8-12-85 PP-13Y TAR-4610

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

004610

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Oncogenicity Studies with Rotenone and Request for Data Waiver. (Reg. No. 16-58T) Tox. Chem. No. 725.

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

THRU: Jane Harris, Ph. D., Section Head JHN 8/12/85
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: Roger Gardner, Toxicologist
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769) Roger Gardner 8-12-85 Reviewed 8/14/85

Actions Requested

1. Review of reports on oncogenicity studies in rats and hamsters (see Appendix).

2. A waiver of any further oncogenicity studies with rotenone.

Recommendations and Conclusions

1. The following two factors limit the hamster study's usefulness:

   a. An insufficient number of females survived to the end of the study.

   b. No histopathology examinations were done in the two mid-dose groups (250 and 500 ppm)

   These factors indicate that the study can only be accepted as supplementary. The results provided by the hamster study are not conclusive with respect to characterizing the oncogenic potential of rotenone. The study also fails to

10/24

establish a no-observed-effect level (NOEL) for other chronic effects. (See Section II. A. and the Appendix.)

2. The rat studies were designed to confirm results obtained in a specific protocol rather than to support registration of a pesticide. Protocol deficiencies included

   a. The test substance was administered for 42 days rather than for the recommended 24 months in rats.

   b. Animals were sacrificed after 14 to 18 months rather than the recommended 24 months.

   (See Section II. B. and the Appendix.)

3. Oncogenicity studies are near completion at the National Toxicology Program (see Sections I. C. and III.) and should be submitted for review after final reports become available. The studies followed standard NTP protocols, and may not provide results on which a NOEL for chronic toxicity can be established.

4. In view of the limitations in the studies described in points 1. and 2., above, the three reports do not satisfy the Agency's requirements for oncogenicity studies in two species.

5. The limitations of the rat and hamster studies and the expected completion of the NTP studies indicate that a data waiver for additional oncogenicity studies should not be granted.

6. Because the NTP study is not a combined chronic/oncogenicity study (see page 5), establishment of a NOEL from the long-term studies described in points 1. through 5. above is unlikely. Therefore, additional chronic toxicity studies are required to support registration (see §158.135, FR Vol. 49, No. 207, October 24, 1984).

I. Background

The U. S. Fish and Wildlife Service (FWS) submitted copies of oncogenicity studies (see Appendix) with a request that they be considered in support of the registration of rotenone as a piscicide. The letter (dated September 24, 1984) accompanying the studies also requested that the Agency waive any further requirements for oncogenicity testing.

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 associated resins to a purity of 99.5%.

B. Regulatory Considerations

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 the three oncogenicity studies submitted by the FWS in a support document entitled Rotenone: Pre-RPAR Review (dated May, 1980). Those studies were described 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.

The support document further recommended that a tolerance should be established in fish as a result of the aquatic use. To support registration of such a use, the Agency recommended that chronic toxicity studies be conducted with rotenone.

C. Other Considerations

Minutes to a meeting of representatives from the FWS and the EPA held on November 3, 1980 contain a discussion of data requirements. That record stated:

> Dr. Rispin (Hazard Evaluation Division, Science Integration Staff),..., will attempt to get the 2-year feeding study in rats (initiated at the National Toxicology Program laboratories in 1980) modified so that it will answer the "no effect level" requirement of EPA. Such items as hematology, organ weight determination, and an extended range of feed intake would have to be added to meet the chronic feeding requirements. If the 2-year feeding study in rats can not be modified, then an extra study...would have to be contracted by the FWS.

In a "Management Status Report" published by the National Toxicology Program on April 5, 1985, the rotenone studies were listed in a category called "Chronic Histopathology in Progress." A telephone converation with Florence Jordan (NTP) on August 2, 1985 indicated that the studies have been submitted for quality assessment, and final reports have not been prepared.

According to a telephone conversation with Dr. Kamail Abdo of NTP on August 6, 1985, the NTP study was not conducted accor-

ing to a protocol for a combined chronic/oncogenicity study Therefore, a NOEL can not be established on the basis of the NTP oncogenicity study.

II. Summary of Submitted Data

Detailed reviews of the rat and hamster studies are included in the Appendix.

A. Carcinogenicity study in hamsters

Groups of 50 male and 50 female hamsters were given diets containing 0, 125, 250, 500, or 1000 ppm rotenone for 18 months (see Appendix below).

The results suggested that rotenone may cause adrenal tumors in female hamsters. However, early mortality caused by an enteric infection and the age-associated nature of the adrenal effects suggests that incidences in each test group should be adjusted before statistical comparisons are made.

Survival in the control and low dose group females was too low at the end of the study (4% and 18%, respectively) to permit valid comparisons with other groups. The authors emphasized this point by describing the control group at necropsies as observations of a younger population than that of other test groups. Therefore, any apparently dose-related trend in the incidence of age-related lesions that spontaneously occur in female hamsters must be confirmed in another study before they can be considered significant.

Mortality in males appeared to be comparable in the 0, 125, and 1000 ppm dose groups, and there were no treatment related increases in histopathological observations.

Hamsters of both sexes that received the 250 and 500 ppm diets were not examined microscopically, and results from preliminary studies as well as data reported in the chronic study did not demonstrate that the 1000 ppm dose was sufficiently high to induce minimal effects (see Appendix below).

Because of the early mortality in females and the absence of histological observations of the two mid-dose groups (250 and 500 ppm), the study can only be accepted as supplementary evidence that rotenone is not likely to be oncogenic in hamsters.

B. Carcinogenicity studies in rats

As mentioned in Section I above and in the Appendix below, these studies were intended to confirm results obtained by Gosalvez

in 1973. They were conducted in accordance with protocols that are different from recommended procedures. The differences included:

1. The test substance was administered for 42 days rather than for the recommended 24 months in rats.

2. Animals were sacrificed after 14 to 18 months rather than the recommended 24 months.

These departures from recommended protocols preclude use of the rat studies to support registration of rotenone.

III. Discussion

As indicated in Section I above, there are studies underway at NTP, and final reports have not been prepared. The studies submitted with the current action are not adequate to fulfill oncogenicity or chronic toxicity data requirements because of their specific purposes or because of deficiencies (see Section II above and the Appendix). Under these circumstances, a data waiver for further oncogenicity studies can not be granted until the NTP studies are completed and reviewed. In addition, further chronic toxicity studies may be needed unless the modifications of the NTP rat feeding study (see Section I. C., above) were made and are found acceptable. The Registrant should be advised that additional information can be obtained from the NTP Management Status Reports or from Florence Jordan, Mail Drop 18-01, NIEHS, P. O. Box 12233, Research Triangle Park, NC 27709.

APPENDIX

This appendix contains Data Evaluation Records for the following reports.

Leber, A. P., and R. L. Persing. January, 1979. Carcinogenic Potential of Rotenone: Phase I: Dietary administration to hamsters. Report published by the Environmental Protection Agency (EPA-600/1-79004a). Submitted by the U. S. Department of the Interior, Fish and Wildlife Service. EPA Acc. No. 255278 (Tab #2).

Leber, A. P., and D. C. Thake. January, 1979. Carcinogenic Potential of Rotenone: Phase II: Oral and Intraperitoneal Administration to Rats. Report published by the Environmental Protection Agency (EPA-600/1-79-004b). Submitted by the U. S. Department of the Interior, Fish and Wildlife Service. EPA Acc. No. 255278 (Tab #3).

Freudenthal, R. I., A. P. Leber, D. C. Thake, and R. L. Baron. April, 1981. Carcinogenic Potential of Rotenone: Subchronic Oral and Peritoneal Administration to Rats and Chronic Dietary Administration to Syrian Golden Hamsters. Report published by the Environmental Protection Agency (EPA-600/1-81-037). Submitted by the U. S. Department of the Interior, Fish and Wildlife Service. EPA Acc. No. 255278 (Tab #4).

004610

DATA EVALUATION RECORD

1. CHEMICAL: Rotenone

2. TEST MATERIAL: The rotenone was 95+ per cent pure.

3. STUDY/ACTION TYPE: Oncogenicity - hamsters

4. STUDY IDENTIFICATION: Leber, A. P., and R. L. Persing. January, 1979. Carcinogenic Potential of Rotenone: Phase I: Dietary administration to hamsters. Report published by the Environmental Protection Agency (EPA-600/1-79-004a). Submitted by the U. S. Department of the Interior, Fish and Wildlife Service. EPA Acc. No. 255278 (Tab #2).

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 8-12-85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane E. Harris
Date: 8/12/85

7. CONCLUSIONS: Because of high mortality caused by an enteric infection (96% in the control group females after 18 months) results from female hamsters are inconclusive. Also the two mid dose groups of both sexes were not evaluated microscopically.

The reproduction phase of the study suggests that rotenone in the diet of hamsters at a level of 500 ppm causes mortality or toxicity in hamster pups during lactation. However, there was no concurrent control or lower dosed groups to evaluate the apparent effects. These results should be confirmed by another study.

Core classification: Supplementary for the carcinogenicity and reproduction studies (See Sections 7. CONCLUSIONS above and 10. DISCUSSION below for reasons).

8. MATERIALS AND METHODS

Test species: Male and female Syrian Golden strain hamsters weighing from 50 to 70 g at the start of the preliminary studies.

Experimental procedure: Preliminary studies. Groups containing 5 male and 5 female hamsters were given control diets for one week. After that period test diets containing 63, 125, 250, 500, or 1000 ppm were given to each group for two weeks followed by two additional weeks on untreated diets. During the 5-week experimental period the animal body weights and feed consumption were measured weekly. At the end of the experiment all animals were sacrificed and subjected to necropsy.

A second preliminary study was conducted under similar conditions with the exception that the test substance was suspended in corn oil prior to blending into the diets. Also gross necropsies were conducted on only 5 of the 1000 ppm group animals and 2 of the 500 ppm group animals.

In the third preliminary study, a group of 5 male and 5 female hamsters was given daily doses of 80 mg/kg by gavage. The rotenone was disolved in corn oil. The animals were treated and observed for 9 consecutive days according to the report. No other details of the methods used were provided.

Reproduction studies: A series of three experiments were briefly reported. In the first study a group of 25 male and 50 female hamsters was given a diet containing 1000 ppm. The diets were fed to the animals for three months before one male was cohabited with two females. Females were examined by the investigators for vaginal plugs after cohabitation. The litter sizes were noted.

A second group of 50 male and 50 female hamsters was maintained on a diet containing no rotenone and mated after three months. In this experiment one male was cohabited with one female. The same observations were made in this experiment as were made in the first. According to the report, there were F_{1a} and F_{1b} litters, but details of mating and other procedures were not described in the report.

A diet containing 500 ppm rotenone and 1% corn oil was fed to a second group that consisted of 50 animals of each sex. Mating procedures and observations followed procedures similar to those used in the previous two experiments according to the report.

8. MATERIALS AND METHODS (continued)

Carcinogenicity study: Groups consisting of 50 male and 50 female hamsters were given diets containing 0, 125, 250, 500, or 1000 ppm test substance for 18 months. The report stated that animals were weighed weekly for the first 6 months of the study, and bi- or tri-weekly thereafter. Food consumption was measured weekly throughout the study, and the animals were observed for mortality, behavior, and appearance daily throughout the study.

After the 18-month feeding period survivors were sacrificed and subjected to necropsy. Animals that died during the feeding period were also subjected to necropsy as soon after their deaths as possible. According to the report, all the animals were examined for appearance of tumors.

The following tissues were removed after gross examination and prepared for histological examination:

Skin	Kidney	Stomach	Thyroid
Mammary gland	Ureter	Duodenum	Parathyroid
Trachea	Bladder	Jejunum	Adrenal
Lung	Gonads	Ileum	Bone
Heart	Uterus	Cecum	Muscle
Aorta	Epididymis	Colon	Brain
Lymph nodes	Prostate	Rectum	Pituitary
Spleen	Seminal vesicle	Pancreas	Spinal cord
Thymus	Salivary gland	Liver	Sciatic nerve
Esophagus	Tongue	Gall bladder	Eyes

The authors stated that initial histological examinations included the 0, 125, and 1000 ppm groups. Because of considerable early mortality (see below), the report stated that microscopic examinations were limited to those animals dying after approximately one year on the study.

No further details of the protocol or conduct of the study were reported.

9. REPORTED RESULTS

Preliminary study: Body weight changes were presented graphically (see Appendix A below). The authors noted that the hamsters given the 1000 ppm diet exhibited a significant decrease in body weight during the first week of dosing. Body weight gain in those animals was greater than that for controls after test diet was replaced with the control diet. The report also stated that no abnormal behavior or signs of

9. REPORTED RESULTS (continued)

64610

toxicity were observed during the course of the preliminary experiment.

At gross necropsy the investigators reported that several hamsters from the 1000 ppm dose group had congested lungs that appeared cherry red. Other gross observations included congestion of the duodenal mucosa, hemorrhagic enteritis in the small intestines, and dilation of the meningeal vessels. The report stated that similar but less extensive lesions were found in the animals given the 500 ppm diet. No lesions were found in groups receiving the 250 ppm or lower dietary concentrations, and no deaths were observed according to the report. No incidence data were included in the report for the preliminary studies.

The authors noted that during the first two days of the gavage dosing there were three deaths. At necropsy they reported an oily substance in the peritoneal cavity without evidence of stomach puncture. After the first of 9 consecutive doses the animals appeared lethargic. Other signs noted by the investigators included diarrhea, eye discharges, lameness, shaggy coat, and mouth ulcerations. Gross observations reported at necropsy included congestion of the lungs, liver, and gastrointestinal tract with redness in the lungs and pleura. Mild diarrhea persisted in those rats given the corn oil vehicle without test substance. There was no tabulation of the incidence for these findings or individual animal data included in the report.

Reproduction studies: In the first trial (with the 1000 ppm diet only), the report stated that body weights showed an increase for the first five weeks. At that time a decrease in food consumption was observed. There were three males and twelve females that died during the first two months of the experiment, and the survivors were reported to exhibit body weight loss and deteriorating physical condition. The investigators noted that the condition of the survivors began to improve during the eighth and ninth weeks as indicated by body weight gains, increased food consumption, and improved appearance (the coats developed a sheen).

The authors stated that no pregnancies were observed and the male hamsters had smaller than normal testicles. No data were presented to support these statements. On the basis of the results the investigators ended the experiment and scheduled a subsequent study at a lower dietary level.

. REPORTED RESULTS (continued)

f the 50 matings of animals given the 0 ppm diet 43 resulted n delivery of live pups according to the report. The average itter size was reported to be 7 and ranged from 2 to 10. ll pups were described as healthy throughout lactation. The uthors noted that one to three of the pups from each litter ere placed in the oncogenicity study (see below).

ccording to the report, a second mating was attempted, but he number of pregnancies were not given. The average litter ize was reported to be 12 with a range from 9 to 17, and the ups were described as healthy through weaning. No further etails were noted.

t the lower dietary level of 500 ppm, there were 45 litters orn in the group of 50 females that were mated. During eaning all but 7 of those litters died. The authors noted hat the average litter size was 9 with a range from 4 to 15, nd they described the pups as smaller than normal without roviding data to support that description. The investigators lso stated that many of the dams cannibalized their young or eglected them during lactation.

second mating of the 500 ppm group animals resulted in the irth of 21 litters with an average size of 12 and ranging rom 7 to 16. The authors did not report a survival rate for hese offspring, but they noted that the dams neglected or annibalized their young as they did after birth of the first itters.

arcinogenicity study: The authors noted that two hamsters n the control group, one in the 500 ppm dose group, and two rom the 1000 ppm group were lost to necropsy. No explanation or these losses or the sex of each animal was indicated in he text of the report.

ubstantial mortality during the first 12 months of the study as also noted by the investigators. These deaths were escribed in the report as follows:

> The deaths were apparently not related to rotenone administration since the losses were as high or higher in the controls than in the treatment groups. Most deaths during the first year of the study were associated with a syndrome of cecal hemorrhage and dilatation which often involved the ileum and colon, and in a few instances, the upper small intestine. The syndrome was characterized clinically

9. REPORTED RESULTS (continued)

by sudden death in apparently healthy animals with no prodromal clinical signs. Intestinal and cecal material from affected animals, when passed through a 45[illegible] filter and injected into the peritoneal cavity of young mice, caused death within 4 to 8 hours in dilutions up to 1:256. Similar filtrates from non-affected hamsters did not produce death in mice. Clostridium perfringens B, C, D, and E antitoxins would not protect against the lethality. The lethal capacity of the filtrates was destroyed by heating at 56° C for 30 minutes. A pathogenic strain of E. coli was isolated from several animals which died of this syndrome.

Because the isolated organism or the enterotoxins were not experimentally evaluated in other hamsters, the authors only speculated that pathogenic E. coli and its associated enterotoxins were the cuase of the early mortality observed.

The authors attributed the deaths after one year to systemic amyloidosis which they described as common in Syrian Golden hamsters.

The survival results were summarized as follows:

Dose (ppm)	Number/sex in each group	At 12 month		At 18 months	
		Males	Females	Males	Females
0	50	30	28	22	2
125	50	35	28	21	9
250	50	33	37	19	20
500	50	35	30	25	18
1000	50	33	34	24	19

Body weight and food consumption data were reported graphically and those results are reproduced in Appendix B below. The authors noted that the 500 and 1000 ppm diets caused decreased group mean body weights which were associated with decreased group mean food consumption.

The incidence of gross lesions was reported as the number of animals (both sexes combined) with a particular finding and a percentage of the total number examined. The percentages reported for the most frequently occuring observations in animals that died during the feeding period are summarized as follows:

9. REPORTED RESULTS (continued)

Observation*	0	125	250	500	1000
Enteritis	28.9	52.8	32.7	40.3	45.6
Typhlitis	43.4	40.0	39.3	57.8	47.3
Colitis	7.8	11.4	13.1	15.7	14.0
Nephrosis	36.8	35.7	27.8	14.0	17.5
Liver centrilobular congestion and necrosis	22.3	18.5	29.5	21.0	15.7
Pulmonary congestion and hemorrhage	38.1	28.5	31.1	35.0	14.0
Testicular atrophy or hypoplasia	1.3	12.8	6.5	1.7	10.5

*The number examined in the 0, 125, 250, 500, and 1000 ppm dose groups were 76, 70, 61, 57, and 57, respectively.

The percentages for the most frequently observed gross lesions in animals sacrificed at termination are summarized as follows:

Observation*	0	125	250	500	1000
Enteritis	12.5	16.6	10.2	--**	--**
Typhlitis	--**	6.6	--**	--**	2.3
Colitis	--**	--**	2.3	--**	--**
Nephrosis	66.6	63.3	69.2	48.8	20.9
Liver centrilobular congestion and necrosis	12.5	16.6	20.5	11.6	2.3
Pulmonary congestion and hemorrhage	37.5	3.3	30.7	6.9	13.9
Testicular atrophy or hypoplasia	8.3	3.3	--	2.3	2.3

*The number examined in the 0, 125, 250, 500, and 1000 ppm dose groups were 24, 30, 39, 43, and 43, respectively.

**No incidence reported.

The most frequently occurring histopathological observation (excluding hyperplastic and neoplastic lesions) was amyloidosis (see Table 1). The report described those lesions as follows:

> ...The pattern of change found in the kidney was retraction of the capsular surface with associated tubular degeneration and regeneration with the

9. REPORTED RESULTS (continued)

deposition of an amyloid material in the tubular interstitial tissues, generally of the cortex. The mesangium of the glomerular tuft was often thickened and contained a similar lightly-stained eosinophilic material; Bowman's capsule was also thickened in some instances. The general picture was that of atrophied misshapened nephron units with dilated collecting tubules filled with brightly staining eosinophilic protein material. The normal liver architecture was altered by rather thick deposits light pink amyloid-related material in and between the hepatocytes and vascular tissues of the portal triads that often completely filled with the pale eosinophilic material with only small lymphoid nodules disturbing the monotonous pink fields. The adrenal cortex was also a deposition site for the lightly staining amyloid-like material, where it was found in or between cortical cells. Female hamsters from all three dosage groups had a higher incidence of amyloid-like material deposited between the follicles of the thyroid than did males.

Table 2 summarizes other frequently reported histopathological observations (exclusive of hyperplasias and neoplasias).

Table 1

Incidence of amyloidosis in rotenone treated hamsters

	Dose (ppm)					
	Males			Females		
Organ	0	125	1000	0	125	1000
No. examined	30	32	32	27	26	33
Kidney	26	15	8	25	21	28
Liver	3	2	2	3	2	2
Spleen	3	3	1	3	3	1
Adrenal	4	4	3	4	4	3
Thyroid	2	1	1	8	11	10

Table 2

Incidence most frequently reported histopathological observations (excluding neoplastic and hyperplastic lesions) (excerpted from Table 5 of the original report)

Observation	0 ppm	125 ppm	1000 ppm
	Males		
No. animals examined	30	32	32
Kidney			
Tubular degeneratin re-generation	24	30	23
Glomerular congestion	8	6	2
Liver			
Hepatocellular vacuo-lization	13	4	7
Hepatic or biliary cysts	3	3	3
Sinusoidal congestion	14	18	6
Bile duct hyperplasia	3	3	4
Centrilobular degenera-tion or necrosis	13	8	5
Extramedullary hema-topoiesis	6	14	5
Spleen			
Lymphocyte depletion	1	2	0
Lung			
Bronchiolar epithelial hyperplasia	3	5	2
Pulmonary edema	3	2	0
Pulmonary congestion	13	9	8
Heart			
Mild myocardial degeneration	5	2	2
Adrenal			
Medullary cell degeneration	9	4	5
Intestines			
Enteritis	8	11	8
Typhlitis	14	13	8
Colitis	7	4	2

Table 2 (continued)

Observation	0 ppm	125 ppm	1000 ppm
Males (continued)			
Lymph nodes			
Adenitis			
Mandibular	0	2	1
Mesenteric	6	6	4
Pituitary			
Pars distalis vacuolar degeneration	13	9	10
Females			
No. animals examined	27	26	33
Kidney			
Tubular degenration regeneration	21	25	25
Liver			
Hepatocellular vacuolization	2	9	17
Hepatic or biliary cysts	11	7	12
Sinusoidal congestion	11	12	22
Bile duct hyperplasia	5	15	20
Centrilobular degeneration or necrosis	2	21	22
Extramedullary hematopoiesis	3	2	0
Spleen			
Lymphocyte depletion	0	5	2
Lung			
Bronchiolar epithelial hyperplasia	1	4	4
Pulmonary edema	10	5	3
Pulmonary congestion	13	5	13
Bronchiolar pneumonia	7	5	4
Interstitial pneumonitis	2	4	7
Heart			
Atrial thrombosis	14	5	2
Ventricular thrombosis	4	2	0
Mild myocardial degeneration	5	3	5

Table 2 (continued)

Observation	0 ppm	125 ppm	1000 ppm
Adrenal			
Medullary cell degeneration	6	3	7
Intestines			
Enteritis	1	14	12
Typhlitis	4	9	14
Colitis	1	3	5
Lymph nodes			
Adenitis			
Mandibular	9	8	3
Mesenteric	5	10	5
Pituitary			
Pars distalis vacuolar degeneration	7	4	8

The authors noted that the most frequently observed hyperplastic or neoplastic lesions involved the adrenals. Table 3 summarizes those results.

Table 3

Reported incidence of hyperplastic and neoplastic lesions in the adrenal glands of hamsters treated with rotenone

	Dose (ppm)					
	Males			Females		
Lesion	0	125	1000	0	125	1000
No. examined	30	32	32	27	26	33
Cortical cell hyperplasia	11	15	19	13	12	15
Cortex adenoma	8	8	8	5	7	6
Cortex carcinoma	0	0	1	0	0	2

Other hyperplastic or neoplastic lesions were reported to occur in one or two animals in isolated groups.

10. DISCUSSION

Preliminary studies: The report stated that there were no signs of toxicity observed in hamsters during preliminary experiments. Although gross lesions were reported by the authors, there were no incidence data or detailed descriptions to indicate that they were dose related. The congestion in the lungs and intestines were also observed in the chronic study. Those lesions were also observed in all test groups at similar incidences.

Since only body weight change is presented graphically without reference to the actual body weights for the hamsters in each group, and because there is no food consumption data presented for the preliminary experiments, the significance of the graphs with respect to rotenone toxicity can not be determined independently.

In the first preliminary feeding study the authors noted a significant decrease in body weight gain in hamsters given the 1000 ppm diet. According to the report, the body weight gain in that group was significantly greater than that for controls during the two weeks following administration of the test substance. The report did not mention a similar decrease in body weight gain when the experiment was repeated using corn oil in the test diet preparation.

Reproduction studies: According to the reported schedule for this phase of the study, the three test groups were not run concurrently (see Appendix D). This factor is important because of the nature of the deaths in the 1000 ppm group. Those deaths were were described in the report as follows:

> The hamsters continued to increase in body weight the first 5 weeks of the study after which time feed consumption decreased. Sixteen (3 males and 12 females) (sic) of the 75 animals on study died during the first two months. Physical condition deteriorated and body weights decreased in the survivors. At 8 to 9 weeks the downward trend in body weight reversed, and animals became more alert, and their coats developed a sheen while feed consumption returned to normal.

There were no more details given to describe the specific nature and timeing of these deaths. However, the temporary decrease in feed consumption implied in the above statement, the apparently corresponding decrease in body weight, and the occurrence of these effects during weeks 5 through 8 of the

10. DISCUSSION (continued)

study suggest that the deaths may have been caused by the enteric infection characterized in the carcinogenicity study (see pages 5 and 6 above). Without a concurrent control group, this conclusion is equivocal, but association of these effects with rotenone administration is also equivocal.

As noted on page 5 above, there were 45 litters born in the first mating for the 500 ppm dose group. During lactation all but 7 of those litters died because of cannibalism or maternal neglect. The average litter size was 9 with a range from 4 to 15, and the pups were described as smaller than normal. However, no pup weight data were provided to support that conclusion. A second mating of the 500 ppm group animals resulted in the birth of 21 litters with an average size of 12 and ranging from 7 to 16. Survival of these offspring was not reported, but the dams again neglected or cannibalized their young. Despite the deficiencies described above, these results suggest that further study of the reproductive effects should be conducted.

Carcinogenicity study: Notable trends indicated by results from adrenal gland observations (see Table 3 above) included:

1. An apparently dose-related increase in the incidence of adrenal cortical hyperplasia in males.

2. The occurrence of adrenal cortical carcinomas only in high dose group animals (one male and two females).

These trends suggest that rotenone treatment may be associated with adrenal tumors in hamsters. However, the authors cautioned that the early mortality caused by an enteric infection should be considered in the interpretation of the adrenal effects. Those effects were described as spontaneous in aging hamsters with incidences similar to those encountered in the treated groups, and incidences in each test group should be adjusted by censoring deaths that occurred before the first diagnosis of a given lesion. After such an adjustment for the bias introduced by early mortality, group comparisons to determine statistical significance of differences can be made.

Survival in the control and low dose group females was too low at the end of the study (4% and 18%, respectively; see page 6 above) to permit valid comparisons with other groups. The authors emphasized this point by describing the control group at necropsy as a younger population than those of other

10. DISCUSSION (continued)

test groups, and in view of the association of lesions considered above with the age of the animals examined, survival in the control group females is not adequate. Therefore, the effects described in point 2. above are, as the authors state, inconclusive with respect to the characterization of the oncogenic potential of rotenone.

Other possibly dose-related trends were found in the livers of females. Those effects included increased incidences of hepatocellular vacuolization, sinusoidal congestion, bile duct hyperplasia, and centrilobular degeneration or necrosis. The authors did not discuss these findings with respect to their occurrence in aging hamsters, but early mortality may have biased these results as well as others discussed herein.

Mortality in males appeared to be comparable in each group according to the reported results, and there were no treatment related increases in histopathological observations reported.

APPENDIX A

Body weights of hamsters in the preliminary studies

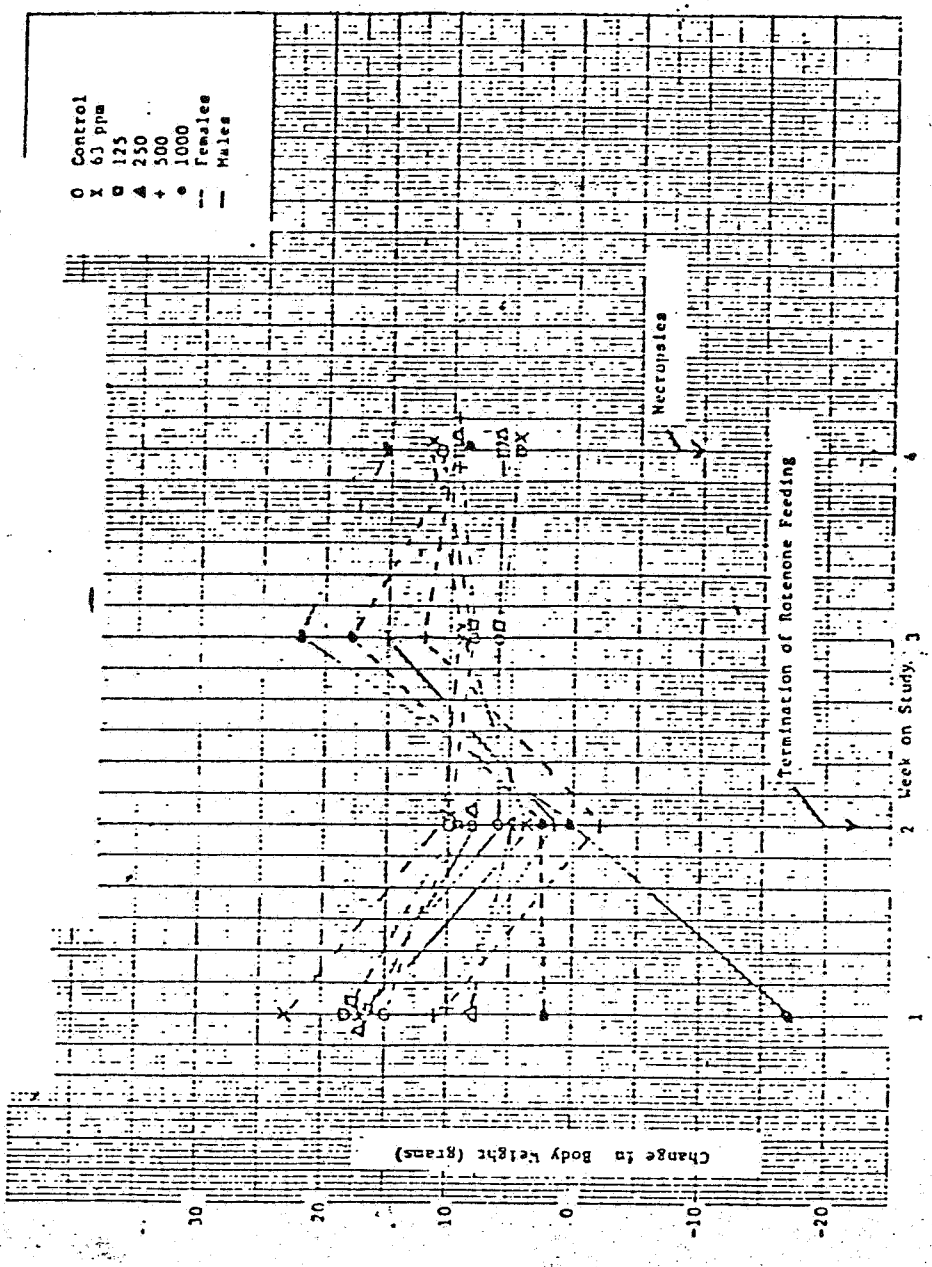

FIGURE 1. Hamster Group Mean Body Weights During Prechronic Study I

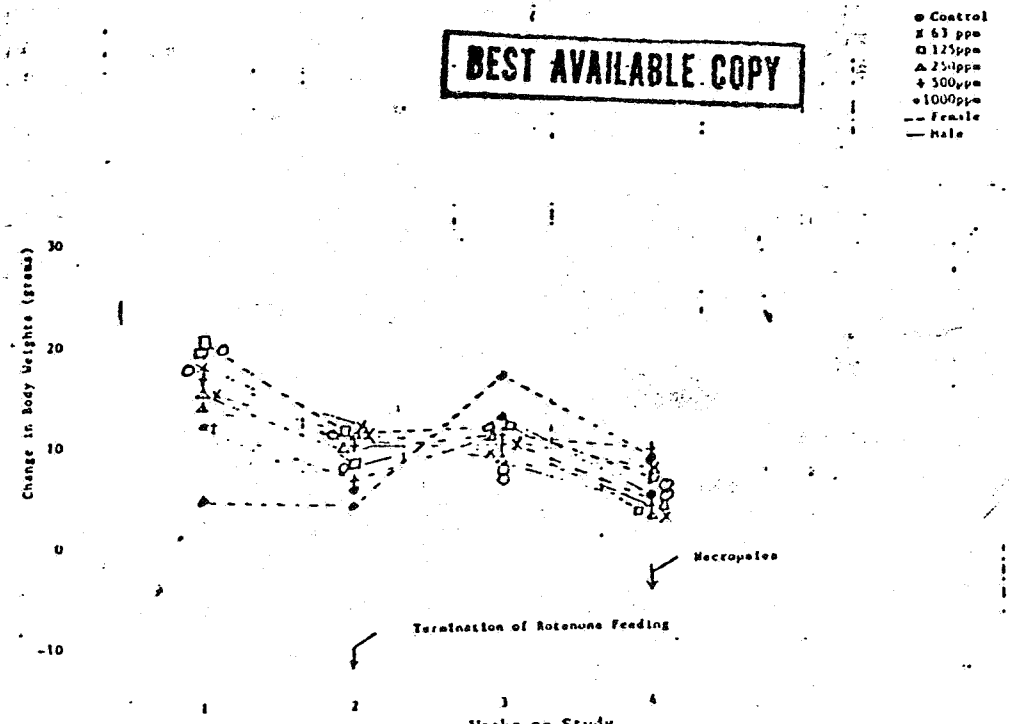

FIGURE 2. Group Mean Hamster Body Weights During Prechronic Study II

APPENDIX B

Body weights and food consumption
of hamsters in
the preliminary studies

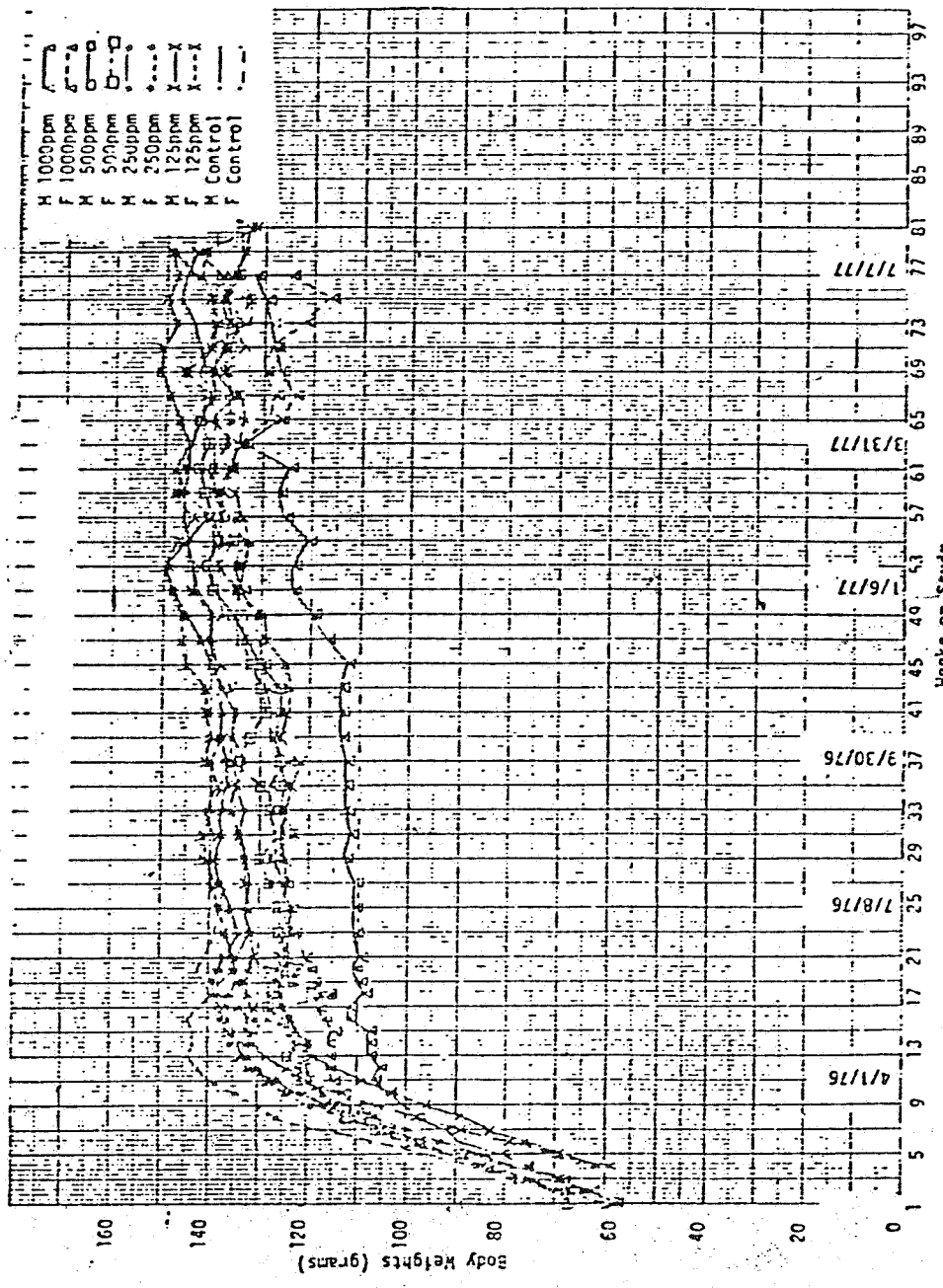

FIGURE 3. Group Mean Body Weights for Hamsters in Carcinogenesis Study

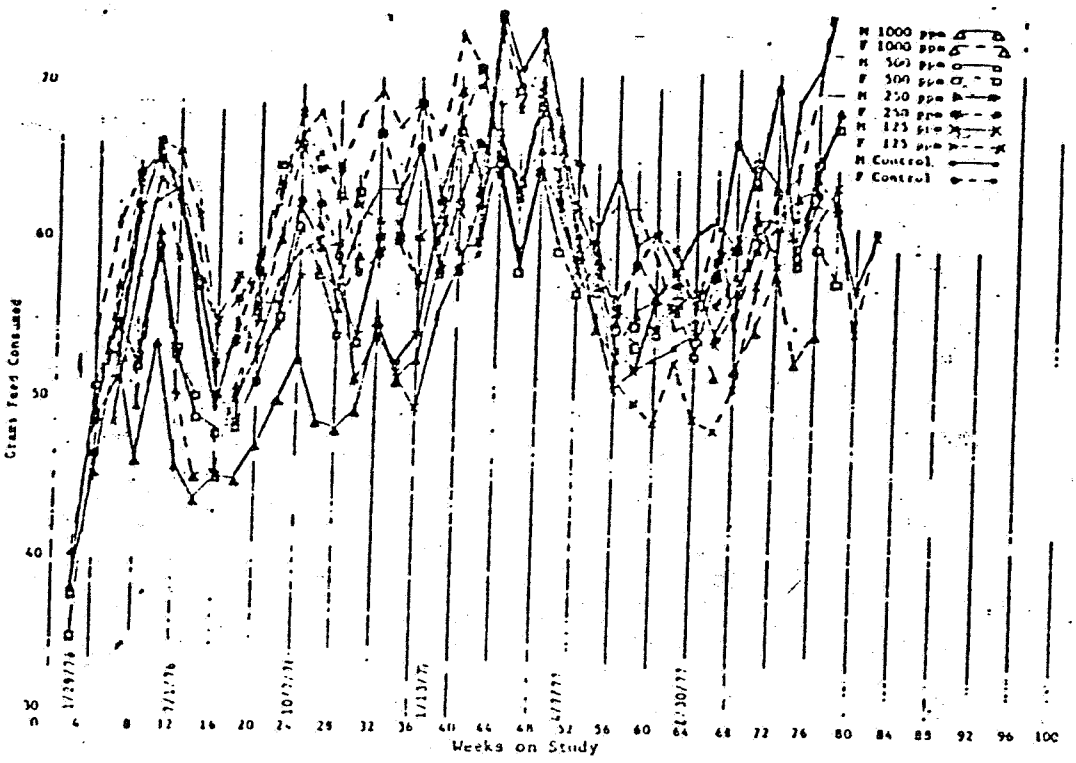

FIGURE 4. Group Mean Rotenone Diet Consumption in Carcinogenesis Study

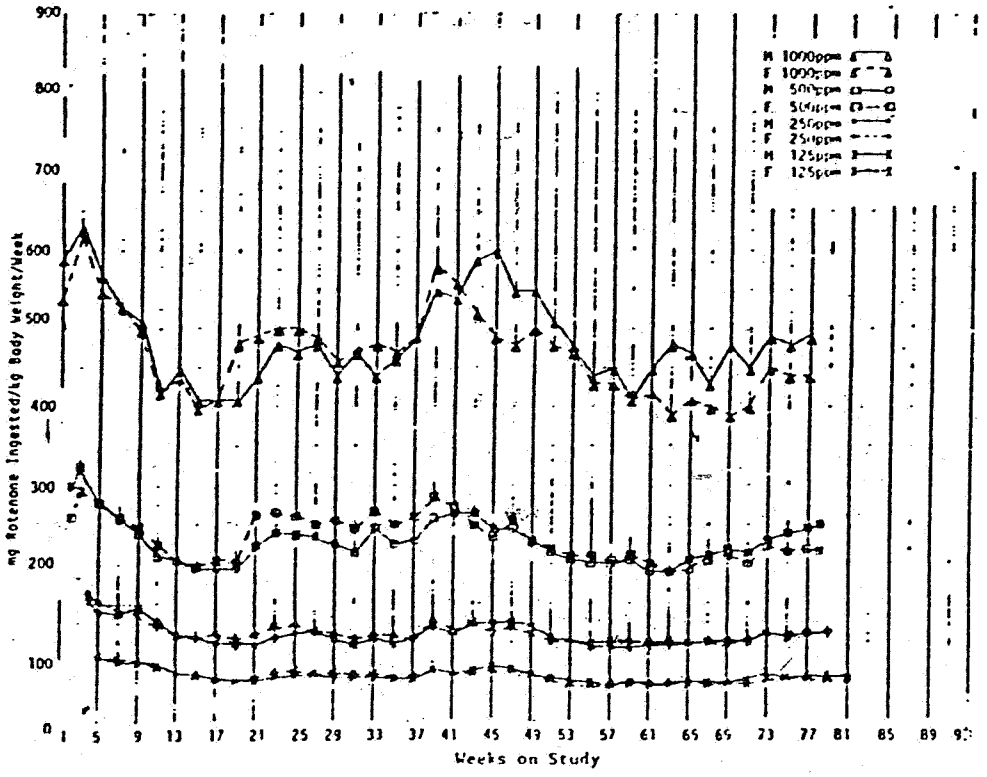

FIGURE 5. Group Mean Rotenone Consumption During Carcinogenesis Study

APPENDIX C

Schedule of studies

OUTLINE OF HAMSTER STUDIES

Title & Description	Dates		
	Receipt of Test Animals	Initiation	Completion
Prechronic Study I To test feed palatability and subacute toxicity of rotenone: rotenone administered 14-days in feed at 63, 125, 250, 500 and 1000 ppm. After 14-day observation period, gross necropsies performed.	8/25/75	9/3/75	10/9/75
Prechronic Study II Repeat of prechronic Study I. This study included addition of corn oil to rotenone-feed mixtures to increase chemical stability.	9/23/75	10/7/75	11/11/75
Oral Dosing Study Rotenone was administered by oral gavage in corn oil for 9 days in a dose of 80 mg/kg. Animals observed for gross toxic signs	9/16/75	9/30/75	10/3/75
Reproduction Study Both males and females maintained on rotenone diets: litter sizes, offspring survival and pregnancy rates were recorded.			
Group Fed 1000 ppm	12/3/75	12/8/75	6/1/76
Group Fed 0 ppm	3/16/76	3/21/76	1/26/77
Group Fed 500 ppm	6/15/76	6/28/76	1/25/77

OUTLINE OF HAMSTER STUDIES

(Continued)

Title & Description	Dates		
	Receipt of Test Animals	Initiation	Completion
Carcinogenesis Study			
Hamsters fed rotenone in diets for 18 months; histopathology performed on 0, 125, and 1000 ppm groups.			
Group Fed 0 ppm	1/13/76	1/26/76	7/28/77
Group Fed 125 ppm	1/27/76	2/9/76	8/17/77
Group Fed 250 ppm	1/21/76	2/2/76	7/29/77
Group Fed 500 ppm	1/6/76	1/19/76	7/22/77
Group Fed 1000 ppm	12/29/75	1/12/76	7/15/77

004610

DATA EVALUATION RECORD

1. CHEMICAL: Rotenone

2. TEST MATERIAL: The rotenone was 95+ per cent pure.

3. STUDY/ACTION TYPE: Oncogenicity - rats and hamsters

4. STUDY IDENTIFICATION: Freudenthal, R. I., A. P. Leber, D. C. Thake, and R. L. Baron. April, 1981. Carcinogenic Potential of Rotenone: Subchronic Oral and Peritoneal Administration to Rats and Chronic Dietary Administration to Syrian Golden Hamsters. Report published by the Environmental Protection Agency (EPA-600/1-81-037). Submitted by the U. S. Department of the Interior, Fish and Wildlife Service. EPA Acc. No. 255278 (Tab #4).

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 8/15/85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane E Harris
Date: 9/12/85

7. CONCLUSIONS: The report cited in Section 4. above is a review of two other reports that are reviewed elsewhere. (see Data Evaluation Records for Leber and Persing, 1979 and Leber and Thake, 1979 and the support document entitled Rotenone: Pre-RPAR Review May, 1980). Conclusions regarding the two other reports can be found in the previous reviews.

8. REFERENCES: Leber, A. P., and R. L. Persing. January, 1979. Carcinogenic Potential of Rotenone: Phase I: Dietary administration to hamsters. Report published by the Environmental Protection Agency (EPA-600/1-79004a). Submitted by the U. S. Department of the Interior, Fish and Wildlife Service. EPA Acc. No. 255278 (Tab #2).

Leber, A. P., and D. C. Thake. January, 1979. Carcinogenic Potential of Rotenone: Phase II: Oral and Intraperitoneal Administration to Rats. Report published by the Environmental Protection Agency (EPA-600/1-79-004b). Submitted by the U. S. Department of the Interior, Fish and Wildlife Service. EPA Acc. No. 255278 (Tab #3).

7. CONCLUSION (continued)

The purpose of the experiments was to confirm results obtained by Gosalvez and Mechan (Cancer Res. 33:3047. 1973) and was not intended for support of registration of rotenone. The following protocol differences preclude use of those rat studies in support of a registration:

1. The test substance was administered for 42 days rather than for the lifespan of the animals.

2. Animals were sacrificed after 14 to 18 months rather than the recommended 24 months.

The study is not evaluated again because of these major differences from recommended protocols for oncogenicity studies in rats (see Pesticide Assessment Guidelines Subdivision F, §83-1 and -2).

Core classification: Supplementary for reasons discussed above.

DATA EVALUATION RECORD

1. CHEMICAL: Rotenone

2. TEST MATERIAL: The rotenone was 95+ per cent pure.

3. STUDY/ACTION TYPE: Oncogenicity - rats

4. STUDY IDENTIFICATION: Leber, A. P., and D. C. Thake. January, 1979. Carcinogenic Potential of Rotenone: Phase II: Oral and Intraperitoneal Administration to Rats. Report published by the Environmental Protection Agency (EPA-600/1-79-004b). Submitted by the U. S. Department of the Interior, Fish and Wildlife Service. EPA Acc. No. 255278 (Tab #3).

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 8-5-85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: Jane E. Harris
Date: 8/12/85

7. CONCLUSIONS: This report has been reviewed previously by the Agency in a support document entitled Rotenone: Pre-RPAR Review (dated May, 1980). The studies were described in that review (submitted under EPA Acc. No. 255278 as Tab #1) as follows:

> ..., 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.