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Project Summary

Carcinogenic Potential of Rotenone: Subchronic Oral and Peritoneal Administration to Rats and Chronic Dietary Administration to Syrian Golden Hamsters

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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.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

This summary describes two series of studies on the carcinogenic potential of rotenone. In the first series, subchronic doses of rotenone were administered to male and female Wistar rats orally and to Sprague-Dawley rats by intraperitoneal injection for 42 consecutive days. The orally treated rats were maintained

for 14 months and the peritoneally treated rats for 18 months. In the second series, chronic dietary studies were performed to evaluate the potential carcinogenicity of rotenone in the Syrian golden hamster. Several ancillary studies were carried out including a preliminary reproduction experiment.

The first series included three studies. The first study was performed to test the palatability of rotenone in the diet of rats. Twenty Sprague-Dawley derived rats (10 male and 10 female rats) were used. Body weights ranged from 125 to 145 g (females) and 150 to 175 g (males) at the start of the feeding period. Rats were individually housed and quarantined for seven 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 feed remaining in feed pans were weighed weekly to obtain body weight and feed consumption values.

The second study was performed to determine the carcinogenic potential of rotenone delivered by intraperitoneal injection. A group of Sprague-Dawley rats (65 male and 65 female Charles River 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 preweighing 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 rotenone/kg 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.

The final study was performed to determine the carcinogenic potential of rotenone delivered by oral gavage. 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 ranges from 75 to 145 g for females and from 81 to 156 g for males. Animals were caged in pairs and quarantined as 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. The rotenone-corn oil mixture was given in a volume of 0.25 ml daily for 42 consecutive days. Doses of 0, 1.7, or 3.0 mg rotenone/kg body weight were administered to groups of 25 males and 25 females. Following 42-day administration period, the rats were observed for 13 months, after which the rats were 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.

The second series of studies concerned the effects of rotenone on Syrian golden hamsters. In a preliminary subchronic trial a group of 10 hamsters were given oral intubations of 80 mg rotenone/kg body weight, suspended in 1.0 ml corn oil, daily for nine days.

Two four-week preliminary dietary studies were conducted 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.

A long-term (18-month) study to evaluate the potential carcinogenicity of rotenone in hamsters was performe Rotenone was incorporated in the fee of the test animals for the duration the study. Groups of 50 male and 5 female hamsters were fed diets cor taining rotenone at concentrations of 125, 250, 500, or 1000 ppm for 1 months. All diets contained 1% corn o to aid the uniform dispersal of rotenon Animals were weighed weekly for th first six months and bi- or tri-weekl thereafter. Feed consumptions wer measured weekly throughout the stud Daily observations were made for mo tality, behavior, and appearance. Ham sters were subjected to complete ne cropsy and examined for evidence c tumors at the time of spontaneou death or, following sacrifice, at th termination of the study.

Tissues examined and removed a necropsy were placed in buffered, neu tral 10% formalin. Tissues from animal in the 0, 125, and 1000 ppm group were processed, sectioned at 5 microns and stained with hematoxylin and eosi for histologic evaluation. The groups fe 250 and 500 ppm rotenone were ex cluded from initial histopathologica examination since these data would b superfluous if either of two situation existed: (1) chemical-related tumor were found in the 125 ppm (low-dosage group or (2) no chemical-related tumor were found in the 125 ppm (low-dosage or the 1000 ppm (high-dosage) groups In the first situation, the tumor-inducin threshold dosage would be below 12 ppm, and data from the 250 and 50 ppm groups would not add to the question of the carcinogenic nature of rotenone. In the second circumstance, 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 intermediate dosages 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 (thoracic and abdominal), mammary gland, trachea, lung, heart, abdominal aorta, bronchial, mandibular and mesenteric lymph nodes, spleen, thymus, kidney, ureter, bladder, ovary, uterus, testicle, epididymis, prostate, seminal vesicle, salivary gland, tongue, esophagus, stomach, duodenu jejunum, ileum, cecum, colon, rectu

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.

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

Results

Mean body weight gains were suppressed in both male and female rats 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 doubled. It is 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.

The intent of the next phase of the project was to duplicate, in a different strain of rats, an earlier study in which mammary tumors were found in rats following intraperitoneal administration of rotenone. Male rats which received 3.0 mg/kg rotenone exhibited up to 25% lower body weights than controls during the 18-month study, while the 1.7 mg/kg group maintained body weights intermediate to those of controls and the 3.0 mg/kg animals. The most highly dosed female rats also experienced reduced body wieghts which were on average not less than 87% of those of the controls.

Table 1 presents the rat survival data for this study. No increases in death 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.

There were numerous mammary gland neoplasms observed both macroscopically and microscopically in animals from the control group and both treatment groups. These were for the most part fibroadenomas and they occurred with similar frequency 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 incidence of all female animals bearing mammary neoplasms was 60, 72, and 43% for the control, low- and high-dosage groups, respectively. The highest incidence of mammary neoplasms in male rats occurred in the control group. Two lymphosarcomas occurred in females given 3.0 mg/kg; all other neoplasms occurred in only one animal for 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.

The only non-neoplastic lesions which occurred with substantially greater frequencies 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. Complete summaries of all neoplastic and non-neoplastic lesions observed in the animals necropsied in this study are given in the full Project Report.

In the second long-term study, where rotenone was administered by oral gavage to Wistar rats, no appreciable differences were 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 previously, the Wistar strain appears to display lower sensitivity to rotenone administration as measured by body weights.

Survival data are presented in Table 2. Again, no significant number of deaths can be attributed to rotenone treatment in this study. Because of the different rates of lesions observed in the control groups, 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. Multiple adenomas (two) were present in two animals, one from the control group and one from the 1.7 mg/kg dosage group. There were other small masses observed grossly in which mammary cysts, ductal or glandular ectasias, or mild hyperplasias 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 as compared to the control group.

Adrenal cortical adenomas occurred in greater numbers in both the 1.7 and

Table 1. Rat Survival Following Rotenone Intraperitoneal Injections

	Dosage Group					
	3.0 mg/kg		1.7 mg/kg		Control	
Month	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

Table 2. *Rat Survival Following Rotenone Oral Gavage*

	Dosage Group					
	3.0 mg/kg		1.7 mg/kg		Control	
Month	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

3.0 mg/kg dosage groups compared to the controls. This was especially 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 groups. Neither fibromas nor fibrosarcomas were observed in control groups in this study.

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 lesions, and other non-neoplastic changes, occurred with similar or greater frequencies in the control groups.

There was a striking difference between the incidence of mammary neoplasia among Wistar rats, treated orally, compared to Sprague-Dawley rats exposed by intraperitoneal administration (Table 3). The incidence of mammary fibroadenomas in the intraperitoneal treatment group was as high in the control animals as in those given 1.7 mg/kg and higher than the group given 3.0 mg/kg. The incidence of animals from the intraperitoneal study with mammary neoplasms 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 were interpreted as showing no evidence that rotenone enhanced or induced mammary neoplasia in animals under the condition of either the intraperitoneal or the oral treatment study.

The significantly higher incidence of mammary neoplasia in animals treated intraperitoneally compared to the oral treatment group may reflect a different incidence of spontaneous mammary neoplasms in Wistar and Sprague-Dawley rats. The large number 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.

Observations were made of 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 only with corn oil 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 being fed 1000 ppm rotenone. This effect was reversed, with observed weight gains greater than normal, after return to the control diet. During the course of this preliminary study, no abnormal behavior or symptoms were observed and none of the hamsters died.

Gross observations at necropsy showed the 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. Preliminary study suggested that 1000 ppm in the diet would be an appropriately high dosage level for the chronic study.

Mortality was evident in the long-term carcinogenesis study. Substantial spontaneous death losses were encountered in all groups of hamsters from this study during the first 12 months of exposure. The deaths were apparently not related to rotenone administration since the losses were as

Table 3. *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 %
Wistar (oral gavage)	Adenoma	1	Control	25	F	4
	Adenoma	1	1.7	24	F	4
	Carcinoma*	1	1.7	24	F	4
	Adenocarcinoma*	1	1.7	24	F	4
Sprague-Dawley (intraperitoneal injection)	Fibroadenoma	8	Control	15	F	53
	Fibroadenoma	3	Control	14	M	21
	Fibroadenoma	13	1.7	25	F	52
	Fibroadenoma	1	1.7	24	M	4
	Fibroadenoma	7	3.0	21	F	33
	Adenoma†	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.

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 and preliminarily suggested that a heat-labile enterotoxin of *E. coli* may have been responsible for early mortality. These observations were not investigated further.

Survival data for the hamsters studied are presented in Table 4. 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, which may have been related to the enteric infections which were prevalent in this group early in the study.

Change in body weight was used as a measure of hamster growth. Both 50 and 1000 ppm groups exhibited depressed body weights relative to other test groups, particularly males fed the higher dosage. 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.

Gross visible lesions encountered at necropsy of hamsters dying spontaneously and of animals sacrificed at study termination are presented in the full Project Report. 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. Several other lesions and alterations were also found.

In this study 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 should be noted.

Two groups of hamsters (50 males and 50 females) were maintained on a control diet throughout the modified reproduction study. Each 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.

The F_1a generation all appeared healthy through weaning. Forty-three litters were delivered from 50 females. Litter sizes ranged from 2 to 10 pups, with an average of 7. 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. The 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

Table 4. *Hamster Survival During the Rotenone Dietary Study*

	Dosage Group									
	1000 ppm		500 ppm		250 ppm		125 ppm		Control	
Month	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

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 again refused 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 at 500 ppm did not substantially affect parental reproduction, survival of offspring was severely affected in hamsters. As these studies were not carried further, more data are needed to evaluate fully the effects of rotenone on hamster reproduction.

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The complete report, entitled "Carcinogenic Potential of Rotenone: Subchronic Oral and Peritoneal Administration to Rats and Chronic Dietary Administration to Syrian Golden Hamsters," (Order No. PB 81-190 936; Cost: $6.50, subject to change) will be available only from:
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