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The Effects of Short Duration Microwave Exposure on the Life Span and the Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila Melanogaster*

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Abstract

In this study, the possible mutagenic and longevity effects of 10 GHz microwave were investigated in the adult fruit fly *Drosophila melanogaster*. Sex-linked recessive lethal assay was performed for mutation research using three brooding processes. The doses were 5 (continuous exposure) and 3+3 h (with half an hour of break in non-continuous exposure). The mean life spans of exposure and control groups were investigated both sex-combined and between sexes. This study concludes that 10 GHz microwave could extend the life span of *Basc* females but no significant difference was detected in the recessive lethal mutation frequency among exposure and control groups.

Key Words: *Drosophila melanogaster*, recessive lethal mutation, life span, microwave, electromagnetic field.

INTRODUCTION

In recent years, microwave (MW) sources have emerged in our environment and their presence suggests that we are exposed to MW frequency more than the normal dose.

There are reports on harmful effects of electromagnetic fields (EM) especially at high levels of force and power on many organisms but conflicting results were obtained in several investigations. There are many conflicting records about the relationship between EM fields and the induction of mutations [1-4].

The sex-linked recessive lethal test (SLRL) is used to assess the mutagenic response of *Drosophila* to EM fields. This test is the best validated *Drosophila* mutagenicity test that detects the lethal mutagenic changes on the X chromosome [5,6].

In researches concerning the relationship between EM fields and the effects on life span, researchers were found statistically significant and insignificant increases in the life span of rats exposed to MW [7, 8]. Furthermore, a decrease in the life span of the treated *D. melanogaster* males was recorded with 2375 MHz.

It was recorded that the power density of MW could affect the life span and 2.45 GHz MW induces a significant decrease in life span if power density is 10 mW/cm^2, while 3 mW/cm^2 power density induces an insignificant increase in the life span [9].

The aim of this study was to investigate the possible effects of 10 GHz EM fields on the life span and determine the possible mutagenic effects on *Drosophila melanogaster*. To determine the genetic damage produced by EM fields in germ cells of

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eucaryotic organisms, this non ionizing radiation for the induction of sex-linked recessive lethal in *D. melanogaster* has tested.

MATERIALS AND METHODS

Culture stocks were maintained in the laboratory by mass culture at 25±1°C in a constant temperature and relative humidity of 60% and in 12+12 h dark and light period. Standard *Drosophila* medium was used in all experiments [10].

1. Microwave doses

All experiments were applied with an EM source (antenna), which was a pulsed square wave (1 kHz), approximately 5 mW (lowered power) and 10 GHz. The empty exposure vials were 1 m away from the antenna. The doses of microwave were determined considering previous publications and autors' pilot experiments. Thereof, we defined the doses as 5 h continuous and 3+3 h discontinuous (30 minutes resting period in between) and continuous at 10 GHz MW.

The control groups were located in the same laboratory with experimental group and they were in empty vials locked in a metal cupboard, approximately 8 meters far away from the antenna. The experimental groups were irradiated by a MW of 0.0156 Watt/m^2 power intensity and SAR=9.8 mW/kg [11]

2. Methods

a. SLRL Tests

In order to investigate the mutagenic effects of 10 GHz EM fields, the SLRL test, which is the standard way to detect the induced lethal mutations on the X chromosome of *D. melanogaster*, was used (5). This test detects recessive lethal mutations on the X chromosome occurring in male germ cells during exposure by making homozygotes. For the SLRL test, *Oregon* wild type males and Muller-5 (*Basc*) females were used as marker stocks. The genetic description of *Basc* stocks is In (1) sc SILsc8R+S, scS1 sc8 wa B (12). *Oregon* (w.t.) males were collected in 1-2 days and they were kept until they were 5-7 days old and placed in empty glass vials (20 fruit fly per vial) before exposure. To test the sensitivity of the different stages of the spermatogenesis of males treated as adults a three times repeated mating brood was followed by transferring males to fresh virgin *Basc* females (5). Treated *Oregon* males and control *Oregon* males were mated with untreated virgin *Basc* females individually (1♂X 3♀) after exposure. The control males were treated in the same environment with the exposure males but they were not exposed to MW frequency. In the experiments, the first brood represented mature sperm cells at the time of the treatment. Each male was removed from its mating vial and was again mated individually with three untreated *Basc* virgin females for 2 days. The second brood represented the spermatid stage at the time of the treatment. The mating process was repeated again after 4 days with three untreated *Basc* virgin females. The third brood represents the spermatocytes and early and late spermatogonia stages at the time of the treatment. F1 heterozygous females from each parental treated male were mated to new virgin *Basc* males, then F2 offsprings counted and lethal mutations were scored. The absence of wild type males in the F2 cultures of the treated males was indicated the presence of SLRL mutations. The significance of the data was calculated using X2 Goodness of Fit Analysis in SPSS 10.0.

b. Life span experiments

Besides the wild type *Oregon* strain, mutant *Basc* strain that is expected to show a different reaction to

any stress factor was selected. The virgin male and female *Oregon* (wild type) and *Basc* (mutant) flies were collected in 2-3 days for life span tests. The flies could be accepted at the same age in first three days [12,13]. The 100 males and 100 females were put in empty vials and were exposed to 10 GHz EM fields as non-continuous 3+3 h with half an hour of break in *Drosophila* standard medium and continuous 5 h. The control groups of two strains were treated like the exposure groups in the same environment but they were not exposed to MW frequency. After exposure, the flies were put in vials (2.5x7.5 cm) each containing 10 individuals and standard medium. The mediums were renewed two times a week. The death flies were recorded until the last fly has dead. The statistic tests were determined using t test and significant control between groups was realized with ANOVA using SPSS 10.0. Survival curves were recorded in Excel.

RESULTS AND DISCUSSION

a. SLRL Test Results

Treated *Oregon* males and Control *Oregon* males were mated with untreated virgin *Basc* females individually (1♂X3♀) after exposure. In order to detect the effect of the recessive lethal mutations occuring in the X chromosome, the female flies were collected in F1 and they were mated individually with three virgin *Basc* males (1♀X3♂). The F2 offsprings were examined carefully. In the 3+3 h experimental group (E3+3), 424 (232+89+103) female flies were collected. In the control group (C1), 418 females were collected. The flies were individually crossed with three virgin *Basc* males taken from the stock. The summarized data of 3+3 h MW irradiation were given in Table 1.

The summarized data of 5 h MW irradiation are given in Table 2. All the collected females were individually mated with three *Basc* males. 360 (158+94+108) females in the 5 h experimental group (E5) and 390 females were collected in the control group (C2). No significant increase in mutation frequency was detected in any experiment group in comparison to control groups.

Table 1. SLRL test results of *Drosophila melanogaster* exposed to 10 GHz MW for 3+3 h (with half an hour break) and non-exposed control groups.

Group	Number of Tested X-Chromo-somes	Number of Produced Lethals	Mutation Rate (Number of Lethals / Number of Tested Chromosomes)
C1			
Brood 1	212	0	0
Brood 2	83	0	0
Brood 3	123	0	0
Total	418	0	0
E-3+3			
Brood 1	232	0	0
Brood 2	89	0	0
Brood 3	103	1	0.0097 (1/103)
Total	424	1	0.0024 (1/424)

C1: Control (non-exposed) group
E-3+3: 3+3 h (with half an hour break) MW exposed group

Table 2. SLRL test results of *Drosophila melanogaster* exposed to 10 GHz MW for 5 h (continuous) and non-exposed control groups.

Group	Number of Tested X-Chromo-somes	Number of Produced Lethals	Mutation Rate (Number of Lethals / Number of Tested Chromosomes)
C2			
Brood 1	167	0	0
Brood 2	76	0	0
Brood 3	147	0	0
Total	390	0	0
E5			
Brood 1	158	2	0.0127 (2/158)
Brood 2	94	0	0
Brood 3	108	1	0.0093 (1/108)
Total	360	3	0.0083 (3/360)

C2: Control (non exposed) group
E-5: 5 hours MW exposed group

b. Life span experiments results

The summerized data concerning the mean life span of *Oregon* w.t. flies irradiated for 3+3 h and 5 h together with their controls are given in Table 3. Statistically the mean life spans of the *Oregon* w.t. strain in all 3+3 h and 5 h exposures and control groups were found significantly different between sexes.

Table 3. Mean life span data of *Oregon* strain in *Drosophila melanogaster* exposed to 10 GHz MW for 5 h (continuous), 3+3 h (with half an hour break) and non-exposed control groups.

Group No.	Group Name	Genotype and Sex	Number of Flies	Mean Life Span (Days)±SE.	Sex Mixed Life span (Days)	Statistical Signifi-cance Control Between Groups
1	C1	*Oregon* M	101	51.46±1.35	58,12	1-2*
2	C2	*Oregon* F	101	64.79±1.57		
3	E-3+3 hrs	*Oregon* M	77	55.42±1.19	64,16	3-4*
4	E-3+3 hrs	*Oregon* F	105	70.57±1.47		
5	E-5 hrs	*Oregon* M	94	52.94±1.12	60,68	5-6*
6	E-5 hrs	*Oregon* F	101	67.89±1.32		

C: Control (non-exposed group), E: Exposed group, S.E: Standard error, M: Male, F: Female,*: p<0,05 significant

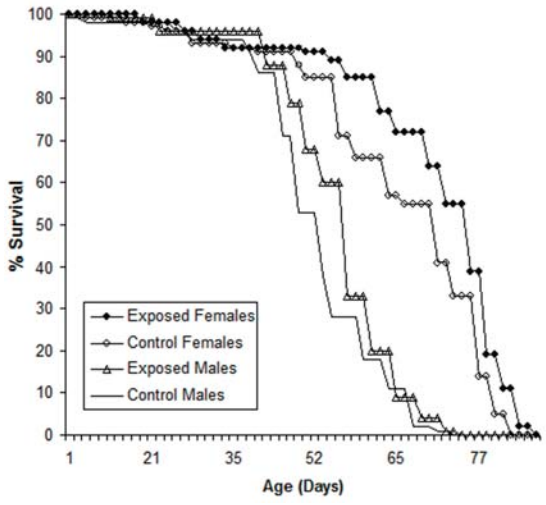

Figure 1. Survival curves of the *Oregon* w.t. females and males exposed to 10 GHz MW for 3+3 h (with half an hour break) and non-exposed control flies.

Females lived significantly longer than males in all exposure and control groups. However, the difference between sex mixed life spans of control and both 3+3 h and 5 h exposure groups were not significant in *Oregon* w.t. strain. The survival curves of this *Oregon* strain are shown in Figures 1 and 2.

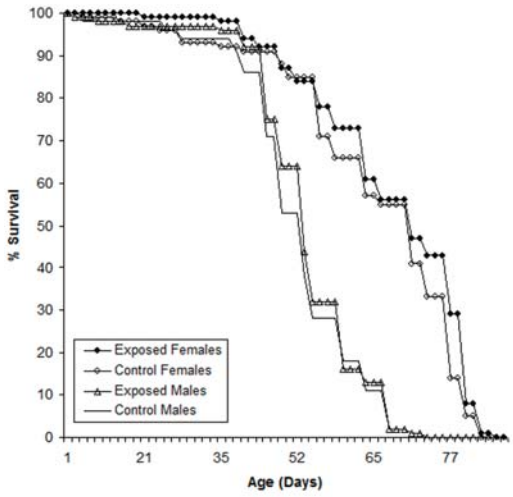

Figure 2. Survival curves of the *Oregon* w.t. males and females exposed to 10 GHz MW for 5 h and non-exposed control flies.

The summerized data concerning the mean life span of mutant *Basc* strain irradiated for 3+3 h and 5 h together with their controls are given in Table 4.The mean life spans of mutant *Basc* strain in all 3+3 h and 5 h MW exposures and control groups were found statistically non significant between sexes. The difference between sex mixed life spans of 3+3 h and 5 h exposure groups were not significant as compared to control groups. However, the mean life spans of 3+3 h irradiated *Basc* females were found longer than that of the control group and the difference was significant at the level of p<0,05. The survival curves of this *Basc* strain are shown in Figure 3 and 4.

This investigation was undertaken in order to understand the possible mutagenic and longevity effects of 10 GHz MW in adult fruit fly *Drosophila melanogaster*. It is well known that there is an interaction among genotype, environmental stress

factors and life span. This also effects adult longevity [14]. In order to determine the genetic damage produced by EM fields in germ cells of eucaryotic organisms, this non ionizing radiation for the induction of sex-linked recessive lethals in *D. melanogaster* is used and the effect of MW frequency to life span of *Drosophila* is tested.

Table 4. Mean life span data of *Basc* strain in *Drosophila melanogaster* exposed to 10 GHz microwave for 5 h (continuous), 3+3 h (with half an hour break) and non-exposed control groups.

Group No.	Strain	Genotype and Sex	Number of Flies	Mean Life Span (Days)±SE.	Sex Mixed Life span (Days)	Statistical Significance Control Between Groups
1	C1	*Basc* M	98	54.74±0.97	50,52	-
2	C2	*Basc* F	100	46.39±1.85		-
3	E-3+3 hrs	*Basc* M	93	49.97±1.29	53,86	1-3
4	E-3+3 hrs	*Basc* F	88	57.81±1.54		2-4*
5	E-5 hrs	*Basc* M	89	53.45±1.,21	50,27	1-5
6	E-5 hrs	*Basc* F	101	47.64±1.87		2-6

C: Control (non-exposed group), E: Exposed group, S.E: Standard error, M: Male, F: Female,*: p<0,05 significant

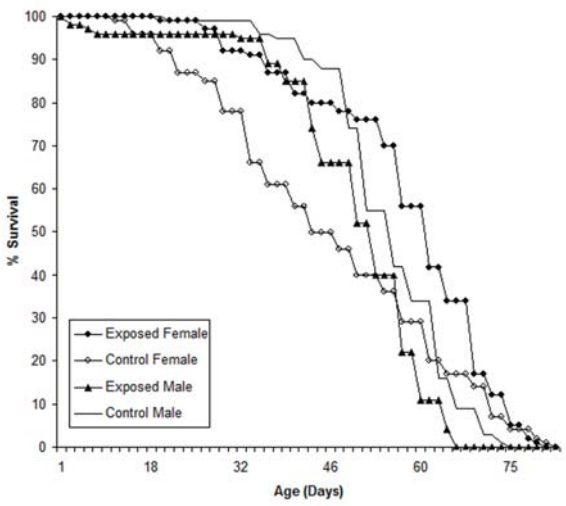

Figure 3. Survival curves of the *Basc* mutant males and females exposed to 10 GHz MW for 3+3 h (with half an hour break) and non-exposed control flies.

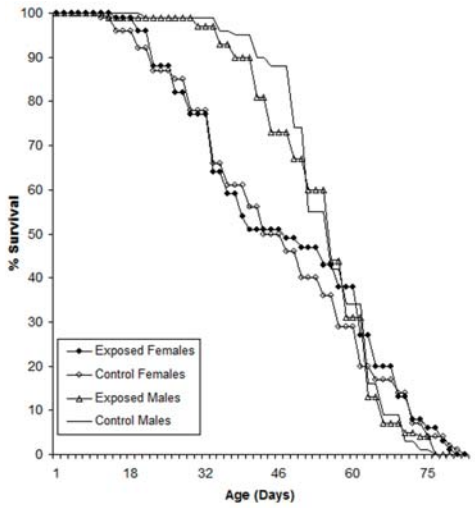

Figure 4. Survival curves of the *Basc* Mutant males and females exposed to 10 GHz MW for 5 h (continuous) and non-exposed control flies.

In the first observation, the mean life spans of *D. melanogaster* w.t. strain of *Oregon* was not affected by the MW frequency in comparison to their own respective controls. There were no significant differences between the mean life spans of irradiated males and control males, irradiated females and control females of *Oregon* w.t.

Nevertheless the results obtained from mutant *Basc* strain were quite different from those of *Oregon* w.t. The mean life span of exposed females of *Basc* strain was lived longer than that of its control females. However, the exposed males of the *Basc* strain were not affected by the MW frequency. Yet, it is not clear why and how MW frequency influences the life span of *Basc* females, but not that of the *Oregon* strain. Assuming that the *Basc* strain is a mutant, EM fields might cause more damage to this genotype in comparison to the wild type *Oregon* strain [17].

The mutagenic effects of MW fields were examined using SLRL test and it is found that short period 10 GHz microwave exposures have no effect on the frequency of SLRL mutations in *D. melanogaster*. The rates of mutation after exposure to 10 GHz EM

fields at 5 h and 3+3 h doses were not significantly different from that of the control group as summarized in Tables 1 and 2.

Moreover, the recessive lethal mutation rate found in the 5 h MW exposure was four times more than that of 3+3 h non-continuous MW exposure as seen in Tables 1 and 2. Under these circumstances, it appears that continuous 5 h exposure could be more effective than non-continuous 3+3 h exposure. In the other words, we could say that as the exposure period increases, the damages increase proportionally. These conclusions are parallel to the findings of other researchers who found that EM fields induce cytogenetic changes [15-19].

The results show that short period exposure of 10 GHz MW exposures was not mutagenic in *D. melanogaster.* Researchers have found that EM fields did not induce mutations in several investigations [2,3,18,19]. However, there are findings that support the view that EM fields might increase mutation frequencies in *D. melanogaster* [1,25].

It should be told once again that the MW frequency was used once in the life span of *D. melanogaster* and the durations were quite short. If such short exposures could affect the life span of adult flies, we could assume that any damage might be induced in genes or cells of the organism. As a result, superoxide radicals, thermal influences, ionic changes, and the damages of DNA or RNA nucleotide bases might also alter the cell structure of the organism [20]. All these probabilities might shed a light on the prolonged life span of *Drosophila* adults.

The results drawn from these experiments suggest that the effect of 10 GHz EM fields is dose dependent but it can not be said that 10 GHz is mutagenic. However, further studies are required to confirm this at higher SAR levels of EM fields [21-24].

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