mg/kg dose groups that was reported to have renal pelvic cavitation. Two litters from the 1.5 mg/kg dose group contained one and three fetuses with the abnormality, and three other fetuses from three litters in the 6 mg/kg dose group also exhibited the renal abnormality. One of those fetuses in the 0.75 mg/kg dose group, three from the 1.5 mg/kg group (in one litter), and three fetuses (one from

9. REPORTED RESULTS (continued)

Table 2

Pregnancy data summary

Parameter	Dose (mg/kg/day) 0	0.75	1.5	3	6
Number mated	25	25	25	25	25
Deaths during dosing	0	0	1	0	3
Dams with implantations	24	25	23	24	22
Litters with live fetuses	24	25	23	24	22
Mean no. live fetuses/litter	13	13	13	14	14
Mean fetal wt. (g)	3.5	3.5	3.5	3.4	2.7*
Sex ratio (% male)	49.1	51.2	46.5	52.0	46.4

*Statistically significantly different from controls; $p<0.05$.

each of three litters) in the 6 mg/kg dose group had distended ureters along with the kidney abnormality.

One fetus from the control group was reported to have dilated ventricles in the brain and microphthalmia; one fetus from the 0.75 mg/kg dose group had cleft palate; and the left carotid artery arose from the innominate in one fetus from the 1.5 mg/kg dose group and one each in two litters from the 3 mg/kg dose group.

Table 3 summarizes the most frequently observed skeletal abnormalities (occurring in >5% of the fetuses examined).

10. DISCUSSION

There were statistically significant decreases in body weight gain (see Table 1 above) in dams given doses of 1.5 mg/kg/day or higher. Those animals had a mean body weight gain that was approximately 10% less than that for the control group animals. At Day 20 the group mean body weight gain for the 1.5 mg/kg dose group was approximately 5% less than that for controls when the weight of the gravid uterus was included, and excluding the uterine weight, the group mean body weight gain was 10% less than that of the controls for the 1.5 mg/kg dose group. At the 3 mg/kg dose level there was approximately 17% less weight gain than that in the controls when uterine weight was excluded, and the group mean body weight gain at Day 20 was 6% less than that of the control animals when uterine weight was included. The mean body weight for the 3 mg/kg dose group excluding the weight of the gravid uterus

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Table 3

Summary of the Incidence of Most Frequently Observed Skeletal Abnormalities

Parameter	Dose (mg/kg/day) 0	0.75	1.5	3	6
No. litters examined	24	25	23	24	22
No. fetuses examined	162	166	159	167	157
Unossified sternebrae					
Litters*	19	20	13	17	21
Fetuses	48	54	26	52	110
Thoracic vertebrae Constricted Centra					
Litters	4	8	6	12	12
Fetuses	5	14	10	17	19

*Number of litters containing one or more fetuses with the abnormality.

10. DISCUSSION (continued)

was 8% less than that for the control group. At the highest dose, the group mean body weight (with or without the uterine weight) was 17% less than that for the controls at Day 20 of gestation, and maternal weight gain (excluding gravid uterine weight) was only 40% of that for the control group. These results suggest that the weight changes reported in Table 1 above were toxicologically significant at 6 mg/kg/day. On that basis, the noobserved-effect level (NOEL) for maternal toxicity suggested by the results is 3 mg/kg.

As shown in Table 2 above, there was a statistically significant decrease in group mean fetal weight at the highest dose level (6 mg/kg/day) when compared with that for control group fetuses (2.7 g in the treated group compared with 3.5 g in the control group). Although the number of litters with one or more fetuses with unossified sternebrae (Table 3 above) was not affected by treatment, the number of fetuses per litter with those effects was increased above control incidences in the highest dosed group. The average number of fetuses per affected litter with unossified sternebrae increased from 2.5 in the control group to 5.2 in the 6 mg/kg/day dose group. These two observations suggest that there are fetal effects at 6 mg/kg/day, and the NOEL for fetal effects is 3 mg/kg/day.

There were no dose-related incidences of terata observed with rotenone administered by gavage at doses as high as 6 mg/kg/day.

DATA EVALUATION RECORD

1. CHEMICAL: Rotenone

2. TEST MATERIAL: Rotenone (94%, Lot No. 100287) was used.

3. STUDY/ACTION TYPE: Teratology - mice (Range finding study)

4. STUDY IDENTIFICATION: Hazleton Raltech, Inc. November 24, 1981. Final Report: Range Finding Study with Rotenone in Pregnant Mice. Unpublished report No. 80049 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254722.

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7. CONCLUSIONS: Based on the results of the range-finding study described below, the highest dose most likely to cause a slight toxicity in a teratology study with mice would be between 12 and 24 mg/kg/day.

Core Classification: Supplementary. The study is a range-finding study.

8. MATERIALS AND METHODS

Test species: Fifty-six-day-old male and female CD-1 strain mice were used. After a two-week acclimation period, each female was mated with a male and was examined daily for a vaginal plug. The day either were found was designated Day 0 of gestation.

Experimental procedures: The test substance was suspended in corn oil and administered by gavage on days 6 through 17 of gestation. Doses of 0, 0.75, 1.5, 3, 6, 12, or 24 mg test substance per kg body weight were given to groups of 7 mated dams.

Each dam was observed at least once daily for occurrence of toxic signs and mortality. Body weight determinations were made on days 0, 6, 9, 12, 15, and 18 of gestation. Food consumption was estimated for days 0 to 5, 6 to 8, 9 to 11,

8. MATERIALS AND METHODS (continued)

11 to 14, and 15 to 18.

Surviving mice were weighed and sacrificed on day 18 of gestation. Gravid uteri from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Live fetuses were grossly examined. Apparently non-gravid uteri were examined for evidence of pregnancy by an unspecified method.

Statistical procedures are discussed below as appropriate. The report noted that animals that died during gestation, aborted, or were not pregnant were not included in the analysis of results.

9. REPORTED RESULTS

Maternal observations: The report noted that there were no deaths in the control, 3, 6, or 12 mg/kg groups. There was one death in each of the 0.75 and 1.5 mg/kg dose groups, and three deaths were reported for the 24 mg/kg dose group.

The only clinical signs reported were pawing and rubbing of the face on the bottom of the cage in one animal from the 24 mg/kg dose group. No other animals exhibited clinical signs according to the investigators.

The only gross pathological changes noted at necropsy included darkened areas on the uteri of one or two mice from the 0.75, 1.5, 6, 12, and 24 mg/kg dose groups (described as probable hematomas), and one fetus from a control group litter with exencephaly.

Reported pregnancy status of test animals is summarized in Table 1.

The report noted no statistically significant differences between the treated and control groups with respect to mean body weight at gestation Day 18, body weight gain during gestation, or mean gravid uterine weights. However, the authors noted that gravid uterine weights were slightly less in the 24 mg/kg/day dose group than control group values (see Table 2)

There was no treatment related effect on food consumption observed according to the report.

Table 3 summarizes the litter observations of test animals. Those results indicated that the 24 mg/kg/day dosage level was associated with increased resorptions and decreased litter size.

8. REPORTED RESULTS (continued)

Table 1

Pregnancy data summary

Parameter	Dose (mg/kg/day) 0	0.75	1.5	3	6	12	24
Number mated	7	7	7	7	7	7	7
Deaths during dosing	0	1	1	0	0	0	3
Not pregnant	1	0	0	2	0	1	1*
Resorbed litters	0	0	0	0	0	0	1*
Delivered before c-section	0	1	1	1	2	4	0
Gravid at c-section	6	6**	6**	4	5	2	5**

*The animal died during the study.
**Includes one animal that died during the study.

Table 2

Summary of maternal weight results

Observation	Test group Control	24 mg/kg
Mean body weight (g) (Day 18)	51.0	45.7
Mean weight gain (g) (Days 0-18)*	19.2	11.3
Mean weight gain (g) (Days 0-18)**	4.6	7.0
Gravid uterine weight (g)	23.8	18.3

*Including gravid uterine weight.
**Excluding gravid uterine weight.

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9. REPORTED RESULTS (continued)

Table 3

Litter data summary

Parameter	Dose (mg/kg/day) 0	0.75	1.5	3	6	12	24
Number examined	7	5	5	6	5	3	4
Dams with implantations	6	5	5	4	5	2	4
Litters with live fetuses	6	5	5	4	5	2	4
Mean litter size	10.8	11.0	11.4	11.8	10.6	11.0	8.2
Dead fetuses per litter	0.3	0.2	0.0	0.0	0.0	0.5	0.0
Resorptions per litter	0.5	0.6	0.4	0.8	0.4	1.0	3.8

10. DISCUSSION

There were adequate data presented by the authors to support the conclusions described in Section 7. above).

DATA EVALUATION RECORD

1. CHEMICAL: Rotenone

2. TEST MATERIAL: Rotenone of unspecified purity was used.

3. STUDY/ACTION TYPE: Teratology - mice

4. STUDY IDENTIFICATION: Hazleton Raltech, Inc. November 24, 1981. Final Report: Teratology Study with Rotenone in Mice. Unpublished report No. 80050 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254724.

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7. CONCLUSIONS: The highest dose (15 mg/kg/day) was selected on the basis of a preliminary study (see Section 10. DISCUSSION below). However, the results described below suggested a NOEL for maternal and fetal effects >15 mg/kg/day. Since there were no dose-related effects observed in the main study, its results should be interpreted along with those from the preliminary study. On that basis, the NOEL for maternal effects (decreased gravid uterine weight and mortality) and fetal effects (decreased litter size with increased resorptions) in mice is 15 mg/kg/day. The lowest effect level (LEL) is 24 mg/kg/day.

Core Classification: Minimum when considered with the range-finding study.

8. MATERIALS AND METHODS

Test species: Fifty-six-day-old female and young adult (proven breeders) male CD-1 strain mice were used. After a two-week acclimation period, each female was mated with a male and was examined daily for a vaginal plug. The day either were found was designated Day 0 of gestation.

Experimental procedures: The test substance was suspended in corn oil and administered by gavage on days 6 through 17 of

8. MATERIALS AND METHODS (continued)

gestation. Doses of 0, 3, 9, or 15 mg test substance per kg body weight were given to groups of 30 mated dams.

Each dam was observed at least once daily for occurrence of toxic signs and mortality. Body weight determinations were made on days 0, 6, 9, 12, 15, and 18 of gestation. Changes in food consumption were monitored visually during the study also.

The mice were weighed and sacrificed on day 18 of gestation. Gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Live fetuses were grossly examined and half of them were prepared for skeletal examination. The remainder were prepared for soft tissue examination, and all abnormalities and variations were noted. Apparently non-gravid uteri were placed in 10% ammonium sulfide and examined to confirm pregnancy status.

Statistical procedures are discussed below as appropriate. The report noted that animals that died during gestation, aborted, or were not pregnant were not included in the analysis of results.

9. REPORTED RESULTS

Maternal observations: One animal from each of three test groups (control, low, and high dose group) died or was sacrificed early because of intubation errors. A fourth animal from the 9 mg/kg dose group was found dead. Its pleural cavity was reported to be filled with blood. Another animal from the control group was sacrificed in moribund condition on Day 13 of gestation, and evidence of another intubation error was reported at necropsy.

There were no dose-related incidences of clinical signs in test animals, and the only signs noted by the investigators included one animal with a wet urogenital area from the mid dose group and another from the high dose group with a dermal lesion in the dorsal cervical region.

Table 1 summarizes reported maternal body weight results.

The report stated that there were 7, 5, 6, and 7 dams that delivered on Day 18 shortly before scheduled sacrifice and necropsy.

Litter observations: The report stated that there were 2, 5, 4, and 4 animals which were not pregnant in the control, low, mid, and high dose groups, respectively. One animal from the low dose group had one early post-implantation resorption,

8. REPORTED RESULTS (continued)

Table 1

Group Mean Maternal Body Weight Data (g)

Weight parameter	Dose (mg/kg/day) 0	3	9	15
Body weight at				
Day 0	30.3	29.4	31.0	29.8
Day 18	53.2	55.0	55.0	55.4
Gravid uterus wt.	18.2	19.7	18.9	19.6
Body wt. excl. uterus at Day 18	35.0	34.9	36.1	35.9
Body wt. gain				
Incl. uterus	22.9	25.5	24.1	25.6
Excl. uterus	4.6	5.2	5.0	5.8

and another from the mid dose group had 11 early resorptions. Related results are summarized in Table 2 below.

One dam from the control group aborted 9 dead fetuses on Day 17 of gestation, and there were 2, 3, and 2 litters with one dead fetus each from the low, mid, and high dose groups, respectively.

There were no significant differences noted with respect to the group mean number of corpora lutea, implantation sites, live fetuses, or resorptions. These results are reflected in the parameters summarized in Table 2 below.

Fetal observations: The only grossly observed abnormalities noted by the investigators were exencephaly in one low-dose group fetus and exencephaly with patent eyes in one mid-dose group fetus. There were no gross abnormalities observed in the control or high dose groups according to the report.

Soft tissue abnormalities did not occur frequently according to the report. There was one fetus from the control group with a missing aortic arch and descending aorta and left carotid and subclavian arteries which were arising directly from the left atrium. There was one fetuse in the mid and high dose groups with cleft palate, and another fetus from the high dose group without testes. Another high dose group fetus exhibited a dark focus on the median lobe of the liver. There were no other soft tissue anomalies observed according to the report.

9. REPORTED RESULTS (continued)

Table 2

Pregnancy data summary

Parameter	Dose (mg/kg/day) 0	3	9	15
Number mated	30	30	30	30
Deaths during dosing	2	1	1	1
Number examined on Day 18	28	29	29	29
Dams with implantations	26	24	25	25
Litters with live fetuses	26	23	24	25
Mean no. live fetuses/litter	11	12	11	12
Mean fetal wt. (g)	1.4	1.4	1.3	1.3
Sex ratio (% male)	51.7	53.8	51.5	50.3

Table 3 summarizes the most frequently observed skeletal abnormalities (occurring in >5% of the fetuses examined).

10. DISCUSSION

The dose levels used in this study were based on results from a range-finding study (Hazleton Raltech, Inc. November 24, 1981. Final Report: Range Finding Study with Rotenone in Pregnant Mice. Unpublished report No. 80049 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254722.) which is reviewed elsewhere. That study indicated that the highest dose most likely to cause a slightly toxic effect in a teratology study with mice would be between 12 and 24 mg/kg/day. The effects noted at a dose level of 24 mg/kg/day were body weight decrease (approximately a 10% decrease below controls) which was associated with a slight decrease in litter size (8.2 live fetuses per litter in a group of 4 pregnant mice compared with 10.2 live fetuses per litter in a control group of 6 animals), an increase in resorptions and the occurrence of 3 deaths at that level compared with no deaths and fewer resorptions in the control group.

The highest dose selected for the main study was 15 mg/kg/day. However, the results of the main study suggested that a slightly higher dose would be more likely to cause slight toxicity in pregnant mice. Since there were no dose-related effects observed in the main study, its results should be interpreted along with those from the preliminary study. Based on reported results from those studies, a no-observed-

Table 3

Summary of the Incidence of Most
Frequently Observed Skeletal Abnormalities

Parameter	Dose (mg/kg/day) 0	3	9	15
No. litters examined	26	23	24	25
No. fetuses examined	130	127	131	145
Skull: Accessory bone				
Fetuses	10	9	9	8
Litters*	8	7	6	5
Rib buds				
Fetuses	30	34	27	20
Litters*	11	16	12	11
Extra ribs				
Fetuses	14	31	16	17
Litters*	6	6	7	4
Unilat. 7th cervical rib				
Fetuses	9	20	21	15
Litters*	8	10	8	10

*Number of litters containing one or more fetuses with the abnormality.

10. DISCUSSION (continued)

effect level (NOEL) for maternal or fetal effects in mice is 15 mg/kg/day. The lowest effect level (LEL) is 24 mg/kg/day based on results from the two studies.

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DATA EVALUATION RECORD

1. CHEMICAL: Rotenone

2. TEST MATERIAL: Rotenone (97.9%) was used.

3. STUDY/ACTION TYPE: Multigeneration study - rats

4. STUDY IDENTIFICATION: Hazleton Raltech, Inc. February 11, 1983. Final Report: Reproduction Study for Safety Evaluation of Rotenone Using Rats. Unpublished report No. 81077 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254726, 254727, and 254728.

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Date: 11/26/85

7. CONCLUSION: Rotenone had no effect on reproduction in rats at dietary levels of 7.5, 37.5, or 75 ppm. The lowest-effect level (LEL) with respect to decreased body weight, and body weight gain was 37.5 ppm in both adult and neonatal rats. The no-observed-effect level was 7.5 ppm.

Core classification: Minimum

8. MATERIALS AND METHODS

Test species: Male and female weanling Charles River CD (SD) BR strain rats were used. The animals were approximately 6 weeks of age when placed on test diets.

Dosages: Each group of 15 males and 25 females was given diets containing 0, 7.5, 37.5, or 75 ppm test substance.

Mating schedule: After 105 days on test diets, one F_0 male was cohabited with two females from the same test group for up to 7 days. Each day after pairing the investigators looked for vaginal plugs or examined vaginal smears for the presence of spermatozoa. The day a plug was found or spermatozoa were disco-

8. MATERIALS AND METHODS (continued)

vered was designated Day 0 of gestation for the appropriate animal. If evidence of successful mating was not observed after 7 days, the male was replaced with another from the same treatment group.

The offspring from the mating of F_0 animals were designated F_{1A} litters, and F_1 parental animals were selected from those litters when the pups were 21 days of age (15 males and 25 females).

After feeding the test diets to F_1 parental animals for 120 days, they were mated in the same manner as the F_0 animals to produce F_{2A} litters.

General observations: The F_0 and F_1 animals were checked daily for the appearance of signs of toxicity, behavioral changes, and mortality. These animals were individualy weighed, and their food consumption was determined at weekly intervals during their maturation periods. During mating males were weighed weekly, and females were weighed on days 0, 7, 14, and 20 of gestation. Females with litters were weighed on days 0, 7, 14, and 21 *post partum*.

The report stated that mating performance was determined by the percentage of cohabited females in each group which mated successfully, and pregnancy rate was determined from the number of successfully mated females which were pregnant (with live pups).

Litter observations: Pups were counted, sexed, and examined for external abnormalities as soon as possible after birth. Litters were observed daily for occurrence of mortalities, and dead pups were removed for *post mortem* examination when possible.

Individual pup weights were obtained on days 1, 4, 7, 14, and 21 after birth. The number of live pups was also determined on each weighing day. Litters were culled to 10 pups at Day 4 of lactation maintaining equal numbers of each sex where possible.

Necropsy: After weaning of their pups, the F_0 males and females were sacrificed and subjected to gross necropsy. The gonads were weighed, and those organs and grossly observed lesions were preserved for subsequent histological examination. At weaning of the F_{1A} pups, five of each sex from those not chosen to be F_1 generation parents were sacrificed and subjected to necropsy and microscopic examination (see below). Five pups of each sex from F_{2A} litters were also subjected to gross and microscopic necropsy.

F_{1A} adults were sacrificed 30 days after weaning of the F_{2A} pups and subjected to gross and microscopic examinations at necropsy. In addition to the gonad weights for these animals, the adrenals, brain, thyroid, heart, kidneys, and liver were weighed.

The following tissues from F_{1A} adults and F_{1A} and F_{2A} weanlings were processed for histological examination:

8. MATERIALS AND METHODS (continued)

Adrenals	Large intestine	Pituitary	Spinal cord
Bone and marrow	Liver	Prostate	Spleen
Brain	Lesions	Salivary gland	Stomach
Esophagus	Lungs with main-stem bronchi	Seminal vesicles	Thymus
Eyes		Skeletal muscle	Thyroid
Gonads	Lymph nodes	Skin with mammary gland	Trachea
Heart	Pancreas		Urinary bladder
Kidneys	Peripheral nerve	Small intestines	Uterus

9. REPORTED RESULTS

Dietary analyses: According to the report, a modified procedure for diet analysis was used periodically beginning with the 9th week of the study. Homogeneity results for the low level dietary mixture were described as unacceptable. The investigators indicated that homogeneity determinations were repeated during week 12 of the study because of the high variability in individual samples and the low recovery during week 9. The apparent decrease in sensitivity of the analytical method was compensated for by the investigators, and homogeneity trials were repeated to assure adequate mixing of test diets. The mean dietary concentrations (ppm) and variability (±%) for week 12 were 6.4 (± 2%), 38.1 (± 21%), and 84.0 (± 10%) for the low, mid, and high dose groups, respectively.

The report stated that stability results indicated that rotenone was stable in test diet mixtures under ambient conditions for 3 days. When diets were kept frozen, the rotenone levels remained stable for 14 days.

Based on these results, fresh diets were presented to test animals twice weekly after the 12th week of the study.

Parental animals: The only clinical observations noted in the report for the F_0 generation animals were described as unrelated to treatment. Exophthalmia appeared in one control group female at week 16, but the animal was not sacrificed until scheduled termination. A second female in the high dose group exhibited alopecia beginning in week 19. Two males from the low dose group and one from the mid dose group were observed to have brownish nasal and/or occular discharges beginning in week 16.

In the F_{1A} generation, alopecia was observed in 1, 3, and 1 females from the low, mid, and high dose groups, respectively. These animals exhibited the sign from week 20 until termination. One female from the mid dose group had an eye lesion during week 17 of the study. There were also some animals with signs of malocclusion (brownish nasal and occular discharges), but the incidence of animals with this sign was small like that for the F_0 generation and was unrelated to treatment. No other clinical observations were noted by the investigators.

During the maturation phase for the F_0 animals, statistically significant body weight decreases were noted during weeks 13, 14, and 15 for high-dose group females and during weeks 14 and 15 for mid-dose group females (see Table 1).

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9. REPORTED RESULTS (continued)

No statistically significant decreases in food consumption for treated females were noted (Table 2), but high-dose group males showed a statistically significant decrease in food consumption during week 15. Body weight gains for the mid-dose group males were statistically significantly less than that for controls during week 14, and for the high dose group, weight gains were significantly less than controls during weeks 13, 14, and 15 (see Table 3). Body weight gains for mid and high dose group females were also significantly less than controls during weeks 13, 14, and 15 (see Table 3).

The report stated that group mean body weights for mid-dose F_{1A} parental males was statistically significantly less than that for controls during weeks 0 through 10 after weaning. High-dose group males had significantly decreased body weights throughout the maturation phase (weeks 0-17 post-weaning). Body weight for the mid and high-dose group F_{1A} females was also significantly less than that for controls throughout the maturation phase of the study. The reported pattern of food consumption decreases and body weight gain differences are reflected in Tables 2 and 3, respectively.

Table 1

Selected group mean body weights for parental animals

Dose (ppm)	F_0 Generation* Males 0	8	15	Females 0	8	15	F_1 Generation* Males 0	8	15	Females 0	8	15
	Treatment week											
0	182	429	517	141	248	292	93	405	509	83	245	289
7.5	191	443	516	141	249	289	93	423	534	84	246	289
37.5	186	425	484	139	242	274†	65††	373†	478	67††	210††	246††
75.0	185	423	468	139	236	253††	47††	302††	390	43††	185††	211††

*The body weights for the F_0 generation are larger for treatment weeks 1 and 5 than those of F_1 animals in correspond treatment weeks because of age differences.

†Statistically significantly different from the control group mean, $p<0.05$.

††Statistically significantly different from the control group mean, $p<0.05$.

Table 2

Selected group mean food consumption (g/animal/week)
for parental animals

Dose (ppm)	Treatment week											
	F_0 Generation*						F_1 Generation*					
	Males			Females			Males			Females		
	1	8	15	1	8	15	1	8	15	1	8	15
0	152	175	177	116	125	128	126	195	194	119	150	137
7.5	157	178	170	119	127	124	129	195	187	114	143	139
37.5	151	173	162	116	128	128†	104†	191	178	102††	136†	127
75.0	149	170	148††	117	123	129	84††	180	159	82††	123††	118††

*The body weights for the F_0 generation are larger for treatment weeks 1 and 5 than those of F_1 animals in correspond treatment weeks because of age differences.

†Statistically significantly different from the control group mean, $p<0.05$.

††Statistically significantly different from the control group mean, $p<0.05$.

Table 3

Selected group mean body weight gain (g) for parental animals

Dose (ppm)	Treatment week											
	F_0 Generation*						F_1 Generation*					
	Males			Females			Males			Females		
	1	8	15	1	8	15	1	8	15	1	8	15
0	46	16	19	22	4	9	50	21	11	37	11	3
7.5	52	14	15	23	4	8	51	26	10	36	10	6
37.5	49	10	15	21	-1†	5†	43†	27	12	34	0	2
75.0	46	12	12†	22	-3††	4††	35††	22	7	32†	8	1

*The body weights for the F_0 generation are larger for treatment weeks 1 and 5 than those of F_1 animals in correspond treatment weeks because of age differences.

†Statistically significantly different from the control group mean, $p<0.05$.

††Statistically significantly different from the control group mean, $p<0.05$.

9. REPORTED RESULTS (continued)

Significant body weight decreases were also observed during gestation and lactation for the F_0 and F_{1A} maternal animals (see Table 4)

Table 4

Selected group mean body wts. (g) for females

Dose (ppm)	Treatment day during Pregnancy 0	14	20	Lactation 1	14	21
			F_{1A} mating			
0	287	329	380	298	320	295
7.5	282	323	370	297	316	294
37.5	261*	300**	348**	274**	289**	286
75	244**	268	305**	244**	248**	250**
			F_{2A} mating			
0	292	329	374	300	315	293
7.5	280	320	372	294	307	276
37.5	246**	282**	330**	259**	278**	273**
75	215**	243**	278**	217**	223**	222**

*Statistically significantly different ($p<0.05$)

**Statistically significantly different ($p<0.01$)

The gonad weights for the F_0 males and females were comparable for all test groups including the control group. Because of reduced terminal body weight for the high dose group males and females, the relative gonad weights were increased but those differences were not considered to be biologically significant. Organ weight changes in the F_{1A} animals reflected the significantly decreased body weights observed at termination of the study (these differences are similar to those provided in Table 1 above), and there were no statistically significant differences between the treated and control groups with respect to organ to body weight ratios.

In the high dose group F_{1A} males, the testes weights averaged 3.061 g compared with 3.705 g in the controls ($p<0.01$), and the testes to body weight ratio (% body weight) for the control and high dose groups averaged 0.630 and 0.677, respectively (no statistically significant difference).

Gonad weight results for F_{1A} female adults showed differences similar to those found in males. The mean ovary weights for the high dose group and controls were 0.142 g and 0.103 g, respectively ($p<0.05$), and the respective ovary to body weight ratios for those two groups were 0.044 and 0.043.

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9. REPORTED RESULTS (continued)

Table 5 summarizes the incidence of mortality and reproductive performance of the F_0 and F_{1A} parental animals. There were no dose-related effects on reproductive performance according to the report.

Table 5

Summary of adult performance

Category	Dose (ppm)							
	F_0 generation				F_1 generation			
	0	7.5	37.5	75	0	7.5	37.5	75
Group size:								
Males	15	15	15	15	15	15	15	15
Females	25	25	25	25	25	25	25	25
	Maturation Phase							
Mortality								
Males	0	0	0	1	0	0	0	3*
Females	0	0	0	0	1	2	1	6**
Females paired	25	25	25	25	25	25	25	24
Females mated successfully	25	24	24	23	24	20	23	23
Males failing to sire a litter	0	2	1	2	1	1	1	0
Females with viable litters	24	19	22	22	21	18	21	23
Females rearing young to weaning	23	19	22	21	20	18	21	21

*All but one died before weaning of the F_{1A} litters. The two weanlings that died were replaced with litter mates.

**All of the deaths occurred before the F_{1A} litters were weaned, the animals (with one exception) were replaced with littermates.

Selected group differences for other weighed organs (F_{1A} adults only) are listed in Table 6. The most frequently observed histopathological lesions in F_0 and F_{1A} adult animals are summarized in Tables 7 and 8, respectively. There were no dose related effects noted by the investigators.

Effects on offspring: Pup weights and litter size were affected at the highest dose tested (see Tables 9 and 10), and the 37.5 ppm diet was associated with decreased pup weights during lactation.

Organ weights in weanlings reflected the reduced body weights for pups in the highest dosed group (see Table 11 below).

Table 6

Selected organ weight (g) and organ to body weight ratio (%) data from F_{1A} adults

	Dose (ppm)					
	Weights (g)			% body weight		
Organ	0	37.5	75.0	0	37.5	75.0
Males						
Body weight	593	543	454**	--	--	--
Brain	2.22	2.15	2.03**	0.378	0.400	0.450**
Heart	1.625	1.519	1.454**	0.275	0.281	0.321**
Liver	21.60	18.17*	18.53**	3.639	3.351	4.077**
Kidney	3.788	3.262**	3.204	0.643	0.603	0.708*
Females						
Body weight	328	274**	238**	--	--	--
Heart	1.159	1.141	1.013**	0.354	0.418**	0.427**
Liver	11.33	10.50	8.48**	3.455	3.832**	3.562
Kidney	2.230	1.968*	1.776**	0.681	0.719	0.749

*Statistically significantly different from the control group mean, $p<0.05$.

**Statistically significantly different from the control group mean, $p<0.05$.

The authors noted that the most frequently observed microscopic lesions were in the livers of weanling rats. These results are summarized in Table 11.

Table 12 summarizes organ weight results for weanling animals, but they did not indicate a treatment-related effect. These results were similar to those reported for adult animals in that they reflected the reduced body weights observed at the mid and high dose levels.

10. DISCUSSION

Shortly after diets were offered twice weekly to the test animals, group mean body weights for mid and high dose animals dropped below that for controls (see page 4 and Tables 1 through 4 above). Food consumption was decreased for the high dose group males (Table 3) which could be associated with the weight losses observed in that group, but there were no similar decreases for high dose group females. The weight differences for the mid and high dose group animals increased during lactation and were somewhat greater during the second generation's maturation than was observed for those two groups in the first. Weight losses persisted until termination of the study (see Table 6), and organ weights were consistently decreased. Mortalities observed in F_{1A} parental animals from the high dose group occurred during lactation or early in maturation

Based on these results, the lowest-effect level (LEL) for parental toxicity is 37.5 ppm, and the no-observed-effect level (NOEL) is 7.5 ppm.

Table 7

Summary of histological observations in parental F_0 animals (only gross lessions and gonads were examined)

Tissue and lesion	Dose (ppm) 0	7.5	37.5	75
[Males]				
Testes*	15	15	15	14
Atrophy/degeneration, bi- and unilateral	1	2	0	1
Mineralization	0	1	0	0
Kidneys*	1	0	0	3
Chronic progressive nephropathy	1	0	0	0
Pelvic dilation	0	0	0	2
Proteinaceous casts	0	0	0	1
Mineralized debris, renal pelvis	0	0	0	1
Thymus*	1	0	0	0
Hemorrhage	1	0	0	0
Eyes*	0	2	0	0
Eyelid*	0	2	0	0
Acanthosis	0	2	0	0
Adipose*	0	0	1	0
Fibrosis	0	0	1	0
Hemorrhage	0	0	1	0
Females				
Ovaries*	25	25	25	25
Kidneys*	4	3	3	2
One examined	1	0	0	0
Chronic progressive nephropathy	3	3	3	1
Pelvic dilation	1	0	0	0
Proteinaceous casts	0	1	0	1
Mineralization	0	2	1	0
Mineralized debris, renal pelvis	0	2	0	0
Mononuclear cell infiltration	0	1	0	0
Uterus*	0	0	0	2
Dilation	0	0	0	1
Cystic endometrial hyperplasia	0	0	0	1
Adipose*	0	0	0	1
Thrombosis	0	0	0	1
Fibrcsis	0	0	0	1

*Number examined.

Table 8

Most frequently reported histopathological observations in F_{1A} parental animals

Tissue and lesion	Dose (ppm) 0	7.5	37.5	75
Males				
Number examined	15	15	15	14
Lungs with bronchi				
Perivascular/peribronchiolar lymphoid hyperplasia	3	6	3	5
Kidneys				
Mononuclear infiltrate, interstitial	11	3	5	7
Liver				
Hepatocytic swelling, periacinar	15	15	12	14
Thyroid				
Ultimobranchial cysts	6	7	5	6
Stomach				
Dilated gastric glands	1	6	7	12
Cecum*				
Parasitism	4	3	2	6
Thymus (no. examined)	13	14	14	14
Hemorrhage	2	6	2	3
Prostate				
Epithelial hyperplasia	0	6	7	2
Females				
Number examined	24	22	24	23
Lungs with bronchi				
Perivascular/peribronchiolar lymphoid hyperplasia	12	14	7	6
Kidneys				
Mineralization	3	6	2	3
Liver				
Hepatocytic swelling, periacinar	16	15	22	16
Bile duct hyperplasia	0	0	0	18
Thyroid				
Ultimobranchial cysts	7	12	5	12
Stomach				
Dilated gastric glands	9	5	7	21
Uterus				
Dilation	4	6	1	6

*Found in other parts of the gastrointestinal tract at similar incidences.

Table 9

Group mean pup weights (g) during lactation

Lactation Day	Dose (ppm) F0 generation 0	7.5	37.5	75	F1 generation 0	7.5	37.5	75
0	6.4	6.5	6.2	5.9†	6.3	6.3	6.2	5.8
4	10.6	11.0	9.3†	8.0†	10.4	10.3	9.4	8.2†
7	16.4	16.8	13.4†	10.8†	15.3	15.2	13.3*	10.9†
14	31.5	31.0	25.2†	17.5†	28.6	28.0	23.7†	17.7†
21	47.5	45.9	37.7†	24.4†	45.6	43.2	36.3†	24.6†

*Statistically significantly different from controls, ($p<0.05$)
†Statistically significantly different from controls, ($p<0.01$)

Table 10

Mean litter size (live pups/litter) during lactation

Lactation Day	Dose (ppm) F0 generation 0	7.5	37.5	75	F1 generation 0	7.5	37.5	75
0	11.8	11.1	11.8	9.7*	11.4	10.7	11.8	9.9*
4[a]	11.7	11.1	11.1	9.3	11.6	10.4	11.3	8.3†
21[b]	9.7	9.6	9.1	8.6	9.5	8.7	9.3	8.0

*Statistically significantly different from controls, ($p<0.05$)
†Statistically significantly different from controls, ($p<0.01$)
[a]Litter size before culling on Day 4 of lactation.
[b]Litter size after culling on Day 4 of lactation.

Table 11

Summary of liver lesions in weanling rats.

Observation	Dose (ppm) Males 0	7.5	37.5	75	Females 0	7.5	37.5	75
F_{1A} Weanlings								
Number examined	5	5	5	5	5	5	5	5
Hepatocytic swelling, periacinar	3	3	3	3	2	4	4	4
Nonsuppurative pericholangitis	0	2	3	0	-*	-*	-*	-*
Fatty change	-*	-*	-*	-*	3	0	0	1
F_{2A} Weanlings								
Number examined	5	5	5	5	5	5	5	5
Hepatocytic swelling, periacinar	3	1	2	1	0	2	1	0
Nonsuppurative pericholangitis	2	0	0	0	-*	-*	-*	-*
Fatty change	1	1	2	2	2	0	0	1
Hepatocellular swelling, diffuse	0	2	1	3	3	2	2	4

*Not observed.

Table 12

Selected organ weight (g) and organ to body weight ratio (%) data from weanlings

Organ	Weights (g) 0	Weights (g) 37.5	Weights (g) 75.0	% body weight 0	% body weight 37.5	% body weight 75.0
			F_{1A} Male Weanlings			
Body weight	46.0	38.4*	25.3**	--	--	--
Brain	1.48	1.46	1.33*	3.249	3.821	5.315**
Heart	0.274	0.285	0.179*	0.593	0.738	0.708
Liver	2.06	1.80	1.29	4.459	4.669	5.000
Kidney	0.650	0.510	0.389**	1.406	1.328	1.524
Gonads	0.283	0.225	0.166**	0.614	0.587	0.649
			F_{1A} Female Weanlings			
Body weight	46.6	35.9*	25.1**	--	--	--
Brain	1.43	1.38	1.24*	3.104	3.865	5.118**
Heart	0.300	0.238	0.180**	0.642	0.666	0.709
Liver	2.29	1.81	1.40	4.797	4.999	5.544
Kidney	0.674	0.546	0.404*	1.425	1.522	1.620
Gonads	0.043	0.033	0.025*	0.094	0.092	0.100
			F_{2A} Male Weanlings			
Body weight	48.7	35.0*	19.5**	--	--	--
Brain	1.49	1.35	1.16**	3.101	3.925	6.005**
Heart	0.285	0.227	0.139**	0.584	0.647	0.715*
Liver	2.27	1.57	1.06**	4.631	4.552	5.355
Kidney	0.665	0.468*	0.293**	1.377	1.350	1.510
Gonads	0.264	0.188	0.113**	0.543	0.538	0.550
			F_{2A} Female Weanlings			
Body weight	47.5	40.5	30.6	--	--	--
Brain	1.40	1.35	1.27	3.046	3.426	4.672*
Heart	0.304	0.253	0.198	0.638	0.647	0.667
Liver	2.46	1.91	1.77	5.098	4.784	6.282
Kidney	0.697	0.550	0.470	1.459	1.381	1.647
Gonads	0.037	0.039	0.028	0.078	0.099	0.093

Dose (ppm)

*Statistically significantly different from the control group mean, $p<0.05$.
**Statistically significantly different from the control group mean, $p<0.05$.

10. DISCUSSION (continued)

There were no effects on mating or fertility in males or females (see Table 5), and rotenone did not affect the proportion of pups born alive (97 to 100% of the pups were born alive). However, group mean litter sizes at birth in the high dose groups for both generations were statistically significantly reduced (see Table 10). Dams in that group had substantially reduced body weights and body weight gain during gestation and lactation (see Table 4), and pup weights were also significantly reduced in both the mid and high dose groups, (see Table 9). Organ weight changes observed in the weanlings examined were consistent with those observed in parental animals in that they reflected the body weight reductions observed in the mid and high dose groups. The similarity of these results to those observed in parental animals suggests that the LEL for toxicity in young animals is 37.5 ppm, and the NOEL is 7.5 ppm in younger rats.

The data presented in the report were adequate to support the conclusions of the investigators that the NOEL for rotenone in the diet of rats is 7.5 ppm. Reduced parental and neonatal weight gain was observed in the 37.5 ppm dosed group, and at the 75 ppm level, reduced litter size was noted. Mating behavior and fertility were unaffected by rotenone treatment.

DATA EVALUATION RECORD

1. CHEMICAL: Rotenone

2. TEST MATERIAL: The test material was 99% rotenone.

3. STUDY/ACTION TYPE: Mutagenicity - differential toxicity in *Escherichia coli*.

4. STUDY IDENTIFICATION: Van Goethem, D., B. J. Barnhart, and S. S. Fotopoulos. April 1, 1981. Mutagenicity Studies on Rotenone: Final Report. Unpublished report no. 7029-E prepared by Midwest Research Institute, Kansas City, MO. Submitted by U. S. Fish and Wildlife Service, Department of the Interior. EPA Acc. No. 254719.

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7. CONCLUSIONS: The report did not included individual data from replicates in each assay, and marginal responses in the liquid suspension assays (see REPORTED RESULTS Section below) cannot be fully assessed because of that deficiency. The report is therefore considered incomplete until individual data for the replicates is submitted.

Core classification: Unacceptable (see Sections 7. and 10.)

8. MATERIALS AND METHODS

Test species: *Escherichia coli* strains W3110 (pol A^+) and p3478 (pol A^-) were used. The latter strain is deficient for a DNA excision repair enzyme.

Culture media: A medium designated HA+T with 1.5% agar was used for plates, and the same medium with 0.75% agar was used for the top agar.

Preparation of S-9 fraction: Male Sprague-Dawley rats (180-200 g) were given a single intraperitoneal injection of 0.5 mg Aroclor 1254 per g body weight. Five days after dosing

8. MATERIALS AND METHODS (continued)

the animals were sacrificed, and the livers were removed and prepared for centrifugation at 9000 X g in 0.15 M KCl (25% w/v). The supernatant (S-9 fraction) was decanted and stored in 2 ml aliquots at -80° C until they were needed.

Each 1 ml sample of the metabolic activation mixture consisted of 4 umoles NADP, 5 ul glucose-6-phosphate, 33 umoles KCl, 8 umole $MgCl_2$, 100 umoles sodium phosphate buffer (pH 7.4), and 100 ul S-9 fraction.

Experimental procedure---Spot test: The test substance was dissolved in dimethylsulfoxice (DMSO) at concentrations of 15, 75, or 150 mg/l, and 10 ul of each solution was used per plate (150, 750, or 1500 ug/plate). Two replicate plates were used for each test substance concentration, and a set was used with or without metabolic activation mixture. One-tenth ml of a log phase culture was added to 2.5 ml molten top agar which was then spread evenly over HA+T agar plates. After the overlay solidified, 6.35 mm filter paper disks saturated with a test solution was placed at the center of each plate. The report stated that control plates included: bacteria alone, 10 ul DMSO, 10 ul of methylmethane sulfonate (MMS, reference mutagen), and 30 ug chloramphenicol (negative control).

All plates were then incubated for 16 hours at 37° C. At the end of incubation, the zones of inhibition were measured, and their widths were noted in mm.

One-half ml of the metabolic activation was added to the 2.5 ml molten agar before spreading on the bottom agar. For these assays, cyclophosphamide was used as the positive control and an S-9 sterility control was included with the other controls mentioned above.

Experimental procedure---liquid assays: For assays in liquid medium, concentrations of 0.1, 1, 10, or 50 ug/ml were used. The report noted that a visible precipitate was formed. The authors further stated that because of the insolubility of the test substance in the broth medium, concentrations capable of killing 50% of the DNA repair proficient strain could not be attained.

Test cultures were diluted to a cell density of approximately 10^4/ml, and 0.5 ml was added in tubes to 100 ul of a test substance solution without metabolic activation and 0.5 ml HA+T medium. For assays with metabolic activation, 0.5 ml of the S-9 mixture was added to the diluted cultures. All of the tubes were then incubated at room temperature and with shaking for 90 minutes.

8. MATERIALS AND METHODS (continued)

At the end of the incubation period, 100 ul aliquots of each culture were added to 2.5 ml molten HA+T top agar. These mixtures were then spread over HA+T plates, and the plates were incubated at 37° C for 24 hours in the dark. Triplicate plates were used for each test substance concentration and control solution. Controls included N-methyl-N-nitroso-nitrosoguanidine (5ug/ml without metabolic activation mixture), 2-aminofluorene (200 ug/ml with metabolic activation mixture), 100 ul DMSO (solvent control), and 15 ug/ml penicillin (negative control).

Interpretation of results: Zones of inhibition in the spot test were measured to the nearest mm, and a mean was calculated for 3 plates at each dose. Differences in the zone diameters for the pol A^+ and pol A^- strains were also noted. If the diameter of the zones of inhibition on the repair deficient lawn is greater than that on the repair proficient lawn for more than one dose, the chemical is considered to cause a positive response.

Plates from the liquid suspension assays were counted after the 24-hour incubation period, and the means for the three plates from each dose group were calculated. These values were then used to calculate survival with respect to the solvent control. Survival indices were calculated as a ratio between the percentage survival of pol A^- and that for pol A^+. The report stated that survival indices of 0.96 to 1.0 indicated an absence of preferential toxicity of the test substance for the DNA repair deficient strain. Indices between 0.86 and 0.95 were considered by the investigators to be a marginal response, and values below 0.85 were considered to be a positive response. A dose-dependent decrease below 0.85 is considered to be confirmatory evidence of a positive result.

Spot test: The authors noted that the test substance did not diffuse from the filter paper disks into the aqueous medium of the agar plates. There were no zones of inhibition around test substance disks.

The diameters of zones of inhibition on the positive control plates were reported as follows:

Test material	Diameter (mm) Pol A^+	Pol A^-
Chloramphenicol*	21	21
Methylmethane sulfonate*	25	51
Cyclophosphamide**	19	13

*Without metabolic activation
**With metabolic activation

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9. REPORTED RESULTS

Liquid suspension assays: The report stated that the 1 ug/ml concentration of rotenone without metabolic activation caused a marginal response (survival index of 0.92), but in a repeated test at that concentration the survival index was found to be 0.98. For the 5 and 10 ug/ml concentrations of rotenone with metabolic activation the responses were marginal (survival indices of 0.94).

10. DISCUSSION

The report did not included individual data from replicates in each study. Because of the marginal responses in the liquid suspension assays (see REPORTED RESULTS Section above), an independent interpretation of the results cannot be made. The report cannot be considered complete until individual data for the replicates is submitted.

DATA EVALUATION RECORD

1. CHEMICAL: Rotenone

2. TEST MATERIAL: Rotenone of >97% purity was used.

3. STUDY/ACTION TYPE: Mutagenicity - yeast assays (including reverse mutation, mitotic recombination, mitotic gene conversion) and a mouse assay (in vivo somatic cell point mutation assay in embryonic melanocytes).

4. STUDY IDENTIFICATION: Litton Bionetics, Inc. June 24, 1981. Mutagenicity Studies on Rotenone: Final Report. Unpublished report no. 22063 prepared by Litton Bionetics, Inc., Kensington, MD. Submitted by the U. S. Fish and Wildlife Service, National Fishery Research Laboratory. EPA Acc. No.254720.

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Signature: [illegible]
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7. CONCLUSIONS: Rotenone did not induce reverse mutations in yeast (Saccharomyces cereviciae strains S138 and S211) at dose levels up to 10,000 ug/ml. No mitotic recombination or mitotic gene conversion was observed in yeast (strains D4 and D5) at the same dose levels.

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 induce 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.

Core classification: Acceptable

8. MATERIALS AND METHODS

Test species: The organisms used for the assays reported are as follows:

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8. MATERIALS AND METHODS (continued)

Assay	Test organism
Reverse mutation assay	Haploid yeast* strains S138 (frameshift) and S211 (base substitution)
Mitotic recombination	Diploid yeast* strain D5 heteroallelic at the ade2-40 and ade2-119 loci
Mitotic gene conversion	Diploid yeast* strain D4 heteroallelic at the ade2 and trp5 loci
Somatic mutation assay	Mouse embryonic melanocytes (in vivo) from mating of T strain males (homozygous recessive alleles at 5 coat color loci) with C57Bl/6J females (homozygous wild type alleles)

*Saccharomyces cereviciae

Vehicle: Ethanol was used as the solvent for the test substance in the yeast studies. DMSO and corn oil were used in the mouse studies.

Reference mutagens: Ethylmethane sulfonate (EMS), dimethyl nitrosamine (DMN), quinacrine mustard, and 2-anthramine (ANTH) were used for yeast assays without or with metabolic activation, respectively. Ethylnitrosourea was used as the positive control in the mouse assay.

Culture media for yeast: Stock cultures of yeast strains were maintained on yeast complete medium slants or plates at 4° C. Stock suspensions were obtained from stationary phase cultures grown at 30° C in yeast complete broth (strains D4 and D5) or in yeast extract peptone (strains S138 and S211).

Selective medium for the D4 strain consisted of minimal yeast medium supplemented with 30 ug/ml adenine. The report stated that the overlay medium consisted of 0.6% purified agar with 0.1M NaCl and a trace amount of tryptophan. Sel.ctive medium for the reverse mutation assay (strains S138 and S211) consisted of minimal yeast medium, and the overlay was 0.6% agar with 0.1M NaCl.

Metabolic activation (S-9) system: Liver microsomal preparations were obtained from Aroclor 1254 induced Sprague-Dawley rats (commercial source). The S-9 mixture also contained the following:

8. MATERIALS AND METHODS (continued)

$MgCl_2$	8 umoles
KCl	33 umoles
NADP (sodium salt)	4 umoles
Phosphate buffer (pH 7.4)	100 umoles
Glucose-6-phosphate	5 umoles
Rat liver homogenate (S-9)	100 uliters

Experimental procedures---Preliminary range-finding assays with yeast: The report stated that all yeast assays were conducted at 4 or 5 concentrations of the test substance.

Reverse mutation assay: A 0.05 ml aliquot of the test substance in ethanol was added to a tube containing the overlay agar, and 0.1 to 0.2 ml of the test strain culture were added. The mixture was swirled gently and poured over the surface of minimal agar plates. These plates were incubated at 30° C for four to seven days. After incubation, the number of methionine revertant colonies on each plate was counted. In tubes used for the activation assay (with the S-9 mix added), 0.5 ml of the liver homogenate mixture was added, but in the assays without the mixture, 0.5 ml phosphate buffer was added. A negative control with ethanol or DMSO was tested with and without the activation mixture, and the positive controls were tested in the appropriate solvent (quinacrine mustard in DMSO, ethylmethane sulfonate used undiluted, and 2-anthramine in DMSO).

Mitotic recombination assay in yeast: A minimum of 5 doses were selected on the basis of preliminary toxicity studies. A 0.05 ml aliquot of the test substance solution, positive control solutions, or solvent control were added to culture tubes. One- to two-tenths ml of the test culture were then added, and 0.5 ml phosphate buffer or S-9 mixture were added. After incubation on a rotary shaker at 30° C for 3 hours, These mixtures were then removed and diluted with 0.15 M saline solutions. They were plated on yeast complete medium and incubated for four days at 30° C followed by 1 to 3 days refrigeration to maximize the appearance of colony coloration. The plates were then screened for pigmentation and sectoring (at least 20,000 colonies per dose level). A solvent control and two positive control solutions were also tested. The positive control for the assay without S-9 activation was EMS, and that for the assay with activation was DMN.

The report states that the phenotypes representing the occurrence of crossover events in D5 strain yeast is the appearance of red-pink cells and red-white or pink-white sectored colonies.

Mitotic gene conversion assay in yeast: A 0.05 ml aliquot of the test substance in ethanol was added to a tube containing

8. MATERIALS AND METHODS (continued)

the overlay agar, and 0.1 to 0.2 ml of the test strain culture were added. The mixture was swirled gently and poured over the surface of minimal agar plates. These plates were incubated at 30° C for four days. After incubation, the number of tryptophan convertant colonies on each plate was counted. In tubes used for the activation assay (with the S-9 mix added), 0.5 ml of the liver homogenate mixture was added, but in the assays without the mixture, 0.5 ml phosphate buffer was added. Negative controls with ethanol or DMSO were tested with and without the activation mixture, and the positive controls were tested in the appropriate solvent (ANTH in DMSO, and EMS used undiluted).

Mouse in vivo somatic cell mutation assay: Female C57Bl/6J mice were mated with T strain male mice. When vaginal plugs were found, each female was randomly assigned to a test group, and each group contained 50 females.

The investigators stated that preliminary trials to determine the lethality of the test material in DMSO or corn oil were used as the basis for dosage and vehicle selection. DMSO was chosen as the vehicle for the main study, and a group of mice was also given 1000 mg/kg doses of the test substance in corn oil. Along with DMSO and corn oil control groups, there were groups given daily doses of 0.05, 0.17, 0.5, or 1.0 mg test substance per kg body weight. Doses were administered by gavage on days 8, 9, 10, and 11 of gestation. A positive control group was given a single intraperitoneal injection of ethylnitrosourea (50 mg/kg) on day 10 of gestation.

A second trial was conducted with the DMSO vehicle diluted to 50% in corn oil. The authors stated that this was done to reduce the volume of DMSO administered.

The mice were observed for the occurrence of toxic signs or mortality during the study. Dams were allowed to deliver their pups, and the pups were examined at birth for gross abnormalities. On day 12 of lactation, the pups were scored for spots. They were examined again for spots at weaning.

The frequency of coat color spots in the test groups were compared with that observed in the control group using Fisher's Exact test. If there were statistically significant increases ($p<0.05$) and a dose-response relationship was seen, the results are considered to be positive according to the report.

Yeast studies: Tables 1, 2, and 3 summarize reported results for the yeast studies which the investigators described as negative (see Section 10. DISCUSSION below for details).

9. REPORTED RESULTS

In vivo somatic mutation study: Table 4 summarizes results from the mouse test (see Section 10. DISCUSSION below for details).

Preliminary results on which dose selection in the main experiment was based were described as follows:

> ...Rotenone was administered to mice by oral gavage both in corn oil (as a suspension) and DMSO (as a solution). The LD50 values obtained were markedly different. Rotenone prepared in corn oil was essentially non-toxic producing death in 1 of 4 females after 14 days following a single dose of 1000 mg/kg and no deaths at 330 mg/kg. Rotenone prepared in DMSO produced death in 6 of 6 female mice dosed acutely with 330 mg/kg, 100 mg/kg, and 33 mg/kg. The LD50 was near 3 mg/kg; however, the data generated in these studies was not amenable to exact calculation of an LD50...

According to the report, several animals in the main study given the 0.5 mg/kg dose were lethargic and clammy. There were more animals from the 1.0 mg/kg dose group appearing lethargic and clammy, and some animals were reported to exhibit convulsions before death. No signs of toxicity were observed in the 0.05 or 0.17 mg/kg/day dosed groups.

10. DISCUSSION

Yeast assays: In the introduction of the report, the authors state:

> It should be noted that activation (reverse mutation) assays with yeast do not typically employ positive activation-dependent controls since compounds giving reproducible responses are not known. We have used compounds such as dimethylnitrosamine or 2-aminoanthracene since these compounds do work occassionally; however, the adequacy of S9 mix is based on other quality control procedures and not on the response in the plate assay. The only positive controls employed for yeast plate technique are nonactivation controls.
>
> ...Strain D5 assays are suspension assays and as such can be conducted with an adequate S9 activation positive control agent.
>
> Studies with D4 were conducted as plate tests and have the same inherent problems with S9 activation

Table 1

Results of the reverse mutation test in yeast
(exerpted from the original report)

Dose (ug/plate)	Without activation: Base substitution[c]	Without activation: Frame shift[d]	With activation: Base substitution[c]	With activation: Frame shift[c]
EtOH[a]	14	3	15	3
EMS[b]	1030	--[b]	--[b]	--[b]
Quinacrine mustard	--[b]	201	--[b]	--[b]
ANTH[d]	--[b]	--[b]	25	4
1	14	2	22	7
10	13	2	12	5
100	16	3	17	2
500	15	1	11	4
1,000	15	5	15	4
2,500	10	2	13	4
5,000	9	3	14	7
10,000	17	3	15	6

Type of mutation (number of reverants/plate)

[a]Vehicle control
[b]Not tested
[c]One trial
[d]Three trials (average count is presented)

Table 2

Summary of the reported frequencies of crossover events in D5 yeast (events per 10^3 surviving cells)

Dose (ug/plate)	Without activation	With activation: Trial 1	With activation: Trial 2
EtOH[a]	0.22	---[b]	0.19
EMS[b]	18.72	---[a]	---[a]
DMN	---[a]	32.50	0.80
1	0.35	0.09	0.08
10	0.38	0.67	0.13
100	0.49	0.39	0.24
1,000	0.22	0.33	0.09
10,000	0.26	0.44	0.18

[a]Not done

[b]No crossover events were detected. The authors stated that they repeated the trial (Trial 2)

Table 3

Mitotic gene conversion results
(tryptophane convertants per plate)

Dose (ug/plate)	Without activation	With activation
EtOH	108	127
EMS	1,103	---
ANTH	---	139
1	90	124
10	103	126
100	116	103
500	118	106
1,000	111	108
2,500	114	119
5,000	118	111
10,000	98	107

10. DISCUSSION (continued)

as the reverse mutation studies. Again, the adequacy of the S9 to activate 2-anthramine was established in advance.

These considerations represent a limitation of the yeast assays with respect to the sensitivity to compounds requiring metabolic activation.

The authors noted that the negative control plates for the first trial in the mitotic recombination assay with strain D5 and S9 activation did not have sufficient growth. Because there were no differences in the responses of all dose groups, the investigators concluded that rotenone had no effect. A second trial with activation was conducted and the conclusion was confirmed. They also noted that the positive control response was slightly less than that expected (approximately 4 times that in the negative control; see Table 2 above).

A third trial with strain D5 was rejected by the investigators because the population on the plates was too sparse to provide an adequate number of colonies for scoring (>20,000).

The preliminary toxicity studies indicated that dose levels up to 10,000 ug test substance per ml medium had relatively low toxicity (approximately 60% for strain S211, and 90 to 95% for strains D4 and D5).

Inconsistent results mentioned above with known mutagens that require activation and the apparently low toxicity of rotenone

9. REPORTED RESULTS (continued)

Table 4

Summary of mortality, reproductive, toxicity, and genetic parameters during treatment of pregnant mice

Observation	Dose (mg/kg/day) 0	0.05	0.17	0.50	1.00	
	DMSO vehicle					
No. died		5	2	7	11	8
No. pregnant/no. mated	10/91	11/91	19/90	9/90	6/50	
No. pups born alive	47	44	90	48	25	
No. pups/litter	4.7	4.0	4.7	5.3	4.2	
No. pups surviving to Day 12	29	36	76	39	13	
No. with melanocyte toxicity[a]	0	2	0	0	0	
No. with recessive coat spots[b]	0	1	3	0	0	
	DMSO/corn oil vehicle					
No. died	---	1	1	---	1	
No. pregnant/no. mated	11/50	16/50	4/25	---	13/49	
No. pups born alive	64	103	24	---	65	
No. pups/litter	7.1	6.4	8.0	---	7.2	
No. pups surviving to Day 12	55	71	22	---	44	
No. with melanocyte toxicity[a]	0	1	0	---	0	
No. with recessive coat spots[b]	3	3	0	---	2	

[a]Indicated by midventral white spots.
[b]Indicated by spots of the following colors or with the following traits: non-agouti, brown, chinchilla, pink eyed dilution, dilute, short ear, pibald

9. REPORTED RESULTS (continued)

Table 4 (continued)

Observation	Corn oil with 1000 mg/kg	ENU Positive control Trial 1	ENU Positive control Trial 2
No. died	11	--	--
No. pregnant/no. mated	9/50	28/90	8/25
No. pups born alive	50	113	61
No. pups/litter	6.3	4.3	7.6
No. pups surviving to Day 12	49	89	52
No. with melanocyte toxicity[a]	7	22	5
No. with recessive coat spots[b]	1	21	5

[a]Indicated by midventral white spots.
[b]Indicated by spots of the following colors or with the following traits: non-agouti, brown, chinchilla, pink eyed dilution, dilute, short ear, pibald

Table 5

Summary of the incidence of coat color changes (no. of 12 day old pups with spots/no. examined) for DMSO and DMSO/corn oil groups combined

Dose (mg/kg/day)	Midventral white spots*	Recessive coat color spots
0	0/84	3/84
0.05	3/107	4/107
0.17	0/98	3/98
0.50	0/39	0/39
1.00	0/57	2/57
1000**	7/49	1/49
ENU***	0/141	26/141

*Indicates toxicity to melanocytes.
**Only group treated with test substance in corn oil
***Positive control, 50 mg/kg dose level.

10. DISCUSSION (continued)

in these assays are two factors suggesting that yeast cells may not be permeable for the test substance. These considerations diminish confidence in the sensitivity of yeast assays which emphasizes the need to use their results along with those from other test systems to form a conclusion about the mutagenic potential of rotenone.

There were adequate results presented in the report to support the conclusion that rotenone has no genotoxicity under the limited conditions of the experiments described.

Somatic cell mutation assay: In a discussion of the results, the authors called attention to the following considerations:

1. A generally lower fertility rate and decreased embryonic development were noted (fertility rates for C57Bl/6 X T matings are usually 30 to 40% according to the report).

2. Rotenone was toxic to the pregnant mice, but that toxicity was not reflected in the increased incidence of midventral white spots (the indicator of melanocyte toxicity) in the pups (except in the group given the 1000 mg/kg dose in corn oil).

3. The number of pups available for scoring was lower than expected because of the decreased fertility rate. However, the authors concluded that the results were clearly negative.

4. The suspension of rotenone in corn oil at a dose of 1000 mg/kg did not increase the incidence of pups with recessive spots.

Results from the preliminary and main mutagenicity studies are generally consistent with those reported in range-finding and full teratology studies with mice that evaluated Rotenone in corn oil (see Section 11., below for citations). A dose of 24 mg/kg/day administered in corn oil by gavage to 7 pregnant mice from day 6 through 19 of gestation resulted in 3 deaths. Results from the two teratology studies suggested that a no-observed-effect level (NOEL) for maternal toxicity in mice is between 15 and 24 mg/kg/day. The choice of DMSO as a vehicle apparently enhances the toxicity of rotenone to pregnant mice.

Litter sizes (mean number of live fetuses per litter) reported in the mouse teratology studies were 11 to 12, while litter sizes in the mutagenicity studies were substantially less in those groups treated with the test substance in DMSO alone

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10. DISCUSSION (continued)

(see Table 4 above). Those animals given the test substance in DMSO/corn oil had a somewhat higher average litter size.

Although the decreased fertility and litter sizes noted in the study reduce the number of pups available for scoring of coat color spots, two trials (one with DMSO as the solvent and one with a DMSO/corn oil vehicle) as well as the addition of a 1000 mg/kg dose group were evaluated to compensate for those limitations. Results shown in Table 4 suggest that the responses are similar with respect to the incidence of midventral white spots and recessive color spots for all test groups (except the 1000 mg/kg dose group). The combined data (see Table 5) for those groups given the test substance in DMSO and DMSO/corn oil provide adequate numbers of pups to support the conclusion that rotenone did not cause somatic mutations in mice under the test conditions.

11. REFERENCES

Hazleton Raltech, Inc. November 24, 1981. Final Report: Range Finding Study with Rotenone in Pregnant Mice. Unpublished report No. 80049 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254722.

Hazleton Raltech, Inc. November 24, 1981. Final Report: Teratology Study with Rotenone in Mice. Unpublished report No. 80050 prepared by Hazleton Raltech, Inc. Submitted by U. S. Fish and Wildlife Service. La Crosse, WI. EPA Acc. No. 254724.

DATA EVALUATION RECORD

1. CHEMICAL: Rotenone

2. TEST MATERIAL: Rotenone of unspecified purity was used.

3. STUDY/ACTION TYPE: Mutagenicity - chromosome damage assays in rats, mice, fruit flies, and horse beans

4. STUDY IDENTIFICATION: Biotech Research Laboratories, Inc. January 10, 1982. Analytical studies for the detection of chromosomal aberration in fruit flies, rats, mice, and horse beans. Unpublished report prepared by Biotech Research Laboratories, Inc. Submitted by the U. S. Fish and Wildlife Service, National Fishery Research Laboratory, La Crosse, Wisconsin. EPA Acc. No. 254721.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch

Signature: Roger Gardner
Date: 11-19-85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch

Signature: Jane C Harris
Date: 11/26/85

7. CONCLUSIONS: 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. Because of the limited solubility of rotenone in aqueous solutions (20 ug/ml), the assays in fruit flies and horse beans are not likely to detect potential genotoxicity under the test conditions described in the report.

Core classification: Acceptable for the rodent studies, and unacceptable for the fruit fly and horse bean studies.

8. BACKGROUND

The above cited report was previously submitted under EPA Acc. No. 246587 and reviewed by the Toxicology Branch (see Memorandum dated June 14, 1982. To: W. Miller, Registration Division. From: R. Gardner. Subject: Review of Mutagenicity Assays with Rotenone. EPA Reg. No. 6704-Q. Acc. No. 246587. Tox. Chem. No. 725.).

8. BACKGROUND (continued)

Experiment 1: In vivo cytogenetics study in rats.

The protocol was described as follows:

> This study was conducted in 8 to 10 week old male Sprague-Dawley rats. The test material was administered by oral gavage in corn oil. A preliminary toxicity study was conducted with groups of 20 rats given single doses of 50, 100, 200, or 300 mg per kg body weight. Based on mortality during a 14-day observation period following treatment, doses for the main study were selected. The doses selected for testing were 0, 0.7, 2.5, or 7.0 mg/kg...

In a letter (to Dr. Fred P. Meyer of the Fish and Wildlife Service from Dr. Thomas M. Li of Biotech Research Laboratory dated August 26, 1982) submitted with the report cited above, one aspect of the protocol was described as follows:

> Three hours before the animals were sacrificed for bone marrow collection, they were injected i. p. with colchicine at 4 mg/kg body weight...

The letter also included a schedule indicating the animals were sacrificed approximately 48 hours after dosing. This information was provided in response to Toxicology Branch comments made in the above cited review.

The Toxicology Branch review further stated:

> Trimethylenemelamine (TEM) was used as the positive control substance.

Experiment 2: Mouse micronucleus test.

The previous Toxicology Branch review described this experiment as follows:

> ...male and female ICR Swiss mice were given doses of rotenone in corn oil by oral gavage. Based on results from a preliminary acute toxicity study doses for the main study were chosen. These doses were 0, 10, or 80 mg/kg. The positive control group was given 1.0 mg TEM per kg body weight.

The following additional information was provided in the letter described above

> Mice...received two consecutive doses of rotenone before sacrifice. The first dose was delivered on June 27, 1981 and the second dose was administered

8. BACKGROUND (continued)

24 hours later. The animals were then sacrificed for the extraction of bone marrow and for making smears 6 hours following the second dose.

Experiment 3: Sex chromosome loss in *Drosophila melanogaster*.

Appendix A contains appropriate exerpts of the previous Toxicology Branch review for this experiment, and Appendix B contains the additional information submitted by the Registrant in the letter described above.

It should also be noted that page 4 of the report cited in item 4., above is missing.

9. MATERIALS AND METHODS

Experiment 1: *In vivo* cytogenetics study in rats.

Experimental procedures: Doses were selected on the basis of a preliminary study (see Section 8. BACKGROUND above). The highest dose was described as equivalent to 1/10th the oral LD_{50} of 71 mg/kg, and the mid and low dose groups were 1/3 and 1/10 of that value.

Three groups containing 10 rats were given single oral doses of 0.7, 2.5, or 7 mg rotenone per kg body weight. The test substance was administered in corn oil by gavage. One group of 10 rats was given corn oil without the test substance, and a second group of the same size was given 1.0 mg trimethylenemelamine (TEM) per kg body weight.

Test animals were sacrificed 48 hours after dosing. Three hours before the animals were sacrificed each was given an intraperitoneal injection of 4 mg colchicine per kg body weight to arrest dividing cells in c-metaphase.

The report stated that bone marrow cells were then harvested from both femurs by aspiration with Hank's Balanced Salt solution. Cells were treated with a hypotonic KCl solution (0.075 M) and fixed with glacial acetic acid:methanol (1:3). Slides were made from these preparations and flame dried. They were stained with Giemsa stain and mounted in Permount for microscopic observation.

The report noted that 400 cells from each slide were examined. Chromosomal abnormalities were classified as chromatid gaps, chromosome gaps, breaks, fragments, minutes, or others.

Cytotoxicity was determined by the mitotic index for each sample.

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9. MATERIALS AND METHODS (continued)

Experiment 2: Mouse micronucleus test.

Experimental procedure: A preliminary acute oral toxicity study was conducted with Swiss albino mice to determine dosage levels to be used in the main study. Single gavage doses of 100, 300, 600, or 1000 mg test substance per kg body weight were administered in corn oil to groups of 20 mice. The animals were observed for mortality for 14 days following dosing. An LD_{50} of approximately 164 mg test substance per kg body weight was estimated.

Groups containing 20 mice were given corn oil, a 0.5 mg/kg dose of TEM or 10 or 80 mg test substance per kg body weight. The animals were sacrificed 24 hours after dosing, and both femurs of each mouse were removed. Marrow was collected, centrifuged, and resuspended in fetal bovine serum. Smears of the bone marrow suspensions were made on glass slides, and the slides were dried and stained with Giemsa stain. One-thousand polychromatic and normochromatic erythrocytes were examined on each slide. Cells containing micronuclei were noted along with any evidence of cytotoxicity.

Experiment 3: Sex chromosome loss in Drosophila melanogaster.

See Section 11. DISCUSSION below.

Experiment 4: Chromosome damage in Vicia faba (horse bean)

See Section 11. DISCUSSION below.

10. REPORTED RESULTS

Preliminary toxicity studies: All 20 of the male rats given the 200 mg/kg dose died within 2 days after dosing. Nine of the animals given the 50 mg/kg dose died. All of the survivors gained weight during the 14 days following dosing, and the LD_{50} was calculated to be 71 mg/kg (the method for calculating the LD_{50} value was not described in the report).

According to the report, 10 or 20 mice of both sexes given 1000 mg/kg died. In the 100, 300, or 600 mg/kg dose groups there were 2, 9, or 6 deaths, respectively. The authors stated that these data were plotted and fitted to a line by an unspecified statistical procedure. They stated that a line was drawn with 95% confidence, and from that an LD_{50} of 640 mg/kg was determined.

Doses for Experiments 1 and 2 were selected according to these results.

Experiment 1: The number of chromosomal abnormalities observed 24 hours after dosing were reported as follows:

10. REPORTED RESULTS (continued)

Type of abnormality	Dose (mg/kg) 0	0.7	2.5	7.0
Chromatid gaps	20	10	31	11
Chromatid breaks	9	4	1	4
Chromosome gaps	1	1	1	0
Chromosome breaks	0	0	0	0
Chromosome fragments	2	0	6	1
Others	3	0	5	1
Total	35	14*	66*	17
Per cent incidence**	0.0088	0.0035	0.0110	0.0043

*These values appear to be discrepancies
**Number of cells with aberration per 4000 cells examined from 10 animals.

The average number of mitotic cells observed per 1000 examined for the 0, 0.7, 2.5, and 7.0 mg/kg dose groups were 263, 233, 287, and 301, respectively. These corresponded to mitotic indices of 26.3, 23.3, 28.7, and 30.1%. Ranges were reported also. For the control, low, mid, and high dose groups those values were 4 to 53, 4 to 45, 6 to 47, and 9 to 62, respectively.

There were no individual animal data or positive control results included in the report.

Experiment 2: The reported results (per cent of cells examined with micronuclei) from the micronucleus test in mice are reproduced as follows:

Cell type	Dose (mg/kg) 0	10	80	TEM*
Polychromatic				
One micronucleus	0.043	0.031	0.029	16.588
Micronuclei	0	0	0	0.013**
Total	0.043	0.031	0.029	16.601
Normochromatic				
One micronucleus	0.043	0.034	0.028	0.338**
Micronuclei	0.001	0.004	0.010**	0.053**
Total	0.044	0.038	0.038	0.391

*Positive control administered at a dose of 1.0 mg/kg.
**Statistically significantly different from controls at $p<0.05$.

Experiments 3 and 4: See Section 10. DISCUSSION below.

11. DISCUSSION

Experiment 1: Although the protocol stated that TEM was used as the positive control substance, there were no results reported for that group of rats. In addition, no individual animal data were included.

Despite these deficiencies, the results suggest that a relatively high dose (1/10th the acute oral LD_{50}) had no effect on the incidence of chromosomal abnormalities or mitotic index in rat bone marrow cells at a dose as high as 7.0 mg/kg.

Experiment 2: There were no treatment-related increases in the occurrence of micronuclei in mono- or polychromatic erythrocytes at dose levels as high as 80 mg/kg in mice.

Experiment 3: The following limitations are described in Appendix A below:

1. Scoring of only three- to four-hundred progeny instead of 5000 usually evaluated.

2. Paired mating of 80 to 90 males instead of a mass mating scheme was conducted.

3. Fertility rate was low (approximately 33% or less).

4. Methyl methansulfonate (MMS) was used as a positive control rather than tetraethyleneimino-1,4-benzoquinone which is more commonly used in such studies. There were no results with MMS to indicate that the test conditions were adequate for detection of genotoxicity.

In addition, the investigators point out (see Appendix B) that MMS induced a significant increase in the number of progeny with sex chromosome loss (14 of 5072 progeny in the positive control group compared to 4 of 6344 progeny in the negative control group) in a pilot experiment which followed the mass mating procedure (see point 2. above). The investigators further stated that the low solubility of the test substance in aqueous solutions indicates that an acceptable feeding method for rotenone should be established.

Under these conditions, the Drosophila assay is not acceptable.

Experiment 4: The report noted that the low solubility of rotenone in aqueous media (20 ug/ml) prevented establishment of an LD_{50} in elongating root tip cells of Vicia fabia (horse bean). The investigators decided that the assay would not be appropriate for evaluating the potential of rotenone to cause chromosomal aberrations in plants.

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APPENDIX A

Excerpts of the previous Toxicology Branch review cited on page 3 above

Experiment 3: Sex chromosome loss in *Drosophila*

Males of the [illegible]125, B/y$^+$ strain of *D. Melanogaster* were used.

The test material was first discolved in acetone, and the solution was then diluted with water so the final concentration of acetone was 0.2% or less. This solution, which contained appropriate concentrations of rotenone, was incorporated into the sucrose-agar medium. The positive control substance was methane sulfonate (MMS) in a sintered glass filter. Flies were exposed to the rotenone medium for 24 hours, while those treated with MMS were exposed for 2 days.

Concentration used in this experiment were 0, 1.0, 3.0, or 10.0 mM. After exposure the treated males were mated with virgin d 63 inscy females (one pair to each 2.5X5.0 mm vial containing 1 ml of sucrose-agar medium).

Mating parameters are as follows:

Group	Number mated	Number with progeny
Negative control	90	37
low dose	91	20
mid dose	91	18
high dose	80	15
MMS	84	37

These data indicated a low fertility rate in all groups. The number of progeny from successful matings was reported as follows:

Group	Males	Females	Average No. offspring per parent
Negative control	736	736	39.8
Low dose	363	383	37.4
Mid dose	395	367	42.3
High dose	747	797	41.7
MMS	301	308	40.6

The normal phenotype of male progeny was normal eyes and apricot body, and that for females was bar-eyes and yellow body according to the authors. The phenotypes used to indicate sex chromosome loss were normal eyes and yellow body in males and bar eyes and apricot body. Chemically induced as well as spontaneous nondisjunction are represented by the same phenotype in males so the incidence of females with bar eye and apricot body resulting from paternal non-disjunction is used to correct for spontaneous nondisjunctions that might occur.

The author reported on 1 incidence of nondisjunction in each of the low and mid dose groups' progeny. None were noted in the high dose and positive control groups.

This assay is limited in scope when compared with a protocol recommended by Brusick (1980). In that protocol evaluation of at least 5000 progeny from each group, mating of at least 100 males according to a mass mating scheme, and use of tetraethyleneimino-1,4-benzoquinone as a positive control substance are the three primary differences. The variation in numbers of progeny scored in each group is large with some groups being twice as large as others, and the low fertility reported by the authors suggests that the study is not well conducted. A larger number of progeny would enable a more appropriate interpretation of the chromosome loss observed in the low and mid dose groups. Since the negative control group is twice the size of either the low or mid dose groups, the apparent significance of the reported results is considerable. In addition, the absence of a response in the positive control group suggests that MMS is an inappropriate choice for a reference substance, or the assay is not sensitive.

In view of these considerations the results of this study are unacceptable.

References

Brunsick., D. 1980. Principles of Genetic Toxicology. Plenum Press, New York.

Evans, H. J. 1976. Cytological methods for detecting chemical mutagens. Ch. 35. In. Hollaender, A. Chemical Mutagens: Principles and Methods for their Detection. Plenum Press, New York. pp. 1-30.

APPENDIX B

Additional information on the *Drosophila* sex chromosome loss assay (Experiment 3)

Experiment 3. Sex Chromosome Loss in Drosophila Melanoganster

This test was done based on 100 single-matings for each group following a revised protocol approved by the U.S. Fish and Wildlife Service (USFWS). This was described in our "Clarification for Technical Proposal" submitted on July 7, 1980, in response to "Questions and Deficiencies for RFP-FWS-9-80-06 for Biotech Research Labs" enclosed in a letter from USFWS dated on June 19, 1980. The adoption of the single-mating method in place of a mass-mating procedure was based upon the advantage of the former system for providing a read-out for possible sterilization effects. However, the single-mating system suffered a generally low fertility rate and also produced only a limited number of F_1 flies per mating pair. In order to raise 5000 or more F_1 males, at least 700-800 single-matings made for each dose level would be necessary. Apparently this was limited by the proposed budget and time period for conducting an experiment of such a scale. However this was not a problem for mass-mating system. In a pilot experiment done prior to the single-matings, the following results from mass-matings were obtained:

Treatment	No. of male flies used for mating	Total F_1 Males produced	No of Sex Chromosome loss	%
Negative (Control)	125	6344	4	0.06
MMS (1mM)	164	5072	14	0.28

The above results show the abundance of progeny produced by both the untreated males and those treated with MMS. The MMS-treated males did produce a significantly higher number of abnormal offsprings. The mass-mating method thus seems to be adequate and practical for such studies, when a proper feeding method with rotenone, due to its extremely low solubility in aqueous solutions, has not been established.

APPENDIX II

Data Evaluation Record for a Subchronic
Toxicity Study in Dogs with Rotenone
(from a previous review cited in Section I. above)

DATA EVALUATION RECORD

004816

(1) CHEMICAL: Rotenone

(2) TYPE OF FORMULATION: Purified Rotenone

(3) CITATION: Ellis, H.V., S. Unwin, J. Cox, I.S. Elwood E.A. Castillo, E.R. Ellis, and J. Carter. 1980. Subchronic oral dosing study for safety evaluation of rotenone using dogs. Final Report to the Department of Interior, Fish and Wildlife Service, National Fishery Research Laboratory, La Cross, Wisconsin. Unpublished report prepared by Midwest Research Institute, Kansas City, Missouri.

(4) REVIEWED BY: [signature] 1-12-81

(5) APPROVED BY: [signature]

(6) TOPIC: This study pertains to Subchronic oral toxicity and relates to the proposed Guidelines data requirement 163.82-1.

(7) CONCLUSION: Rotenone was administered orally to dogs in capsules at doses of 0, 0.4, 2, or 10 mg/kg/day for 26 weeks. It was found to be toxic at the highest dose level and caused emesis and diarrhea. Other effects at this level included decreases in body weight and food consumption hematocrit and hemogdlohin; serum glucose, cholesterol and total lipid levels were decreased below those value in controls. Rotenone was less toxic at the mid dose level with no significant effects on the hematocrit, hemoglobin, serum glucose, cholesterol, or total lipids. Emesis was less frequent and body weight reductions reflected adaptation of the dogs in the mid dose group to rotenone's gastrointestinal effects. No gross or microscopic changes could be associated with treatment. The lowest dose had no effect on the dogs.

The authors stated that the gastrointestinal effects were probably intensified by the offering of feed an hour after the dogs were dosed.

The effects observed suggest that a nutritional problem developed in high-dose dogs which resulted in decreased hemoglobin production and low levels of glucose, cholesterol and total lipids in the serum. The no-observable-effect level was established as 0.4 mg/kg/day for the 6 month treatment period.

CORE CLASSIFICATION: Guideline. This study meets the Agency's most recently proposed guideline requirements for subchronic toxicity testing.

(8) MATERIALS AND METHODS

004816

Chemical: Rotenone was obtained by the Fish and Wildlife Service and provided to the investigators. The report states that two samples of Lot No. 578-RSP-1424 were used in a pilot study and the main study. A third sample was used in the main study and was identified as being from Lot No. 100287 which was obtained from the Aldrich Chemical Company. These samples were 99% pure as determined by high performance liquid chromatography.

Animals: Four to five month old beagle dogs were obtained from Laboratory Research Enterprises (Kalamazoo, Michigan). They were quarentined for at least two weeks prior to the beginning of the experiments. The dogs were housed individually in metal cages. During the study Purina Dog Chow was offered to the dogs for one hour beginning an hour after dosage administration. Ten dogs of each sex were divided into 5 groups containing one of each sex for the range finding portion of the study, while dogs were divided into 4 groups containing 6 of each sex for the main study.

Doses and dose selection: Each group of two dogs was given daily doses of 0.08, 0.4, 2, 10, or 50 mg/kg in gelatin capsules for 4 weeks. The results of this range finding study provided the investigators with a basis for selecting the middle three doses (0.4, 2, and 10 mg/kg/day) for the main study. In the main study each of the four groups of dogs was given daily doses of 0, 0.4, 2, or 10 mg/kg for 26 weeks (6 months).

In all cases, the dogs were weighed before rotenone administration began and at the end of each week during the test to determine the amount needed in the gelatin capsules for the following week. This procedure ensured a constant mg/kg dose for each dog throughout the study.

Observations: Daily observations included feed consumption, behavior (emphasizing effects on the autonomic nervous system) and water consumption. Body weights were measured each week. Blood samples were taken from each dog's jugular vein once a week for two weaks prior to treatment and at weeks 2,4,6,13,17,21, and 26 of treatment. These samples were examined hematologically (erythrocytes, reticulocytes, hematocrit, hemoglobin, methemoglobin, Wintrobe indexes, platelet, total and differential leukocyte counts, and nucleated-erythrocytes), and clinical chemistry (fasting blood glucose, total protein, albumin, globulin, oxaoacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, lactic dehydrogenose, blood urea nitrogen, creatine phosphokinase, creatinine bilirubin, calcium and potasium) was also done on the blood samples. Urinalysis (glucose, protein, pH, bilirubin and urobilinogen, specific gravity, and presence of erythrocytes, leukocytes, epithelial cells, and casts) was also performed. Urine was collected by Catheterization except in cases of difficulties which required that cage urine be analyzed.

At terminal sacrifice liver, kidneys, heart, brain, and gonads were weighed. Tissues examined histologically included eyes, skin, mammary gland, trachea, lung, tongue, salivary gland, liver, gall bladder, pancreas, esophagus, fundic and pyloric stomach, duodenum, jejunum, ileum, colon, kidneys, urinary bladder, gonads and accessory organs, diaphragm, skeletal muscle (gracilis), pituitary, thyroid with parathyroid, adrenals, tonsils, thymus, spleen, prescapular and mesenteric lymph nodes, rib bone with marrow, spinal cord, brain (coronal sections of cerebrum, cerebellum, and brainstem), sciatic nerve, and any lesions, tumors or other abnormalities observed at necropsy.

Statistical Analyses: Bartlett's test for homogeneity was used. Heterogeneous data were analyzed by methods the authors described as appropriate nonparametric tests. Dunnett's multiple comparison procedures were used, and the Fisher's exact test was used to determine significant differences (when p less than 0.05) for incidences of lesions.

(9) REPORTED RESULTS: The most severe effects were noted in the 10mg/kg group. During the first week emesis was observed frequently, but during subsequent weeks of the study the emesis subsided. Soft stools or diarrhea were commonly noted throughout the study in the dogs of this group also. Body weights of these dogs decreased during the first two months of the study and stablilized thereafter. Feed consumption was lower in these dogs than in control dogs throughout the study. However, during the last part of the study it increased from the level seen during the first two months of treatment. The following summarizes significant findings in these high dose animals.

Results expressed as mean ± S.D.[a/]

Parameter	Control group	High dose group
Males (n)	6	6
Hematocrit (Vol.%)	46.3±1.5	40.7±1.0 [b/]
Hemoglobin (mg%)	15.9±0.5	13.9±0.3
Glucose (mg%)	92.0±3.6 (104.8±1.1) [d/]	84.5±3.4 [c/]
Cholesterol (mg%)	129±13 (164±8) [d/]	120_14 [c/]

Females (n)	6	6
Hematocrit (Vol.%)	47.3+1.7	38.7+1.0 b/
Hemoglobin (g%)	16.3+0.3	13.1+0.3 b/
Glucose (mg%)	90.5+3.5 (97.8+1.4) d/	79.8+3.1 c/
Cholesterol (mg%)	149+8 (153+7) d/	99+5 b/ c/
Total lipids (mg%)	524+19	410+21 b/

a/ These results are from 26th week blood samples.

b/ Significantly different from controls

c/ Significantly different from baseline data

d/ Baseline results.

Although the absolute weights of the major organs were comparable to the controls and other treated groups, their relative weights were higher. This increase in organ-to-body weight ratio is attributable to the body weight loss of the high dose dogs.

The toxicity of the 2mg/kg/day dose was less severe. After the second month of treatment the mid-dose dogs had body weights comparable to the control dogs. Food consumption was less in females of this group than those receiving no rotenone, while the males of these two groups were comparable. The only effect on clinical chemistry parameters in the mid dose males was decrease in blood glucose levels in comparison to that measured as baseline. These respective values are 86.3 (+2.0) mg% and 101.9 +3.0 mg% (the control value for this parameter at 25 weeks was 92.0 +3.6 mg%). The mid-dose females exhibited similar results for serum blood glucose. The baseline was 101.5 +2.5 mg%; while the control and mid dose values were 90.5+3.5 and 84.5+3.0 mg%, respectively.

There were no compound-related effects seen in the control low-dose dogs. There were also no effects observed in any treatment group on urinalysis parameters or the incidences of gross and microscopic lesions.

(10) DISCUSSION: The authors concluded that the primary target organ in dogs is the gastrointestinal tract which resulted in a condition described as mild starvation. The condition was most evident in the 10mg/kg/day group as suggested by decreased hemoglobin, glucose, cholesterol and total lipids in the blood. The authors also stated that offering food an hour after administration of rotenone could have intensified the effect on the gastrointestinal tract.

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These effects are commonly observed in dogs, and the results described here are limited to characterizing effects on the gastrointestinal tract of dogs. Doses capable of inducing other types of systemic toxicity could not be administered because of the gastrointestinal response.

APPENDIX III

Toxicology Branch "One-liners"
for Rotenone

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic - in vivo cytogenetic - rat; Biotech Res. Lab. Inc.; 7/20/81	Rotenone 98% Pure	246587			Unacceptable 001981
Mutagenic - micronucleus mouse; Biotech Res. Lab. Inc.; 7/20/81	Rotenone 98% Pure	246587			Unacceptable 001981
Mutagenic - sex chromosome loss in Drosophila Biotech Res. Lab. Inc.; 7/20/82	Rotenone 98% Pure	246587			Unacceptable 001981
Acute oral LD50 - rat; MB Research Lab; #82-[illegible]A; 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

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute dermal LD_{50} rabbit ; # MB 82-6509B MB Research Labs;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
Primary eye irritation - rabbit; # MB 82-6509D; MB Research Labs; 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

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Primary dermal irritation -rabbit;#MB82-6509C MB Research Labs; 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	Severe erythema/eschar at 72 hours, slight edema (4 hr exposure). Irritation had healed at 14 day.	II	Minimum 003117

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
18 Month oncogenic-hamster;Battelle;Report No. EPA-600/1-79-040a; January, 1979	Rotenone (95+% a. i.)	255278	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
Oncogenic - rat; Battelle; Report No. EPA-600/1-79-040b; January, 1979	Rotenone (95+% a. i.)	255278	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
Reproduction-hamster; Battelle; Report No. EPA-600/1-79-040a; January, 1979	Rotenone (95+% a. i.)	255278	500 ppm caused mortality in pups during lactation. No concurrent control group. Only one generation. 1000 ppm - no litters were produced. 3 males and 12 females died during treatment period before mating. No concurrent control group. Only one generation.		Supplementary 004610

004816

	No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute oral LD_{50} - rat; Hazleton Labs., Inc.; Proj. No. 419-137; Oct. 16, 1984	99.23% a. i.	253333 258849	LD_{50} for males = 102 $\pm$ 12.6 mg/kg LD_b0 for females = 39.5 $\pm$ 2.21 mg/kg Doses tested = 10, 25, 50, 75, and 150 mg/kg (10 per sex/dose)	I	Minimum 004652 004653
Metabolism - rat; Hazleton Labs., Inc.; Proj. No. 419-137; Oct. 16, 1984	94.64% a. i. spec. activ. 32.8479 uCi/mg	253333 258849	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

:tion/Reviewer 6/Gardner
:e Assigned 9/27/84
Pack # ________
TECH 446
Record # 130154
Date In 8-22-84
:well # 725

STUDIES PER PACK

JDY

A		O A	
	Acute Oral LD_{50}		Chronic Non-rodent Feeding
	Acute IP LD_{50}		Chronic Rodent Feeding
	Acute Dermal LD_{50}	/	Reproduction
	Acute I.V. LD_{50}		Dermal Absorption
	Acute Dermal Irritation		Metabolism
	Acute Eye Irritation		Mouse Oncogenic or Mouse Onco/Feeding
	Antidote Study		Inert clearance
	Acute Inhalation LC_{50}		Section 18
	Photo Sensitization		Protocol
	21 Day Dermal		SOP 3068.2 (Me Too)
	21 Day Inhalation		Company response
	Sensitization		
/	Subchronic Feeding/Intubation/Inhalation		
	Cataract In Ducks		
3	Teratology Study		
5	Mutagenic Study		
	Neurotoxicity		

:ated 4/22/85 - ssr