FINAL REPORT

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ANALYTICAL STUDIES FOR THE DETECTION OF CHROMOSOMAL ABERRATIONS IN FRUIT FLIES, RATS, MICE, AND HORSE BEAN

SUBMITTED TO

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SUMMARY

This project was designated to evaluate the clastogenic effects of rotenone on the 4 test systems: sex chromosome loss in Drosophila melanogaster, chromosomal changes in rat bone marrow, micronucleus induction in mice and chromosomal aberrations in root tip cells of Vicia fabia. Prior to each of the genetic studies, a toxicity test was performed. A LD_{50} value for each of the systems was derived from data obtained from the corresponding toxicity test. Based upon these values sub-lethal dose levels were determined for the genetic studies. The LD_{50} values estimated were 3.4 mM for male fruit flies, 71 mg/kg b.w. for male Sprague Dawley rats, and 164 mg/kg b.w. for swiss albino mice. Due to the low solubility of rotenone in aqueous medium, a LD_{50} value for inhibiting root tip elongation of Vicia fabia was not obtainable. The clastogenic effects of rotenone were tested on Drosophila melanogaster at 0.1, 0.3 and 3.0 mM, on male rats at 0.7, 2.5 and 7.0 mg/kg b.w. and on mice at 10 and 80 mg/kd b.w. Dosing was achieved by oral administration. Negative results were obtained from all of these tests. It is concluded that rotenone at the sub-lethal dose levels used did not affect the normal chromosomal behavior of these organisms.

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I. INTRODUCTION

Rotenone, a root derivative from several plants such as Derirs ellaptica, has been used as a pesticide and fish toxicant, with an annual global consumption ranging from 10,000 to 20,000 tons. As a pesticide, rotenone is mainly used to control garden and house-hold pests including rodents. As a fish poison, it is generally used to eliminate unwanted fish species in fisheries and to regulate fish populations in lakes, reservoirs and streams (1). Due to the extended use of this toxic substance, low levels of rotenone have been detected in home-grown vegetables and in drinking water. This has caused great concern about possible hazardous effects on human health at sub-lethal levels of rotenone. The carcinogenicity of rotenone has been studied by several groups of workers. Gosalvez and Merchen found that chronic exposure to low doses of rotenone led to the development of mammary tumors in rats (2). They suggested that the specific induction of mammary tumors in these animals by rotenone was due to the modification of the synthesis and function of estrogen, a key factor in mammary carcinogenesis (3). However, similar studies carried out on hamsters (4) and rats (5) by Leber and colleaques and on two strains of mice by Innes, et al. (6) showed no significant increase in incidence of tumors in any of these animal species. In spite of these controversial reports, evidence has been provided recently that rotenone interferes with the biosynthesis and function of spindle fibers in certain types of cells (7-9). These facts tend to suggest that sublethal doses of rotenone may be clastogenic. The present work was designated to examine such possibilities using the following test systems:

1. Cytogenetic effects on rat bone marrow
2. Micronucleus formation in mice

3. Sex chromosome loss in Drosophila melanogaster

4. Chromosomal changes in Vicia faba

Tests on the horse bean root tip cells could not be performed due to the low solubility of rotenone in aqueous medium. We found that the maximum concentration of rotenone which could be dissolved in Hoagland's salt solution, a nutrient medium for growing the horse bean plants, was 20 mg/l. Precipitation appeared in this solution 12-16 hours later upon standing at room temperature. Since this concentration was not found to affect root development of these plants, a LD_{50} value of rotenone was not obtainable. Therefore, a genetic test using this concentration might not lead to the production of meaningful information concerning its clastogenicity on this test system. Test results from the 2 animal systems and the insect system are presented in this report.

II. MATERIALS AND METHODS

A. Maintenance of Animals and Drosophila

1. Rats: Dublin Sprague-Dawley (random bred Dub) rats 8-10 weeks old were supplied by Flow Laboratories, Inc. (McClean, VA). They were housed in plastic cages with stainless steel bar tops with 5 rats to a cage. The rats were maintained in a room with constant temperature and a 12/12 hours of light/dark condition. They were fed with Purina Laboratory Chow and water ad libitum.

2. Mice: Male and female random bred Dub (ICR Swiss) mice of 7-8 weeks old were supplied by Flow Laboratories, Inc.. Male and female out bred swiss mice were purchased from M.A. Bioproducts, Inc. (Walkersville, MD). They were housed in plastic cages with 4 mice to a cage under identical environmental conditions as the rats. Male and female mice were housed in separate cages.

3. Drosophila melanogaster: Strains of d/63 Inscy and FI25, B/y+ were obtained from the Mid-American Drosophila Stock Center at the Bowling Green State University, Bowling Green, Ohio. The stock cultures were raised in 6 oz. glass bottles (Urine specimen bottles, Professional Specialties Co.), at 23°-25°C and fed with the Carolina Biological instant medium without dyes (formula 4-24, Carolina Biological Supplies, CBS, Burlington, N.C.). The medium was fortified with dry yeast powder for controlling bacterial growth. The bottles were plugged with sterile styrofoam plugs. Under these conditions the life cycle of the flies, from egg to adult, was approximately 9-10 days.

B. Chemicals

Rotenone was provided by the U.S. Fish and Wildlife Services and stored

50, 100, 200 and 300 mg/kg of body weight (b.w.). Twenty rats were treated at each dose level. An additional 20 rats were dosed with corn oil alone as negative controls. Three dose levels were used for cytogenetic studies on rats with 10 animals for each dose. These dose levels were chosen based upon LD_{50} values obtained from the toxicity tests. The high dose was 1/10 of the LD_{50} concentration, of and the intermediate and low doses were 1/3 and 1/10 of the high dose, respectively.

For mice, 4 dose levels were also employed for toxicity studies: 100, 300, 600 and 1000 mg/kg b.w. with 20 animals per dose. Twenty animals were treated with corn oil alone as negative controls. For the micronucleus test, 2 doses of rotenone were chosen based on LD_{50} value obtained. The high dose was 1/8 of the LD_{50} dose and the low dose was 1/8 of the high dose. Eight animals were tested at each of the dose levels. Two groups of 8 each were separately treated with corn oil and TEM at 0.5 mg/kg b.w. (11), as negative and positive controls, respectively.

Both the rats and mice were conditioned and observed for at least 2 weeks prior to each test. The body weight was measured individually at the beginning and the end of the observation period. Animals shown to have uniform weight gains were selected and randomized into various groups for the test.

2. Dose and Dosing of Fruit Flies: For toxicity testing, 8 concentrations of rotenone were used: 0.001, 0.003, 0.01, 0.03, 0.10, 0.30, 1.00 and 3.00 mM. Data was plotted and a LD_{50} value was estimated from the survival curve. For the genetic study, 3 dose levels of rotenone were chosen based upon the LD_{50} values obtained as above. The high dose was 1/10 of the LD_{50} concentration and the 2 lower doses were 1/3 and

1/10 of the high dose concentrations, respectively. Negative controls were treated with the sucrose-agar medium alone and MMS at 10 mM was used as the positive control. One hundred male flies were used for each of the dose levels in both the toxicity and genetic testings.

Male flies of F125 (B/y+) were dosed by feeding on sucrose-agar medium according to Wetzel and Barnett (12). Rotenone was first dissolved in acetone to 0.1 M and then was gradually diluted in sucrose solutions to the desired concentrations. Two hours before feeding, equal volumes of these solutions were quickly mixed with an agar solution. The agar solution was melted by boiling and then cooled to 50°C in a water bath before mixing. The final concentrations of agar and sucrose were 1.5 and 5%, respectively. MMS was given to the flies using a sinter glass filter submerged in an aqueous solution at a concentration of 1.0 mM, acccording to Vogel (13). The flies were treated for 24 hours with rotenone and for 2 days with MMS. The flies were treated in groups of 25 in 25 x 95 mm glass vials with 10 ml of the treating medium.

D. Recording of Rotenone Toxicity

Death of rats and mice was recorded every day for 2 weeks following rotenone dosing. The death rate of Drosophila melanogaster was determined 72 hours after dosing. The main criterion for toxicity of rotenone to rats and mice was change in body weight. Each animal was weighed prior to treatment, and the surviving animals were weighed at the end of the 1st and 2nd week. Weight was measured with an Ainsworth top-loading electronic balance (Fisher, model 2000). Reduction in activity was also recorded as a reference during the 2-week period. Survival curves for treated animals were constructed based on the lethality data obtained

and these curves were statistically evaluated acording to Litchfield and Wilcoxon (14). An LD_{50} value for each of these organisms was estimated from the survival curve.

E. Extraction of Bone Marrow

Rats were sacrificed by CO_2 and the mice were sacrificed by cervical dislocation. Immediately after sacrifice, both femurs of each animal were removed in toto. After removal of the surrounding muscles, the femurs were shortened until a small opening to the marrow canal became visible. The marrow was aspirated using a 5 ml plastic syringe half filled with warm Hank's balanced salt solution. The marrow mixture was then transferred into a 15 ml centrifuge tube and suspended completely. For the micronucleus analysis, the marrow was aspirated with a 1 ml syringe and transferred to a 15 ml conical centrifuge tube containing 5 ml of fetal bovine serum (FBS) for further processing.

F. Preparation of Slides for Chromosome Studies

The bone marrow suspension was centrifuged immediately at 1000 rpm in a table-top centrifuge for 10 minutes. The pellet was resuspended in 8 ml of 0.075 M KCl for hypotonic treatment at 37°C in a water bath for 30 minutes. After centrifugation, the cells were fixed in a fresh mixture of methanol and acetic acid, 3:1, v/v at 4°C for 2 hours. After a change with the same fixative the cells were left at 4°C overnight. The fixative was again centrifuged out and the cells suspended in a small volume of fresh fixative. The cell suspension was then deposited on glass slides, pre-chilled in ice-water. Due to the majority of the mitotic cells failing to spread properly, nearly all of the slides made were flamed to burst the chromosomes for better spreading. The slides

were kept overnight at room temperature and then stained in 2% Geimsa stain in Sorensen's buffer, pH 6.8, for 12-15 minutes and rinsed with distilled water. After drying, the slides were cleaned in xylene for 10 minutes and mounted with a cover glass in Permount. Slides from each specimen were blindly coded before microscopic analysis.

G. Preparation of Smears for Micronucleus Analysis

Bone marrow collected in PBS was immediately centrifuged at 1000 rpm for 10 minutes at room temperature. After the removal of the supernatant, the pellet was suspended and smeared onto a glass slide and air dried overnight. Differential staining was obtained by the procedure of Gollpudi and Kamra (15). In brief, the air-dried slides were first fixed in methanol for 5 minutes, rinsed twice with distilled water, and then stained with a solution of Geimsa stain (1:6 in distilled water) for 10 minutes. After drying the slides were mounted in Permount.

H. Scoring of Chromosome Aberrations and Micronuclei Formation

For each specimen, 400 mitotic spreads showing well scattered chromosomes were analysed under a light microscope (Olympus/Vanox, Japan) for chromosome number and structural abnormalities. The criteria for the abnormalities used were those of Kilian et al. (16). Each category of abnormalities observed was recorded separately and the total incidence was calculated. Data obtained from this study were treated statistically using the Student's T Test (17). In addition, 1000 lymphocytes from each specimen were scored for the relative number of mitoses present and a mitotic index was calculated based upon these results.

For the bone marrow smears of mice, 1000 polychromatic and normochromatic erythrocytes were scored from each specimen for the presence

of micronuclei. The results obtained were treated statistically using the probit analysis (19).

I. Drosophila Procedures and Scoring Method

The flies were rendered inactive with CO_2 for convenience during transfer and scoring. Virgin females were collected from new cultures of d 63 inscy for mating. Mating was conducted in 2.5 x 5.0 cm glass vials with styrofoam plugs and containing approximately 1 ml of medium. Single pairs were mated for 24 hours. After mating, the male flies were removed from the vials and the females were kept in the same vials to allow laying eggs for 2 days. Newly hatched adults from the mating were scored for sex chromosome loss. The regular flies in the progeny should be male with normal eyes and apricot body and females with bar eyes and yellow body. Sex chromosome loss is characterized by male offspring phenotype of normal eyes and yellow body (17). However, male flies with this phenotype will also be produced when spontaneous non-disjunction occurs in the treated males. This can be corrected based on the number of female offspring with bar eyes and apricot body since that phenotype will also be produced as a consequence of paternal non-disjunction. Therefore, both the two abnormal types of flies were scored if present in the progeny.

III. RESULTS

A. The Toxic Effects of Rotenone

1. Toxicity to Male Sprague-Dawley Rats: Mortality and weight gains of male Sprague-Dawley rats after treatment of rotenone are summarized in Table I. A survival curve was constructed with this data collected on the 14th day of dosing. Statistical analysis of the fitness of the line made showed it to be significant at 95% of confidence. A LD_{50} value of rotenone for the male Sprague-Dawley rats was then derived from this curve and found to be 71 mg/kg b.w. Death of the animals in nearly all of the cases was found to be within the first 2 days of oral administration. Growth repression in all of the surviving animals treated with rotenone, in terms of weight gains, were observed on the 7th day following the drug administration. However they recovered in this aspect one week later.

2. Toxicity to Swiss Albino Mice: The toxicity of rotenone was investigated in two strains of swiss albino mice, DUB (ICR) and Mai (s) BR. Rotenone failed to produce a 100% lethality in these mice at doses used up to 1 g/kg b.w. However, a survival curve plotted with data obtained from an experiment using both sexes of Mai (s) BR mice was found to be of a good fit statistically. A LD_{50} value for these animals was thus estimated from this curve as 640 mg/kg b.w. Similar to that observed in the rats, death of the mice mainly took place within the first 2 days of dosing. Physical sicknes became conspicuous a couple of hours later in all of the animals receiving rotenone treatment. However, no apparent reduction in weight gain was found in any of the survivors as measured on the 7th day following dosing. No sex differences in response

to both the lethal and toxic effects of rotenone were observed in animals at each of the dose levels. Table II summarizes the results of these experiments.

3. Toxicity to Drosophila Melanogaster: The lethality of rotenone to male flies was investigated using concentrations between 0.01 and 3.0 mM of the substance dissolved in a sucrose-agar medium. The results are shown in Table III. A LD_{50} of 3.4 mM was estimated for the male flies from a surviving curve plotted with the mortality date obtained. Reduction in activity of the flies was conspicuous, within a few hours of feeding with this drug, particularly in groups receiving the high doses. The phenomenon was no longer noticeable after 48 hours following dosing.

B. Cytogenetic Effects of Rotenone on Rat Bone Marrow

The relative incidences of various chromosomal aberrations observed in bone marrow specimens of male Spraque-Dawley rats treated orally with 3 sublethal doses of rotenone, 0.7, 2.5 and 7.0 mg/kg, are shown in Table IV. In general, the total incidence of cells with chromosomal aberrations in all groups of the specimens was low, ranging from 0.035 to 0.110 per 1000 cells analysed. No correlation between concentration and incidence aberrations were observed among different groups of samples. Of all the various types of aberrations found, the chromatid type gaps were most common, followed by chromatid type breaks. Neither aneuploidy nor polyploidy were observed in any cells of any of the 40 specimens analysed.

In order to see if rotenone would affect mitotic progression in bone marrow, one thousand cells from each specimen were analysed for mitotic

distribution. The results obtained from this analysis are listed in Table V. The average mitotic index estimated in all of the 4 groups of specimens was in the range between 23.3 and 30.1%. Variations in mitotic index existed only between individual animals within a group but not between the untreated and the 3 treated groups. Rotenone, then, did not affect mitotic progression on those tests.

C. Induction of Micronuclei in Bone Marrow of Swiss Mice

The effects of rotenone on micronuclei formation in mice bone marrow was tested at 2 sub-lethal doses, 10 and 80 mg/kg of body weight. Results obtained from this test are presented in Table VI. Micronuclei were detected in both polychromatic and normochromatic erythrocytes. These micronuclei were mainly single, or occasionally double in number, round in shape, and intensively stained. The total incidence of micronucleated cells in untreated samples (control) was less than 1 (0.087%) per thousand erythrocytes, with nearly equal distribution in both types of the red blood cells. This figure thus represents as the spontaneous level of micronuclei formed in these animals under the experimental condition. The incidence of micronucleated cells observed in the TEM-treated animals was much greater than that found in the untreated ones. Treatment with this known clasogenic compound led to an approximately 2,000 fold increase (17%) in total micronucleated cells. Micronuclei formed in these samples were predominately in the polychromatic cells. Those formed in normocytes accounted for less than 3% of the total micronucleated cells. Administration of rotenone to mice did not seem to affect the formation of micronuclei in their bone marrow. The level of total micronucleated

cells found in these samples was not significantly different from that of the untreated samples. Besides the ordinary micronuclei observed as above, a small fraction of cells were found to contain multiple nuclear bodies which were smaller in size and much less intensively stained. In the untreated and rotenone-treated samples, in contrast to the ordinary type, these multiple micronuclei were only found in normochromatic cells. The incidence of the multiple micronucleated normocytes found in the controlled samples was only 0.001%, but tended to increase in number in accordance with the increase in Rotenone dosage. At 80mg/kg b.w. of rotenone, the incidence of these cells increased from 0.001% to 0.01%. The multiple micronuclei were detected in both types of cells in the TEM- treated samples. However they were found to be preferentially formed in the matured erythrocytes.

D. Sex Chromosome Loss in Drosophila Melanogaster

The effects of rotenone on sex chromosome loss in Drosophila melanogaster were tested at 3 sub-lethal concentrations, 0.1, 0.3 and 1.0 mM. The results obtained from this test are shown in Table VIII. As the results show, the fertility of male flies was not affected by this drug at any of the concentrations tested. This is evidenced by the lack of concentration-related changes in fertility rates between different groups and the similarity in average size of progeny produced by the fertile mating pairs in all of the treatment groups. However, the general fertility rate is low, only 18-46% of the single mating pairs yielding progeny.

Sex chromosome loss was detected in the progeny of the two groups

treated with 0.1 and 0.3 mM of rotenone, but not in the group treated with a higher concentration of this drug, 1.0 mM. However, only one such event was scored in each of these groups. The total number of offspring produced by the two groups was approximately 750 and that produced by the higher concentration and the untreated (control) groups was around 1500. The sex chromosome loss observed was apparently not due to rotenone treatment but to spontaneous production in these flies. No chromosome loss was detected in the group treated with MMS, a known mitogen which has been shown to cause sex chromosome loss in drosophila (3). The reason for the failure of MMS to cause sex chromosome loss in these tests is not known.

IV. DISCUSSION

In order to obtain background information for the appropriate dose levels for studies on the clastogenic effects of rotenone on albino rats and Swiss mice, and also on Drosophila melanogaster, the lethality of this substance was examined. LD_{50} values of rotenone obtained from this study were 71 mg/kg b.w., 640 mg/kg b.w. and 3.4 mM for the male rats, mice and the male flies, respectively. The reported LD_{50} value for rats was 61 mg/kg b.w. (20) and that for mice was 2.8 mg/kg b.w. (21). The LD_{50} value for rats established in the present study is in good agreement with the previous finding but that found for mice is far greater than the reported value. Many toxic compounds are known to be detoxified enzymatically by $NADPH_2$-dependent, mixed function oxidases in the mammalian liver microsome fraction. This enzyme system biotransforms rotenone into several metabolites with various toxic potentials. For instance, the toxicity of 8'-hydroxyrotenone, one of the metabolites of rotenone formed in mice or houseflies is approximately 30 times more toxic to mice than the other metabolites, such as rotenolone II (21,22). The great discrepancy between the two LD_{50} values for mice obtained here and by the previous study may be the result of strain differences in mice used in the two different studies. Information concerning rotenone toxicity to Drosophila melanogaster has not been found in the literature to date. The present findings thus provide a general idea about the sensitivity of the organism to this substance.

The results obtained from the genetic study of rotenone on Drosophila melanogaster were negative. Rotenone at the 3 sub-lethal doses tested did not affect the fertility of the male flies nor cause sex chromosome loss in this organism. MMS also caused no sex chromosome loss in drosophila in these

tests. Since MMS induced sex chromosome loss has been reported by only one group (13) further study of the effect of MMS on drosophila may be indicated.

Results obtained from the cytogenetic analysis on bone marrow cells of rats treated with sub-doses or rotenone also failed to show enhancement of chromosomal aberrations. This suggests that rotenone is not clastogenic, and that it does not cause chromosomal breaks, at least at the dose levels tested. When the rotenone treated group was compared to the untreated group, no significant difference in the frequency of micronuclei in both polychromatic and normochromatic erythrocytes were found. The elevated levels of the micronucleated cells induced by TEM, a known clastogenic agent, indicates that the experimental conditions used for this test were valid. In view of the known mechanism underlying micronuclei formation (18), the lack of enhanced production of micronuclei in the rotenone-treated animals also indicates that rotenone does not cause damage to chromsomes in a direct mode. This argument seems to be supported by findings of an earlier study that rotenone does not react with DNA and/or cause detectable damage to the genetic material (23). Increases in the frequency of multiple micronuclei for both young and mature erythrocytes were found in the TEM studies. Also, a small increase over background in the level of multiple micronuclei was observed in normochromatic cells in animals treated with rotenone. Multiple micronuclei were not reported by previous workers with either TEM (11,24,25,26) or any other drug-treated animals (18,27,28). Their preferential appearance in normocytes as observed in the present study tends to imply that they are products of stage-related damages inflicted by a specific action shared by both toxic substances. It has been suggested that the appearance of micronuclei in

polychromatic or normochromatic erythrocytes reflects cellular damages induced by a clastogenic agent in the course of the last replication cycle of erythropoiesis. Damages induced in S phase appear in the young erythrocytes and those induced in later stages, G_2 or M, appear in the mature cells (27). Nevertheless, the exact nature of multiple micronuclei formation is not clear. The toxicity of rotenone has been shown to be attributed to its anti-metabolic action, antagonizing cellular oxidation (29). In addition, it was also shown to inhibit tubulin synthesis (7), disrupt microtubal assembly (8) and thus interferes with the normal function of the spindle fiber apparatus (9). These facts along with the lack of apparent chromosome-breaking effects of rotenone tend to suggest that the production of multiple micronuclei might be a result of a distortion of the spindle fiber apparatus. Due to the extremely low incident of this type of damage produced by rotenone, the possible genetic impact of this damage may be minimal.

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TABLE I. MORTALITY AND WEIGHT GAINS OF MALE SPRAGUE-DAWLEY RATS AS AFFECTED BY ROTENONE

DOSE (mg/kg. b.w.)	NO. OF DEATH/ NO. OF RATS TREATED	% OF MORTALITY**	WEIGHT GAIN (g)*	
			ON DAY 7	ON DAY 14
0	0/20	0	+40.0	+65.4
50	9/20	45	+32.0	+65.2
100	13/20	65	+27.0	+62.9
200	20/20	100	-	-

* In reference to day 0

** The mortality rate was calculated on the 14th day after dosing.

TABLE II. EFFECTS OF ROTENONE ON THE SURVIVAL AND WEIGHT GAIN IN SWISS MICE (mai(s)) BR strains)

DOSE (mg/kg. b.w.)	NO. OF DEATH/ NO. OF MICE TREATED	% OF MORTALITY**	WEIGHT GAIN (g)*	
			ON 7th DAY	ON 14th DAY
0	0/20	0	+2.3	+12.3
100	2/20	10.0	+1.9	+ 9.7
300	9/20	45.0	+2.2	+11.2
600	6/20	30.0	+2.3	+11.8
1000	10/20	50.0	+2.4	+12.0

* Pooled data of both sexes in reference to day 0

** The % of mortality was calculated 14 days following drug administration

TABLE III. LETHALITY OF ROTENONE TO MALE DROSOPHILA MELANOGASTER

CONCENTRATION (mM)	% OF SURVIVAL	P(L5%)
0	99.0	
0.01	96.0	
0.03	92.0	
0.10	96.0	
0.30	89.0	*
1.00	81.0	*
3.00	69.0	*

* Significant at P<5%

TABLE IV. INCIDENCES OF CHROMOSOME ABERRATIONS INDUCED BY ROTENONE IN RAT BONE MARROW CELLS*

ROTENONE DOSES (mg/kg. b.w.)	CHROMATID GAPS	CHROMATID BREAKS	CHROMOSOME GAPS	CHROMOSOME BREAKS	CHROMOSOME FRAGMENTS	OTHERS	TOTAL ABERRATIONS	% INCIDENCE
0	20	9	1	0	2	3	35	0.0088
0.7	10	4	1	0	0	0	14	0.0035
2.5	31	1	1	0	6	5	66	0.0110
7.0	11	4	0	0	1	1	17	0.0043

* Number of cells with chromosome aberrations per 4000 metaphase cells analyzed from 10 animals

TABLE V. EFFECTS OF ROTENONE ON MITOTIC INDEX OF RAT BONE MARROW

ROTENONE DOSES (mg/kg. b.w.)	AVERAGE NO. OF MITOTIC CELLS/1000	RANGE	INDEX (%)	P(5%)
0	263	4-53	26.3	
0.7	233	4-45	23.3	
2.5	287	6-47	28.7	
7.0	301	9-62	30.1	*

* Significant at P<5%

TABLE VI. EFFECTS OF ROTENONE ON THE FORMATION OF MICRONUCLEI IN MICE BONE MARROW ERYTHROCYTES

TREATMENT	% MICRONUCLEATED POLYMACHROMATIC ERYTHROCYTES			% MICRONUCELATED NORMOCYTES			TOTAL
	MONO-	MULTIPLE	SUB-TOTAL	MONO-	MULTIPLE	SUB-TOTAL	(%)
CONTROL	0.043	0	0.043	0.043	0.001	0.044	0.087
ROTENONE (1)							
10 mg/kg	0.031	0	0.031	0.034	0.004	0.038	0.069
80 mg/kg	0.029	0	0.029	0.028	0.010*	0.038	0.067
TEM (2)							
1.0 mg/kg	16.588	0.013*	16.601	0.338*	0.053*	0.391	16.990*

* Significant at $p<5\%$

(1) Dosed orally in corn oil

(2) Dosed i.p. in PBS

TABLE VII. SEX CHROMOSOME LOSS DETECTED IN DROSOPHILA MELANOGASTER TREATED WITH VARIOUS CONCENTRATION OF ROTENONE

DOSE*(mM)	NO. PAIRS MATED	NO. YIELDED PPROGENY	NO. OF PROGENY MALE	FEMALE	TOTAL	NO. WITH SEX CHROMO. LOSS	AVER. NO. OFFSPRING PRODUCED PER PAIR
0	90	37 (41.1)	736	736	1472	0	39.8
0.1	91	20 (22.0)	363	383	747	1	37.4
0.3	91	18 (19.8)	395	367	762	1	42.3
0.0	80	37 (46.3)	747	797	1544	0	41.7
MMS (0.5 E)	84	15 (17.9)	301	308	609	0	40.6

* Male flies were treated for 24 hours with rotenone with 48 hours with MMS