Freudenthal et al. (1981)

EPA 600/1-81-037
April 1981

PB81-190936

CARCINOGENIC POTENTIAL OF ROTENONE: SUBCHRONIC ORAL AND PERITONEAL ADMINISTRATION TO RATS AND CHRONIC DIETARY ADMINISTRATION TO SYRIAN GOLDEN HAMSTERS

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/1-81-037	ORD Report	PB81 190936

4. TITLE AND SUBTITLE	5. REPORT DATE
Carcinogenic Potential of Rotenone: Subchronic Oral and Peritoneal Administration to Rats and Chronic Dietary Administration to Syrian Golden Hamsters	April 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) R.I. Fruedenthal, A P. Leber, D.C. Thake and R.L. Baron	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Stauffer Chemical Co. PPG Battelle Memorial Institute U.S. Environmental Protection Agency	10. PROGRAM ELEMENT NO. 1EA615 11. CONTRACT/GRANT NO. 68-02-1705
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES
Project Officer: Ronald L. Baron

16. ABSTRACT Three long-term studies were performed to evaluate the carcinogenic potential of the pesticide rotenone in hamsters and rats. Rotenone was administered orally to Wistar rats and by intraperitoneal injection to Sprague-Dawley rats, which were maintained and observed for 14 and 18 months, respectively. Syrian golden hamsters were maintained for 18 months on diets containing rotenone in concentrations up to 1000 ppm. Following these studies the animals were subjected to extensive necropsy.

No evidence of an increased incidence of mammary or any other type of neoplasm was noted in the two rat studies. At all dosage levels in the hamster dietary study, no gross or histopathological evidence was obtained to suggest that rotenone induced the formation of mammary tumors. Three adrenal cortical carcinomas were observed in 65 hamsters from the highest dosage group; while suspicious, it is questionable that this occurrence was related to rotenone treatment. There were no other indications of neoplastic events.

In ancillary studies there was preliminary evidence that rotenone at levels of 500 ppm in the maternal diet was embryo-toxic and resulted in cannibalism of the young by the maternal animals. A level of 1000 ppm led to sterility in one or both sexes. Hamsters fed rotenone displayed reduced feed consumption and diminished weight gains during the first few months of administration.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Rotenone Carcinogenicity		

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	47
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risks of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

In this report the long-term carcinogenic potential of rotenone is tested in two rat strains administered subchronic dosages and in hamsters fed chronic amounts. The carcinogenic potential of rotenone in mammals is extremely important because of the chemical's wide distribution as a pesticide.

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ABSTRACT

Three long-term studies were performed to evaluate the carcinogenic potential of the pesticide rotenone in hamsters and rats. Rotenone was administered orally to Wistar rats and by intraperitoneal injection to Sprague-Dawley rats, which were maintained and observed for 14 and 18 months, respectively. Syrian golden hamsters were maintained for 18 months on diets containing rotenone in concentrations up to 1000 ppm. Following these studies the animals were subjected to extensive necropsy.

No evidence of an increased incidence of mammary or any other type of neoplasm was noted in the two rat studies. At all dosage levels in the hamster dietary study, no gross or histopathological evidence was obtained which suggested that rotenone induced the formation of mammary tumors. Three adrenal cortical carcinomas were observed in 65 hamsters from the highest dosage group. While this occurrence was suspicious, it is questionable that it was related to rotenone treatment. There were no other indications of neoplastic events.

In ancillary studies there was preliminary evidence that rotenone at levels of 500 ppm in the maternal diet was embryo-toxic and resulted in cannibalism of the young by the maternal animals. A level of 1000 ppm led to sterility in one or both sexes. Hamsters fed rotenone displayed reduced feed consumption and diminished weight gains during the first few months of administration.

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SECTION 1

INTRODUCTION

In a brief communication, Gosalvez and Merchan (1973) described experiments in which mammary tumors were found in rats which were administered rotenone. In these studies, four series of female albino rats of a strain which reportedly has a natural mammary tumor incidence of 0.5 tumors per 1000 rats per year were given intraperitoneal injections of rotenone (1.7 mg/kg body weight) dissolved in 0.1 ml sunflower oil on 42 consecutive days. Control animals received 0.1 ml of sunflower oil alone. The rats were observed for up to 19 months after treatment. In one series, 8 out of 8 rats exhibited mammary tumors between months 6 and 11 while 10 controls had no tumors after 10 months. Several of these tumors were histologically diagnosed as "adenomas with accentuated interstitial fibrosis and showed localized areas with adenocarcinomatous transformation and one was diagnosed as a differentiated adenocarcinoma. All tumors were encapsulated and did not show metastasis." Four to five successful tumor transplants out of 30 trials were made to normal rats. Primary and transplanted tumors developed slowly, requiring 7 to 12 months after initial detection before full development was achieved. In three other series of 10 rotenone-treated rats, a 60% incidence of mammary tumors was reported 10 months after the end of the treatment. No mammary tumors were noted in controls.

Based on these observations, studies were initiated to evaluate the carcinogenic potential of rotenone. In an attempt to verify the results of Gosalvez and Merchan (1973), two long-term studies were conducted in which subchronic levels of rotenone were administered orally to Wistar rats and intraperitoneally to Sprague-Dawley rats daily for six weeks, and the animals were monitored for 14 and 18 months, respectively, At the end of each study, samples from 40 different tissues for each animal were examined for abnormalities or tumors. This report also presents results of a chronic feeding study which was performed to investigate the carcinogenic potential of rotenone in Syrian golden hamsters. Included are observations made in ancillary acute toxicity studies and preliminary studies on the effects of rotenone on hamster reproduction.

SECTION 2

MATERIALS AND METHODS

Rotenone Preparation

The rotenone for all phases of these studies was obtained from S. S. Penick and Company. The material was reported by the supplier to be 98+ percent pure with traces of other rotenoids. High pressure liquid chromatographic (HPLC) analyses performed according to the method of Freudenthal et al. (1977) demonstrated the rotenone to be 95+ percent pure. Rotenone was stored in desiccators under nitrogen at -20°C until used.

Rotenone was prepared for oral gavage and dietary incorporation by adding appropriate amounts to corn oil and stirring the mixture to suspend the chemical. Corn oil (Mazola) was purchased locally and gavage suspensions were prepared daily. In a rotenone palatability study, a suspension of rotenone in corn oil was poured onto the surface of pre-weighed amounts of Purina Rat Chow Meal in a twin shell blender and mixed for 30 minutes. Initial feed analyses indicated that rotenone degraded very rapidly, with a half-life of less than 2 days, following its direct addition to feed. However, when rotenone was suspended in corn oil and then added to feed (with a final corn oil concentration of 1%), 92% of the rotenone could be recovered after one week. Subsequently, all rotenone diets were made weekly and included 1% corn oil for stability and to aid in maintaining dietary uniformity.

Animal Care and Examination

Sprague-Dawley derived rats for the dietary palatability study were obtained from Laboratory Supply Company. Wistar and Sprague-Dawley derived rats used in the oral and intraperitoneal studies were obtained from the Charles River Company. Syrian golden hamsters were obtained from Charles River Lakeview Hamster Colony.

Animals were housed individually throughout all studies except during mating periods in the reproduction study. Hamsters and rats were housed in 19 x 10.5 x 6.2 inch polycarbonate cages and cage bedding was changed weekly. Environmental conditions in the individual animal rooms were maintained under conditions suitable for the species and included a temperature of 70° to 74°F, relative humidity of 45 to 60%, and a 12-hour light, 12-hour dark cycle. Water was provided *ad libitum* throughout. Animal cages were equipped with feeders that were suitable for periodic feed consumption determinations. Hamsters were identified by a toe clipping-ear punching scheme.

Animals were observed daily for mortality and signs of abnormal behavior and were weighed and evaluated for food consumption on a weekly basis. Necropsies were performed on animals which died spontaneously and on those which were terminated at the end of the exposure period. Weights for the following organs were obtained at necropsy: heart, pituitary, adrenal and thyroid glands, brain, testicles, ovaries, spleen, liver, and kidneys.

Tissues were placed in a 10% neutral buffered formalin solution for fixation prior to processing and slide presentation. Slides were stained with hematoxylin and eosin. The following tissues were preserved for microscopic examination: abdominal skin, mammary gland, trachea, lung, heart, bronchial lymph node, mandibular lymph node, mesenteric lymph node, spleen, thymus, eye, bone marrow, tonsil, kidney, urinary bladder, ovary, uterus, testicle, epididymis, prostate, seminal vesicle, salivary gland, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, muscle, pancreas, liver, thyroid, parathyroid, adrenal, rib and femur, brain, spinal cord, and sciatic nerve. Microscopic examinations of tissues from these animals were performed by board-certified or board-eligible pathologists.

SECTION 3

EXPERIMENTAL PROCEDURES

Preliminary Hamster Palatability Studies

In a preliminary subchronic trial, a group of five male and five female hamsters were given oral intubations of 80 mg rotenone/kg body weight in 1.0 ml of corn oil daily for nine days. Their behavior and appearance were observed and compared with those of hamsters administered corn oil only.

Two four-week preliminary dietary studies were carried out to provide information on the palatability of rotenone-containing feeds and to evaluate gross toxicity in the treated animals. Rotenone was blended with Purina Hamster Chow Meal to yield concentrations of 0, 63, 125, 250, 500, and 1000 ppm for the subacute studies. Hamster weights ranged from 50 to 70 g at the beginning of the study. Animals were given access to control feed for one week prior to dosing.

In the preliminary trials, hamsters in groups of 10 animals (5 males and 5 females) were fed rotenone-containing feeds for 14 days followed by 14 days of control feed. Animal body weights and feed consumption were determined weekly. Gross necropsies were performed at the end of the 14-day control diet feeding period.

Preliminary Rat Palatability Study

Twenty Sprague-Dawley derived rats (10 male and 10 female rats) were used to test the palatability of rotenone in the diet of rats. Body weights ranged from 125 to 145 g (females) and 150 to 175 g (males) on day 0 of the feeding period. Rats were individually housed and quarantined for 7 days prior to dietary exposure to rotenone.

Five rats of each sex were assigned to each of two dosage groups. Feeds containing 1% corn oil were dosed with either 500 or 1000 ppm rotenone. Feed was presented to the rats for two weeks followed by one week of exposure to control diets (no rotenone or corn oil). Individual rats and uneaten feed were weighed weekly to obtain body weight and feed consumption values.

Rat Intraperitoneal Injection Carcinogenesis Study

A group of 65 male and 65 female Charles River Sprague-Dawley rats were used in this study. Body weights on the first day of dosing were 67 to 156 g for females and 110 to 169 g for males. Rats, housed in groups of two per cage, were held in quarantine for 13 days prior to dose initiation. Following

quarantine, the rats were assigned to one of three treatment groups. Rotenone was prepared for injections by pre-weighing amounts of the chemical which were determined to be appropriate for the following week. These amounts were calculated from the mean weights of the rats in a specific group, using the previous week's body weights and the weight increases estimated for the following week from historical growth-rate data. All samples were suspended in corn oil on the day of dosing and administered by intraperitoneal injection in a volume of 0.1 ml. Test groups of 25 males and 25 females each received doses of 1.7 or 3.0 mg of rotenone per kilogram body weight. Control rats, 15 males and 15 females, received 0.1 ml corn oil injections. Injections were made on 42 consecutive days. Following the dosing period, the rats were observed for a period of 17 months, after which the surviving animals were sacrificed and necropsied.

Rat Gavage Carcinogenesis Study

A group of 150 Charles River Wistar rats (75 of each sex) were used in this study. Body weights on the first day of dosing ranged from 75 to 145 g for females and from 81 to 156 g for males. Animals were caged in pairs and quarantined as defined above. Following quarantine, rotenone was administered by gavage with a stainless steel feeding needle. The rotenone suspension was prepared as described above for the 18-month study and was administered in a volume of 0.25 ml daily for 42 consecutive days. Doses of 0, 1.7, or 3.0 mg rotenone per kilogram body weight were administered to groups of 25 males and 25 females. Following the 42-day administration period, the rats were observed for 13 months and were then sacrificed and necropsied.

In both long-term rat studies, growth was recorded weekly for three months and biweekly for the remainder of the study. At the conclusion of the studies, gross and microscopic pathology was performed on each animal.

Long-Term Hamster Dietary Carcinogenesis Study

A long-term (18-month) study to evaluate the potential carcinogenicity of rotenone in hamsters was performed. Rotenone was incorporated in the feed of the test animals for the duration of the study.

Groups of 50 male and 50 female hamsters were fed diets containing rotenone at concentrations of 0, 125, 250, 500, or 1000 ppm for 18 months. All diets contained 1% corn oil to aid in the uniform dispersal of rotenone. Animals were weighed weekly for the first 6 months and bi- or tri-weekly thereafter. Feed consumptions were measured weekly throughout the study. Daily observations were made for mortality, behavior, and appearance.

Hamsters were subjected to complete necropsy and examined for evidence of tumors at the time of spontaneous death or, following sacrifice, at the termination of the study. Tissues examined and removed at necropsy were placed in buffered, neutral 10% formalin. Tissues from animals in the 0, 125, and 1000 ppm groups were processed, sectioned at 5 microns, and stained with hematoxylin and eosin for histologic evaluation. The groups fed 250 and 500 ppm rotenone were excluded from initial histopathological examination since these data would be superfluous if either one of two situations existed:

(1) chemical-related tumors were found in the 125 ppm low dose group or (2) no chemical-related tumors were found in either the 125 ppm low dose or the 1000 ppm high dose groups. In the first situation, the tumor-inducing threshold dose would be below 125 ppm, and data from the 250 and 500 ppm groups would not add to the question of the carcinogenic nature of rotenone. In the second circustance, lack of tumors in the high-and low-dosage groups would suggest that the tumor-inducing threshold for rotenone is above 1000 ppm and that formation of tumors by the intermediate dose would be extremely unlikely. If tumors were found in the 1000 ppm rotenone group, but none in the 125 ppm group, the preserved tissues could have been processed and examined for the establishment of a dietary threshold.

The following tissues were removed at necropsy: skin (thoractic and abdominal), mammary gland, trachea, lung, heart, abdominal aorta, bronchial, mandibular and mesenteric lymph node, spleen, thymus, kidney, ureter, bladder, ovary, uterus, testicle, epididymis, prostate, seminal vesicle, salivary gland, tongue, esophagus, stomach, duedenum, jejunum, ileum, cecum, colon, rectum, pancreas, liver, gall bladder, thyroid, parathyroid, adrenal, rib, femur, muscle, brain (cerebrum, cerebellum and medulla), pituitary, spinal cord, sciatic nerve, and eyes.

Selected sections of kidney, spleen, liver, adrenal, and thyroid were stained by the Bennhold Congo red method for amyloid detection. Other selected samples of adrenal were stained by the Gomori chromaffin staining technique for chromaffin granule detection. Microscopic examination of tissues was performed by board-certified or board-eligible pathologists.

Hamster Reproduction Study

In an ancillary study, an effort was made to evaluate the effects of rotenone on hamster reproduction. Male and female animals were fed rotenone, incorporated into the diet, at 0, 500, or 1000 ppm for three months and mated twice at appropriate times to evaluate the effects on reproduction. Groups of 50 females and 25 males (50 males were used in the controls) were used in this effort.

SECTION 4

RESULTS AND DISCUSSION

Preliminary Hamster Palatability Studies

Observations were made of 10 hamsters administered rotenone orally on a subacute basis for nine days. The animals became lethargic soon after the initial dose. Three animals died during the first two days of the study and exhibited an oily substance in the peritoneal cavity, although no stomach punctures were found. Other animals displayed signs of diarrhea, eye discharge, lameness, shaggy coats, and mouth ulcerations. The lungs, liver, and G.I. tract were congested and redness was observed in the lungs and pleura. Intussusceptions of the small intestine and rectal prolapses were commonly seen. Hamsters treated with corn oil only exhibited mild diarrhea which subsided after four days.

In preliminary dietary studies, the mean body weight data showed a substantial reduction in growth of males during the first week of feeding 1000 ppm rotenone. This effect was reversed, with weight gains greater than normal observed after return to control diet. During the course of the preliminary study, no abnormal behavior or symptoms were observed and none of the hamsters died.

Gross observations at necropsy showed lungs of several 1000 ppm hamsters to be congested and colored cherry red. Several small petechial hemorrhagic areas were present on the lung surface. Also seen were congestion of the duodenal mucosa, dilation of meningeal vessels, and hemorrhagic enteritis in the small intestines. Similar but less extensive lesions were present in animals given 500 ppm. No lesions were found in the 250 ppm or lower dosage groups. This preliminary study suggested that 1000 ppm rotenone in the diet would be an appropriate high dosage level for the chronic study.

Preliminary Rat Palatability Study

The mean body weights and feed consumption data for the palatability study are presented in Figure 1. Mean body weight gains were suppressed in both males and females fed either 500 or 1000 ppm rotenone in the diet. Females appeared to be more sensitive to rotenone than male rats in this respect. Male rats fed 1000 ppm rotenone experienced smaller weight gains than those on 500 ppm diets.

Feed consumption was slightly higher for rats fed 500 ppm rotenone compared to those fed 1000 ppm (days 7-14). When rats were returned to normal diets (days 14-21), feed consumption increased by a factor of 2.

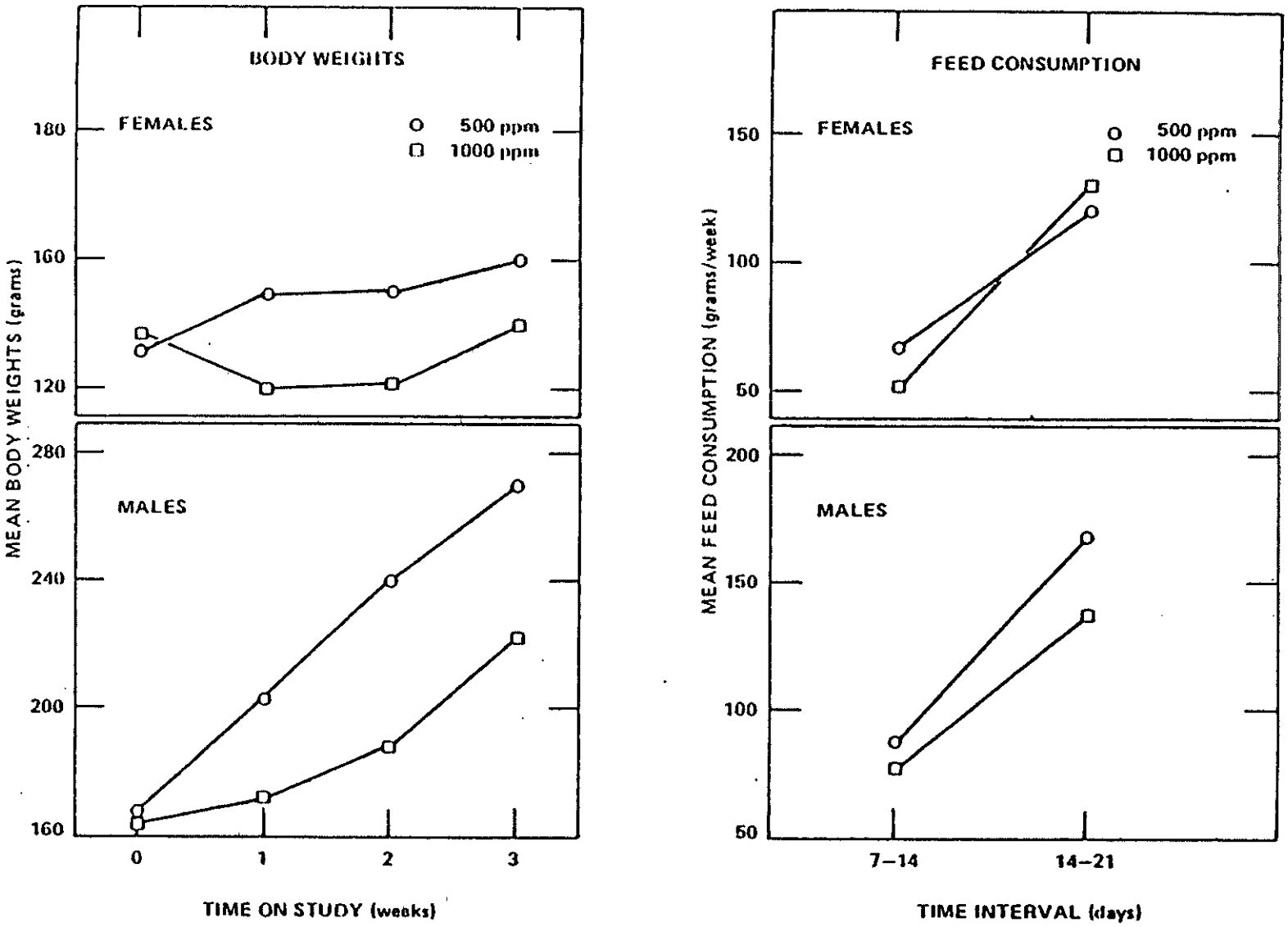

Figure 1. Mean body weights and feed consumption for rats fed rotenone.

It was concluded that feed containing rotenone at a concentration of 500 ppm is less palatable to rats than normal feed and dietary ingestion at this concentration leads to depressed body weight gains in immature rats. The use of rats for dietary carcinogenicity bioassays may be limited by the dosage levels that can be used in long-term studies.

Rat Intraperitoneal Injection Carcinogenesis Study

The intent of this phase of the project was to duplicate, in a different strain of rats, an earlier study (Gosalvez and Merchan, 1973; see INTRODUCTION) in which mammary tumors were found in rats following intraperitoneal administration of rotenone.

The mean body weight data are presented in Figure 2. Male rats which received 3.0 mg rotenone/kg exhibited up to 25% lower body weights than controls during the 18-month study, and the 1.7 mg/kg group maintained body weights intermediate to those of controls and the 3.0 mg/kg animals. The high-dosage female rats also experienced reduced body weights which were on average never less than 87% of those of the controls.

Table 1 presents the rat survival data for the study. No increases in deaths resulted from rotenone exposure. Control, low-dose, and high-dose groups experienced 23, 16, and 30% mortalities, respectively, within the 18-month study period. No substantial sex difference in mortality was noted.

TABLE 1. RAT SURVIVAL FOLLOWING ROTENONE INTRAPERITONEAL INJECTIONS

Month	Dosage Group					
	3.0 mg/kg		1.7 mg/kg		Control	
	Male	Female	Male	Female	Male	Female
0	25	25	25	25	15	15
11	21	21	24	25	15	15
12	21	21	24	24	15	15
13	21	21	24	24	14	14
14	20	21	24	23	12	14
15	18	20	24	23	12	13
16	18	19	23	23	12	13
17	17	19	23	23	11	12
18	17	18	22	20	11	12

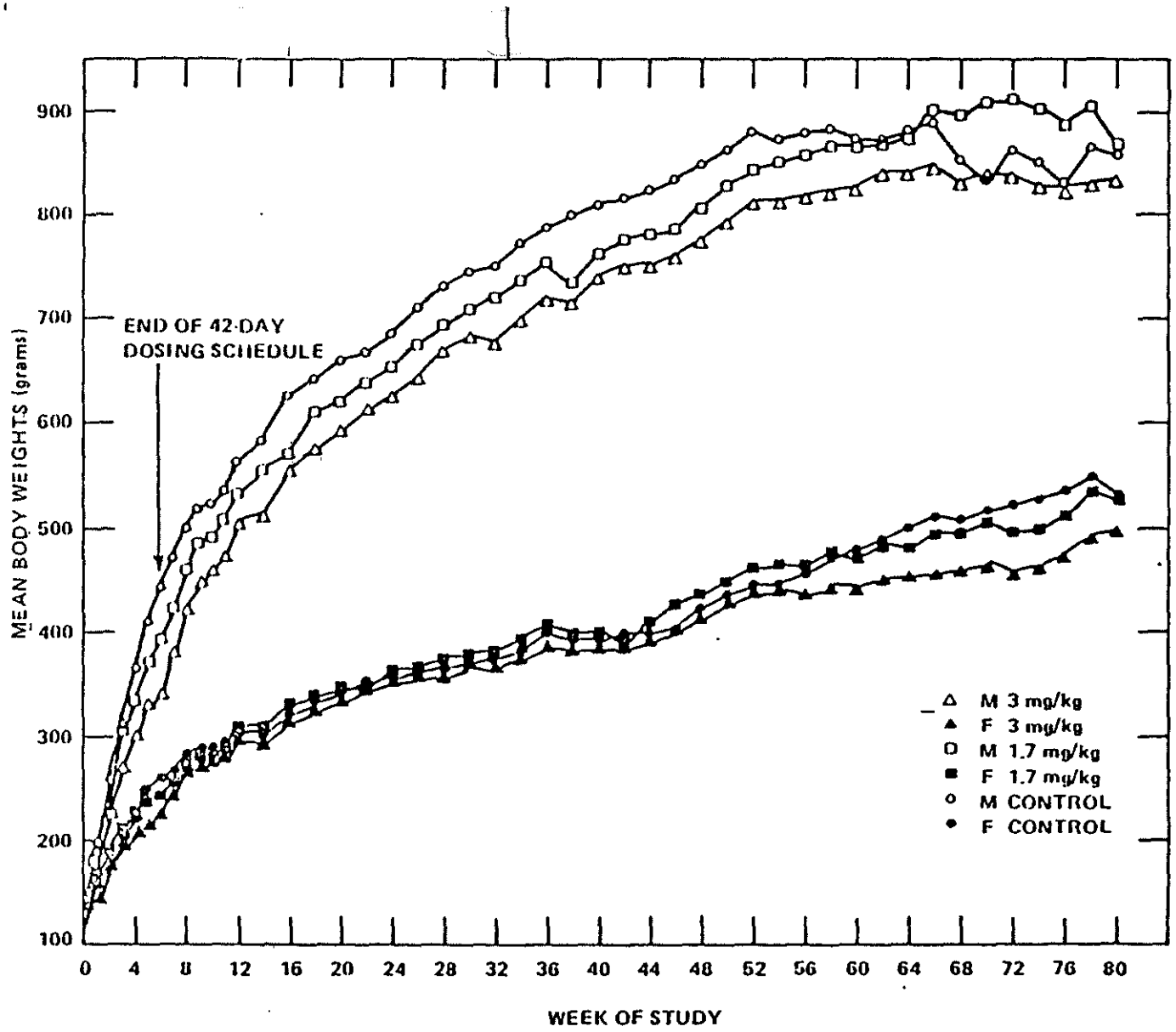

Figure 2. Mean body weights of rats in the rotenone intraperitoneal injection study.

There were numerous mammary gland neoplasms observed both macroscopically and microscopically in animals from the control group and both treatment groups (Table 2). These were for the most part fibroadenomas and they occurred with similar frequencies among control and treatment groups. In addition to the fibroadenomas, one adenoma occurred in females given 3.0 mg/kg and three in females given 1.7 mg/kg; the latter occurred in conjunction with fibroadenomas. Mammary carcinoma was present in one female given 3.0 mg/kg. Several fibroadenomas were also present in males from the control and 1.7 mg/kg groups. Fibroadenomas occurred in control rats with a frequency equal to or greater than that of treated groups, and the incidences of all female animals bearing mammary neoplasms were 60, 72, and 43% for the control, low- and high-dosage groups, respectively (Table 2). The highest incidence of mammary neoplasms in male rats occurred in the control group. Other neoplasms which occurred in rats from this study are listed in Table 2. Two lymphosarcomas occurred in females given 3.0 mg/kg; all other neoplasms occurred in only one animal from any given dosage group with the exception of chromophobe adenomas of the pituitary gland and adrenal cortical adenomas which occurred with similar frequency among control and treatment groups.

Table 3 presents a summary of the non-neoplastic lesions observed in three animals. The only non-neoplastic lesions which occurred with substantially greater frequency in rotenone-treated animals compared to controls included myocardial fibrosis and/or lymphoreticular myocarditis in both the 1.7 and 3.0 mg/kg groups and cystic ductular dilatations in mammary glands of females given 3.0 mg/kg.

Rat Gavage Carcinogenesis Study

Figure 3 depicts the mean body weights of the rats during the 14-month gavage study. No appreciable differences are seen in body weights between the different groups throughout the study. Although this study was not as lengthy nor did it use the same route of administration as that described in the second part of this report, the Wistar strain appears to display lower sensitivity to rotenone administration as measured by body weights.

Survival data are presented in Table 4. Again, no significant number of deaths can be attributed to rotenone treatment in this study. Because of the different routes of administration, it is difficult to make comparisons on the lethality of rotenone in Wistar and Sprague-Dawley rats.

Neoplasms were observed in mammary glands of three female rats from this study, as indicated in Table 5. Multiple adenomas (two) were present in two animals, one from the control group and one from the 1.7 mg/kg dosage group. A small early carcinoma and adenocarcinoma were present in two different mammary glands from one animal in the 1.7 mg/kg dosage group. There were other small masses observed grossly in which mammary cysts, ductal or glandular ectasias, or mild hyperplasia were observed microscopically. Ductal or glandular ectasias and cysts were slightly more prevalent in females from the 1.7 or 3.0 mg/kg dosage groups compared to the control group.

Adrenal cortical adenomas occurred in greater numbers in both the 1.7 and 3.0 mg/kg dosage groups as compared to the controls. This was especially

TABLE 2. INCIDENCE OF NEOPLASTIC/ HYPERPLASTIC LESIONS IN RATS IN THE ROTENONE INTRAPERITONEAL INJECTION STUDY

Organ and Lesion	Dosage Group					
	3.0 mg/kg IP		1.7 mg/kg IP		Control	
	Male (n=23)	Female (n=21)	Male (n=24)	Female (n=25)	Male (n=14)	Female (n=15)
Kidney						
Hyperplasia, pelvic epithelium	0	1	0	0	0	0
Bladder						
Transitional cell papilloma	0	0	1	0	0	0
Thyroid						
Adenoma	0	0	1	0	1	0
Pituitary						
Chromophobe hyperplasia	2	1	4	2	0	3
Chromophobe adenoma	1	2	1	2	0	2
Adrenal						
Cortical adenoma	0	2	0	5	0	4
Spleen						
Extreme hematopoietic hyperplasia	0	1	0	1	0	1
Lymph Node						
Lymphoplasmatic hyperplasia	2	1	0	0	3	0
Salivary Gland						
Ductular hyperplasia	0	0	1	0	0	1
Stomach						
Pseudoepitheliomatous hyperplasia of squamous epithelium	0	0	0	1	0	1
Pancreas						
Islet hyperplasia	0	1	1	0	0	0
Islet adenoma	0	0	2	0	0	0
Ductular hyperplasia	0	0	1	1	0	1
Liver						
Hepatocellular carcinoma	1	0	1	0	0	0
Focus of cellular alteration	0	0	0	1	0	0
Biliary hyperplasia	0	0	1	2	0	2
Uterus						
Adenocarcinoma with metastasis	-	1	-	0	-	0
Testicle						
Interstitial cell tumor	0	-	1	-	0	-
Skin						
Keratoacanthoma	0	0	0	0	1	0
Polyp	0	1	0	0	0	0
Squamous cell carcinoma	0	0	1	0	0	0
Pseudoepitheliomatous hyperplasia	0	0	0	1	0	0
Fibroma	0	0	1	0	0	0
Fibrosarcoma	0	0	1	0	0	0
Sebaceous adenoma	0	0	1	0	0	0
Mammary Gland						
Fibroadenoma	0	7	1	13	3	8
Glandular hyperplasia	0	0	0	2	0	0
Adenoma	0	1	0	3	0	1
Carcinoma	0	1	0	0	0	0
Bone (rib)						
Osteosarcoma	0	0	0	0	1	0
Lymph Node, Spleen, Liver, Ovary						
Lymphosarcoma	0	2	0	0	0	0

TABLE 3. INCIDENCE OF NON-NEOPLASTIC LESIONS IN RATS IN THE ROTENONE INTRAPERITONEAL INJECTION STUDY

Organ and Lesion	Dosage Group					
	3.0 mg/kg IP		1.7 mg/kg IP		Control	
	Male (n=23)	Female (n=21)	Male (n=24)	Female (n=25)	Male (n=14)	Female (n=15)
Lung						
Pneumonia	2	0	2	0	0	0
Bronchiectasis	0	0	1	0	0	0
Thrombosis	0	0	0	0	1	0
Heart						
Myocardial fibrosis, lymphoreticular myocarditis	11	4	22	8	4	0
Vessels						
Pyogranulomatous vasculitis	0	0	1	0	0	0
Kidney						
Nephritis (all developmental stages)	16	2	20	4	12	6
Bladder						
Cystitis	0	2	0	0	0	0
Stomach						
Granulomatous gastritis	0	0	0	1	0	0
Liver						
Hepatic necrosis	0	0	0	1	0	2
Vacuolar degeneration	0	0	0	2	0	1
Ovary						
Follicular cyst	-	2	-	0	-	1
Uterus						
Endometritis	-	2	-	0	-	0
Cystic glandular dilatation/ hydrometra	-	1	-	1	-	1
Prostate						
Prostatitis	0	-	1	-	0	-
Muscle						
Myositis	0	0	1	1	0	0
Atrophy	0	0	1	0	0	0
Skin						
Dermatitis (all types)	3	0	2	1	4	0
Epidermoid cyst	2	0	1	0	0	0
Dilated hair follicles	1	0	0	0	0	0
Subcutis						
Granulomatous steatitis	0	0	1	2	0	0
Fat necrosis (encapsulated, lipoma?)	0	0	0	1	0	2
Mammary Gland						
Cystic ductular dilatations	1	3	1	11	0	6
Eye						
Retinal atrophy	0	0	1	0	0	0

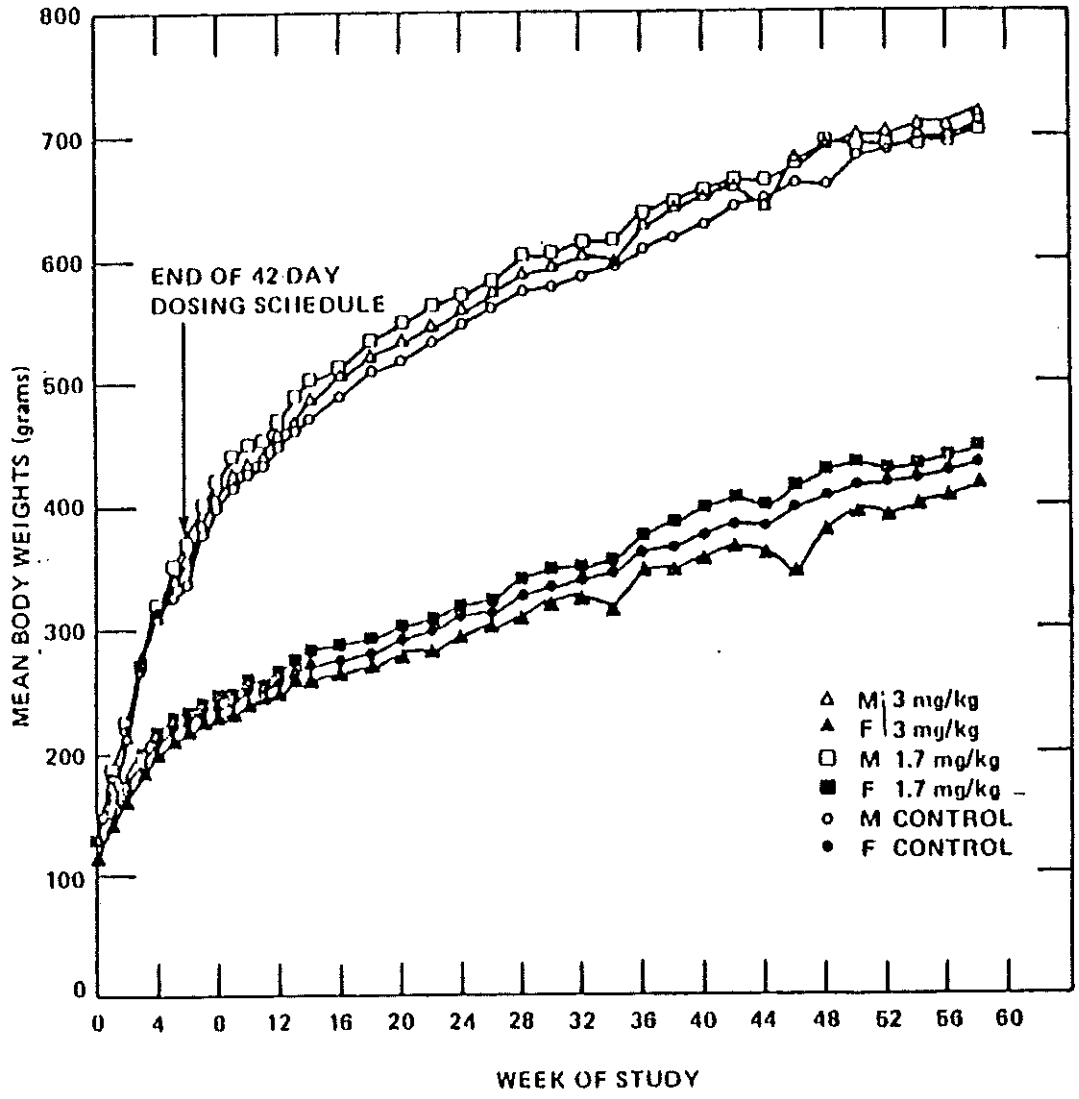

Figure 3. Mean body weights of rats in the rotenone oral gavage study.

TABLE 4. RAT SURVIVAL FOLLOWING ROTENONE ORAL GAVAGE

Month	Dosage Group					
	3.0 mg/kg		1.7 mg/kg		Control	
	Male	Female	Male	Female	Male	Female
0	25	25	25	25	25	25
7	25	24	25	24	25	25
8	25	24	25	24	25	25
9	25	24	25	24	25	25
10	24	24	25	24	25	25
11	24	24	25	24	25	25
12	23	24	25	24	25	25
13	23	24	24	24	25	25
14	23	24	24	24	25	25

noticeable in the females and occurred with similar frequency among females from both the high- and low-dosage groups.

Fibrosarcomas occurred in subcutaneous sites in three males from the 3.0 mg/kg dosage group and one fibroma was observed in a male rat from the 1.7 mg/kg dosage group. Neither fibromas nor fibrosarcomas were observed in the control group.

Bile duct hyperplasias were observed in three females and one male from the high-dose group and one male from the control group. These were extremely mild changes. All other neoplastic or hyperplastic lesions occurred with similar or greater frequencies in the control groups compared to treated groups.

Other non-neoplastic changes (Table 6) generally occurred with similar frequencies in the control and treated groups. Chronic renal disease, consisting of glomerular scleroses, thickening of tubular basement membranes with associated renal tubular epithelial regenerative changes, and in some instances chronic interstitial inflammatory changes (some or all of these changes) occurred in high percentages of male rats from all dosage groups and to a lesser extent in females. Likewise, respiratory disease was common in many animals: peribronchial and peribronchiolar lymphoid nodules were present in essentially all animals and were not listed in the diagnoses unless

TABLE 5. INCIDENCE OF NEOPLASTIC AND OTHER GROWTH CHANGES (INCLUDING MAMMARY GLAND CYSTS AND ECTASIAS) IN RATS IN THE ROTENONE ORAL GAVAGE STUDY

Organ and Lesion	Dosage Group					
	Control		1.7 mg/kg		3.0 mg/kg	
	Female (n = 25)	Male (n = 25)	Female (n = 24)	Male (n = 25)	Female (n = 24)	Male (n = 25)
Adrenal Gland						
Cortical adenoma	3	1	7	1	6	3
Cortical carcinoma			1			
Cortical hyperplasia	4	12	5	5	4	4
Pituitary Gland						
Chromophobe adenoma	4		4		4	3
Chromophobe hyperplasia	3	3	5		6	1
Focus of cellular alteration	2		2		1	
Adenoma, pars intermedia				1		
Thyroid Gland						
Follicular adenocarcinoma					1	
Follicular hyperplasia		2				1
Medullary carcinoma		1				
Mammary Gland						
Cysts	4		3		6	
Adenoma	1		1			
Adenocarcinoma*			1			
Carcinoma*			1			
Ductal or glandular ectasia	1		4		6	
Hyperplasia			1		2	
Skin and Soft Tissue						
Fibroma				1		
Trichoepithelioma						1
Fibrosarcoma						3
Mesenteric Lymph Node						
Lymphosarcoma, focal	1					
Multiple Organs						
Lymphosarcoma		1				
Liver						
Neoplastic nodule		1			1	
Focus of cellular alteration		1				1
Bile duct hyperplasia		1			3	1
Diaphragm						
Carcinoma metastatic (origin unknown)		1				

*These neoplasms occurred in the same animal.

TABLE 6. INCIDENCE OF NON-NEOPLASTIC LESIONS IN RATS IN THE ROTENONE ORAL GAVAGE STUDY

Organ and Lesion	Dosage Group					
	Control		1.7 mg/kg		3.0 mg/kg	
	Female (n = 25)	Male (n = 25)	Female (n = 24)	Male (n = 25)	Female (n = 24)	Male (n = 25)
Heart						
Myocardial degeneration and/or necrosis	1			1	2	2
Myocardial fibroplasia	1					
Myocarditis, acute or subacute	1	2	3	3	3	
Coronary artery, dystrophic mineralization			1			1
Papillary muscle, ectopic bone						2
Pericardial fat, necrosis and granulomatous inflammation with crystalline deposition	1					
Kidney						
Chronic renal disease*	5	19	6	22	5	18
Nephritis, acute						1
Cortex, medulla or pelvis mineralization	4		9	1	2	
Medulla, granulomatous inflammation					3	
Hydronephrosis	1				1	
Congestion	5	1		1		
Pyelitis, suppurative			2			
Nephritis, chronic active with papillary necrosis and abscessation		1				
Dilatation of Bowman's capsule			1			
Focal subcapsular cyst				2		
Cortical or pelvic lymphocytic infiltrates		2	1	1		
Liver						
Bile duct hyperplasia		1			3	2
Congestion		1	1		3	6
Hepatocyte degeneration			2	1		
Centrilobular necrosis				2		1
Focus of cellular alteration		1				1
Hepatitis, subacute			4	2		1
Extramedullary hematopoiesis					3	
Hepatocyte vacuolation	1	1	1	5		
Lung						
Congestion	2	3	3	3		
Granulomatous inflammation					1	2
Edema						1
Interstitial pneumonia	8	9	15	10	11	8
Hemorrhage		1				
Atelectasis		1				

*See text for definition of chronic renal disease.

TABLE 6. (Continued)

Organ and Lesion	Dosage Group					
	Control		1.7 mg/kg		3.0 mg/kg	
	Female (n = 25)	Male (n = 25)	Female (n = 24)	Male (n = 25)	Female (n = 24)	Male (n = 25)
Lung (continued)						
Emphysema		1				
Developmental anomaly		1				
Alveolar macrophage accumulations	1					
Prostate Gland						
Prostatitis, suppurative, focal				1		
Prostatitis, subacute				1		2
Prostatitis, chronic, active, with necrosis				1		
Stomach						
Fundic mucosa, mineralization						1
Submucosa, edema						1
Submucosal cyst	1					
Gastritis, chronic, eosinophilic, with fibrosis		1				
Intestine						
Enteritis, subacute					4	3
Colon, parasitosis	1	2				
Jejunum, congestion			1			
Lymph Nodes						
Mandibular lymph node, hemorrhage, hemosiderin congestion and/or lymphadenitis	4	3	2	6	10	4
Mandibular lymph node, fibroplasia				1		
Mesenteric or mandibular lymph node, sinusoidal dilation				3		
Mesenteric lymph node, hemorrhage, hemosiderin, congestion, and/or lymphadenitis			1	1	1	6
Pancreatic lymph node, hemorrhage and hemosiderin			1			
Bronchial lymph node, ectopic tissue of uncertain origin				1		
Pituitary Gland						
Hemosiderin deposition	2	1				
Pars distalis chromophobe hyperplasia	2	2	1			
Pars nervosa, eosinophilic crystalline deposition	1					
Neurohypophyseal cleft, protein deposition				1		

TABLE 6. (Continued)

Organ and Lesion	Dosage Group					
	Control		1.7 mg/kg		3.0 mg/kg	
	Female (n = 25)	Male (n = 25)	Female (n = 24)	Male (n = 25)	Female (n = 24)	Male (n = 25)
Urinary Bladder						
Cystitis	1				1	1
Pancreas						
Acinar cell atrophy	1		2			3
Focal ductal ectasia					1	
Fibrosis		1				
Pancreatic islets, fibrosis		5		4		
Pacreas or pancreatic islets, lymphocytic infiltrates	1	1		2		
Hemosiderin deposition		2				
Epididymis						
Lymphocytic infiltrate				1		
Testicle						
Artery mineralization		1				
Degeneration, fibrosis and/or atrophy		1		1		3
Ovary						
Follicular cysts	2					
Multilocular cysts					1	
Paraovarian cyst			1			
Thymus						
Branchial arch cysts					1	
Skin						
Subcutis, necrotizing phlebitis						1
Mammary gland, dermatitis, nonsuppurative, mild			1			
Adrenal Gland						
Cortex, cytoplasmic vacuolation	3	1	1			
Cortical hyperplasia	4	12	5	5	4	4
Cortex, vascular ectasia	7		6		13	
Cortex, focus of cellular alteration	1					
Cortex, congestion	3	1	3	1		
Cortex, lymphocytic infiltrate	1					
Medulla, sinusoidal ectasia						2
Capsular fibrosis	1					

TABLE 6. (Continued)

Organ and Lesion	Dosage Group					
	Control		1.7 mg/kg		3.0 mg/kg	
	Female (n = 25)	Male (n = 25)	Female (n = 24)	Male (n = 25)	Female (n = 24)	Male (n = 25)
Thyroid Gland						
Parafollicular cell hyperplasia						3
Follicular hyperplasia		3				1
Cyst	2					
Eye						
Corneal vascularization and degeneration of Bowman's capsule					2	
Spleen						
Siderotic nodule						1
Trachea						
Submucosa, inflammatory infiltrate						1
Uterus						
Endometrial gland dilatation	2					
Hemosiderin deposition	1					
Endometrium, lymphocytic infiltrate	1					
Endometrial glands, epithelial, hyperplasia			1			
Pulmonary Artery						
Medial hypertrophy	1					
Mammary Gland						
Ductal or glandular ectasia	1		4		5	
Cysts	4		3		6	
Mastitis, granulomatous	1					
Hyperplasia			1		1	
Mastitis, chronic				1		
Parathyroid Gland						
Fibrosis	1					
Mandibular Salivary Gland						
Hyperplasia with cytomegaly		1				
Atrophy			1			
Degeneration, focal			1			
Hemosiderin deposition		1				
Meninges						
Cerebellum, fibrosis				1		

TABLE 6. (Continued)

Organ and Lesion	Dosage Group					
	Control		1.7 mg/kg		3.0 mg/kg	
	Female (n = 25)	Male (n = 25)	Female (n = 24)	Male (n = 25)	Female (n = 24)	Male (n = 25)
Brain						
Encephalitis, nonsuppurative, multifocal				1		
Cerebrum, thromboembolic meningitis and encephalitis						1
Subependymal hemorrhage	1					
Sciatic Nerve						
Mineralization		1		1		
Femur						
Marrow cavity, fibrosis		1		5		

associated with other changes. Subacute enteritis, hepatic congestion, and lymphadenitis of mesenteric and mandibular lymph nodes occurred with slightly greater frequencies in the 3 mg/kg dosage group than in controls.

There was a striking difference between the incidence of mammary neoplasia among rats in the groups treated orally compared to those exposed by intraperitoneal administration (Table 7). The incidence of mammary fibroadenomas in the intraperitoneal treatment control group was as high as in those given 1.7 mg/kg and higher than the group given 3.0 mg/kg. The incidence of animals with mammary neoplasms from the intraperitoneal study was similar for rats in the 1.7 mg/kg and control groups since the three adenomas occurred in animals which also had fibroadenomas. The lowest incidence was recorded in those rats given 3.0 mg/kg. These data have been interpreted as showing no evidence that rotenone enhanced or induced mammary neoplasia in animals under the conditions of either the intraperitoneal or the oral treatment study.

The significantly higher incidence of mammary neoplasia in animals from the intraperitoneal versus the oral treatment group may reflect a difference in the incidence of spontaneous mammary neoplasms in Wistar and Sprague-Dawley rats. The large numbers of spontaneous fibroadenomas commonly present in Sprague-Dawley rats was evident in this study and was in sharp contrast to the absence of this tumor type in the Wistar rats.

The absence of any evidence of increased mammary tumor incidence in rotenone-treated animals from either the oral or intraperitoneal study is significantly different from the results of Gosalvez and Merchan (1973), who reported a 60 to 100% incidence of mammary tumors in female albino rats which developed 6 to 11 months following intraperitoneal injections of 1.7 mg/kg of rotenone in 0.1 ml of sunflower oil for 42 days. The reason for the variation in test results is not known.

The significance of three fibrosarcomas and one fibroma which occurred in male rats from the 3.0 and 1.7 mg/kg dosage groups in the oral study is not clear. One fibroma and one fibrosarcoma also occurred in male rats given 1.7 mg/kg in the intraperitoneal study. This is suspicious but inconclusive evidence that the fibromas and fibrosarcomas were induced by the rotenone.

A greater incidence of adrenal cortical adenomas occurred in the treated groups compared to control groups from the oral study. The significance of these differences is difficult to interpret in view of the small number of animals included in these groups and the relatively high incidence of these changes which were present in control groups.

Long-Term Hamster Dietary Carcinogenesis Study

Mortality was evident in the long-term hamster dietary study. Substantial spontaneous death losses were encountered in all groups of hamsters in this study during the first 12 months of exposure. The deaths were apparently not related to rotenone administration since the losses were as high or higher in controls than in the treatment groups. A pathogenic strain of E. coli was isolated from several animals dying during this period,

TABLE 7. COMPARISON OF INCIDENCE OF MAMMARY GLAND NEOPLASIA IN RATS ADMINISTERED ROTENONE ORALLY AND INTRAPERITONEALLY

Species and Route	Type of Neoplasm	No.	Treatment Level (mg/kg)	No. in Group	Sex	Incidence %
	Adenoma	1	Control	25	F	4
Wistar	Adenoma	1	1.7	24	F	4
(oral gavage)	Carcinoma*	1	1.7	24	F	4
	Adenocarcinoma*	1	1.7	24	F	4
	Fibroadenoma	8	Control	15	F	53
	Fibroadenoma	3	Control	14	M	21
	Fibroadenoma	13	1.7	25	F	52
Sprague-Dawley	Fibroadenoma	1	1.7	24	M	4
(intraperitoneal injection)	Fibroadenoma	7	3.0	21	F	33
	Adenomat	3	1.7	25	F	12
	Adenoma	1	3.0	21	F	5
	Adenoma	1	Control	15	F	7
	Carcinoma	1	3.0	21	F	5

*Occurred in same animal.
†All three adenomas in this group occurred in animals which also had fibroadenomas.

which suggested that a heat-labile enterotoxin of E. coli may have been responsible for the early mortalities. These observations were not investigated further.

The mean values for hamster growth as evidenced by body weight changes are presented in Figure 4. Both the 500 and 1000 ppm groups, and particularly males fed the high dose, exhibited depressed body weights relative to other test groups. This trend may have resulted in part from the decreased feed consumption for these groups during the first six months of the study as noted with rats. Data depicting rotenone consumption are presented in Figure 5 (expressed as milligrams of rotenone consumed per kilogram of body weight per week).

Survival data for the hamsters studied are presented in Table 8. Spontaneous deaths were not dose-related for rotenone nor were there sex-related differences for mortalities in the test groups. Female control animals experienced a high death rate during the final five months of the study; this may have been related to the enteric infections which were prevalent in this group early in the study.

Gross visible lesions encountered at necropsy of hamsters dying spontaneously are presented in Table 9. Notable lesions encountered in animals dying spontaneously were nephrosis due to apparent amyloid deposition, centrilobular congestion and necrosis of the liver, vegetative thrombosis generally in the left atrium of the heart, pulmonary congestion, hemorrhage, and pneumonia as well as atrophy or hypoplasia of the testicles.

Several tumors were also apparent at necropsy. Ovarian tumors were noted in one female of the 1000 ppm group and in one of the control group. Masses involving leg muscles were found in one female of the 1000 ppm group and in one female of the control group. A renal tumor was suspected in one male of the 500 ppm group, and a cystic adenoma was found in one female of the 125 ppm group; possible thyroid tumors were found in one female hamster of the 125 ppm group and two females of the control group. An adrenal tumor was suspected in one female of the 1000 ppm group and a lymphoid neoplasm was present in several tissues from one female of the 250 ppm group.

Gross lesions observed in hamsters sacrificed at the termination of the study are described in Table 10. These hamsters had fewer and less severe inflammatory intestinal and pulmonary lesions than animals that died spontaneously. Amyloid-induced nephrosis occurred more frequently and severely in hamsters terminated at the end of the study than in hamsters dying spontaneously. The incidence of hepatic or biliary cysts in the liver also increased in those animals that survived longest in the study. Grossly visible tumors or focal hyperplasia of the adrenal glands were noted in two male hamsters of the 1000 ppm group, one female of the 500 ppm group, two males of the 125 ppm group, and one male of the control group. A chondroma of of the costochondral junction of the rib was found in one female of the 1000 ppm group. An increase in thyroid and adrenal congestion was noted at 1000 ppm.

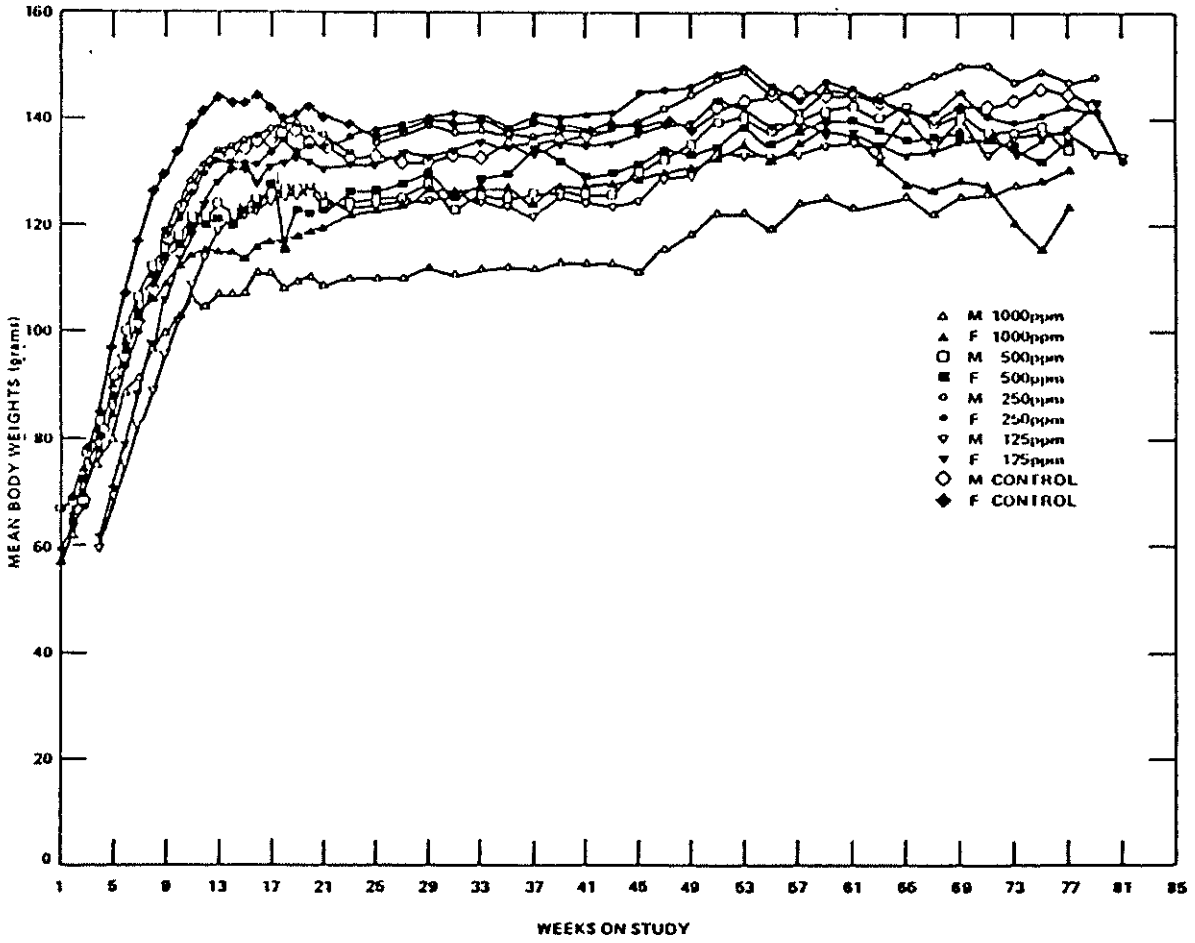

Figure 4. Mean body weights of hamsters fed rotenone.

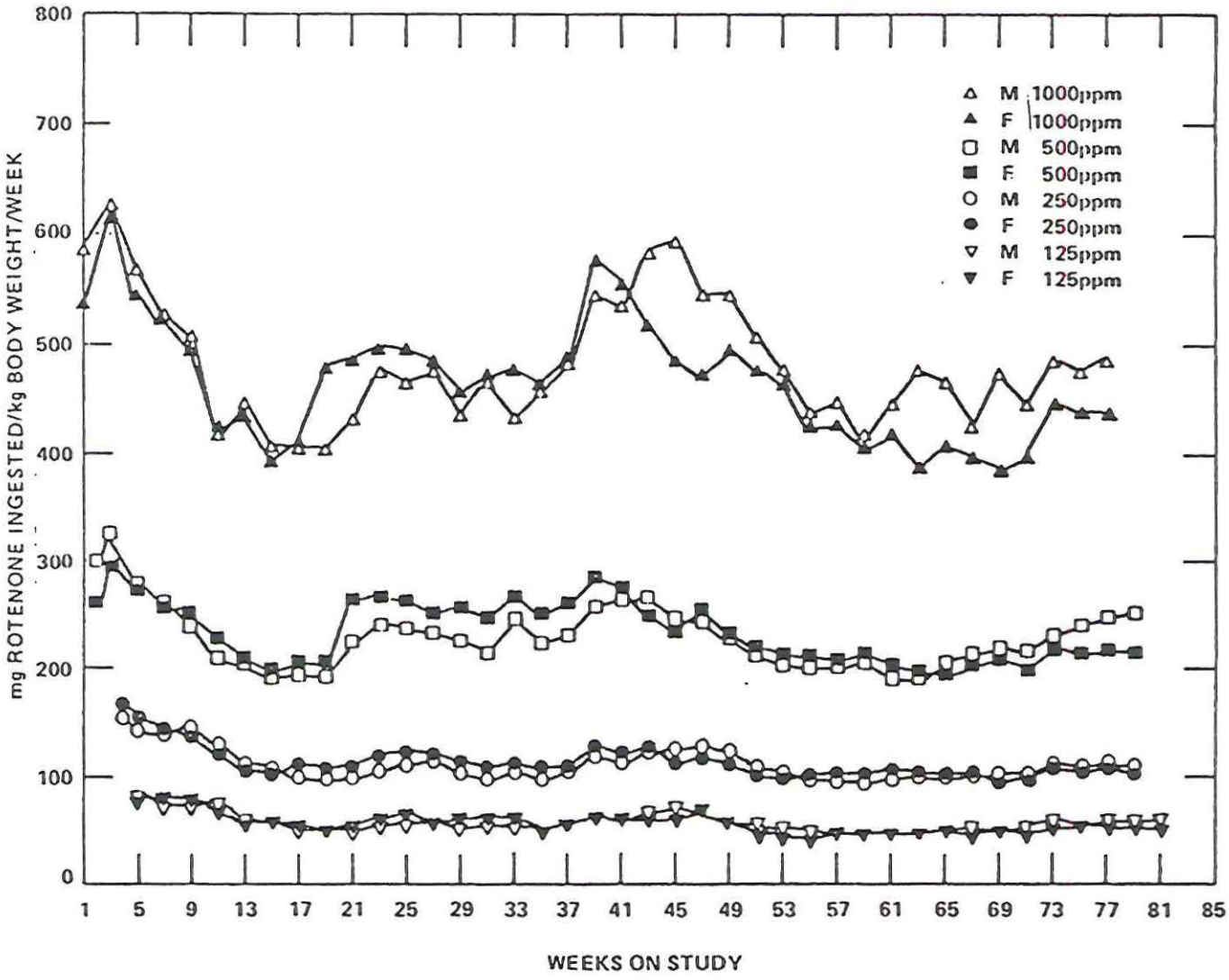

Figure 5. Mean rotenone consumption for hamsters in the dietary carcinogenesis study.

TABLE 8. HAMSTER SURVIVAL DURING THE ROTENONE DIETARY STUDY

Month	Dosage Group									
	1000 ppm		500 pm		250 ppm		125 ppm		Control	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
0	50	50	50	50	50	50	50	50	50	50
12	33	34	35	30	33	37	35	28	30	28
13	32	33	34	28	31	34	32	26	30	27
14	29	30	33	26	27	30	27	25	30	23
15	27	28	32	26	27	30	26	24	29	20
16	26	27	31	23	25	28	25	22	28	14
17	25	24	29	21	24	27	24	19	25	11
18	25	19	26	20	20	24	24	14	22	6
19 (until necropsy)	24	19	25	18	19	20	21	9	22	2

TABLE 9. GROSS LESIONS IN HAMSTERS DYING SPONTANEOUSLY DURING THE ROTENONE DIETARY STUDY

Diagnosis	Dosage Level and Number in Group									
	1000 ppm		500 ppm		250 ppm		125 ppm		Control	
	57	%	57	%	61	%	70	%	76	%
Enteritis	26	46	23	40	20	33	37	53	22	29
Typhlitis	27	47	33	58	24	39	28	40	33	43
Colitis	8	14	9	16	8	13	8	11	6	8
Nephrosis	10	18	8	14	17	28	25	36	28	37
Liver centrilobular congestion and necrosis	9	16	12	21	18	30	13	18	17	22
Pulmonary congestion and hemorrhage	8	14	20	35	19	31	20	28	29	38
Pneumonia	10	18	4	7	8	13	1	1	4	5
Heart arterial or ventricular thrombosis	4	7			4	6	5	7	7	9
Liver hepatic or biliary cysts	2	4	2	4	3	5	5	7	10	13
Gastric ulceration	2	4	1	2	6	10	5	7	1	1
Rectal prolapse and intussusception					1	2	1	1	2	3
Testicular atrophy or hypoplasia	6	10	1	2	4	6	9	13	1	1
Ovarian tumor or cyst	1	2			1 cyst	2	1 cyst	1	1	1
Tumor (muscle of leg)	1	2							1	1
Renal tumor			1	2			1	1		
Spleen focal hyperplasia or tumor mass	1	2			1	2			2	3
Thyroid focal hyperplasia or tumor mass							1	1	2	3
Adrenal focal hyperplasia or tumor mass	1	2								
Salivary gland focal hyperplasia or tumor tumor mass							1	1		
Tumor, lymphosarcoma					1	2				

TABLE 10. GROSS LESIONS IN HAMSTERS SACRIFICED AT THE END OF THE ROTENONE DIETARY STUDY

Diagnosis	Dosage Level and Number in Group									
	1000 ppm		500 ppm		250 ppm		125 ppm		Control	
	43	%	43	%	39	%	30	%	24	%
Enteritis					4	10	5	17	3	12
Typhlitis	1	2					2	7		
Colitis					1	2				
Nephrosis	9	21	21	49	27	69	19	63	16	67
Liver centrilobular congestion and necrosis	1	2	5	12	8	20	5	17	3	12
Pulmonary congestion and hemorrhage	6	14	3	7	12	31	1	3	9	38
Heart atrial or ventricular thrombosis			4	9	2	5				
Liver hepatic or biliary cysts	10	23	5	12	10	26	3	10	5	21
Gastric ulceration	1	2								
Testicular atrophy or hypoplasia	1	2	1	2			1	3	2	8
Ovarian cysts					2	5	1	3		
Eye microophthalmic appearance	1	2			1	2				
Spleen focal hyperplasia or tumor	2	5			5	13	3	10		
Thyroid congestion	3	7								
Adrenal congestion	2	2								
Adrenal focal hyperplasia or tumor	3	7	1	2			3	10	1	4
Rib tumor chondroma	1	2								
Epididymus sperm granuloma									1	4
Skin focal hyperplasia of dermis									1	4

The distribution of animals from the 1000, 125, and 0 ppm dosage groups which died or were sacrificed and subjected to histopathological examination is summarized in Table 11, and the distribution of non-neoplastic and non-hyperplastic lesions from these groups is shown in Table 12.

TABLE 11. DISTRIBUTION BY CAUSE OF DEATH FOR HAMSTERS EVALUATED MICROSCOPICALLY IN THE ROTENONE DIETARY STUDY

	Dosage Level					
	1000 ppm		125 ppm		Control	
Cause of Death	Male	Female	Male	Female	Male	Female
Spontaneous	8	14	11	17	8	25
Sacrifice	24	19	21	9	22	2
Total	32	33	32	26	30	27

The predominant non-neoplastic microscopic alterations encountered in all groups reviewed were associated with changes due to age-related amyloid deposition in the kidneys, liver, spleen, and adrenals. The pattern of change found in the kidneys was retraction of the capsular surface with associated tubular degeneration and regeneration with the 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 misshapen nephron units with dilated collecting tubules filled with brightly stained eosinophilic protein material. The normal liver architecture was altered by rather thick deposits of light pink amyloid-related material in and between the hepatocytes and vascular tissues of the portal triads that often become confluent between adjacent triads. The splenic red pulp was 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 follicles in the thyroid than did males. Thrombosis of the left atrium was present in all female groups but was not found in any male reviewed microscopically. Pulmonary lesions ranged from mild alveolar sputal congestion to bronchiolar pneumonia of a moderate severity. Varying degrees of enteritis, typhlitis and colitis were observed in all groups. Testicular atrophy was present to some extent in all three groups of male animals. Numerous other lesions of lower incidence, less significance, or random occurrence are listed in Table 12 and will not be discussed further.

TABLE 12. NON-NEOPLASTIC AND NON-HYPERPLASTIC LESIONS IN HAMSTERS FROM THE ROTENONE DIETARY STUDY

Diagnosis	Control				125 ppm				1000 ppm			
	Female		Male		Female		Male		Female		Male	
	No. Affected (27 total)	%	No. Affected (30 total)	%	No. Affected (26 total)	%	No. Affected (32 total)	%	No. Affected (33 total)	%	No. Affected (32 total)	%
Kidney amyloidosis	25	92	26	87	21	81	15	47	28	85	8	25
Kidney tubular degeneration and regeneration	21	78	24	80	25	96	30	94	25	76	23	72
Kidney glomerular congestion			8	27			6	19			2	6
Liver amyloidosis	21	78	3	10	23	88	2	6	20	61	2	6
Liver hepatocyte vacuolation	2	7	13	43	9	35	4	12	17	54	7	22
Liver hepatic or biliary cysts	11	41	3	10	7	27	3	9	12	36	3	9
Liver sinusoidal congestion	11	41	14	47	12	46	18	25	22	67	6	19
Liver bile duct hyperplasia	5	18	3	10	15	58	3	9	20	61	4	12
Liver centrilobular degeneration and necrosis	2	7	13	43	21	81	8	25	22	67	5	16
Liver extramedullary hematopoiesis	3	11	6	20	2	8	14	44			5	16
Liver hepatitis suppurative mulifocal			1	3			1	3				
Spleen amyloidosis	14	52	3	10	21	81	3	9	18	54	1	3
Spleen lymphoid depletion			1	3	5	19	2	6	2	6		
Spleen lymphocyte depletion			9	30	13	50	7	22	10	30	2	6
Lung bronchiolar epithelial hyperplasia	1	4	3	10	4	15	5	16	4	12	2	6
Lung pulmonary edema	10	37	3	10	5	19	2	6	3	9		
Lung pulmonary congestion	13	48	13	43	5	19	9	28	13	39	8	25
Lung bronchiolar pneumonia	7	26	1	3	5	19	2	6	4	12		
Lung interstitial pneumonitis	2	7			4	15			7	21		
Lung parabronchiolar fibrosis focal											1	3
Heart arterial thrombosis	14	52			5	19			2	6		
Heart ventricular thrombosis	4	15	1	3	2	8						
Heart myocardial degeneration, mild	5	18	5	17	3	12	2	6	5	15	2	6
Adrenal cortex amyloidosis	12	44	4	13	17	65	4	12	18	54	3	9
Adrenal medullary cell degeneration	6	22	9	30	3	12	4	12	7	21	5	16
Eye lenticular degeneration vacular	1	4	2	7	1	4	4	12	3	9	2	6
Intestine lamina propria amyloidosis	5	18			3	12	1	3	2	6		
Enteritis	1	4	8	27	14	54	11	34	12	36	8	25
Typhlitis	4	15	14	47	9	35	13	41	14	42	8	25
Colitis	1	4	7	23	3	12	4	12	5	15	2	6
Thyroid amyloidosis	8	30	2	7	11	42	1	4	10	30	1	3
Heart dystrophic mineralization	3	11					1	4	2	6		
Lymph node mandibular lymphadenitis	9	33			8	31	2	6	3	9	1	3
Lymph node mesenteric lymphadenitis	5	18	6	20	10	38	6	19	5	15	4	12
Heart atrial-ventricular valve thickening	7	26	6	20	9	35	1	4	6	18	3	9
Heart atrioventricular valve thrombosis									1	3	1	3
Ovary abtess	1	4										

TABLE 12. (Continued)

Diagnosis	Control				125 ppm				1000 ppm			
	Female		Male		Female		Male		Female		Male	
	No. Affected (27 total)	%	No. Affected (30 total)	%	No. Affected (26 total)	%	No. Affected (32 total)	%	No. Affected (33 total)	%	No. Affected (32 total)	%
Brain cerebrum paravascular cuff mild	3	11										
Granuloma NOS			1	3								
Kidney, adrenal cortex subcapsular rest			1	3								
Seminal vesicle, mucosa papillary hyperplasia			1	3								
Eye, microophthalmia with distortion									1	3		
Passive congestion generalized									1	3		
Ectopic adrenal rest, brown fat									1	3		
Skeletal muscle, necrosis									1	3		
Mammary gland, acinar cell hyperplasia focal									1	3		
Tongue glossitis											1	3
Adrenal cortico-medullary cysts	1	4	1	3					1	3		
Ovary, cystic follicle					2	8						
Fallopian tube cyst					2	8						
Liver, bile duct ectasia (cholangiomatosis)					3	12						
Pituitary pars distalis vacuolar degeneration	7	26	13	43	4	15	9	28	8	24	10	13
Parathyroid cysts multilocular	1	4										
Cerebrum ischemic necrosis	1	4										
Heart atrium bone marrow			1	3								
Bone marrow rib cellular arrest			1	3	1	4			1	3		
Salivary gland acinar cell atrophy	1	4										
Salivary gland adenitis	1	4										
Ovary cytoplasmic vacuolar alteration	3	11										
Lymph node mandibular granuloma			1	3								
Epididymis sperm granuloma			1	3								
Seminal vesicles mineralization			1	3								
Brain choroid plexus distrophic mineralization			1	3								
Ileum ileitis proliferative			2	7								
Stomach fundic mucosa mineralization			1	3			1	4				
Thyroid follicular cysts multilocular					3	12	2	6	4	12	1	3
Stomach gastritis					2	8			1	3		
Testicle atrophy and seminiferous cell degeneration			4	13			8	25			2	6
Stomach gastric ulceration									1	3	1	3
Stomach, cardia, papillary fronds					2	8						
Pancreas acinar cell atrophy					2	8						

TABLE 12. (Continued)

Diagnosis	Control				125 ppm				1000 ppm			
	Female		Male		Female		Male		Female		Male	
	No. Affected (27 total)	%	No. Affected (30 total)	%	No. Affected (26 total)	%	No. Affected (32 total)	%	No. Affected (33 total)	%	No. Affected (32 total)	%
Cecum proliferative cystic typhlitis					2	4	3	9				
Pancreas branchial arch cysts					1	4						
Mass, inflammation					1	4						
Tongue, dystrophic mineralization							2	6				
Heart, coronary artery mineralization							1	3				
Heart, coronary artery sclerosis							3	9				
Testicle, necrotizing vasculitis focal							1	3				

Tables 13 and 14 list the tumors and various types of hyperplasias which occurred in the different dosage groups. Adrenal cortical carcinomas were observed in the group given 1000 ppm (two in females, one in a male). Adenomas and hyperplasias of the adrenal cortex were common in all dosage groups and were the most consistent lesions noted in the male and female groups examined. Three animals from the medium dosage groups had tumors which were evident at necropsy. After microscopy, these were diagnosed as renal nephroblastoma for one male hamster at 500 ppm, lymphosarcoma in one female at 250 ppm, and splenic hemangioma in one female at 250 ppm.

Acinar cell hyperplasia occurred in the mammary tissue of one female from the 1000 ppm group. There were no neoplastic or other lesions present in mammary tissue from hamsters in this study. Adrenal cortical adenomas and hyperplasias occurred with similar frequencies in all groups. However, three adrenal cortical carcinomas occurred in the group that received 1000 ppm rotenone in their diet; one male and one female lived until study completion while another female died two months prior to study termination. Analysis of these data by a chi-square 2 x 3 contingency scheme indicated that the appearance of this tumor in the high-dosage group (compared to its non-occurrence in the 0 and 125 ppm groups) is significant between the 90 and 95% confidence levels. However, because the control group represented a younger population of hamsters due to a larger number of early deaths, this comparison is not valid since these tumors are known to develop spontaneously in aging hamsters (Pour et al., 1976b). The significance of these carcinomas in the highest dosage group and their absence from other groups is not clear.

The lesions encountered in this 18-month study are similar in nature to those reported by Pour et al. (1976a, b, c, and d), Chesterman (1972), and Gleiser et al. (1971) on larger hamster colonies retained for lifetime studies. Aging hamsters are predisposed to development of renal, splenic, hepatic, and adrenal lesions related to amyloid deposition.

From the current study it is concluded that no substantial histopathological differences were seen between treatment groups. Males in this study had less amyloid depositions in the liver, spleen, adrenals and thyroid than females. No profound differences in the incidence of non-neoplastic pathologic lesions were observed between rotenone-treated animals at various dose levels and the control group. No pathologic alterations of note were seen in any dose group relative to mammary gland changes. Under the conditions of this bioassay, rotenone is not carcinogenic to the Syrian golden hamster. However, the occurrence of increased levels of adrenal cortical tumors in hamsters (as well as in rats as observed above), should be noted.

Hamster Reproduction Study

Two groups of hamsters (50 males and 50 females) were maintained on a control diet throughout the entire study. One female was caged with one male for mating. Pregnancies occurred, offspring were delivered, weaned and discarded. The process was repeated to produce a second litter.

TABLE 13. NEOPLASTIC AND HYPERPLASTIC LESIONS IN HAMSTERS FROM THE ROTENONE DIETARY STUDY

Diagnosis	Control				125 ppm				1000 ppm			
	Female		Male		Female		Male		Female		Male	
	No. Affected (27 total)	%	No. Affected (30 total)	%	No. Affected (26 total)	%	No. Affected (32 total)	%	No. Affected (33 total)	%	No. Affected (32 total)	%
Leg muscle fibrosarcoma	1	4										
Leg muscle undifferentiated sarcoma metastatic									1	3		
Ovary granulosa cell tumor	1	4			1	4			2	6		
Thyroid parafollicular cell adenoma	1	4	2	7								
Rib chrondroma									1	3		
Pituitary adenoma	2	7							1	3		
Spleen hemangioma											1	3
Thyroid follicular cell carcinoma							1	3			1	3
Trachea mucosal carcinoma											1	3
Adrenal medulla pheochromocytoma											1	3
Para thyroid adenoma	1	4					1	3				
Adrenal cortical cell hyperplasia	13	48	11	37	12	46	15	47	15	45	19	59
Adrenal cortex adenoma	5	18	8	27	7	27	8	25	6	18	8	25
Adrenal cortex carcinoma									2	6	1	3
Spleen reticuloendothelial cell sarcoma							1	3				
Thyroid parafollicular cell hyperplasia	1	4	2	7								
Skin sebaceous gland hyperplasia			1	3								
Adrenal medulla hyperplasia							1	3				
Thyroid follicular cell adenoma cystic					1	4						
Kidney papillary cystic adenoma cortex					1	4						

TABLE 14. MICROSCOPIC DIAGNOSES OF NEOPLASIA IN HAMSTERS FROM THE ROTENONE DIETARY STUDY

Lesion	Dosage Level					
	Control		125 ppm		1000 ppm	
	Male	Female	Male	Female	Male	Female
Adrenal cortex adenoma	9	4	8	7	8	6
Adrenal cortex carcinoma	0	0	0	0	1	2
Adrenal medulla pheochromocytoma	0	0	0	0	1	0
Trachea mucosa carcinoma	0	0	0	0	1	0
Ovary granulosa cell	0	1	0	1	0	2
Spleen hemangioma	0	0	0	0	1	0
Kidney papillary cyst adenoma	0	0	0	1	0	0
Rib chondroma	0	0	0	0	0	1
Thyroid cystic follicular adenoma	0	0	0	1	0	0
Thyroid follicular carcinoma	0	0	1	0	1	0
Thyroid parafollicular cell adenoma	2	1	0	0	0	0
Parathyroid adenoma	0	1	1	0	0	0
Spleen reticulum cell sarcoma	0	0	1	0	0	0
Pituitary pars distalis adenoma	0	2	0	0	0	1
Fibrosarcoma leg	0	1	0	0	0	0
Undifferentiated sarcoma NOS	0	0	0	0	0	1

The F_1a generation all appeared healthy through weaning. Forty-three litters were delivered from 50 females. The average litter size was 7, and ranged from 2 to 10 pups. One to three pups were retained from each litter and observed for tumor formation over an extended period. The second group of offspring (F_1b) also appeared to be healthy through weaning. There was an average of 12 pups per litter which ranged in size from 9 to 17 pups.

A group of animals (50 females and 25 males with mean body weights of 45 g for both sexes were maintained on a 1000 ppm rotenone diet containing 1% corn oil for three months and mated. The parental hamsters continued to increase in body weight for the first five weeks of the study, after which time feed consumption decreased. Fifteen (3 males and 12 females) of the 75 animals studied died during the first two months. Physical conditions deteriorated and body weights decreased in the survivors. After eight to nine weeks, the downward trend in body weights reversed, the animals became more alert, their coats developed a sheen and food consumption returned to normal.

Females were observed daily according to the Orsini technique to monitor estrus cycles. During the fourth month of the study, mating was initiated by housing one male with two females at the end of the first day of the estrus cycle, which is normally four days in duration. Although several vaginal plugs were observed, it was soon determined that one or both sexes were infertile since no pregnancies occurred. Males were observed to have smaller than normal testicles. This group was discontinued when pregnancies failed to occur.

Another group of hamsters (25 males and 50 females) were maintained on a 500 ppm rotenone diet for three months prior to and during the mating periods. Mating was carried out as described above. The first generation of offspring (F_1a) consisted of 45 litters from 50 females. Only seven litters survived through weaning as the dam often cannibalized or totally neglected her young. The pups were all smaller than normal. The average litter size was 9 pups and ranged from 4 to 15 offspring. The second mating of the 500 ppm group yielded 21 litters ranging in size from 7 to 16 pups with an average of 12. The dams continued to refuse to nurse their young and often cannibalized them.

Six months after the beginning of the 500 ppm rotenone feeding study, and 10 months for the control group, the studies were terminated because of high toxicity at the 500 ppm level, exhibited by large numbers of fetal deaths, maternal deaths, cannibalism of offspring pups, and offspring death which resulted in a small number of hamsters surviving weaning. While rotenone did not affect substantially parental reproduction at 500 ppm, survival of offspring was severely affected in hamsters. As these studies were not carried further, further data are needed to fully evaluate the effects of rotenone on hamster reproduction.

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