SOMATIC AND GENETIC EFFECTS OF LOW SAR 2.45 GHz MICROWAVE RADIATION ON WISTAR RATS

A Ph.D THESIS

BY

USIKALU, MOJISOLA RACHAEL
MATRIC NO: CUGP050144

August, 2010

SOMATIC AND GENETIC EFFECTS OF LOW SAR 2.45 GHz MICROWAVE RADIATION ON WISTAR RATS

A Ph.D THESIS

BY

USIKALU MOJISOLA RACHAEL

(CUGP050144)

Submitted to the School of Post Graduate Studies of Covenant University, Ota
In partial fulfillment of the requirements for the award of degree of
Doctor of Philosophy (Ph.D.) in Radiation and Health Physics

AUGUST, 2010

CERTIFICATION

This is to certify that Mrs. USIKALU, Mojisola Rachael (Matric No: CUGP050144) carried out this research work in partial fulfillment of the requirements for the award of Doctor of Philosophy (Ph.D.) degree in Physics (Radiation and Health Physics) of Covenant University, Ota, under our supervision.

Dr. M. A. Aweda (Main Supervisor)	Signature	Date …….
Professor Guangming Zhou (Co-Supervisor)	Signature	Date …….
Professor E.B. Babatunde (Co-Supervisor)	Signature	Date …….

DECLARATION

I hereby declare that this thesis entitled “SOMATIC AND GENETIC EFFECTS OF LOW SAR 2.45 GHz MICROWAVE RADIATION ON WISTAR RATS” was compiled and written by me and that it is a record of my research work. It has not been presented in any previous application for a higher degree. All quotations and the sources of information are specifically acknowledged by means of references.

_____

USIKALU, Mojisola R.

DEDICATION

This work is dedicated to the **Almighty God** the giver and source of all knowledge. Also to my dearly beloved husband, **Oladipo Victor** and God's perfect gift **Ayomikun**

ACKNOWLEDGEMENTS

I am grateful to God Almighty the giver of power, wisdom, and strength, from whom all mercies flow for seeing me through the research work and this dissertation.

I wish to express my profound gratitude to Dr. M.A. Aweda and Professor Guangming Zhou for their words of encouragement and for thorough supervision of this work. I appreciate very much their guidance, making their libraries available to me and for their support. I appreciate them also for their readiness to attend to my questions on this work at any time.

The support of Third World Organization for Women in Science (TWOWS) Trieste, Italy is gratefully acknowledged for the TWOWS fellowship granted me to complete this research work in the Institute of Modern Physics, Chinese Academy of Sciences, China.

The support of Institute of Modern Physics, Chinese Academy of Sciences, China, is also gratefully acknowledged for granting me access to their laboratories and equipment. I am particularly grateful to all staff of the institute most especially radiobiological group for their care and affection throughout my stay in Lanzhou, China.

I wish to show my gratitude to the management of Covenant University for the staff development program and for granting me study leave to conclude the research in Institute of Modern Physics, Chinese Academy of Sciences, China under TWOWS fellowship.

I acknowledge with thanks the encouragement received from members of staff of Department of Physics, Covenant University Ota under the leadership of Dr. Uhuegbu C.C. I also appreciate Professor E.B. Babatunde, my co-supervisor for his support and fatherly advice.

Finally, I wish to express my deep gratitude to my husband, Dipo, perfect gift of God, Ayomikun and my family members for their love, prayers, understanding, word of encouragement and for standing by me during the period of this study.

TABLE OF CONTENTS

CHAPTER ONE

INTRODUCTION

CHAPTER TWO

LITERATURE REVIEW

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER FOUR

RESULTS AND DISCUSSION

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

ABSTRACT

The somatic and genetic effects of 2.45 GHz Microwave radiation (MWR) on male and female Sprague Dawley rats were studied. Two hundred rats were used for this study. They were grouped into control and exposed according to the parameters under consideration. The animals were exposed to various levels of specific absorption rate (SARs) using the microwave generator, model ER660E, Serial No MX704CCR from Toshiba UK Ltd. All animals were kept in healthy, and radiation free environment with water and feed provided *ad libitum.* The study is divided into three parts; firstly, the effects of MWR on the anxiolytic and exploratory behaviour of the animals. MWR produced dose-dependent significant decrease in the cumulative time spent in the open arms of maze in the first twelve days after exposure and gradually increased towards the control value before the end of two weeks. It also affects the exploratory behavoiur of the animals, for the first two weeks after exposure but by the third week there was no significant difference between the control and the exposed animals. The modifications in the behaviour were not sex dependent as there were no significant differences between male and female rats. Secondly, the effects of MWR on the vital organs of male and female most especially, male reproductive functions were studied. The sperm count, gross motility and sperm morphology were determined using standard methods. The results showed that 2.45 GHz MWR reduced the sperm concentration, gross motility while increasing the percentage abnormal sperm cells. There was a decrease in reproductive organ weights and increased dead sperm cells as large numbers of the sperm cells membranes took up the eosin-nigrosin vital stain in the exposed group. Lastly, the genotoxic effects of MWR were studied using Chromosomal aberrations, single cell gel electrophoresis (comet assay) and direct amplification of length polymorphisms (DALP). There was a significant increase in the frequency of chromosomal aberrations between control and

exposed groups. The exposure of vital mammalian cells to 2.45 GHz radiation altered the band patterns of the exposed animals, especially in the range 40 – 120 bp. Starting with appearance of additional peaks compared with the control which was not seen in their tail DNA before exposure to MW and these were confirmed through the densitometric gel analysis. There was a statistically significant difference in the Olive moment and % DNA in tail of the exposed animals compared with control ($p < 0.05$). Thus, these findings support the hypothesis that exposure to 2.45 GHz MWR at studied SARs may have potential somatic and genetic effects.

CHAPTER ONE

INTRODUCTION

1.1 Preamble

Microwaves (MW) are non-ionizing electromagnetic waves in the centimeter range, with a wide frequency band between 300 MHz and 300 GHz. They are a very important component of the electromagnetic spectrum as demonstrated by the increasing scope of applications. Nevertheless, they pose considerable health hazards to humans upon exposure (EPA, 1984). They have relatively short wavelengths and high frequencies compared to the extremely low frequency fields. They therefore have a greater energy which is sufficient to cause heating in conductive materials (Fig. 1). Close to transmitting antennae, high frequency fields may be harmful to man by producing damaging thermal effects that may not be easily reversed by thermoregulation processes e.g. cataract (Cleary *et al*., 1965; Hollows and Douglas, 1984). Unlike the ultraviolet, X-rays and gamma rays, which are ionizing, MW interaction with matter does not result in removal of orbital electrons. Rather, such interactions are known to cause effects like atomic excitations and increased atomic and molecular vibrations and rotations and heat production (Aweda, 2000). As a result of the nature of MW, its interaction mechanisms and the biological effects have previously not been associated with production of free radicals, oxidative modification of cell membranes, lipid peroxidation. These mechanisms are associated with ionizing radiations.

Interest in the study of MW interaction with biological systems has been sustained for several decades. Interactions with tissues arise as a result of three processes: (i) penetration by electromagnetic waves and their propagation into the living system; (ii) primary interaction of the waves with tissue; and (iii) possible secondary effects arising from the primary interaction. The first person to explore the bioeffects of MW fields was Antonin Gosset in 1924, when he and his co-workers used short waves to destroy tumours in plants with no damage to the plant itself (Rowbotton, 1984). Subsequently, in 1926, Joseph Williams began to investigate such effects on animals and reported that he could kill flies with his “short-wave” equipment (Rowbotton, 1984).

In the 1920s and 1930s, researchers' interests were on whether the effects were due primarily to heating, athermal effects or whether they were simply caused by a back heating of specific sites of microscopic dimensions. D'Arsonval demonstrated that the high-frequency MW in the long wave region were beneficial for the treatment of certain forms of rheumatism, arthritis and gout (Bren, 1996).

It was observed from early experiments that MW energy at 2.45 GHz was absorbed by water nearly 7000 times more strongly than at the commonly used short-wave diathermy with a frequency of 27 MHz. This led to the decision in 1946 to assign this frequency (2.45 GHz) for use in physical medicine based on its assumed therapeutic effect (Bren, 1996). Moreover, due to the easy generation of this frequency, many industries and domestic equipment are designed to work at this frequency, e.g., microwave ovens and therapeutic equipment such as those used in cardiology, urology, surgery, ophthalmology, cancer therapy etc. This, therefore, explains the choice of this frequency for this study, since it is the most probable frequency to which man is exposed.

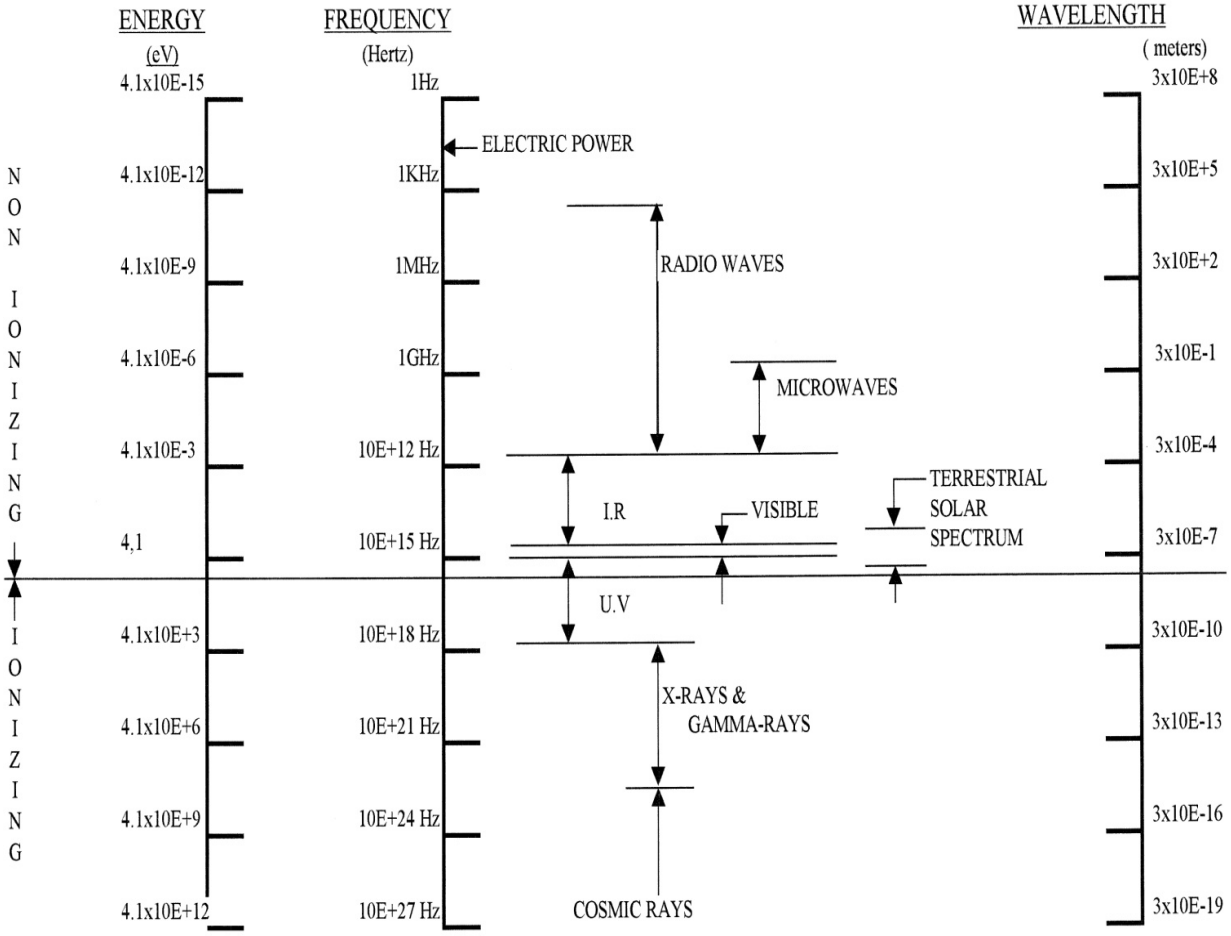


Fig. 1.1: Electromagnetic Spectrum (Verschaeve and Maes, 1997).

However, the aforementioned studies did not account for factors such as the geometry of the body, the depth of penetration and orientation, which may be more important factors than bulk absorption coefficients alone (Bren, 1996). Therefore, in 1941, a team of physicians and engineers measured, to within 5% precision, the energy absorbed by patients during short-wave diathermy, the results of the study were expressed in terms of energy absorbed per unit volume of tissues (W l^{-1}), which is similar to the current exposure metric, the specific absorption rate (SAR) measured in W kg^{-1} (Guy, 1984). In addition, the rapid development of microwave technology, especially in the late 1940s after the World War II, the increasing public concern and the growing controversy over the potential hazardous effects on biological systems have stimulated research in this direction. However, the mechanisms of the interactions of microwaves with living system continue to be of concern to many investigators. Some researchers suggested that the thermal and athermal interactions occur whenever the radiations interact with biological systems, leading to absorption of the radiation energy. Thermal interaction became the better understood of these processes.

The molecular phenomena involved in the conversion of field energy to heat and the possible biological implications have been reported (Schwan and Foster, 1980). It was suggested that the interactions occur through the stimulation of the excitable membranes of nerve and muscle cells, interactions associated with heating leading to perturbations of biochemical reactions, current flow and destruction of the cell membranes (Pichard, 1978; Barsoun and Pickard, 1982; Aweda, 2004). These interactions resulted in some measurable physiological effects, such as induction of increased immune response (Veyret, 1991) and activation of peritoneal macrophages to a viricidal state (Rao *et al*., 1983). There was then the question of how MW exposure actually leads to oxidative stress and causes both somatic and genetic effects. It is known that in biological systems, initiators of oxidative stress are usually excess (free) radicals, which are chemical species with one or more unpaired electrons and capable of separate existence. However, the association of MW exposure with free radical production and with the induction of cancer has generated some controversy in the field of radiation biology (Foster 1996; Aweda *et al*., 2003).

1.2 Statement of the Problem

Increasing applications of MW radiation has led to concerns globally due to the suspected health effects associated with its exposure. Due to inconclusive investigations from literature on the somatic effects as well as genetic effects, this study focuses on these effects. This work is therefore aimed at investigating the hypothesis that the exposure of living systems to MW may cause (i) behavioural modification, (ii) modification of tissues, and (iii) damage to deoxyribonucleic acid (DNA), which should be observed in the measurable associated parameters.

1.3 Rationale of the Research Work

There are numerous and increasing applications of MW energy and technology in the industries, in homes, in medical, research institutions etc., and there is greater awareness and concern of the public over the suspected potential health hazards associated with such exposures. There is therefore, a need for deeper understanding of the bioeffects of exposure to this radiation.

Most studies published so far did not demonstrate convincingly any direct DNA damage after acute or chronic exposure of biological systems to RF (Radiofrequency) fields (Leonards *et al*., 1983; WHO, 1993), in particular when the temperatures were maintained within normal physiological limits. It is important that we have an understanding of this type of interaction, where synergistic effects and mechanisms of investigation are highlighted. The data available at present are very scarce with regard to the potential health effects, especially long-term effects. There is need for further investigations in order to arrive at valid conclusions.

1.4.1 Aim and Objectives

The primary aim of this research is to verify the hypothesis that the exposure of living systems to MW radiation may induce and / or promote changes in DNA, chromosomal aberrations, free radical production and oxidative stress. Thus, a “cause and effect” relation may exist between the changes and observable effects in some measurable physiological quantities.

1.4.2 Specific objectives are:

i. To investigate the effects of whole body exposures of wistar rats to 2.45 GHz MW radiation on

a) behavioural and health status changes;

b) reproductive functions;

c) histology of the various vital tissues;

d) chromosomes;

e) Deoxyribonucleic Acid (DNA)

ii. A study of the sex dependence (if any) of the effects in (i) above.

CHAPTER TWO

LITERATURE REVIEW

2.1 Microwave Origins and Spectrum

The existence of electromagnetic waves, of which microwaves are a part, was first predicted by James Clerk Maxwell in 1864 using a set of equations (given in section 2.5). In 1888, Heinrich Hertz was the first to demonstrate the existence of electromagnetic waves by building an apparatus that produced and detected MW in the UHF region. The design necessarily used horse-and-buggy materials, including a horse trough, a wrought iron point spark, Leyden jars, and a length of zinc gutter whose parabolic cross-section worked as a reflection antenna. In 1894, J. C. Bose publicly demonstrated radio control of a bell using millimeter wavelengths, and conducted research into the propagation of MW (Presman, 1968).

Microwaves are a group of non-ionizing electromagnetic waves. Non-ionizing radiations (NIR) have wavelengths equal to or longer than 10^{-7}m. The non-ionizing radiations are given in Fig.1.1 in terms of wavelength, frequency and photon energy which are inter-related. NIR have photon energies lower than approximately 12 eV, which is considered to be the boundary with ionizing radiations. This energy is too low to break even the weakest chemical bond (Verschaeve and Maes, 1997).

The processes of deactivation of the atomic and molecular excited states, and the friction resulting from atomic and molecular rotations and vibrations lead to the production of the heat employed in physiotherapy. Actually, the mode of heat production differs for different wavelength bands. While the radiofrequency band causes resistive tissue heating at the interfaces between electrodes and tissue, MW forms an electromagnetic field in the surrounding tissues in which dipoles in cells oscillate. The damping of the dipole moments associated with molecules, cells or blocks of tissue results in the heating effect which rate is measured by the temperature rise (Johnson *et al*., 1974). The consequences of the temperature rise include changes in chemical reaction rates, fluid viscosities, thermal conductivity, diffusion coefficient, electrical conductivity and dielectric constants.

2.2 Generation and uses of Microwaves

MW can be generated as direct radiation from electric sparks across gaps by applying a high electric potential. The spark gap can also be part of a very high frequency oscillating circuit, which generates EM waves. MW can also be derived from the thermal radiation from heated bodies. All these are unsatisfactory because of lack of purity of the wave and the low power of the radiation. Modern MW generators are electric devices, which produce continuous wave oscillations of a single tunable frequency. Some important ones are the klystrons, magnetrons and travelling wave-guide oscillators. The power output ranges from microwatts to thousands of kilowatts, depending upon the type and design of the generator and the operating frequency.

2.2.1 Telecommunications

Before the advent of fiber optic transmission, most long distance telephone calls were carried via MW point-to-point links through sites like the AT&T (American Telephone and Telegraph) Long Lines. Starting in the early 1950s, frequency division multiplex was used to send up to 5,400 telephone channels on each microwave radio channel, with as many as ten radio channels combined into one antenna for the *hop* to the next site, up to 70 km away (IEEE, 2006).

1. Wireless LAN (Local Area Network) protocols, such as Bluetooth and the Institute of Electrical and Electronics Engineers (IEEE 802.11) specifications, also use microwaves in the 2.4 GHz ISM band, although (802.11a) uses ISM (Industrial, Scientific and Medical) band and Unlicensed National Information Infrastructure (U-NII) frequencies in the 5 GHz range. Licensed long-range (up to about 25 km) Wireless Internet Access services can be found in many countries (but not the USA) in the 3.5–4.0 GHz range (IEEE, 2006).

2. Metropolitan Area Networks: MW is employed in MAN protocols, such as WiMAX (Worldwide Interoperability for Microwave Access) based in the IEEE 802.16 specification. The IEEE 802.16 specification was designed to operate between 2 and 11 GHz. The commercial implementations are in the 2.3GHz, 2.5 GHz, 3.5 GHz and 5.8 GHz ranges (IEEE, 2006).

3. Wide Area Mobile Broadband Wireless Access: MBWA protocols based on standards specifications such as IEEE 802.20 or ATIS/ANSI HC-SDMA (e.g. iBurst) are designed to operate between 1.6 and 2.3 GHz to give mobility and in-building penetration characteristics similar to mobile phones but with vastly greater spectral efficiency (IEEE, 2006).

4. Cable TV and Internet access on coaxial cable as well as broadcast television use some of the lower MW frequencies. Some mobile phone networks, like GSM, also use the lower frequencies (IEEE, 2006).

5. MW radio is used in broadcasting and telecommunication transmissions because, of the short wavelengths, highly directive antennas are smaller and therefore more practical than they would be at longer wavelengths (lower frequencies). There is also more bandwidth in the MW spectrum than in the rest of the radio spectrum; the usable bandwidth is wide between 300 MHz and 300 GHz. Typically, microwaves are used in television news to transmit a signal from a remote location to a television station from a specially equipped van (IEEE, 2006).

2.2.2 Remote sensing

Radar uses MW radiation to detect the range, speed, and other characteristics of remote objects. Development of radar was accelerated during World War II due to its military utility. Now radar is widely used for applications such as air traffic control, navigation of ships, and speed limit enforcement.

1. A Gunn diode oscillator and waveguide are used as a motion detector for automatic door openers (although these are being replaced by ultrasonic devices).

2. Most radio astronomy uses MW.

2.2.3 Health

MW radiation is used in imaging such as Breast cancer diagnosis, Hemodynamic monitoring and Brain lesion detection.

2.2.4 Navigation

MW is also used in Global Navigation Satellite Systems (GNSS) including the Chinese Beidou, the American Global Positioning System (GPS) and the Russian GLONASS broadcast navigational signals in various bands between about 1.2 GHz and 1.6 GHz.

2.2.4 Power

A MW oven passes (non-ionizing) radiation at a frequency of 2.45 GHz through food, causing dielectric heating through the absorption of energy by water, fats and sugar contained in the food. MW ovens became common kitchen appliances in Western countries in the late 1970s, following the development of inexpensive cavity magnetrons.

1. MW heating is used in industrial processes for drying and curing products.

2. Many semiconductor processing techniques use microwaves to generate plasma for such purposes as reactive ion etching and plasma-enhanced chemical vapor deposition (PECVD).

3. MW can be used to transmit power over long distances, and post-World War II research was done on the possibilities. NASA worked in the 1970s and early 1980s to research the possibilities of using solar power satellite (SPS) systems with large solar arrays that would beam power down to the Earth's surface via microwaves.

Less-than-lethal weaponry exists that uses millimeter waves to heat a thin layer of human skin to an intolerable temperature so as to make the targeted person move away. A two-second burst of the 95 GHz focused beam heats the skin to a temperature of 130 °F (54 °C) at a depth of 1/64th of an inch (0.4 mm). The United States Air Force and Marines are currently using this type of Active Denial System.

2.3 Interactions of Microwave with Biological Materials

The interactions can be considered at various levels of organization of a living organism: atomic, molecular, subcellular, cellular, organ, entire organism. One of the first fundamental steps in evaluating the effects of a certain exposure to radiation in a living

organism is determination of the induced internal electromagnetic field and its spatial distribution. Further, various possible biophysical mechanisms of interaction can be applied. Any such interactions, which may be considered primary, elicit one or more secondary reactions in the living system. For instance, when microwave energy absorption results in a temperature increase within certain parts of the body (a primary interaction), the activation of the thermoregulatory, compensatory mechanism is a possible secondary interaction (Baranski and Czerski, 1976). While the primary interactions are becoming better understood (Cleary, 1977), there is still insufficient attention devoted to the interaction mechanisms involving molecular and the entire body.

Studies on the biological effects of microwaves reveal several areas of established effects and mechanisms on the one hand and speculative effects on the other. There are known thermal and athermal interaction mechanisms of microwaves with biological systems.

2.3.1 Thermal Mechanisms of Interaction

Absorption of RF energy may cause an increase in tissue temperature. The initial rate of temperature increase is proportional to the SAR, as given in equation (2.29). Schwan and Foster (1980) and Stuchly (1979) reported molecular phenomena involved in the conversion of RF energy to thermal energy and their implications in terms of biological effects. Biological tissues exhibit three strong relaxation phenomena responsible for α-, β-, γ-dispersion, and a weak dispersion known as δ. The molecules responsible for the α-dispersion are least understood, appearing to be related to relaxation of counter ions, but in some tissues intracellular structures such as the tubular apparatus may also contribute (Schwan and Foster, 1980). The β-dispersion is due to heterogeneous structures such as membranes and their interfacial polarization. The γ-dispersion is due to the relaxation of free water, while δ-dispersion results from the relaxation of bound water, amino acids and the charged side groups of proteins (Schwan and Foster, 1980).

Bowman (1981) and ICNIRP (1994) reported on thermoregulatory responses and stated that the deposition of RF energy in the body may not necessarily lead to an increase in temperature. When RF energy deposition and conversion to thermal energy in a

biological body exceed its heat dissipation capabilities, an increase in temperature occurs. It has been shown that biological effects, such as the heat killing of cells (Sapareto and Devey, 1984), depend on the temperature profile in time. A convenient reference temperature for the heat killing of cells is 43^{o}C, and thus the thermal dose may be expressed in minutes equivalent to heating at 43^{o}C. However, this reference temperature may vary, particularly for different chosen end points, and may be relative to the normal physiological temperature of the tissue (Lau, 1979). The relationship between temperature (T) and time needed to obtain an equivalent biological effect (cell killing) can be written as

$$t_1 = t_2 R(T_1 - T_2) \qquad 2.1$$

where R is the gas constant, t is the time and T_1 *and* T_2 is the initial and final temperature respectively,

2.3.2 Athermal (Non-thermal) Mechanisms of Interaction

A well-established athermal mechanism of interaction at frequencies below a few tens of MHz is through electrical stimulation of excitable membranes of nerve and muscle cells (Bernhardt, 1979; Bernhardt, 1983). RF fields can induce currents sufficient to stimulate excitable tissue at frequencies below 1 MHz (Bernhardt, 1979). The threshold current densities for stimulation, and approximate relationships between the strengths of the RF exposure fields and the induced current densities are available (Bernhardt, 1979; Bernhardt, 1983). Very high field strengths are necessary to reach the stimulation thresholds (electric field strength above 100 kV m^{-1}, magnetic field strength above 1.5 kA m^{-1}) (Bernhardt, 1983).

Some experimental results can be interpreted to mean that biological effects may occur without significant changes in temperature well below the stimulation threshold, and should be attributed to non-thermal mechanisms at the molecular level. At low levels, experimental demonstrations include behavioural changes in continuous-wave or modulated fields (Shandala, 1977), altered efflux of calcium from brain tissue exposed to extremely low frequency modulated waves (Blackman, *et al*., 1979; Blackman, 1980), changes in EEG waves (Takashima, 1979), impaired killing ability of lymphocytes (Lyle, 1983), chromosomal changes in developing mouse sperm cells, changes in

intracellular enzyme activity, altered firing rate of molluscan pacemaker neurons (Seaman and Wachtel, 1978), and changes in growth rate of yeast cells. Furthermore, athermal mechanisms are proposed for rectification by cell membranes (Pickard and Rosenbaum, 1978), which has been observed in plant cells at frequencies below 10 MHz; vibrational resonance in DNA molecules (Edwards, 1984); and structural transformations from random to coiled configurations in polymeric proteins. Careful measurements recently performed in various laboratories have not confirmed the earlier claims of resonance in DNA molecules (Foster, 1987; Gabril, 1987). In addition, the RF hearing phenomena may be considered athermal or microthermal since the temperature rise is very small (as low as 10^{-5} ^{o}C). However, the effects of pulsed microwaves on the auditory system are described by a model in which rapid thermal expansion launches an acoustic wave in the head (Swicord and Poscow, 1986).

Davidov (1982) proposed that the longitudinal vibrations along the axis of alpha helical molecules could form solitary wave quasiparticles (soliton) as a means of energy transport over molecular distances. In application of soliton concept, Lawrence and Adey (1982) reported the existence of soliton in the membrane-spanning channel proteins. Moreover, they proposed that interaction of the solitons with electromagnetic waves was a mechanism for bioelectromagnetic effects.

2.4 Macroscopic Interactions

Macroscopic interactions implies that the phenomena expressed as in equation 2.1 can be described in terms of classical electrodynamics (i.e., Maxwell's equations) rather than quantum mechanics (Johnson *et al*., 1974)

$$\nabla \times \vec{H} = \sigma \vec{E} + \frac{\partial \vec{D}}{\partial t} \qquad 2.2$$

$$\nabla \times \vec{E} = -\frac{\partial \vec{B}}{\partial t} \qquad 2.3$$

$$\vec{D} = \varepsilon_o \vec{E} + P \qquad 2.4$$

$$\vec{B} = \mu_o (\vec{H} + M) \qquad 2.5$$

Where ***E*** is the electric field, ***H*** is the magnetic field, ***D*** and ***B*** are the electric and magnetic displacement vectors, ε_o and μ_o are the dielectric constant and magnetic permeability for free space respectively, P and M are the electric and magnetic polarizabilities and ***J*** is the current density.

From the macroscopic point of view the interaction can be defined in terms of the absorbed power, which in turn is determined by the electric permittivity and magnetic permeability, and internal field intensity. The magnetic permeability (μ) of biological materials is equal to that of vacuum and therefore, can be neglected in considerations of the energy absorbed. The electric permittivity of biological tissue depends on the type of tissue, water content, temperature and the frequency given as (Johnson *et al*., 1974)

$$\hat{\varepsilon} = \varepsilon_o(\varepsilon' - j\varepsilon'') \qquad 2.6$$

where ε_o is the permittivity of vacuum, ε' is the dielectric constant ε'' is the relative dielectric permittivity. The real part of the relative permittivity ε' is called dielectric constant. The imaginary part of the relative dielectric permittivity ε'' is related to the conductivity (σ) in the following way:

$$\sigma = 2\pi f \varepsilon_o \varepsilon'' \qquad 2.7$$

where f is the frequency. The dielectric properties of biological materials for frequencies up to 10 GHz have been studied extensively by (Cook *et al*., 1951 and Schwan *et al*., 1963) while only more recently have these been measured at millimeter wavelengths (Gandhi *et al*., 1978; Szwarnowski and Sheppard 1977). The values of both the dielectric constant (ε') and loss factor (ε'') or conductivity (σ) vary substantially in the frequency range from 10 MHz to 10 GHz. For the high water content tissue such as muscle, or skin, the dielectric constant ranges from about 160 to 40, and the conductivity from 0.6 to 10 S/m. For the low water content tissue such as bone or fat, it varies between 7.5 and 4.5, and the conductivity between 0.02 and 0.5 S/m (Johnson and Guy, 1972). The dielectric permittivity and frequency determine also how far the electromagnetic wave penetrates into the body. The penetration depth varies from a small fraction of a millimeter at the upper frequencies of microwave range (above 100 GHz), to a few centimeters for high water content tissue at frequencies of a few GHz and for low water content tissue at frequencies of a few tens of GHz to over 1 m for low

water content tissue at 10 MHz. Power absorbed in a unit volume of a homogeneous tissue, for a constant electric field intensity (E) within this volume is equal to

$$P = \frac{\sigma}{2}\vec{E}^2 = \pi f \varepsilon_o \varepsilon'' \vec{E}^2 \qquad 2.8$$

The quantification of the internal fields in complicated biological medium such as in man or animal body is, in general a difficult task due to the shape irregularity and heterogeneity of the material properties. Nevertheless, several sophisticated and useful models of the human body or its parts have been developed and analytically and experimentally analyzed (Shapiro *et al*., 1971; Guy *et al*., 1977). These methods permit an estimation of total power absorbed in the human and animal body in a wide range of frequencies, for various field polarizations under a plane wave (far-field) exposure conditions (Johnson *et al*., 1975).

A concept of the relative absorption cross section that expresses the percentage of the energy absorbed in relation to the energy incident was introduced by Schwan and his colleagues (Anne *et al*., 1961). From the analysis of several phantoms of the human body it was concluded (Salati *et al*., 1962) that an adult man absorbs 50-200% of the power that is calculated as the incident power density times surface area (one side) of the man. More recently, it was shown that the average specific absorption rate can be substantially higher at so-called resonant frequencies (Gandhi *et al*., 1978). Considerable attention has been devoted by many researchers (Shapiro *et al*., 1971; Guy *et al*., 1977) to determine approximate distribution of the energy maxima ("hot spots") within the human and animal bodies and their important parts, e.g., the head. The total power absorbed and its spatial distribution within a human body are functions of the following parameters:

1. Electromagnetic field frequency, with the maximum total absorption in the whole body occurring at a certain ratio of the body dimensions to the wavelength; when the long body axis is parallel to the electric field, the maximum (resonant) absorption occurs for the ratio of the body length to the wavelength equal to about 0.4, while in other orientations (the long body axis parallel to the direction of propagation or the magnetic field) the maximum absorption is for the body length approximately equal the wavelength.

2. Body to radiation source configuration - far-field versus near-field exposure conditions.

3. Exposure environment; presence of other objects in the vicinity of the source and/or the exposed body.

4. Electrical properties (dielectric constant and conductivity) of the tissue. These depend critically on the water content and on relative amounts of free and bound water present.

The initial rate of temperature increase resulting from the absorption of microwave power P (the conduction process neglected) can be given as (Johnson *et al*., 1974)

$$\frac{\Delta T}{\Delta t} = kP \qquad 2.9$$

where ΔT is the temperature increase in time Δt and k is the proportionality factor dependent on the tissue specific heat and density. An understanding of the factors influencing the body temperature resulting from absorption of MW radiation involves a study of the different heat pathways within the body. Body heat can be transferred to the environment only after it is first transferred to the body surface. That transfer can be accomplished by three mechanisms: conduction, circulatory convection, and radiation (Emery *et al*., 1976).

The conduction process in the body involves the flow of heat through a body tissue due to a temperature gradient. The body tissues are relatively poor thermal conductors with typical values of conductivity between 2 and 10 Sm^{-1}. Forced convection via the blood circulation is of primary importance in the heat transfer to the surface. In well perfused tissues, the heat transfer is rapid due to small distances separating the cells and blood vessels. Temperature differences which would exist in the absence of blood flow and as small as a few tenths of a degree are equalized by the blood flow. The blood flow also controls the effective body insulation through constriction or expansion of the cutaneous capillaries, so that the distance that heat has to flow through the superficial layer to the superficial epidermis is increased or decreased accordingly. The amount of heat transferred from the body to the environment (or vice versa), is governed by the operation of the thermoregulatory control.

Heat reaching the body surface is lost to the environment by the usual modes of conduction, convection, radiation, passive diffusion of water vapour through the skin

and respiration. In contrast to the passive diffusion, sweating or "sensible perspiration" involves an active secretion of fluid from sebaceous glands located in the skin. The heat loss though sweating depends on the amount of sweat secreted and the sweat evaporation rate. Under normal rest conditions for a naked man, convection accounts for 40% of the total heat loss, radiation 45 %, respiration 8 %, and passive diffusion 7 %. Under some environmental (high temperature) or physiological conditions (e.g., heavy exercise), sweating becomes the dominant heat loss mechanism. If heat loss from sweating does not compensate for the heat build-up in the body in some animals an evaporative heat loss mechanism can maintain the internal body temperature at safe levels. In many animals this is accomplished by the regulated evaporation of water from the respiratory tract by panting. Sweating is controlled by the central neural integrative mechanism which receives signals from the thermo-sensitive sites within the body. The hypothalamus and spinal cord are regarded as equivalent core temperature sensors since their thermo-receptors' signals are additive.

Various models of human thermoregulation were applied in evaluating effects of MW power absorption. It was concluded (Emery *et al*., 1976) that different sweat models give substantially different results when applied to MW energy absorption. The disparity indicates a need for better sweat models to be developed before reliable simulation of the thermal behaviour of humans exposed to MW power radiation can be achieved. In general, the absorption of MW power by the tissue (human or animal) leads to thermal effects which are either compensated for by the thermoregulatory action and result in no temperature change or are accompanied by a local or overall temperature increase. A non-uniform distribution of the absorbed power is a well established fact, which may lead to involved interactions. In some exposure situations only certain parts of the body are absorbing MW power. Some insight into the possible phenomena is given by Emery *et al*., (1976) If 10 W is deposited in the head, which corresponds to exposure to a plane wave of 1 W/cm^2 at 100 MHz, and no power deposited in the remaining parts of the body, there is only a small rise in the temperature of the head core tissue since it is well protected by the perusing blood, but a significant decrease in the surrounding tissue temperature occurs because of the sweating caused by the hypothalamic temperature increase. When on the other hand 100 W is deposited in the body (no power deposited in the head), which corresponds to the exposure of 120 mW/cm^2 at 100 MHz, or 590

mW/cm^2 at 20 MHz, the hypothalamic temperature does not rise, the sweating is inhibited and the other tissue temperatures have to rise considerably to facilitate the heat transfer.

Non-uniform heating, resulting from exposure to RF or microwave radiation, generally referred to as formation of "hot spots" may cause a variety of secondary interactions (Michaelson, 1977; Baranski and Czerski, 1976). Preferential heating of the hypothalamus may affect thermoregulation and elicit aberrant neurophysiological responses even at relatively low power density levels, which are not accompanied by an increase in the whole body temperature. The temperature sensitivity of the thermo-sensitive neurons of the preoptic nucleus of the hypothalamus is such that a temperature increase of only 0.1 °C may result in a 3% increase in the firing rate of such cells (Cleary, 1977). However, relatively large power densities may be required to cause such increase in the temperature.

One of the most prominent thermally induced effects where the temperature increases are very small is the microwave hearing effect (Guy *et al*., 1975; Lebovitz and Seaman, 1977). Exposure to one pulse of electromagnetic radiation results in a perception of "a click", and exposure to pulsed electromagnetic radiation results in hearing of a buzzing or hissing sound. The threshold of perception depends on radiation frequency, pulse peak power and pulse duration. The average power density sufficient to induce microwave hearing is relatively small (approx. 0.1 mW/cm^2), while an estimated threshold SAR is approximately 16 mJ/kg for humans. This energy is capable of increasing the tissue temperature by only 5×10^{-6} °C (Guy *et al*., 1975). The mechanism of interaction is as follows: the electromagnetic radiation causes rapid temperature increase which generates thermal expansion pressure in the brain matter which then launches the acoustic wave of pressure that is detected by cochlea (Lebovitz and Seaman, 1977). The cochlea microphonic frequency is independent of the MW frequency and the absorption pattern (Lin, 1978).

2.5 Microscopic Interaction -- Molecular Mechanisms

Evaluation of possible biological effects of electromagnetic exposure at low intensities energies corresponding to radio and microwaves of frequencies between 10 MHz and 300 GHz are respectively between 0.4×10^{-7} and 1.2×10^{-3} eV. This is considerably

lower than energies required to produce any intramolecular alterations or intermolecular bond breaking (Cleary, 1977), and is even one order of magnitude lower than thermal motion energy *(kT,* k-Boltzman's constant, T-temperature in °K) at 37 °C. Therefore, direct MW effects at a molecular level can be ruled out unless some cooperative mechanisms can be invoked. One of the basic mechanisms underlying interactions of MW with biological systems at molecular level is the field-induced rotation of polar molecules. Such molecules as water and proteins experience a torque when placed in an external electric field and attempt to align with the field to minimize the potential energy of the dipoles. Orientation is hindered by the thermal (Brownian) motion of the molecules. Frictional forces, that are dependent upon the rate of orientation (the frequency of the applied field) and the relaxation time, determine the degree of orientation. The relaxation time depends on the viscosity of the solvent, the size and shape of the dipoles, temperature and the nature of the solute-solvent bonds. Only partial orientation takes place when moderate values of the electric field are applied.

The degree of orientation is proportional to the strength of the directing field until the saturation occurs (BSttcher *et al*., 1978). The number of dipoles oriented along a specific direction θ and $\theta + \Delta\theta$ in respect to the z axis when the electric field direction is along the z axis is (assuming uniform field of a relatively low frequency) (Spiegel, 1984)

$$\Delta N = A\exp(\mu E_r \cos\theta / KT)\Delta N_o \qquad 2.10$$

where: A is a constant, μ is the permanent dipole moment, E_r is the directing field, k is the Boltzmann's constant, T is the temperature in °K, and ΔN_o is the number of dipoles oriented in the direction $\theta + \Delta\theta$ without an external electric field.

$$\Delta N_o = \frac{1}{2} N Sin\,\theta \Delta\theta \qquad 2.11$$

where N is the total number of dipoles. The directing field is smaller than the internal electric field due to the reaction field of the dipole, and the internal electric field is never greater than the applied field. The dipoles are directed by the field to the extent determined by the average value of the function *Cos* θ (for a random distribution it is zero, for a perfect alignment it is one). This function called the Langevin function is equal to (BSttcher *et al*., 1978):

$$L\left(\frac{\mu E_r}{kT}\right) = \sqrt{\cos\theta} = Co\,\tanh\left(\frac{\mu E_r}{kT}\right) - \frac{kT}{\mu E_r} \qquad 2.12$$

For small values of $\mu E_r / kT$, $\left(\mu E_r / kT < 1\right)$ the Langevin function is linear and given by

$$L\left(\frac{\mu E_r}{kT}\right) = \frac{\mu E_r}{3kT} \qquad 2.13$$

The degree of orientation of the dipoles in the directing field decreases with the frequency of the field above a certain frequency owing to the phase lag between the motion of the dipoles and the oscillations of the field. At these frequencies the combined effects of the viscosity, molecular size and shape, and solute-solvent bonding prevent the molecular motion from being in phase with the applied field. As the frequency is further increased a stage is reached where none of the dipoles is able to keep up with the field and the system then behaves like a non-polar material i.e., the value of ε' will lie between 2-5 and ε'' will diminish to zero. Within the range at which dielectric dispersion occurs lies the relaxation frequency f_r at which ε'' (the absorption of energy per cycle of applied field) reaches a maximum. However, the transfer of energy from the field to the medium per unit volume of medium (specific energy absorption does not peak at f_r but increases with frequency to a limit value σ_∞ (Equation 2.6) and remains fairly stable at millimeter wavelengths). Close to the far infra red region a further increase is observed (Illinger, 1969). The relaxation time T is the reciprocal of the angular relaxation frequency w_r, which is equal to $2\pi f_r$. The orientation of the molecules and their rotation in the electric field are only partial in directing field of order of a few V/cm (BSttcher *et al*., 1978). The rotational energy is converted into heat. This type of interaction is not suggestive of irreversible effects in biological media other than those resulting from the increase in temperature.

An interesting insight into the extent of molecular interactions resulting from electromagnetic fields in RF and MW range can be gained by looking at the electric field intensities due to polar molecules and ions. The field strength on the axis of a haemoglobin dipole (assuming a point shaped dipole) is given as (BSttcher *et al*., 1978).

$$E = \mu / r^3 \qquad 2.14$$

and at a distance of 1 μm is 1.26 kV/cm, while still even at a distance of 10 μm it is 126 V/cm. The field intensities due to a mono-valent ion are 140 kV/cm and 1.4 kV/cm at a distance of 1 μm and 10 μm, respectively.

The molecular rotational interaction is described by the frequency behaviour of the dielectric permittivity which (from the macroscopic interaction point of view), may be considered without needing insight into the actual molecular phenomena. Between 10 MHz and 300 GHz several rotational phenomena take place. Water, which constitutes about 70% of the total weight of humans exhibits rotational relaxation at MW frequencies. Water appears in two forms as free water and bound water. The relaxation of free water takes place at a frequency of approximately 25 GHz at 37 °C, and the relaxation frequency is a function of temperature. An increase in the temperature causes an increase in the relaxation frequency. The rotational motion of cell free water, which may be converted into translational and vibrational excitations by collision leads to an increase in the cell temperature. However, no specific biological effects are expected from this mechanism since the molecular structure remains unchanged (Rabinowitz, 1973). At high microwave power levels the rotational relaxation of free water can lead to alterations in the biological structure due essentially to the heating effect (Illinger, 1969).

Bound water has many definitions according to the experimental techniques being employed to investigate it. A suitable general designation would be to refer to bound water as molecules held in non-random orientations at or near the surface of a macromolecule. Considerable differences of opinion exist as to how much water in the vicinity of the macromolecule may be considered to be different from free water. Dielectric evidence (Grant *et al*., 1978) indicates that only two molecular layers are involved, however some workers believe that the bound water layer extends much further (Hazlewood, 1977).

The relaxation frequency of bound water depending on the molecule the water is attached to and the solvent viscosity occurs between 100 and 1000 MHz (Grant *et al*., 1978; Schwan, 1973). In the dispersion region of bound water more or comparable amounts of the energy per unit volume is absorbed by the bound water than by the free water in a biological tissue subjected to electromagnetic fields. The bound water constitutes about 30% of the total weight of protein molecules (Schwan, 1973). However, most tissues contain substantially more free water than bound water although for lens material recent dielectric measurements show that as much as 15% of the water

may be in the bound state. In view of the vulnerability of the eye to microwave radiation this observation might be significant.

The rotational motion of biological molecules such as amino acids, peptides, and proteins have been researched extensively, including pioneering work by Oncley (1942) and many others as reviewed by Grant *et al*. (1978); Takashima and Minakato (1973) . Protein molecules are highly charged with the number of positive and negative charges nearly equal and the charge distribution on the surface highly uniform. Due to large dimensions of the molecules a relatively small asymmetry in the charge distribution results in a large dipole moment. Typical values of dipole moments are 200-1000 Debye units (1 D = 3.33 x 10^{-30} C/m). The relaxation frequencies are between 100 kHz and 50 MHz (Grant *et al*., 1978; Schwan, 1973). For many proteins more than one relaxation time have been observed and attributed to non-spherical shape of the molecules (Oncley, 1942). The relaxation frequencies for amino acids and peptides are typically between 0.5 and 3 GHz (Grant *et al*., 1978). As indicated by earlier calculations moderate field intensities at radio and microwave frequencies cause partial reorientations of dipoles and are very small compared with either the intensities required for saturation or the intensities due to the polar molecules themselves. Therefore, no direct, specific effects other than those resulting from the temperature increase are envisioned to result from rotational relaxation of biopolymers (Illinger, 1969; Schwan, 1973).

At millimeter waves (frequencies above 100 GHz) three possible field induced motions in biopolymers have been suggested (Illinger, 1969), namely: terminal group rotation (-OH, -NH_2, etc.), inversions (-NH_2) and ring deformation, and proton tunneling in H-bonded systems. However, no experimental evidence has been provided. Another suggestion was made by Frfhlich (1975) that at frequencies above 100 GHz sections of macromolecules undergo vibrational excitation that may lead to positional or conformational changes (Frthlich, 1975).

2.5.1 Cellular Interactions

An essential function is played by cell membranes, which apart from maintaining the integrity of the interior of the cell, regulate the exchange of molecules and fluids between the interior and exterior, in a manner specific to the type of cell (Schwan, 1958). The essential electrical parameters of the membrane are the capacitance per unit

area and conductance. For a large number of membranes the unit capacitance is 1 $\mu F/cm^2$ and conductance is 10^4-10^5 S/m^2 (1-10 S/cm^2). The membranes separate the regions of different dielectric properties, so that charged interfaces are formed. When subjected to high frequency electromagnetic fields, they exhibit a frequency relaxation behavior referred to as the Maxwell-Wagner effect (Grant *et al*., 1978). This relaxation occurs between a few tens of kHz and a few hundred MHz. Biological implications of this phenomenon are similar to those of rotational relaxation.

An excitation of biological membranes should be considered as a potential interaction mechanism. A comprehensive description of the electrical behavior of the membrane was given by Hodgkin and Huxley (1952) and several papers by Schwan (1977) considered the membrane behavior at RF and microwave frequencies (Schwan, 1973; 1977). Above 100 MHz the membranes are short circuited due to their capacitance and no electrical potential can be induced. It was shown (Schwan, 1977), that when an alternating electric field of a few V/cm, is applied perpendicularly to the membrane surrounding a nerve cell, an electrical potential of a few mV (which may excite the nerve) can be formed across the membrane below 1 MHz. This potential decreases rapidly with frequency, and at frequencies above 10 MHz becomes very small compared with the resting potential of nerve cells. Speculative theoretical interaction mechanisms at the membrane level have recently been suggested (Barnes and Hu, 1977; Pickand and Rosenbaum, 1978), however their validity has to be further established and experimentally confirmed. Recent experimental data (Bawin *et al*., 1975; Blackman *et al*., 1977) on changes in the calcium ions efflux in isolated cerebral tissue of neonatal chicks due to exposure to low intensity amplitude modulated at (5-25 Hz) radiofrequency fields or low frequency fields (5-25 Hz) suggests a possible interaction on the cellular level. The electrochemical equilibrium between ions, macromolecules and glycoproteins of the cell surface could be disrupted by small variations of surrounding ionic concentrations or local electric gradient (Bawin *et al*., 1975; Adey, 1975). Further investigations should provide more experimental data that would aid in establishing the interaction mechanism responsible for the phenomenon.

2.5.2 Other Interaction Mechanisms

It was postulated by Presman (1968) that electromagnetic fields of various frequencies transmit biological information between various component parts of a human and animal body. The existence of some sort of telecommunication link between cellular components within a cell and between various cells within a multi-cellular organism is assumed. Individual organisms are in turn sensitive to electromagnetic fields in the environment, so that man-made electromagnetic fields may directly interfere with normal functions of the organisms.

2.6 Temperature Effects

The temperature rise is determined by equating the rate at which energy is supplied to the tissue to the rate at which it flows out. Heat energy is transported away from the biological tissue by conduction, convection and radiation. In most cases, the cooling by radiation is insignificant. The characteristics of the biological tissue interaction can be assessed by using short enough electromagnetic pulses, so that both convection and radiation cooling can be neglected. In that case, the rate of change of temperature is given by:

$$\frac{\partial T}{\partial t} = \frac{P}{\rho c} - \frac{T - T_o}{\tau} \qquad 2.15$$

where T is the temperature, T_o is the initial temperature, t is time, P is the power absorbed,τ is the thermal time constant, ρ is the density and c is the specific heat capacity of the tissue. The thermal time constant, τ, is dependent on the geometry. For a sphere,

$$\tau = \frac{r}{4K'} \qquad 2.16$$

where r is the radius and K' is the thermal diffusivity (Barnes and Hu, 1980). For very short pulses, all the losses can be neglected and

$$\Delta T = \frac{Pt}{\rho c} \qquad 2.17$$

The most important of these are the changes in biochemical reaction rates, which in turn affect a large number of physiological functions (Yariv, 1985). Other bioeffects are the physiological and proliferation phenomena in various enzymes, cells, tissues, organs and organisms. Typical biochemical reactions can be described by an equation of the form:

$$\frac{d[S]}{dt} = -k[S] \qquad 2.18$$

where $[S]$ is the concentration of the material undergoing the chemical reaction, t is the time and k is the reaction rate constant (Kenneth, 1984). k is often given by:

$$k = \frac{k_B T}{h} \exp\left(\frac{+\Delta H' - T\Delta S'}{RT}\right) \qquad 2.19$$

where k_B is the Boltzmann constant, T is the absolute temperature, H' is the enthalpy of activation, S' is the entropy of activation, h is Planck's constant, R is the gas constant (Johnson *et al*., 1974). The significance feature is the reaction constant, k, which varies exponentially with temperature, since $\Delta H'$ and $T\Delta S'$ are large numbers (Johnson *et al*., 1974). Thus, very small changes in temperature can lead to large changes in chemical reaction rates. The critical temperature is in part controlled by the length of time that the temperature is elevated above 37.5^{o}C. A rule of thumb suggested by Barnes (1989) to estimate whether significant biological changes are likely is to see if ΔT < 10^{o}C for periods of 10^{-6} s, or 5^{o}C for 1 s, or 2^{o}C for hours. If the ΔT is large, then it can be expected to lead to important changes in the biological system.

In addition to the magnitude of temperature change, it can be shown that the rise $\frac{dT}{dt}$, is important and can induce current to flow across membranes. Taking the time derivation of the equation, which describes the passive equilibrium potential across a membrane for a single ion, it was shown that a current is proportional to the temperature derivative (Barnes 1984).

$$I = -qV_1C_1\left(\frac{\phi}{\Theta_T}\right)\left(\frac{\dot{\phi}}{\phi} - \frac{\dot{T}}{T}\right) \qquad 2.20$$

where q is the charge on the ion, V is the volume of the cell, C is the concentration of ions in the cell, ϕ is the resting potential, Θ_T is the voltage equivalent temperature, Θ_T = (Kt/q), $\dot{\phi}$ is the derivative of the membrane potential with respect to time, T is the temperature, and $\dot{T}$ is the temperature derivative with respect to time. In order to get the magnitudes of the heating effect rate rise, for a sphere of radius a, and density ρ with impulse heating, the following equation can be used.

$$\Delta T_{\max} = \left(\frac{3}{2\pi e}\right)^{32} \frac{P_2}{\rho C a^2} \qquad 2.21$$

where e is the base for natural logarithm (Hu and Barnes, 1970). The rate of temperature rise is approximately 13 $^{o}Cs^{-1}$, assuming no conduction or diffusion heat losses and the thermal capacity of water. For this high field, a significant temperature rise requires about 0.5 s however, the rate of rise has been shown to be significant in exciting a brain slice from mouse with pulses as short as 10^{-2} s (Adey *et al*., 1983).

The changes in chemical reaction rates with temperature and the changes in physical properties of the biological materials can be primary effects of the changes in temperature. These are followed by many biological responses, such as changes in the firing rate of nerve cells, changes in growth rates, increases in metabolism, sweating etc. The exponential sensitivity of many processes in the body to changes in temperature is a possible explanation, for the many regulatory feedback processes that allows humans to maintain body temperature to ±0.5 ^{o}C over a wide range of external condition (Barnes, 1989).

2.6.1 Effects: High – Frequency Fields Force

Fields at RF are quantified in terms of electric field strength (***E***) and magnetic field strength (***H***). In the SI system, ***E*** is expressed in volts per metre (V m^{-1}) and ***H*** as amperes per metre (A m^{-1}).

Both are vector fields, i.e., they are characterized by magnitude and direction at each point (INRCIRPA, 1985; NCRP 1981). Electromagnetic waves are waves of electric and magnetic fields, where a wave motion is defined as a propagation of disturbances in a

physical system. A change in the electric field is accompanied by a change in the magnetic field, and vice versa. The basic expression for the force exerted on a free charge in such field is given by:

$$\mathbf{F} = q\,(\mathbf{E} + \mathbf{v} \times \mu\mathbf{H}) \qquad 2.22$$

where q is the electric charge, moving with velocity **v** perpendicular to the magnetic field, and μ is the constant of proportionality called the magnetic permeability in a vacuum and in air, as well as in non-magnetic (including biological) materials. When a sinusoidal varying electric field is applied in a homogeneous or symmetrical environment, a charged particle simply oscillates about its starting position. The amplitude builds up until the damping leads to a rate of energy loss equal to the rate of energy input. If the resorting forces are asymmetrical or there is a gradient in the applied fields, then a net force is exerted on the charged body.

An asymmetrical field can lead to nonlinearities, including rectification and harmonic rectification and harmonic generation. However, because the mean time between collisions is so short and the rate of energy loss is high, direct force effects on ions or molecules are usually not observed at frequencies above 10 MHz, even though they could be quite important at low frequencies. Upon the application of an electric field to a slab with dielectric constant ε and conductivity σ, torques will be applied to the dielectric slab in order for it to take on the lowest energy configuration, which will either be at right angles or parallel to the electric field, depending upon the ratio ε_1 to ε_2. The torque on the slab can be given as

$$\tau = -\frac{E_o{}^2 v}{2}\left(\frac{\varepsilon_1{}^2 - \varepsilon_2{}^2}{\varepsilon_2}\right)\sin 2\theta_o \qquad 2.23$$

Where E_o is the electric field in the bulk material, ε_1 is the dielectric constant in the bulk material, ε_2 is the dielectric constant of the slab, θ is the angle between the field and the long axis of the slab, v is the volume of the slab, and if we assume Brownian motion to be the disordering force, then the threshold field for the observation of the alignment of the dielectric slab is given as (Barnes, 1989).

$$E_{th} = \sqrt{\frac{2kT\varepsilon}{(\varepsilon_o - \varepsilon^2)v}} \qquad 2.24$$

It is possible to observe the motion of particles with induced dipole moments placed in an electric field gradient. In this case, the induced polarization is assumed to be given by $P = \alpha v E$, where v is the volume and α is the polarizability. The force in this case is given as (Barnes, 1989)

$$F = \alpha \, v \, (E.\nabla E) \qquad 2.25$$

The direction of the force is independent of the sign of $\boldsymbol{E}$. These forces must be used to separate bodies with two different dielectric constants. If the body to be moved can be approximated by an insulating sphere with a dielectric constant ε_m in medium, the force is given by Barnes (1989) as

$$F_d = \frac{3}{2} v \varepsilon_1 \frac{\varepsilon_2 - \varepsilon_1}{\varepsilon_2 + 2\varepsilon_1} \nabla |E|^2 \qquad 2.26$$

Another use for these forces is to bring cells in contact with an electric impulse, which breaks down the cell membrane for genetic transfer from one cell to the next. Some experimental work has been done on the effect of short pulses microwaves on cells (Pyle *et al*., 1975; Fout 1980). There are electron micrographs that indicate that the cell membranes and the mitochondria membranes are broken or destroyed by the large microwave fields in a way that is not observed when cells are raised to the same or higher temperatures by heating in hot water for the same length of time. Similar work on transformed brain cells in a different exposure system shows similar membrane damages for exposures to fields greater than 1.7 x 10 Vm^{-1} and calculated temperature rise of less than 4 ^{o}C (Webber *et al*., 1980). The calculated temperature rise for the cells as a whole is a function of the geometry of the exposure system, the peak power input, the pulse repetition rate, and the average cell conductivity and dielectric constant. Additionally, the estimated threshold temperature for death is lowered when the cell temperature before is lowered to 0 ^{o}C (Barnes, 1989). The time constant for a possible differential temperature rise for the membrane above the surrounding high-water-content material can be obtained as

$$\tau = \frac{\chi^2}{k'} \qquad 2.27$$

where χ is the thickness of the membrane and k' is the thermal conductivity.

2.7 Radiometry of Non-ionizing Radiation

In the application and interpretation of the effects of NIR on biological systems, a precise knowledge of the dosimetry is indispensable. There is a need for a standardized way of quantifying NIRs and unifying their units. Exposure of an organism to RF fields results in induction of RF fields and currents inside the organism. The internal, rather than the external, fields and currents are responsible for interactions with biological systems independently of whether these interactions are thermal or athermal. A dosimetric quantity that is widely used in quantifying the interactions of RF fields with biological systems is the specific absorption rate, SAR. It is defined as the rate of energy transfer to the body, tissue or cells per unit mass of the body (NCRP, 1981).

$$SAR = \frac{d}{dt}\left(\frac{\Delta W}{\Delta m}\right) = \frac{d}{dt}\left(\frac{\Delta W}{\rho \Delta V}\right) \qquad 2.28$$

where ΔW is the energy transferred to a mass Δm; ΔV is the volume containing the mass Δm and ρ is its density. For sinusoidal varying fields, the SAR in a small volume of tissue thoughout the electric field is constant and is given as:

$$SAR = \frac{\sigma \vec{E}_{in}^{\,2}}{\rho} \qquad 2.29$$

where σ is the tissue conductivity and $\mathbf{E}^2_{\text{in}}$ is the mean square magnitude of the electric field in the tissue. The SAR is expressed in units of watts per kilogram (W/kg). Absorption of NIR energy leads to thermal effects resulting in a temperature increase as in the relation:

$$SAR = \frac{C \Delta T}{\Delta t} \qquad 2.30$$

Here T is temperature, t is the duration of exposure and C is the specific heat capacity of the tissue. It is a complex function of the NIR source configuration, the shape and size of the object, the orientation of the object with respect to the source, and the source frequency. The SAR is not only an index of the absorbed radiation energy but also that of the internal electric field strength. It can often be determined from electromagnetic theory, but in most cases one has to rely upon electrical instrumentation such as field survey meters, electric probes, thermocouples, thermistors, optical fibres, thermographic and calorimetric devises for quantifying SAR in tissues or phantoms.

2.8 Free Radicals

These are species of atoms and molecules with one or more unpaired electrons that are capable of independent existence (hence the term "free"). Energy created by this unstable configuration is released through reactions with adjacent molecules, such as inorganic or organic chemicals –proteins, lipids, carbohydrates particularly with key molecules in membranes and nucleic acid. Free radicals initiate autocatalytic reactions whereby molecules with which they react are converted into free radicals to propagate the chain of damage (Cotran *et al*., 1999). The damaged molecules, which are important for cellular functions, lead to a total loss of such cellular functions.

2.8.1 Hydroxyl radical

The chemical reactivity of free radicals varies. One of the most reactive is the hydroxyl radical ($OH^{\cdot}$) (Von, 1987). Exposure of living organisms to ionizing radiation causes fission of O-H bonds in water to give H^+ and OH^- (Riley, 1994). Hydroxyl radicals react at diffusion controlled rate with almost all molecules in living cells (Von, 1987).

$$H_2O \rightarrow H^+ + OH^- \qquad 2.31$$

2.8.2 Nitric oxide

It is synthesized from the amino acid L-arginine in the presence of molecular oxygen, NADDH and other cofactors by the enzyme nitric synthetase (Nathan, 1997) by vascular endothelial cells, phagocytes, certain cells in the brain and many other cell types (Moncadas, 1993). Nitric oxide is a vasodilatory agent and possibly an important neurotransmitter. It can act as free radicals and can be converted to high reactive peronitrite anion ($ONOO^-$) as well as NO_2 and NO_3^- (Cotran *et al*., 1999).

2.8.3 Superoxide (O_2^-)

Superoxide is the one electron reduction product of oxygen. It is produced by phagocytic cells (neutrrophills, monocytes, macrophages, eosinophils) and helps them to inactivate virus and bacteria (Babior, 1990). O_2^- is also produced in vivo by several cell types other than phagocytes, including lymphocytes (Maly, 1990) and fibroblasts (Meler *et al*.,

1990): superoxide produced by such cells is often thought to be involved in intercellular signaling and growth regulation (Bundon *et al*., 1989). There are many reports (Arroyo *et al*., 1990; Babs, 1991; Britigan *et al*., 1992) that vascular endothelial cells generate O_2^-. They are also produced during normal respiration. In addition to deliberate metabolic production of O_2^- this radical can also emanate from what may be called accidents of chemistry. Superoxide and hydrogen peroxide may be generated by 'auto-oxidation' reactions, in which compounds such as catecholamines, tetrahyrofolates and reduced flavin reacts directly with O_2 to form O_2^-. Superoxide may be made in the mitochondria. The mitochondria electron transport chain is a gradient of redox potential from highly reducing NADH/NAD^+ coupled to the oxidizing O_2^-.

2.8.4 Hydrogen peroxide

This is not a free radical by definition, it is a biological important oxidant because of its ability to generate the hydroxyl radical, and extremely potent radical (Aruoma, *et al*., 1991). Protonation of O_2^- results in the formation of perhydroxyl radical, (HO_2) is a much stronger radical than O_2^-. Acts as a Bronsted base in aqueous solutions to shift the acid-base equilibrium to form a hydroperoxyl radical, thereby forming H_2O_2 in acidic environment (Yu, 1994)**.** Because of its non-ionized and low charged, H_2O_2 is able to diffuse though hydrophilic membrane as seen with the leakage of H_2O_2 from mitochondria (Yu, 1994). The hydroxyl radical (OH^-) is formed not only by the reduction of hydrogen peroxide but also though the interaction of superoxide with hydrogen peroxide and reduced form of metal ions, that is copper and iron (Ross, 1991) as seen in Fenton reaction.

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH \qquad 2.32$$

It is equally produced by action of several oxidase enzymes in vivo, including amino acid oxidases and enzyme xanthine oxidase (Granger, 1988). It has a half life of about 1 x 10^{-9} s^{-1} at 37^{o}C. The complete reduction of O_2 is summarized in the following reactions:

$$O_2 + e \rightarrow O_2^- \qquad 2.33$$

$$O_2^- + H_2O \rightarrow H_2O + OH^- \qquad 2.34$$

$$HO_2 + e^- + H \rightarrow H_2O_2 \qquad 2.35$$

$$H_2O_2^- + e^- \rightarrow OH + OH^- \qquad 2.36$$

H_2O_2 generated in the thyroid gland is used by a peroxidase enzyme to iodinate the thyroid hormones (Dupuy *et al*., 1991). H_2O_2 may up-regulate the expression of certain genes (directly or indirectly) leading to displacement of an inhibitory subunit from the cytoplasmic genes transcription factor to migrate to the nucleus and activate many different genes by binding to specific DNA sequences in enhancer and promoter elements (Scheck *et al*., 1992).

2.8.5 Trichloromethyl (CCI_3)

It is a carbon centered radical. It is formed during metabolism of tetrachloromethane (CCI_4) in the liver and contributes to the toxic effect of this solvent (Recknagel *et al*., 1989). Carbon centered radicals usually reacts fast with O_2 to make peroxyl radicals given as

$$CCl_3 + O_2 \rightarrow CCl_3 + O_2^- \qquad 2.37$$

2.8.6 Free Radicals and Lesion in DNA

Simie and Javanovic (1986) were able to show that reactions with thymine in nuclear and mitochondrial DNA produce single stranded breaks in DNA. The DNA damage has been implicated in 'cell aging and malignant transformation of cells (Arnes, 1989; Harman, 1993). Excess formation of $H_2O_2^-$ and O_2 will damage the DNA. Singlet O_2 attacks guanine preferentially.

2.9 Plausible Biological Mechanisms

Plausible biological mechanisms are already identified that can reasonably account for most biological effects reported for exposure to RF and ELF at low-intensity levels (oxidative stress and DNA damage from free radicals leading to genotoxicity, molecular mechanisms at very low energies are plausible links to disease, e.g., effect on electron transfer rates linked to oxidative damage, DNA activation linked to abnormal biosynthesis and mutation). Traditional public health and epidemiological determinations do not require a proven mechanism before inferring a causal link

between EMFs exposure and disease (Hill, 1971). Many times, proof of mechanism is not known before wise public health responses are implemented. "Obviously, melatonin's ability to protect DNA from oxidative damage has implications for many types of cancer, including leukemia, considering that DNA damage due to free radicals is believed to be the initial oncostatic event in a majority of human cancers (REFLEX, 2004). In addition to cancer, free radical damage to the central nervous system is a significant component of a variety of neurodegenerative diseases of the aged including Alzheimer's disease and Parkinsonism. In experimental animal models of both of these conditions, melatonin has proven highly effective in forestalling their onset, and reducing their severity (Henshaw and Reiter, 2005).

Oxidative stress though the action of free radical damage to DNA is a plausible biological mechanism for cancer and diseases that involve damage from ELF to the central nervous system.

2.10 Non-humans Studies

2.10.1 Thermoregulatory Responses to RF radiation

Thermoregulatory effectors such as peripheral vasodilation, evaporation, metabolism and behavior may be activated during exposure to RF radiation exposure. Many responses are activated in the absence of any measurable change in deep-body temperature during RF radiation exposure (Gordon, 1984). Most thermoregulatory responses, as well as other biological effects, have been recorded in laboratory animals exposed to RF radiation at normal room conditions of temperature, humidity, air flow, etc. Thus, it is reasonable to predict that the threshold for effects due to RF heating are lower to ambient conditions that exacerbate thermal effects (Elder, 1984). Due to heat stress from absorbed RF energy, dose rates of 3.6-7 W/kg are lethal to rats, rabbits, dogs and rhesus monkeys exposed for 1-4 hours at normal laboratory conditions of temperature and humidity (Lotz, 1985).

2.10.2 Reproductive Effects

Most of the studies on reproduction and development of small mammals exposed to radiofrequency and microwave radiation have shown effects that can be related to an

increase in temperature. Several studies have shown, for example, that acute microwave exposure can affect the spermatogenic epithelium, and thus male fertility, though a rise of the testicular temperature (Kowalczuk *et al*., 1983; Saunders *et al*., 1991). The primary spermatocytes were often identified as a particularly sensitive stage and the response identified as identical to convectional heating.

RF radiation was found to be teratogenic at exposure conditions that approached lethal levels for the pregnant animals (Berman, 1984). Lary *et al*., (1982) reported teratogenic effects on rats exposed to SARs 11.1-12.5 W/kg for 20-40 minutes. Reduced fetal weight seemed to occur consistently in rodents exposed gestationally to SARs greater than 4.8 W/kg. Berman (1980) found temporary sterility in male rats at SAR of 5.6 W/kg, which caused a significant increase in testicular temperature.

Berman *et al*., (1974) reported that sufficiently elevated body temperatures are teratogenic to a number of mammalian species, including primates. As microwave-induced teratogenic effects were usually accompanied by a significant rise in maternal body temperature, they hence could be ascribed to this thermal effect. Yet the results of studies on induced fetal loss and developmental malformations were sometimes inconsistent with others. This is most probably due to species differences with regard to their thermo-susceptibility. Overall, the conclusion should be that the evidence suggests that only exposures that have an appreciable heating effect are likely to affect the embryo adversely (WHO, 2007; IEEE, 2002).

2.10.3 Effects on the Blood-forming and Immune Systems

The release of inflammatory substances, such as histamine is well-known to cause skin reactions, swelling, allergic hypersensitivity and other conditions that are normally associated with some kind of defense mechanism. The human immune system is part of a general defense barrier that protects against harmful exposures from the surrounding environment. When the immune system is aggravated by some kind of attack, there are many kinds of immune cells that can respond. Anything that triggers an immune response should be carefully evaluated, since chronic stimulation of the immune system may over time impair the system's ability to respond in the normal fashion.

Measurable physiological changes (mast cell increases in the skin, for example that are markers of allergic response and inflammatory cell response) are triggered by ELF and RF at very low intensities. Mast cells, when activated by ELF or RF, will break (degranulate) and release irritating chemicals that cause the symptoms of allergic skin reactions. There is very clear evidence that exposures to ELF and RF at levels associated with cell phone use, computers, video display terminals, televisions, and other sources can cause these skin reactions. Changes in skin sensitivity have been measured by skin biopsy, and the findings are remarkable. Some of these reactions happen at levels equivalent to those of wireless technologies in daily life. Mast cells are also found in the brain and heart, perhaps targets of immune response by cells responding to ELF and RF exposures, and this might account for some of the other symptoms commonly reported (headache, sensitivity to light, heart arrythmias and other cardiac symptoms). Chronic provocation by exposure to ELF and RF can lead to immune dysfunction, chronic allergic responses, inflammatory diseases and ill health if they occur on a continuing basis over time.

Specific findings from studies on exposures to various types of modern equipment and/or EMFs report over-reaction of the immune system; morphological alterations of immune cells; profound increases in mast cells in the upper skin layers, increased degranulation of mast cells and larger size of mast cells in electrohypersensitive individuals; presence of biological markers for inflammation that are sensitive to EMF exposure at non-thermal levels; changes in lymphocyte viability; decreased count of NK cells; decreased count of T lymphocytes; negative effects on pregnancy (uteroplacental circulatory disturbances and placental dysfunction with possible risks to pregnancy); suppressed or impaired immune function; and inflammatory responses which can ultimately result in cellular, tissue and organ damage (Lowenthal *et al*., 2007; WHO, 2007)

Liburdy, (1980) found effects of RF radiation on the blood-forming and immune systems similar to those caused by glucocorticoid-mediated stress responses. Effects on the blood-forming and immune systems have been reported at SARs greater than 0.4 W/kg however, there is a lack of convincing evidence for RF radiation effects on these systems without some form of thermal involvement. Many reports, particularly those describing effects of acute exposure, show an association between RF-induced thermal

loading or increased body temperature and changes in blood, blood-forming and immune systems (Rudnev and Goncher, 1985).

2.10.4 Nervous System

Acute or chronic continuous or pulsed-wave irradiation of animals at SARs greater than 2 W/kg can produce morphological alterations in the central nervous system. These changes were qualitatively similar after acute or chronic exposure at different SARs, but quantitatively more alterations occurred in the affected neural structure at higher SARs and after chronic exposure. The changes were found less frequent in animals allowed to survive several days to weeks after exposure ceased (Gage and Albert, 1984).

Von Klitzing *et al*., (1995) were the first to report that cell phone radiation affected electroencephalogram (EEG) alpha activity during and after exposure. Mann and Roschke (1996) reported that cell phone radiation modified rapid eye movements sleep (REM sleep) EEG and shortened sleep onset latency. Rosche *et al*., (1997) found no significant change in spectral power of EEG in subjected exposure to cell phone radiation for 3.5 minutes. Eulitz *et al*., (1998) reported that cell phone radiation affected brain activity when subjects were processing task-relevant target stimuli and not for irrelevant standard stimuli. Freude *et al*., (1998) found that preparatory slow brain potential was significantly affected by cellular phone radiation in certain regions of the brain when the subjects were performing a cognitive complex visual task. The same effects were not observed when subjects were performing a simple task. Urban *et al*., (1998) reported no significant change in visual evoked potentials after 5 minutes of exposure to cell phone radiation. Wagner *et al*. (1998, 2000) reported that cell phone radiation had no significant effect on sleep EEG.

Borbely *et al*., (1999) found that the exposure induced sleep and also modified sleep EEG during the non-rapid eye movement (NREM) stage. Freude *et al*, (2000) confirmed their previous report that cellular phone radiation affected slow down brain potentials when subjects are performing a complex task. However, they also reported that the exposure did not significantly affect the subjects in performing the behavioral task. Huber *et al*., (2000) reported that exposure for 30 minutes to a 900-MHz field at 1 W/kg peak SAR during waking modified EEG during subsequent sleep. Kramarenko *et al*., (2003) reported abnormal EEG slow waves in awake subjects exposed to cell phone

radiation. Marino *et al*., (2003) reported an increased randomness of EEG in rabbits. Croft *et al*., (2002) reported that radiation from cellular phone altered resting EEG and induced changes differentially at different spectral frequencies as a function of exposure duration. D'Costa *et al*., (2003) found EEG effect affected by the radiation within the alpha and beta bands of EEG spectrum. Huber *et al*., (2003) reported EEG effect during NREM sleep and the effect was not dependent on the side of the head irradiated. They concluded that the effect involves subcortical areas of the brain that project to both sides of the brain. Dosimetry study shows that the SAR in those area during cell phone use is relatively very low, e.g., 0.1 W/kg at the thalamus. Recently, Aalto *et al*., (2006), using PET scan imaging, reported a local decrease in regional cerebral blood flow under the antenna in the inferior temporal cortex, but an increase was found in the prefrontal cortex.

2.10.5 Behavioural Effects

Changes in locomotor behaviour occurred after continuous-wave exposures at an SAR as low as 1.2 W/kg (D,Andrea *et al*., 1979). Reductions in conditioned behaviour were reported during exposure at an SAR of 2.5 W/kg and such behaviour ceased at an SAR of 10 W/kg (D,Andrea *et al*., 1976). Behavioural alterations were reported to be reversible with time after termination of exposure (Gage, 1984). Bornhausen and Scheingrahen (2000) found no significant change in operant behaviour in rats prenatally exposed to a 900-MHz RF. Sienkiewicz *et al*., (2000) reported no significant effect on performance in an 8-arm radial maze in mice exposed to a 900-MHz RF pulsed at 217 Hz at a whole body SAR of 0.05 W/Kg. Dubreuil *et al*. (2002, 2003) found no significant change in radial maze performance and open-field behavior in rats exposed head only for 45 min to a 217-Hz modulated 900-MHz field at SARs of 1 and 3.5 W/kg. Yamaguichi *et al*., (2003) reported a change in T-maze performance in the rat only after exposure to a high whole body SAR of 25 W/kg.

Lovely and Guy (1975) reported that rats that were exposed to continuous-wave 918-MHz RF for 10 min at > 25 mW/cm^2 (SAR ~22.5 W/kg) and then allowed to drink saccharin solution, showed a significant reduction in saccharin consumption when tested 24 h later. No significant effect was found in rats exposed to RF at 5 or 20 mW/cm^2. Rudnev *et al*., (1978) studied the behavior of rats exposed to 2375-MHz RF at 0.5 mW/cm^2 (SAR 0.1 W/kg), 7 h/day for 1 month. They reported decreases in food intake,

balancing time in a treadmill and inclined rod, and motor activity in an open-field after 20 days of exposure. Interestingly, the open-field activity was found to be increased even at 3 months post-exposure. In a long-term exposure study (Johnson *et al*., 1983), rats were exposed to pulse 2450-MHz RF (10 μs pulses, 800 pps) from 8 weeks to 25 months of age (22 h/day). The average whole body SAR varied as the weight of the rats increased and was between 0.4-0.15 W/kg. Open field activity was measured in 3-min sessions with an electronic open-field apparatus once every 6 weeks during the first 15 months and at 12 week intervals in the final 10 weeks of exposure. They reported a significantly lower open field activity only at the first test session and a rise in the blood corticosterone level was also observed at that time. The authors speculated that RFR might be minimally stressful to the rats.

2.10.6 Molecular, Subcellular and Cellular Effects

For most molecular or subcellular systems exposed in vitro, no consistent biological effects have been demonstrated that can be attributed to RF specific interactions, exceptions include chain-length-dependent microwave absorption by DNA (Swicord *et al*., 1983), conformational transitions in a model protein and sodium and potassium ion transport across red blood cell membranes. No consistent effects have been demonstrated on the growth and colony-forming ability of single cells such as bacteria that can be attributed to RF-specific interactions (Allis, 1983). However, there are reports of frequency-specific alterations in growth rates of yeast cells exposed at 41-42 GHz (Grundler and Keilmann, 1983).

2.11 Genetic Toxicology

Possible effects on DNA or chromosome structure in somatic cells are considered to be very important as these changes could be associated with cell death or, possibly, with the development of cancer. Furthermore, such effects on male or female germ cells are important, as surviving mutations might be passed on to the next generation. A large number of studies have been carried out to investigate the effects of electromagnetic field (EMF) exposure on DNA and chromosomal structures. The single-cell gel electrophoresis (comet assay) has been widely used to determine DNA damages: single and double strand breaks and cross-links. Studies have also been carried out to investigate chromosomal conformation and micronucleus formation in cells after

exposure to RF. Genetic effects in somatic as well as in germ cells, therefore, have already been conducted in many different cell and animal systems some with significant effects while some recorded no significant effects.

Lagroye *et al*., (2004a) reported no significant change in DNA strand breaks in brain cells of rats exposed for 2 h to 2450-MHz field at 1.2 W/kg. Lagroye *et al*., (2004b) found no significant increases in DNA-DNA and DNA-protein cross-link in C3H10T(1/2) cells after a 2-h exposure to CW 2450 MHz field at 1.9 W/kg. Li *et al*., (2001) reported no significant change in DNA strand breaks in murine C3H10T(1/2) fibroblasts after 2 hs of exposure to 847.74 and 835.02 MHz fields at 3-5 W/kg. Stronati *et al*., (2006) showed that 24 h of exposure to 935-MHz GSM basic signal at 1or 2 W/Kg did not cause DNA strand breaks in human blood cells. Tice *et al*., (2002) measured DNA single strand breaks in human leukocytes using the comet assay after exposure to various forms of cell phone signals. Cells were exposed at 37±1°C, for 3 or 24 h at average specific absorption rates (SARs) of 1.0-10.0 W/kg. Exposure for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes. Vershaeve *et al*., (2006) long-term exposure (2 hs/day, 5 days/week for 2 years) of rats to 900 MHz GSM signal at 0.3 and 0.9 W/kg did not significantly affect levels of DNA strand breaks in cells. Vijayalaximi *et al*., (2000) reported no significant increase in single strand breaks in human lymphocytes after 2 hs of exposure to 2450-MHz field at 2 W/kg. Zeni *et al*., (2005) reported that a 2-h exposure to 900-MHz at 0.3 and 1 W/kg did not significantly affect levels of DNA strand breaks in human leukocytes. Vijayalaximi *et al*., (2001b) reported that there is no evidence for the induction of chromosomal aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 835.62 MHz RF radiation at SARs of 4.4 or 5.0 W/kg. Vijayalaximi *et al*., (2001c) reported no evidence for induction of chromosome aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 847.74 MHz RF radiation (CDMA) at SARs of 4.9 or 5.5 W/kg.

Aitken *et al*., (2005) exposed mice to 900 MHz RF at a specific absorption rate (SAR) of 0.09 W/kg for 7 days at 12 h per day. DNA damage in caudal epididymal spermatozoa was assessed by quantitative PCR (QPCR) as well as alkaline and pulsed-field gel electrophoresis post-exposure. Gel electrophoresis revealed no significant change in single or double DNA strand breakage in spermatozoa. However, QPCR revealed

statistically significant damage to both the mitochondrial genome ($p < 0.05$) and the nuclear-globin locus ($p < 0.01$). Diem *et al*., (2005) exposed human fibroblasts and rat granulosa cells to 1800 MHz; SAR 1.2 or 2 W/kg; different modulations; during 4, 16 and 24 h; intermittent 5 min on/10min off or continuous). RF exposure induced DNA single and double-strand breaks as measured by the comet assay. Effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations.

Belyaev *et al*. (1996) studied the effect of millimeter waves on the genome conformational state of E. coli AB1157 by the method of anomalous viscosity time dependencies in the frequency range of 51.64-51.85 GHz. Results indicate an electron-conformational interactions. Belyaev *et al*. (2005) investigated response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to microwaves from GSM mobile phone (915 MHz, specific absorption rate 37 mW/kg), and power frequency magnetic field (50 Hz, 15 microT peak value). Changes in chomatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chomatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 ^{o}C. No significant differences in responses between normal and hypersensitive subjects were detected. Garaj-Vrhovac *et al*. (1991) exposed V79 Chinese hamster fibroblast cells to continuous wave 7.7 GHz radiation at power density of 0.5 mW/cm^2 for 15, 30 and 60 min. There was a significantly higher frequency of specific chromosome aberrations such as dicentric and ring chromosomes in irradiated cells.

Gandhi and Anita (2005) reported increases in DNA strand breaks and micronucleation in lymphocytes obtained from cell phone users. Garaj-Vrhovac *et al*., (1990) reported changes in DNA synthesis and structure in Chinese hamster cells after various durations of exposure to 7.7 GHz field at 30 mW/cm^2. Lai and Singh (1995; 1996; 1997a; 2005) and Lai *et al*., (1997) reported increases in single and double strand DNA breaks in brain cells of rats exposed for 2 h to 2450 MHz field at 0.6-1.2 W/kg. Lixia *et al*., (2006) reported an increase in DNA damage in human lens epithelial cells at 0 and 30 min after 2 h of exposure to 1.8 GHz field at 3 W/kg.

D'Ambrosio *et al*. (1995) reported some evidence of genetic effects and ascribed this to hyperthermia, as exposure conditions were clearly or most probably thermal in nature. He found an increased incidence of micronuclei in microwave-exposed human lymphocytes. However, the exposure was accompanied by a thermal increase of 5 ^{o}C. Reviews of assays used to detect DNA alterations have shown that such sporadic positive responses are indeed obtained from time to time (Brusick, 1995).

2.12 Epidemiological Investigations

Some epidemiological investigations were conducted in the past with regard to other radiofrequency fields and applications. These studies were, among others, related to occupational exposures to radar, populations living near military installations and near broadcasting towers, amateur radio operators and users of hand-held traffic radar devices (Bergqvist, 1997; Rothman *et al*., 1996; Verschaeve, 1995). Most studies were rather small and were criticized, e.g. on grounds of wrong or insufficient data collection, the absence of any or adequate dosimetry, and the failure to investigate potential confounders. In a study on RF-exposed US Embassy personnel in Moscow, no deleterious effects were found, but the limited numbers of cancer cases make this study rather non-informative in comparison with other studies (Lilienfield *et al*., 1978). Milham (1988) found an increased standard mortality ratio (SMR) for certain cancer sites in radio amateurs, but there was no adjustment for confounders and most subjects were also professionally exposed to (other) electric and magnetic fields. Armstrong *et al*. (1994) investigated electric utility workers in France and Quebec (Canada) who were exposed to different non-ionizing radiation frequencies, including radiofrequencies. They found a relationship with lung cancer in one of the utilities (Quebec), whereas a less independent indicative relationship was found stomach cancer in another utility (France). These variations from one utility to another detract somewhat from the credibility of these findings regarding the connection between electromagnetic fields and cancer.

Szmigielski (1997) examined the cancer morbidity in Polish career military personnel. He found an excess occurrence of cancers at several cancer sites but no individual assessment was made of exposure levels or duration and, apart from age, adjustments for other factors, e.g., possible carcinogen exposures, were not made. These and other epidemiological investigations concerned many different modes of exposure and

different frequencies. For several studies, the link between the subject's occupation and actual exposure was questionable and, very often, exposure was also to extreme low-frequency fields and different chemicals. Therefore, taking the limited epidemiological evidence together, it must be concluded that no definite conclusion can be drawn so far, either about the mobile telephone or about other radiofrequency fields. A number of investigations are now being conducted to find out whether MW can cause cancer (Verschaeve and Maes, 1997) and other disorders.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Calibration of Microwave (MW) Source

A microwave generator, model ER660E, Serial No MX704CCR from Toshiba UK Ltd available in the Department of Radiation Biology and Radiotherapy, College of Medicine, University of Lagos was used for irradiation. The detector of MW used was the non-interacting thermistor RS 141, which has a resistance of 4.7 kΩ at 25^{o}C. The thermometer was calibrated in a 12 cm x 6 cm x 4 cm size water phantom with the aid of a digital multimeter as readout and mercury-in-glass thermometer as reference (Aweda *et al*. 2000). The thermistor response values as indicated by the digital multimeter were recorded against the corresponding readings from the thermometer in ^{o}C.

3.2 Determination of Specific Absorption Rate (SAR)

Measurement of the SAR in a biological system exposed to MW radiation is very important so that any biological changes can be correlated with energy deposited by the radiation and extrapolation of results from different species of animals to humans can be accomplished. This was done by inserting the thermistor probe into the animals' rectum during exposure (Guy *et al*., 1987). The irradiation chamber surfaces were lagged with 0.5 litre water bags (a product of Medilag Consult, Lagos) in order to minimize the reflective properties, which may increase the heating rate (Bren, 1996). The generator was operated at room temperature 25 ± 2^{o}C and 56 ± 4% relative humidity. The exposure conditions were whole body irradiation with the animals at 12 cm away from the antenna of the MW source of dimension 12 cm x 5 cm. The SARs were obtained using equation 2.29 with the value of C taken as 3334 Jkg^{-1}K^{-1} given by Durney *et al*., (1980); Mc Ree and Davies, (1987); Aweda *et al*., (2003).

3.3 Animal and Sample Preparations

The rats and their care were conducted in conformity with international, national and institutional guidelines for care and use of laboratory animals in Biomedical Research as promulgated by the Canadian Council of animal care (Canadian Council, 1984; NIH,

1985). Two hundred (200) wistar rats of both sexes, 6-8 weeks old weighing 90 -130 g were purchased from the Animal House, College of Medicine, University of Lagos, as specimens. The rats were fed with standard rat chow (Livestock feeds, Ikeja, Lagos, Nigeria) and had free access to drinking water. The animals were maintained at standard laboratory conditions (12 / 12 h dark / light cycle). These specimens were grouped in fives. Five groups (male) and five groups female served as control at various times. All the other groups except the control groups were exposed to MW radiation and irradiated at different times to obtain various SARs.

3.4 Behavioural Studies

After exposure of the rats to MW radiation of five different SARs values (0, 0.48, 0.95, 1.43, 1.91 and 2.39 W/kg), the exploratory and anxiolytic activities were studied.

3.4.1 Exploratory Behavioural Activity Study

This study was carried out by using a white painted hole- wooden board (40 cm x 40 cm) The board was constructed with four equidistant holes according to specification (1 cm diameter x 2 cm depth as shown in Figure 3.1). Each rat was placed at one corner of the board, able to move about, and dipping its head into the hole, indicating exploratory behaviour (Yemitan and Adeyemi, 2003). The number of dips in 7.5 minutes was recorded (Files and Wardrill, 1975). This was carried out 1 h, repeated for days and weeks after exposure.

3.4.2 Anxiolytic Activity Study

Anxiolytic study was carried out using elevated plus maze and Y-maze as describe below

3.4.2.1 Elevated Plus Maze Study

The elevated plus maze (EPM; 120 cm x 12 cm x 12 cm each arm, as shown in Figure 3.2) is made of wood consisting of two open and two closed arms across, at 60 cm above the ground level. Each rat was placed in the central position facing an open arm and the cumulative time spent in open and closed arm within a period of 5 min was recorded. This was carried out 1 h, repeated for days and weeks after exposure.

3.4.2.2 Y- maze Study

The Y – maze (YM) is a Y – shaped wood, consisting of wooden runway designed with one of the two arms closed (YM; 120 cm x 12 cm x 12 cm Figure 3.3). This was placed at 60 cm above the ground level. Each rat was placed at the centre of the Y – shaped wooden runway.

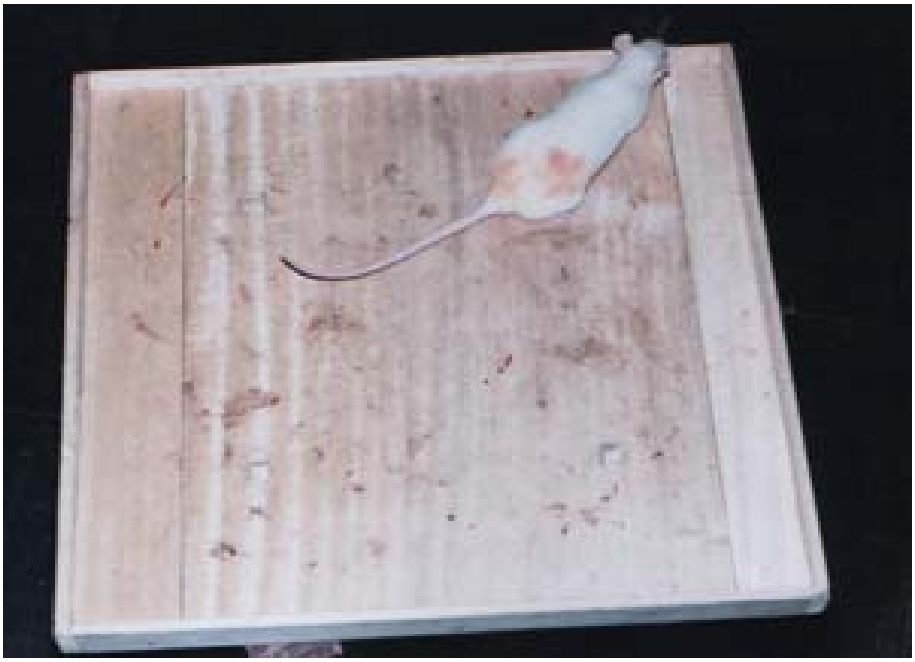


Figure 3.1: White painted hole-board used for exploratory study

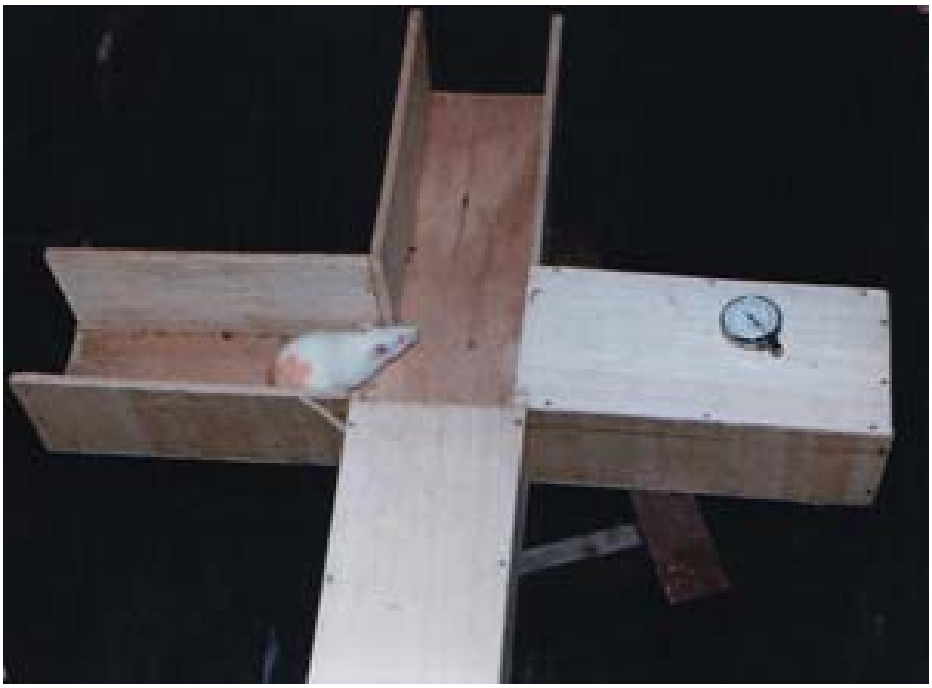


Figure 3.2: Elevated Plus Maze

Figure 3.3: Elevated Y Maze

3.5 Fertility Studies

After exposure of the rats to MW radiation of five different SARs (0, 0.48, 0.95, 1.43, 1.91, 2.39 W/kg), the initial and final weights were measured, so also are the organ weights. The sperm motility, sperm morphology and sperm counting were determined as described in the next sections.

3.5.1 Weight of the Specimens

The average body weight of the rats at 1 week interval for four weeks were taken, with Mettler weighing balance (Mettler – Toledo Type BD 6000, Greifensee, Switzerland) , and percentage weight gain from initial weight per group were calculated and recorded. After sacrificing the rats their average organ weights were taken using electronic organ weighing balance (Citizen Model MP 300, USA).

3.5.2 Semen Collection

The abdomen of each animal was cut opened and the left testes were removed along with the epididymis. The epididymis was carefully separated from the testes and the caput sever from the remaining part of the epididymis. The caput was quickly transferred on to a pre-warmed slide 27 ^{o}C and lacerated with a razor to release some semen sample onto the slide surface.

3.5.3 Sperm Motility Study

Some drops of warm normal saline 27 ^{o}C were added to the semen sample on the slide to potentiate full motility of the spermatozoa (Tumer and Giles, 1982). The average gross motility was scored under the microscope using the 40 x objectives (Oyeyemi *et al*., 2000).

3.5.4 Sperm Morphology Study

Morphology study was done using two drops of eosin – nigrosin stain. This was added to the semen sample collected on the slide. The sample was then drawn into a film by a coverslip held at an angle 45^{o} to dry. After drying, the film was mounted under a coverslip using Canada or Depex mounting fluid and examined under the microscope

with a 40 x Objective. Different type of abnormalities found in the sperm cells were counted using the method of Oyeyemi *et al*., (2000). These were grouped under primary and secondary abnormalities. Abnormal sperm cells were counted in 5 fields and average calculated for each group.

3.5.5 Sperm Count

The caput from the epididymis was immersed in 5 ml normal saline in a measuring cylinder and its volume measured. It was matched into suspension, from which the count was done. Sperm count was done under microscope using improved Neubauer hemocytometer. Count in 5 large Thomas square was taken and adjustment was made for the volume of the normal saline added. The count was therefore calculated as;

Count / ml = No. of sperm cell in 5 large Thomas square x 32 000 x dilution

3.5.6 Histopathological Studies on the Reproductive Organs

The abdomen of each animal was carefully opened up and the following organs were removed. For male: testes, epididymis, seminal vesicle, prostate gland, liver, kidney and heart; for female: ovaries along with the fallopian tube, liver, kidney and heart. The sectioning of the tissue was done in the Department of Morbid Anatomy, College of Medicine, University of Lagos. The tissue samples were fixed in 10 % neutral buffered formalin; these were dehydrated with ethanol, and embedded in paraffin. Tissue sections of 5 to 7 μm thickness were cut and stained with hematoxylin and eosin (H & E) for microscopic examination.

3.6 Chromosomal Aberration Study from Rats Bone Marrow

After exposure of the specimens to MW radiation of five various SARs (0, 0.95, 1.43, 1.91 and 2.39 W/ kg) and left for 2 days, the chromosomal aberration study was carried out. Each rat was injected with colchicines 0.6 mg/kg (anti-mitotics inhibitor) 2 h prior to sacrifice. This was done in order to arrest the chromosome at the metaphase. At 48 h post irradiation, the specimens were sacrificed by cervical dislocation and bone marrow cells were prepared from the femoral bone marrow by the conventional method (Brusick, 1980). Briefly, bone marrow cells were flushed from the femurs in 2.2 % Sodium Citrate, and the cells were centrifuged at 1500 rpm for 10 minutes. Pelleted

bone marrow cells were then resuspended in 5 ml of a hypotonic solution of 0.075 M KCl for 20 min at 37 ^{o}C. The cells were centrifuged again and fixed with thee changes of 5 ml each of ice – cold Carnoy's fixative (Methanol – Acetic acid, 3:1 v/v) for 30 min at 25 ^{o}C. The cells were then dropped onto clean, grease free microscope slides which were air – dried and stained with 5 % Giemsa for 15 minutes (Alimba *et al*., 2006). All slides were blindly evaluated in oil-immersed x 100 objectives for structural chromosomal aberrations. Fifty well spread complete metaphases were scored per slide and 6 slides were prepared per specimen at each SAR.

3.7 DNA Extraction and Washing of the Blood Cells (Suounou *et al*., 1993)

The rats were exposed to MW radiation of 10 different SARs (0, 0.48, 0.95, 1.43, 1.91, 2.39, 2.9, 3.4, 3.8 and 4.3 W/kg). Immediately after exposure each animal was anesthetized by placing in a glass jar containing cotton dipped in anesthetic ether; different organs were extracted and isolation of DNA was done.

This involved three steps: lysis of red blood cells, lysis of organs (liver, lung, spleen, brain, kidney, testes, ovary, prostate, and thyroid), phenol extraction and precipitation of DNA.

2.5 µL of 0.1 % saponin was added to 0.5 ml of whole blood. The mixture was vortexed using autovortex mixer SA2 (Stuart Scientific, UK) and left to stand for 2 min at room temperature to allow for lysis. The contents were centrifuged at 13000 rpm for 5 min, this was repeated for 2 to 3 times until the samples were fully cleaned.

3.7.2 Lysing of WBC and other Organs

The organs were teased and the pellets suspended in 25 µL in lysis buffer (40 mM Tris PH 8.0, 80 mM EDTA PH 8.0, 2 % SDS, Proteinase K 5 mg/ml). Polymerase Chain Reaction (PCR) clean water was added to a final volume of 100 µL and incubated overnight in a water bath (Uniscope SM801A by Surgifriend Medicals) at 37 ^{o}C.

3.7.3 Phenol Extraction and Precipitation of DNA

300 µL of distilled water was added to the extracted sample and mixed by vortexing. An equal volume of phenol – chloroform isoamyl – alcohol (25:24:1) was added, vortexed and then centrifuged at 13000 rpm for 10 min, after which the lower layer was removed leaving the interphase and the aqueous layer. This was repeated, then an equal volume of chloroform was added, vortexed and the lower layer removed, this step was also repeated for three more times. The aqueous layer was transferred to another tube, 45 µL of 3.0 M sodium acetate PH 5.0 was added and twice the volume of cold absolute ethanol was added. The contents of the tube were mixed by rapid inversion of the tube. DNA may become visible at this stage as fine strands or it may not. The tubes were left overnight at -20 ^{o}C, the DNA was recovered by centrifuging at 13000 rpm at room temperature. 1 ml of 70 % ethanol was used to wash the DNA. The DNA was recovered by centrifuging at 13000 rpm and the supernatant was carefully removed by gently inverting the tube and left to dry. The DNA was reconstituted in 20 µL TE solution.

3.8 Quantification of DNA Sample

6 µL of sample was added to 114 µL de-ionized water (ddH_2O) to give 20 dilution of the solution, this was mixed properly. 50 µL of the mixture was added to cap of Eppendorf Biophotometer (AG 2331 Harmburg, Germany) and the mean digital outputs were presented in the Appendix.

3.9 Primers

Each reaction was carried out with a combination of one of the selective primers spanning the 16-kb rat genome forward, 5′-GTTTTCCCAGTCACGACGC-3′, and reverse, 5′-TTTCACACAGGAAACAGCTATGAC-3′ (Takara Biotechnology Co., Dalian).

3.10 Amplification and Electrophoresis

The polymerase chain reactions (PCR) were carried out in a final volume of 15 µl starting from 1 µl DNA sample, 1 µl of each primer 7.5 µl of Premix Ex-taq (Takara biotechnology co, Dalian) using biometra thermocycler (12014, Germany). The cycling

parameters were 94 ^{o}C, 10 min; 94 ^{o}C, 1.5 min; 50 ^{o}C 1min; 72 ^{o}C, 2 min; 29 more cycles to step 2 and 72 ^{o}C, 10 min; 4 ^{o}C pause. 5 µl formamide loading dye was added to the PCR products, this is essential to achieve sufficient resolution between very close bands in the multi-locus pattern.

Electrophoresis was performed on 10 % gels, prepared with 6 ml acrylamide/ bisacrylamide (29:1); 2 ml 10 x TBE (PH 8.0); 11.7 ml ddH2O; 200 µl APs and 10 µl TEMED in an Hoefer VE (Vertical Electrophoresis System) connected to Electrophoresis power supply (Amersham pharmacia biotech, sweeden) and run at 120 V for 2 h. The staining started by transferring the gel into $AgNO_3$ solution in the dark and shake for 7 min, washed twice in distil water for 1 min. Stained using staining solution (3.5 g NaOH, 0.0475g $Na_2B_4O_7$.10H2O and 1 ml formaldehyde in 250 ml distil water) until the bands are visible; then washed thrice in distil water for 1 min each. The gel photographs were taken using both digital camera and mutilmage light cabinet connected to computer system using Chemilmager software (Alpha Innotech Corporation, USA). Densitometric analysis of the tracks was carried out on Image J gel analyzer software developed by National Institute of Health, USA (NIH).

3.11 Single Cell Gel Electrophoresis (Comet Assay)

Comet assay also referred to as single cell gel electrophoresis (SCGE), was used to determine DNA damage in terms of single strand break (SSB) in brain, liver, lung, spleen and testis of the animals after exposure to 2.39 W/kg MW radiation. This assay has been used extensively in toxicological studies for DNA damages (Fairbairn *et al*. 1995, McKelvey-Martin *et al*. 1993) and found to be sensitive especially for assessing SSB DNA damage. Immediately after exposure each animal was anesthetized by placing it in a glass jar containing cotton dipped in anesthetic ether and tissues were dissected out immediately for DNA strand break assay. Whole tissue was washed four times with phosphate buffered saline (PBS) (1.37 mM NaCl, 4.3 mM Na_2HPO_4, 2.7 mM KCl, 1.4 mM KH_2PO_4, pH 7.4) to remove red blood cells (RBC).The cells were minced into small pieces by adding 1 ml PBS in a medimachine (Becton Dickinson, Italy) and a single cell suspension was collected using a 200 µl pipette.

The comet assay was performed as described by Singh, with minor modifications (Singh *et al*., 1995). Normal melting point agarose (Ameresco, NMA) and low melting point

agarose (Ameresco, LMA) were suspended in PBS at 37 ^{o}C. Then, 100 µl of 1 % NMA was added to comet slides, and the slides were allowed to solidify. From the cell suspension, 22.5 µl of its suspension was mixed with 67.5 µl, 1 % agarose (3:1) added on the solidified gel and cover with cover slip for 5 min in the refrigerator. After removing the cover slips, the slides were submersed in the lysing solution (2.5 M NaCl, 100 mM EDTA-Na_2, 10 mM Tris–HCl, pH 10; 1 % Triton X-100 and 10 % DMSO, pH 10) for 2 h in the dark. The slides were then placed in unwinding buffer (1 mM EDTA and 300 mM NaOH, pH 13) for 25 min. In the same buffer electrophoresis was carried out at 4 ^{o}C for 25 min at 25 V using Amersham Pharmacia Biotech power supply and adjustment of current to 302 mA by modulating the buffer level. After electrophoresis, the slides were neutralized via three washings with neutralization buffer (400 mM Tris–HCl, pH 7.4) 5 min each. Slides were immersed in 70% ethanol for 10 min to precipitate the DNA and dehydrate the gels. Slides were left in vertical position and stained with 50 µl of 10 µg/ml ethidium bromide (EB). The slides were examined using a Roper Scientific (RS) image analysis system (Alpha Innotech Corporation, USA) fitted with an Olympus BX51 fluorescence microscope equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm. For each cell, two slides were prepared and each 50 randomly chosen cells (total 100 cells) were scored. DNA damage was evaluated by calculating the olive moment, % DNA in head and tail using comet score software 1.5 (TriTek Cor., Virginia). In order to compare each sample of the tissue with its control, two-tailed paired t-test was used and P values lower than 0.05 was regarded as statistically significant.

Figure 3.5: Electrophoresis Set up

Figure 3.6: Gel Staining Set up

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Calibration and Determination of Specific Absorption Rate (SAR)

The variation of thermistor resistance with temperature is presented in Figure 4.1. This was obtained to determine the corresponding values of increase in temperature with the thermistor. Figure 4.1 is the calibration curve whose slope gives the calibration factor 0.16 $k\Omega^{o}C^{-1}$. Figure 4.2 presents the mean variation of rats' rectal temperature with time. The plot represent the measured values in order to obtain the corresponding thermistor value from the thermometer which is to be inserted during exposure of the rats, from the calibration it was deduced that the change in temperature throughout the experiment is 1.44 ± 0.11 ^{o}C. The variation of SAR with mass is presented in Figure 4.3 as the plot of the measured values.

4.2 Effects of Microwave Radiation on the Behaviour of Rats

4.2.1 Effects of Microwave on the Exploratory Behaviour

Figures 4.4 and 4.5 represent the measured number of head dips as a function of time over 3 weeks for male and female rats respectively, after being exposed to different SARs of MW. 1 h after exposure to SAR of 0.48 W kg^{-1}, the number of head dips reduced from a mean value of 15.6 ± 4.88 to 8.5 ± 0.58 in males and then decreased till the third day after which it increased again gradually to attain the control value after about 3 weeks. One hour after exposure to SAR 0.48 W kg^{-1}, the number of head dips reduced from a mean value of 14.8 ± 1.51 to 8.3 ± 0.44 in females, and decreased till the fourth day after which it increased again gradually to attain the control value after about 3 weeks. The variation in the measured value is from a minimum of 1.1 in females exposed to 2.39 W kg^{-1}, 6 days post exposure with maximum of 15.4 in males exposed to 0.48 W/kg, 3 week post exposure, while in the control groups throughout the study period, it ranged between 14.5 ± 0.44 and 16.2 ± 0.48 for both males and females. Similar trend was observed in all other exposed groups up to SAR value of 2.39 Wkg^{-1}.

The results obtained correlate with the effects reported by Yamaguichi *et al*., (2003). In which, MW altered the exploratory behaviour in the exposed male and female rats compared with control. There is no significant difference between the male and female in the time spent in closed and open arms, indicating that the effects are not sex-dependent. For a given SAR value, the number of head dips increased with time to approach the value for the control at the end of the third week. The same trend of variation was observed in both male and female changes in the exploratory behaviour in terms of SAR and time variation over a 3-week period after exposure, there is high correlation between the number of dips in both sexes (C = 0.94). Some other reported consequences of MW exposures include changes in locomotive behaviour due to SAR as low as 1.2 Wkg^{-1} (D,Andrea *et al*., 1979). Reductions in conditioned behaviour due to SAR of 2.5 Wkg^{-1} and such behaviour ceased at SAR of 10 Wkg^{-1} (D, Andrea *et al*., 1976). Behavioural alterations were reported to be reversible with time (Gage, 1984).

4.2.2 Effects of Microwave Radiation on the Elevated Plus Maze and Y-Maze Study

Figures 4.6 and 4.7 show the variation in the percentage of duration of cumulative time spent in the open arms of the EPM with the different applied MW SARs for males and females respectively. The overall variation is from a minimum of 3.92 % (Tables 4.1 and 4.2) with SAR of 2.39 W kg^{-1} in males 1 h post exposure, to 75.11 % with same SAR in females after a period of 15 days. The control values ranged from 74.93 % to 75.77 %. 1 h after exposure to SAR of 0.48 W/kg the percentage of time reduced from a mean of 75.30 % to 26.61 % in males, and it increased gradually with time to attain the control value after about 15 days. One hour after exposure to the same SAR, the percentage of time reduced from a mean value of 79.13 % to 28.45 % in females, and it increased gradually with time to attain the control value after 15 days. Similar trend was observed in all other exposed groups up to SAR value of 2.39 Wkg^{-1}. The shortest percentage time was found in males is 3.92 % with SAR of 2.39 W kg^{-1} 1 h after exposure, while the longest was 75.11 % with SAR 0.48 W kg^{-1} at 15 days post exposure. By implication, the longest cumulative time spent in the closed arms of the

EPM is obtained in the group exposed to 2.39 Wkg^{-1} 1 h after exposure, while the shortest time was found in the group with SAR of 0.48 Wkg^{-1} 15 days after exposure.

Figs. 4.8 and 4.9 show the variation in the percentage of duration of cumulative time spent in the open arms of the YM with the different applied MW SARs for males and females respectively. The overall variation is from a minimum of 3.49 % with SAR of 2.39 W kg^{-1} found among the males (Tables 4.3 and 4.4) 1 h post exposure, to 73.94 % with same SAR also in males after 15 days. The control values ranged from 74.22 % to 78.88 %. One hour after exposure to SAR of 0.48 W/kg, the percentage of time reduced from a mean of 74.22 % to 25.85 % in males, and it increased gradually with time to attain the 73.94 % after 15 days. 1 h after exposure to the same SAR, the percentage of time reduced from a mean value of 78.55 % to 26.75 % in females, and it increased gradually with time to attain 73.60 % after 15 days. Similar trend was observed in all other exposed groups up to SAR value of 2.39 Wkg^{-1}. The shortest percentage time was found in males for the YM study is 3.49 % with SAR of 2.39 Wkg^{-1} 1 h after exposure, while the longest was 73.94 %, also among males with SAR 0.48 Wkg^{-1} at 15 days post exposure. By implication, the longest cumulative time spent in the closed arms of the YM is obtained in the group exposed to 2.39 Wkg^{-1} 1 h after exposure, while the shortest time was found in the group with SAR of 0.48 Wkg^{-1} 15 days after exposure.

MW produced a significant SAR- and time-dependent cumulative time spent in the closed arms of EPM and elevated Y-maze compared with the control, which indicate that MW causes fear and anxiety to open and elevated areas, which suggests that exposure to this frequency can induce fear and anxiety in those exposed to it. Bornhausen and Scheingrahen (2000) did not record any significant change in operant behaviour in rats prenatally exposed to a 900-MHz. Sienkiewicz *et al*., (2000) reported no significant effect on performance in an 8-arm radial maze in mice exposed to a 900-MHz MW pulsed at 217 Hz at a whole body SAR of 0.05 Wkg^{-1}. Dubreuil *et al*. (2002, 2003) found no significant change in radial maze performance and open-field behaviour in rats with heads exposed only for 45 min to a 217-Hz modulated 900 MHz MW at SARs of 1 and 3.5 Wkg^{-1}. Yamaguichi *et al*., (2003) reported a change in T-maze performance in the rats only after exposure to a high whole body MW radiation. Rudnev *et al*., (1978) studied the behaviour of rats exposed to 2375-MHz RF at 0.5 mW/cm^2 (SAR 0.1 Wkg^{-1}), 7 h/day for 1 month. They reported decreases in food intake,

balancing time in a treadmill and inclined rod and motor activity in an open-field after 20 days of exposure. Interestingly, the open-field activity was found to increase even at 3 months post-exposure. In a long-term exposure study (Johnson *et al*., 1983), rats were exposed to pulse 2.450 GHz MW (10 μs pulses, 800 pps) from 8 weeks to 25 months old (22 h/day). The average whole body SAR varied as the weight of the rats increased and was between 0.4-0.15 W/kg. Open field activity was measured in 3-min sessions with an electronic open-field apparatus once every 6 weeks during the first 15 months and at 12 week intervals in the final 10 weeks of exposure. They reported a significantly lower open field activity only at the first test session and a rise in the blood corticosterone level was also observed.

Absorption of MW energy may cause an increase in tissue temperature. The initial rate of temperature increase was proportional to the SAR, according to Schwan and Foster, (1980). Separato *et al*. (1984) showed that biological effects, such as the heat killing of cells, depend on the temperature profile in time. A well-established athermal mechanism of interaction at frequencies below a few tens of MHz is through electrical stimulation of excitable membranes of nerve and muscle cells (Bernhardt, 1979; Bernhardt, 1983). RF fields can induce current sufficient to stimulate excitable tissue for frequencies below 1 MHz (Bernhardt, 1979).

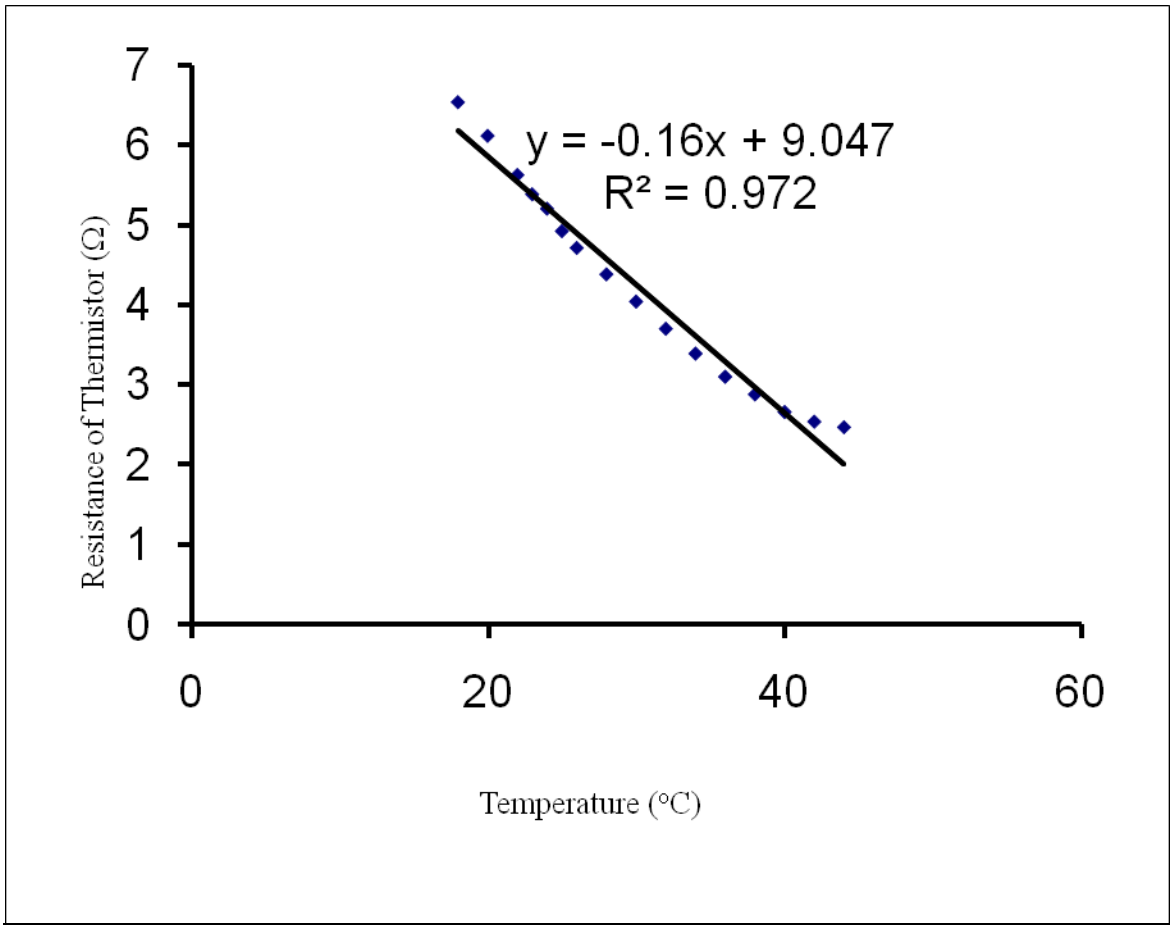


Fig. 4.1: Variation of Thermistor Resistance with Temperature (Calibration Curve)

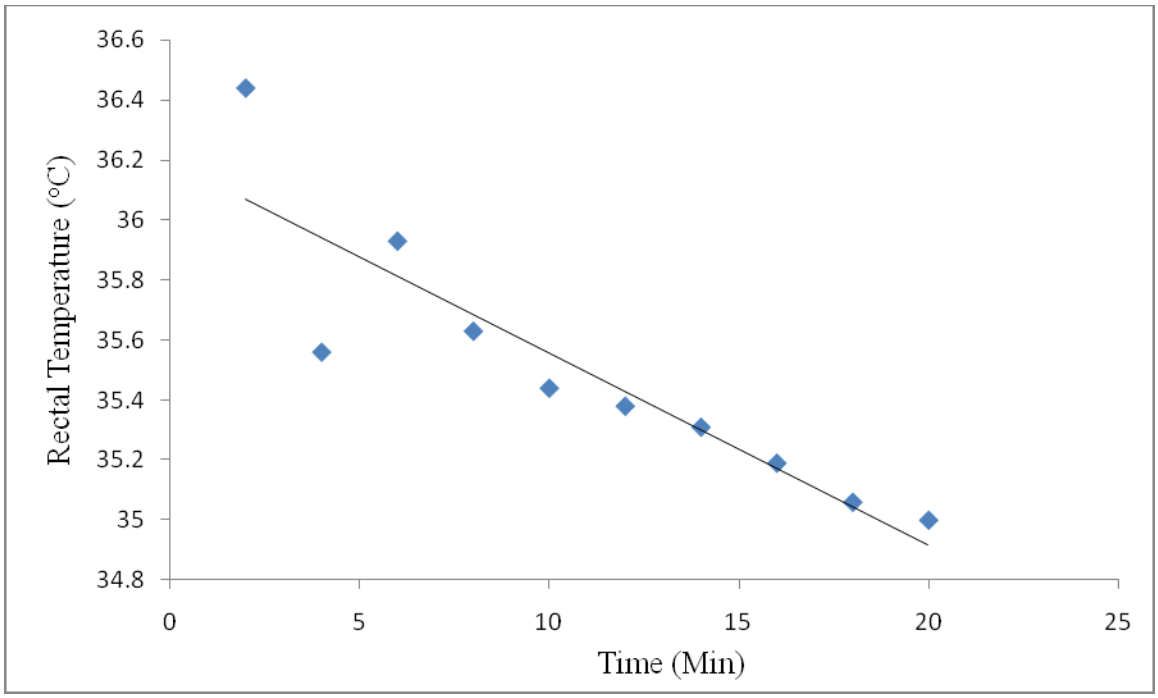


Fig. 4.2: Variation of Rectal Temperature with Time

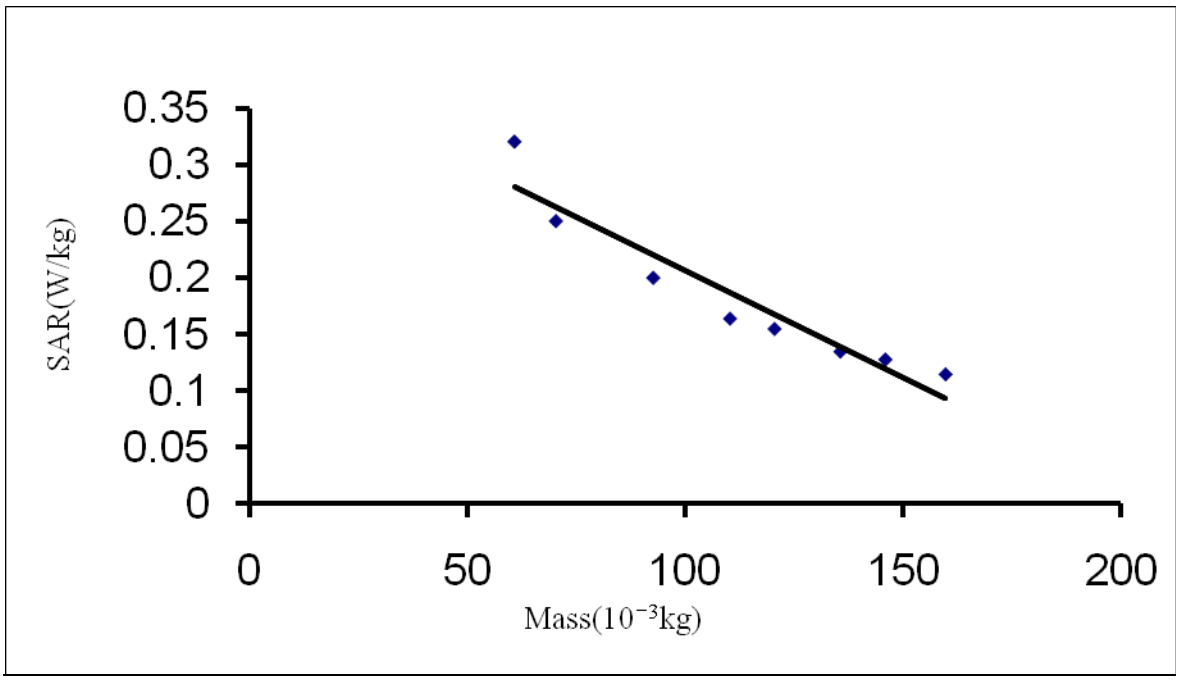


Fig. 4.3: Variation of SAR with Mass

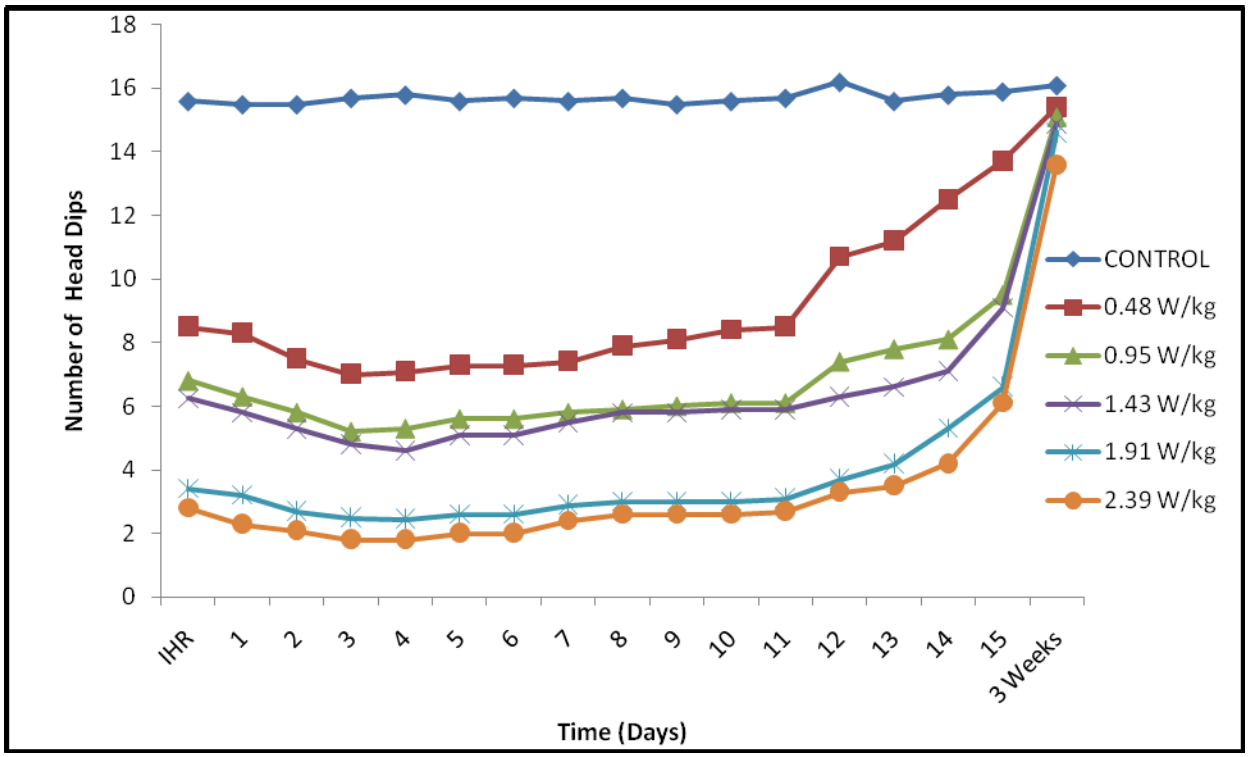


Fig.4.4: Microwave Effects on the Exploratory Behaviour in Male Rats

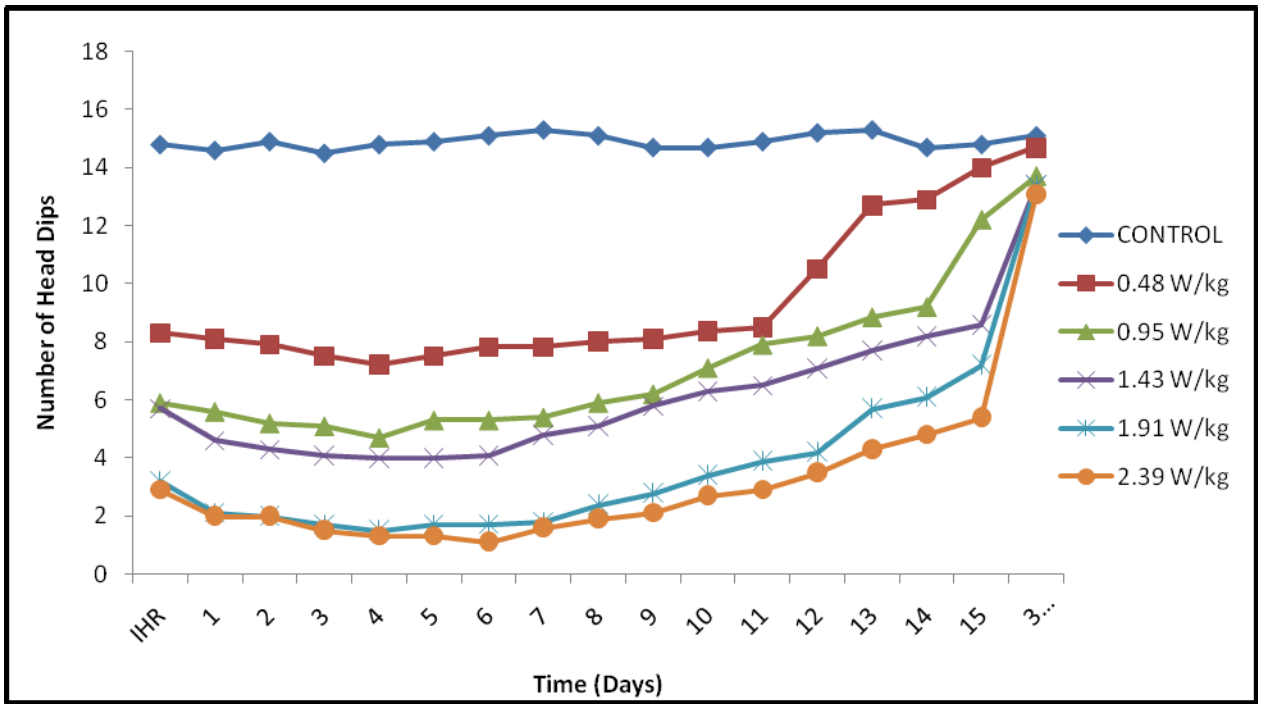


Fig. 4.5: Microwave Effects on the Exploratory Behaviour in Female Rats

Table 4.1: Cumulative Time Spent by the Male rats in the Elevated Plus Maze

Time (Days)		1 H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.48 W/kg	Open	79.83 ± 6.78	82.32 ± 8.42	93.69 ± 1.99	113.35 ± 5.98	125.35 ± 7.23	141.65 ± 8.22	155.15 ± 6.14	163.73 ± 5.24	171.47 ± 6.28	182.58 ± 7.13	195.44 ± 2.54	207.23 ± 3.59	213.41 ± 13.39	217.3 ± 4.97	220.69 ± 6.74	223.25 ± 5.19
	Closed	220.17 ± 6.78	217.68 ± 8.42	206.31 ± 1.99	186.65 ± 5.98	174.65 ± 7.23	158.35 ± 8.22	144.85 ± 6.14	136.27 ± 5.24	128.53 ± 6.28	117.42 ± 7.13	104.56 ± 2.54	92.77 ± 3.59	86.59 ± 13.39	82.7 ± 4.97	79.31 ± 6.74	76.75 ± 5.19
0.95 W/kg	Open	79.2 ± 3.92	81.33 ± 0.97	88.37 ± 4.11	97.21 ± 5.13	101.37 ± 2.35	119.25 ± 5.39	123.64 ± 5.17	142.82 ± 6.07	147.22 ± 9.25	155.13 ± 8.11	161.52 ± 10.01	173.27 ± 9.76	193.35 ± 4.40	201.2 ± 11.97	211.81 ± 15.19	216.49 ± 5.61
	Closed	220.8 ± 3.92	218.67 ± 0.97	211.63 ± 4.11	202.79 ± 5.13	198.63 ± 2.35	180.75 ± 5.39	176.36 ± 5.17	157.18 ± 6.07	152.78 ± 9.25	144.87 ± 8.11	138.48 ± 10.01	126.73 ± 9.76	106.65 ± 4.40	98.8 ± 11.97	88.19 ± 15.19	83.51 ± 5.61
1.43 W/kg	Open	71.53 ± 39.27	73.35 ± 12.47	77.10 ± 8.34	85.23 ± 10.22	97.70 ± 11.23	104.6 ± 14.31	116.8 ± 9.88	120.16 ± 7.09	128.4 ± 1.25	139.5 ± 8.97	153.8 ± 11.15	171.22 ± 5.77	187.25 ± 4.18	196.17 ± 11.25	207.45 ± 13.14	215.23 ± 10.73
	Closed	228.47 ± 39.27	226.65 ± 6.51	222.9 ± 8.34	214.77 ± 10.22	202.3 ± 11.23	195.4 ± 14.31	183.2 ± 9.88	179.84 ± 7.09	171.6 ± 1.25	160.5 ± 8.97	146.2 ± 11.15	128.78 ± 5.77	112.75 ± 4.18	103.83 ± 11.25	92.55 ± 13.14	84.77 ± 10.73
1.91 W/kg	Open	25.06 ± 9.45	29.41 ± 6.51	31.94 ± 4.35	48.35 ± 1.78	61.81 ± 10.11	66.25 ± 7.18	74.18 ± 5.53	87.30 ± 8.08	93.70 ± 3.29	112.63 ± 4.28	115.37 ± 14.19	132.8 ± 9.15	139.5 ± 2.97	146.2 ± 19.23	152.28 ± 8.66	167.22 ± 7.90
	Closed	274.94 ± 9.45	270.59 ± 6.51	268.06 ± 4.35	251.65 ± 1.78	238.19 ± 10.11	233.75 ± 7.18	225.82 ± 5.53	212.70 ± 8.08	206.3 ± 3.29	187.37 ± 4.28	184.63 ± 14.19	167.2 ± 9.15	160.5 ± 2.97	153.8 ± 19.23	147.72 ± 8.66	132.78 ± 7.90
2.39 W/kg	Open	11.76 ± 4.06	23.75 ± 2.10	25.20 ± 4.77	34.85 ± 6.70	39.22 ± 1.16	52.88 ± 10.25	58.40 ± 7.90	67.90 ± 12.07	83.15 ± 5.11	101.48 ± 11.09	109.26 ± 22.12	113.30 ± 4.08	116.45 ± 8.94	128.05 ± 9.41	135.70 ± 12.81	142.50 ± 14.16
	Closed	288.24 ± 4.06	276.25 ± 2.10	274.8 ± 4.77	265.15 ± 6.70	260.78 ± 1.16	247.12 ± 10.25	241.60 ± 7.90	232.10 ± 12.07	216.8 ± 5.11	198.52 ± 11.09	190.74 ± 22.12	186.70 ± 4.08	183.55 ± 8.94	171.95 ± 9.41	164.3 ± 12.81	157.50 ± 14.16
C	Open	225.90 ± 14.49	225.28 ± 12.30	226.04 ± 10.32	226.40 ± 9.50	224.92 ± 11.31	226.25 ± 10.50	227.10 ± 8.70	226.9 ± 13.25	225.75 ± 7.85	226.04 ± 11.50	226.84 ± 12.11	225.88 ± 16.70	224.72 ± 12.24	227.32 ± 15.21	226.44 ± 11.40	224.8 ± 12.78
	Closed	74.10 ± 14.49	74.72 ± 12.30	73.96 ± 10.32	73.60 ± 9.50	75.06 ± 11.31	73.75 ± 10.50	72.90 ± 8.70	73.10 ± 13.25	74.28 ± 7.85	73.96 ± 11.50	73.16 ± 12.11	74.12 ± 16.70	75.28 ± 12.24	72.68 ± 15.21	73.56 ± 11.40	75.20 ± 12.78

Table 4.2: Cumulative Time Spent by the Female rats in the Elevated Plus Maze

Time (Days)		1 H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.48 W/kg	Open	85.35 ± 5.06	86.15 ± 1.25	91.40 ± 2.43	98.23 ± 10.23	105.35 ± 0.36	115.34 ± 1.43	135.73 ± 3.92	143.30 ± 3.77	153.40 ± 2.97	169.05 ± 2.22	163.45 ± 2.03	173.24 ± 7.18	185.13 ± 1.29	201.79 ± 4.80	214.55 ± 5.21	225.34 ± 12.34
	Closed	214.65 ± 5.06	213.85 ± 1.25	208.60 ± 2.43	201.77 ± 10.23	194.65 ± 0.36	184.66 ± 1.43	164.27 ± 3.92	156.70 ± 3.77	146.60 ± 2.97	130.95 ± 2.22	136.55 ± 2.03	126.76 ± 7.18	114.87 ± 1.29	98.21 ± 4.80	85.45 ± 5.21	74.66 ± 12.34
0.95 W/kg	Open	80.28 ± 9.45	83.14 ± 6.29	88.68 ± 0.23	86.32 ± 2.33	92.07 ± 0.87	97.15 ± 7.21	101.33 ± 9.11	110.72 ± 4.36	113.93 ± 1.20	118.27 ± 3.93	120.71 ± 1.21	128.43 ± 0.76	135.79 ± 10.05	154.30 ± 11.12	165.52 ± 7.22	185.35 ± 2.41
	Closed	219.72 ± 9.45	216.86 ± 6.29	211.32 ± 0.23	213.68 ± 2.33	207.93 ± 0.87	202.85 ± 7.21	198.67 ± 9.11	189.28 ± 4.36	186.07 ± 1.20	181.73 ± 3.93	179.29 ± 1.21	171.57 ± 0.76	164.21 ± 10.05	145.70 ± 11.12	134.48 ± 7.22	114.65 ± 2.41
1.43 W/kg	Open	77.47 ± 6.17	80.36 ± 1.18	83.92 ± 3.73	81.33 ± 0.25	89.28 ± 1.29	90.97 ± 0.47	95.53 ± 0.97	98.76 ± 0.39	104.01 ± 0.24	110.47 ± 1.75	117.45 ± 9.28	118.64 ± 0.37	121.03 ± 11.75	126.14 ± 13.43	135.56 ± 10.83	142.88 ± 3.27
	Closed	222.53 ± 6.17	219.64 ± 1.18	216.08 ± 3.73	218.67 ± 0.25	210.72 ± 1.29	209.03 ± 0.47	204.47 ± 0.97	201.24 ± 0.39	195.99 ± 0.24	189.53 ± 1.75	182.55 ± 9.28	181.36 ± 0.37	178.97 ± 11.75	173.86 ± 13.43	164.44 ± 10.83	157.12 ± 3.27
1.91 W/kg	Open	38.96 ± 2.05	39.48 ± 1.10	42.64 ± 13.34	40.18 ± 1.23	44.54 ± 0.86	52.75 ± 7.38	68.11 ± 2.55	73.41 ± 4.33	83.54 ± 10.97	89.69 ± 0.53	91.38 ± 4.75	97.56 ± 7.60	103.57 ± 1.92	115.30 ± 0.59	117.81 ± 2.15	125.28 ± 1.14
	Closed	261.04 ± 2.05	260.52 ± 1.10	257.36 ± 13.34	259.82 ± 1.23	255.46 ± 0.86	247.25 ± 7.38	231.89 ± 2.55	226.59 ± 4.33	216.46 ± 10.97	210.31 ± 0.53	208.62 ± 4.75	202.44 ± 7.60	196.43 ± 1.92	184.70 ± 0.59	182.19 ± 2.15	174.72 ± 1.14
2.39 W/kg	Open	21.55 ± 1.77	23.63 ± 0.47	31.20 ± 1.94	33.46 ± 2.26	32.41 ± 3.01	38.13 ± 1.63	41.75 ± 1.39	52.15 ± 2.47	58.34 ± 3.29	62.29 ± 2.07	69.69 ± 5.58	78.11 ± 2.41	89.50 ± 1.33	97.61 ± 2.38	105.77 ± 9.36	110.22 ± 0.27
	Closed	278.45 ± 1.77	276.36 ± 0.47	268.80 ± 1.94	266.54 ± 2.26	267.59 ± 3.01	261.87 ± 1.63	258.25 ± 1.39	247.85 ± 2.47	241.66 ± 3.29	237.71 ± 2.07	230.31 ± 5.58	221.89 ± 2.41	210.50 ± 1.33	202.39 ± 2.38	194.23 ± 9.36	189.78 ± 0.27
C	Open	237.41 ± 6.15	236.12 ± 3.13	235.50 ± 0.37	236.34 ± 2.59	229.47 ± 12.25	230.77 ± 13.14	234.38 ± 2.41	235.29 ± 5.31	237.09 ± 13.11	235.37 ± 10.13	236.68 ± 8.28	236.08 ± 7.28	236.31 ± 4.38	237.63 ± 1.13	235.55 ± 2.17	234.90 ± 1.87
	Closed	62.59 ± 6.15	63.88 ± 3.13	64.50 ± 0.37	63.66 ± 2.59	70.53 ± 12.25	69.23 ± 13.14	65.62 ± 2.41	64.71 ± 5.31	62.91 ± 13.11	64.63 ± 10.13	63.32 ± 8.28	63.92 ± 7.28	63.69 ± 4.38	62.37 ± 1.13	64.45 ± 2.17	65.10 ± 1.87

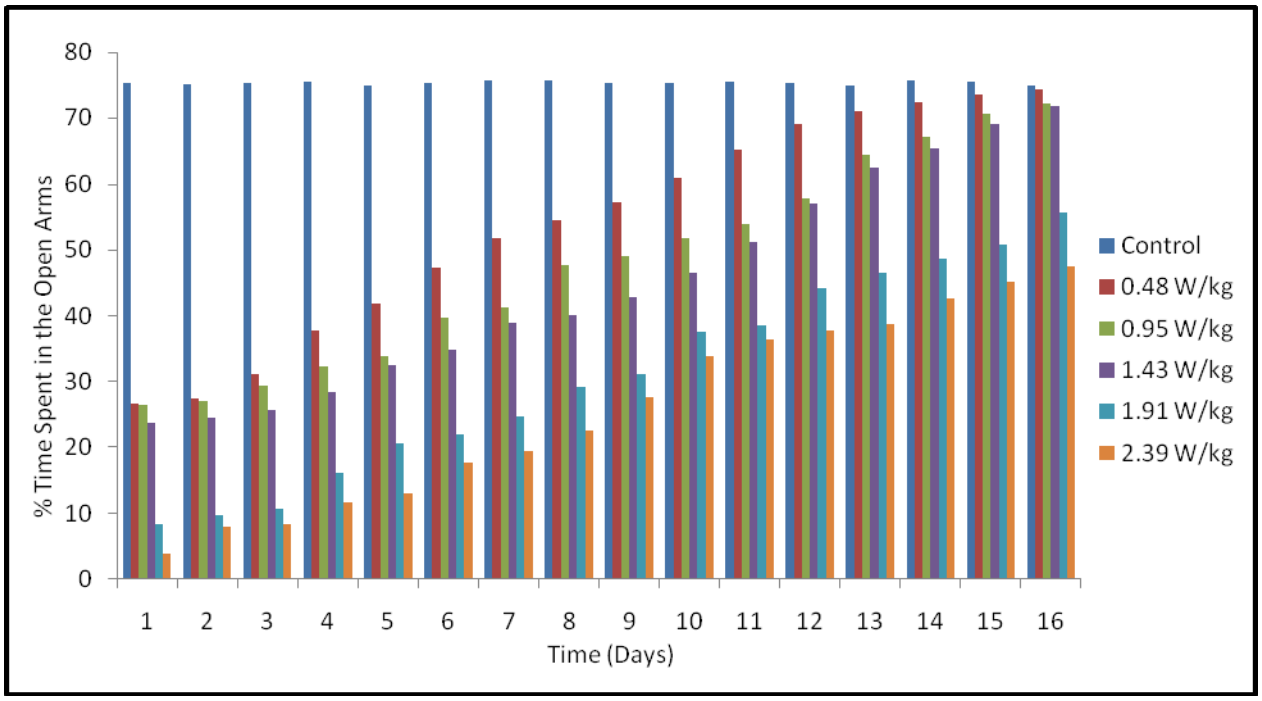


Fig. 4.6: % Time Spent in Open Arms of EPM by Male Rats

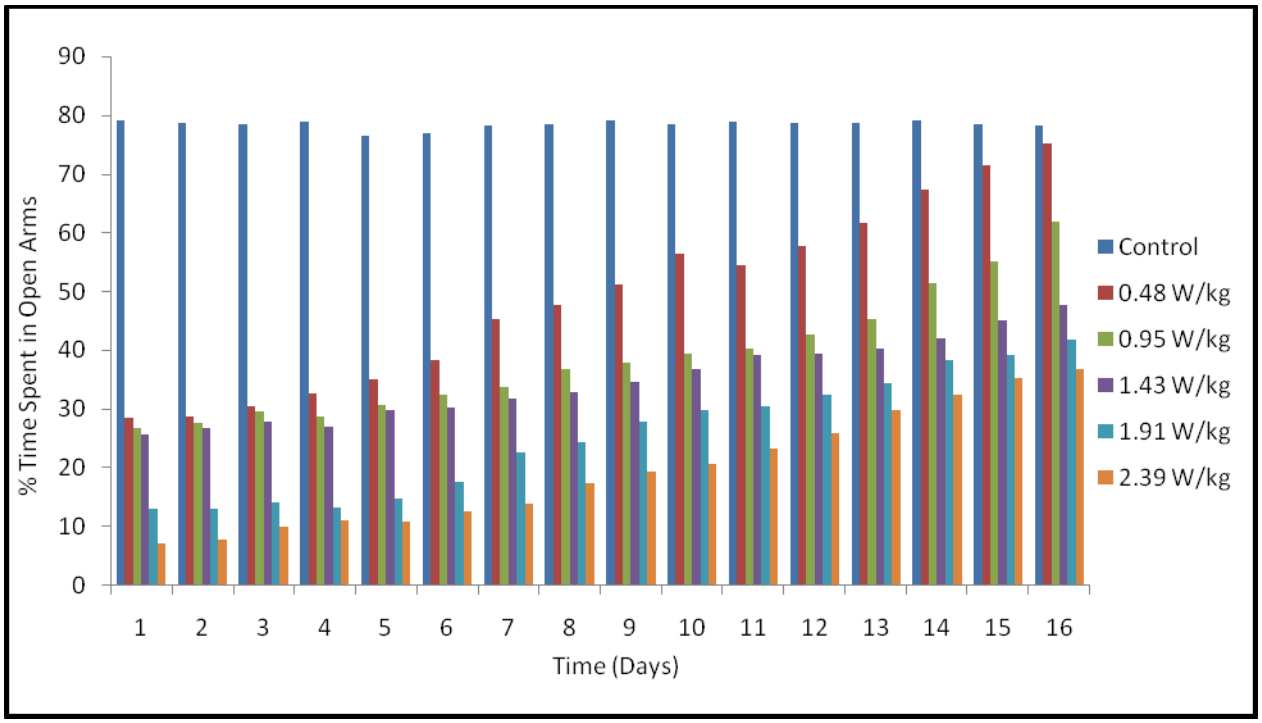


Fig. 4.7: % Time Spent in Open Arms of EPM by Female Rats

Table 4.3: Cumulative Time Spent by the Male rats in the Y-Maze

Time (Days)		1 H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.48 W/kg	Open	77.54 ± 0.51	79.12 ± 5.12	97.53 ± 0.31	109.52 ± 5.04	115.40 ± 3.64	155.64 ± 7.26	152.84 ± 4.17	168.36 ± 1.59	179.83 ± 2.38	196.41 ± 8.19	201.58 ± 5.70	204.01 ± 12.72	208.43 ± 2.04	211.37 ± 8.91	217.42 ± 3.12	221.81 ± 9.10
	Closed	222.46 ± 0.51	220.88 ± 5.12	202.47 ± 0.31	190.48 ± 5.04	184.6 ± 3.64	144.36 ± 7.26	147.16 ± 4.17	131.64 ± 1.59	120.17 ± 2.38	103.59 ± 8.19	98.42 ± 5.70	95.99 ± 12.72	91.57 ± 2.04	88.63 ± 8.91	82.58 ± 3.12	78.19 ± 9.10
0.95 W/kg	Open	76.63 ± 1.94	74.28 ± 8.32	81.97 ± 1.72	87.61 ± 3.84	98.40 ± 10.92	99.47 ± 2.14	115.84 ± 13.75	127.12 ± 2.35	141.21 ± 5.12	167.42 ± 1.53	165.89 ± 5.76	174.11 ± 1.11	185.74 ± 1.47	188.64 ± 13.41	197.43 ± 7.46	215.77 ± 0.74
	Closed	223.37 ± 1.94	225.72 ± 8.32	218.03 ± 1.72	212.39 ± 3.84	201.60 ± 10.92	200.53 ± 2.14	184.16 ± 13.75	172.88 ± 2.35	158.79 ± 5.12	132.58 ± 1.53	134.11 ± 5.76	125.89 ± 1.11	114.26 ± 1.47	111.36 ± 13.41	102.57 ± 7.46	84.23 ± 0.74
1.43 W/kg	Open	68.21 ± 15.60	65.67 ± 8.69	73.24 ± 1.58	79.07 ± 2.34	91.22 ± 5.32	95.85 ± 0.72	108.15 ± 1.84	117.72 ± 5.62	124.51 ± 6.12	143.75 ± 2.13	147.23 ± 3.07	164.62 ± 2.35	176.24 ± 9.21	192.45 ± 1.10	207.45 ± 13.14	215.23 ± 10.73
	Closed	231.79 ± 15.60	234.33 ± 8.69	226.76 ± 1.58	220.93 ± 2.34	208.78 ± 5.32	204.15 ± 0.72	191.85 ± 1.84	182.28 ± 5.62	175.49 ± 6.12	156.25 ± 2.13	152.77 ± 3.07	135.38 ± 2.35	123.76 ± 9.21	107.55 ± 1.10	92.55 ± 13.14	84.77 ± 10.73
1.91 W/kg	Open	28.33 ± 2.06	29.54 ± 5.77	43.02 ± 6.12	42.66 ± 0.89	56.28 ± 1.65	58.98 ± 4.26	75.93 ± 4.88	83.42 ± 12.83	88.04 ± 8.01	102.75 ± 5.85	127.48 ± 10.41	141.32 ± 3.58	145.82 ± 0.54	155.53 ± 11.23	163.71 ± 2.55	169.15 ± 10.22
	Closed	271.67 ± 2.06	270.46 ± 5.77	256.98 ± 6.12	257.34 ± 0.89	243.72 ± 1.65	241.02 ± 4.26	224.07 ± 4.88	216.58 ± 12.83	211.96 ± 8.01	197.25 ± 5.85	172.52 ± 10.41	158.68 ± 3.58	154.18 ± 0.54	144.47 ± 11.23	136.29 ± 2.55	130.85 ± 10.22
2.39 W/kg	Open	10.48 ± 1.09	17.88 ± 0.47	21.29 ± 0.81	29.05 ± 2.35	33.41 ± 6.80	48.94 ± 7.92	60.07 ± 1.46	75.33 ± 13.16	81.59 ± 2.38	97.74 ± 3.42	105.34 ± 9.11	121.58 ± 1.63	128.69 ± 7.25	133.12 ± 2.87	143.55 ± 10.22	149.76 ± 4.78
	Closed	289.52± 1.09	282.12 ± 0.47	278.71 ± 0.81	270.95 ± 2.35	266.59 ± 6.80	251.06 ± 7.92	239.93 ± 1.46	224.67 ± 13.16	218.41 ± 2.38	202.26 ± 3.42	194.66 ± 9.11	178.42 ± 1.63	171.31 ± 7.25	166.88 ± 2.87	156.45 ± 10.22	150.24 ± 4.78
C	Open	222.66 ± 7.03	227.85 ± 2.44	215.23 ± 5.73	220.96 ± 11.27	225.75 ± 9.51	227.16 ± 3.68	225.07 ± 7.52	227.33 ± 10.54	228.28± 0.63	225.54 ± 5.51	227.56 ± 1.56	226.17 ± 13.77	226.72 ± 4.75	226.90 ± 8.02	227.20 ± 13.89	225.36 ± 12.78
	Closed	77.34 ± 7.03	72.15 ± 2.44	84.77 ± 5.73	79.04 ± 11.27	74.25 ± 9.51	72.84 ± 3.68	74.93 ± 7.52	72.67 ± 10.54	71.72 ± 0.63	74.46 ± 5.51	72.35 ± 1.56	73.83 ± 13.77	73.30 ± 4.75	73.10 ± 8.02	72.80 ± 13.89	74.64 ± 12.78

Table 4.4: Cumulative Time Spent by the Female rats in the Y-Maze

Time (Days)		1 H	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.48 W/kg	Open	80.24 ± 1.25	81.41 ± 0.97	88.52 ± 0.11	98.10 ± 1.73	102.24 ± 1.30	116.23 ± 2.41	128.33 ± 1.55	143.45 ± 2.34	163.24 ± 1.43	175.12 ± 0.89	183.42 ± 1.03	182.17 ± 2.07	197.34 ± 1.99	204.13 ± 2.18	214.13 ± 7.90	220.80 ± 8.10
	Closed	219.76 ± 1.25	218.59 ± 0.97	211.48 ± 0.11	201.90 ± 1.73	197.76 ± 1.30	183.77 ± 2.41	171.67 ± 1.55	156.55 ± 2.34	136.76 ± 1.43	124.88 ± 0.89	116.58 ± 1.03	117.83 ± 2.07	102.66 ± 1.99	95.87 ± 7.32	85.87 ± 7.90	79.20 ± 8.10
0.95 W/kg	Open	79.52 ± 2.55	79.55 ± 8.10	83.22 ± 1.74	87.09 ± 9.41	91.53 ± 8.20	103.10 ± 10.03	114.03 ± 0.97	112.30 ± 1.25	118.11 ± 2.04	129.07 ± 0.88	134.80 ± 1.08	163.25 ± 1.13	166.14 ± 7.21	185.13 ± 0.91	193.10 ± 12.13	209.41 ± 9.01
	Closed	220.48 ± 2.55	220.45 ± 8.10	216.78 ± 1.74	212.91 ± 9.41	208.47 ± 8.20	196.90 ± 10.03	185.97 ± 0.97	187.70 ± 1.25	181.89 ± 2.04	170.93 ± 0.88	165.20 ± 1.08	136.75 ± 1.13	133.86 ± 7.21	114.87 ± 0.91	106.90 ± 12.13	90.59 ± 9.01
1.43 W/kg	Open	72.74 ± 6.41	74.31 ± 0.93	76.43 ± 1.26	81.37 ± 0.63	86.80 ± 9.20	93.03 ± 1.22	103.31 ± 3.27	109.37 ± 4.09	114.22 ± 1.34	117.21 ± 0.33	110.25 ± 0.11	121.37 ± 2.21	135.21 ± 8.09	143.10 ± 1.63	169.70 ± 12.09	197.41 ± 9.23
	Closed	227.26 ± 6.41	225.69 ± 0.93	223.57 ± 1.26	218.63 ± 0.63	213.20 ± 9.20	206.97 ± 1.22	196.69 ± 3.27	190.63 ± 4.09	185.78 ± 1.34	182.79 ± 0.33	189.75 ± 0.11	178.63 ± 2.21	164.79 ± 8.09	156.90 ± 1.63	130.30 ± 12.09	102.59 ± 9.23
1.91 W/kg	Open	33.85 ± 0.78	37.23 ± 1.07	44.33 ± 1.26	55.29 ± 2.11	57.21 ± 0.71	61.22 ± 1.99	71.33 ± 2.22	85.34 ± 1.69	96.21 ± 2.34	101.31 ± 2.39	100.29 ± 3.93	109.23 ± 4.25	111.90 ± 0.25	125.30 ± 2.41	137.80 ± 0.97	157.23 ± 1.33
	Closed	226.15 ± 0.78	262.77 ± 1.07	255.67 ± 1.26	244.71 ± 2.11	242.79 ± 0.71	238.78 ± 1.99	228.67 ± 2.22	214.66 ± 1.69	203.79 ± 2.34	198.69 ± 2.39	199.71 ± 3.93	190.77 ± 4.25	188.10 ± 0.25	174.70 ± 2.41	162.20 ± 0.97	142.77 ± 1.33
2.39 W/kg	Open	17.79 ± 0.51	18.25 ± 2.35	21.43 ± 0.81	35.97 ± 1.71	33.64 ± 0.34	48.37 ± 0.13	62.33 ± 2.01	66.03 ± 9.07	69.24 ± 12.29	78.55 ± 13.41	81.73 ± 3.10	87.40 ± 4.97	95.43 ± 0.97	107.09 ± 9.71	113.43 ± 4.24	137.20 ± 10.03
	Closed	282.21 ± 0.51	281.75 ± 2.35	278.57 ± 0.81	264.03 ± 1.71	266.36 ± 0.34	251.63 ± 0.13	237.67 ± 2.01	233.97 ± 9.07	230.76 ± 12.29	221.45 ± 13.41	218.27 ± 3.10	212.60 ± 4.97	204.57 ± 0.97	192.91 ± 9.71	186.57 ± 4.24	162.80 ± 10.03
C	Open	235.65 ± 2.54	225.73 ± 1.44	224.94 ± 9.72	231.02 ± 2.34	235.54 ± 13.39	225.14 ± 10.24	224.83 ± 9.04	233.55 ± 7.08	235.27 ± 12.31	228.12 ± 0.76	231.42 ± 0.36	234.53 ± 7.93	235.95 ± 8.33	236.63 ± 2.43	235.75 ± 1.93	224.98 ± 3.98
	Closed	64.35 ± 2.54	74.27 ± 1.44	75.06 ± 9.72	68.98 ± 2.34	64.46 ± 13.39	74.86 ± 10.24	74.86 ± 9.04	66.45 ± 7.08	64.73 ± 12.31	71.88 ± 0.76	68.58 ± 0.36	65.47 ± 7.93	64.05 ± 8.33	63.37 ± 2.43	64.25 ± 1.93	75.02 ± 3.98

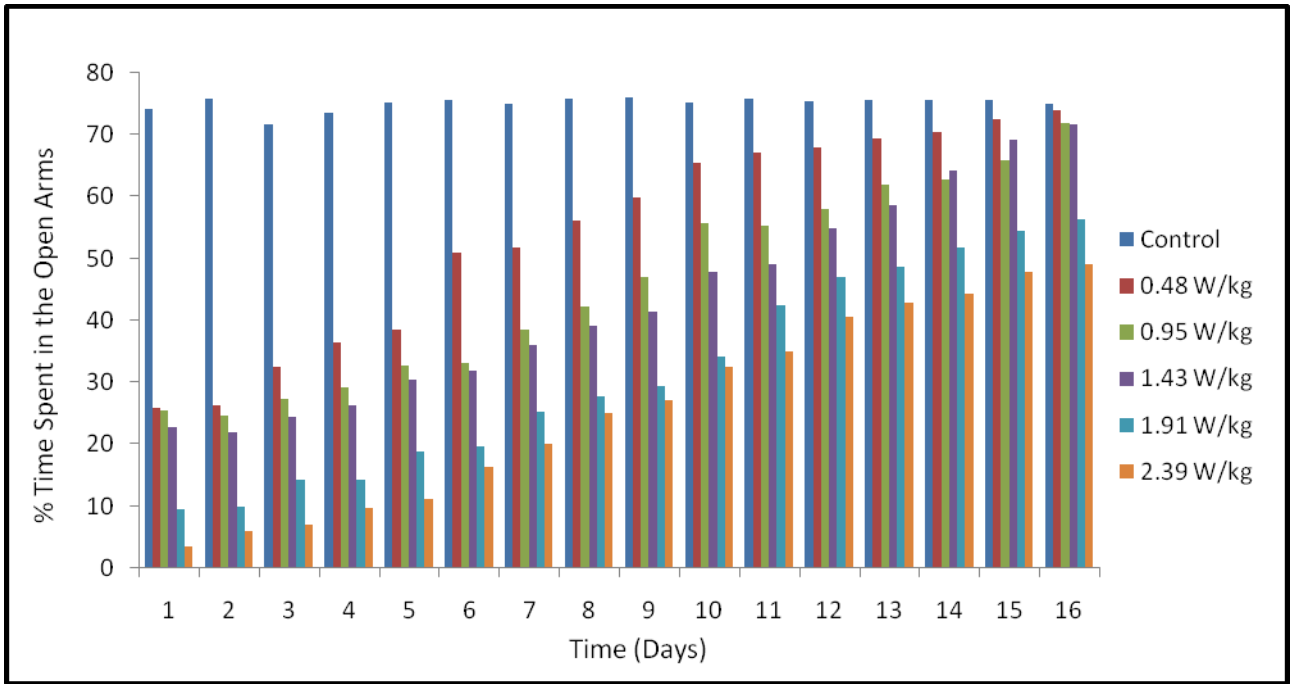


Fig. 4.8: % Time Spent in Open Arms of Y-Maze by Male Rats

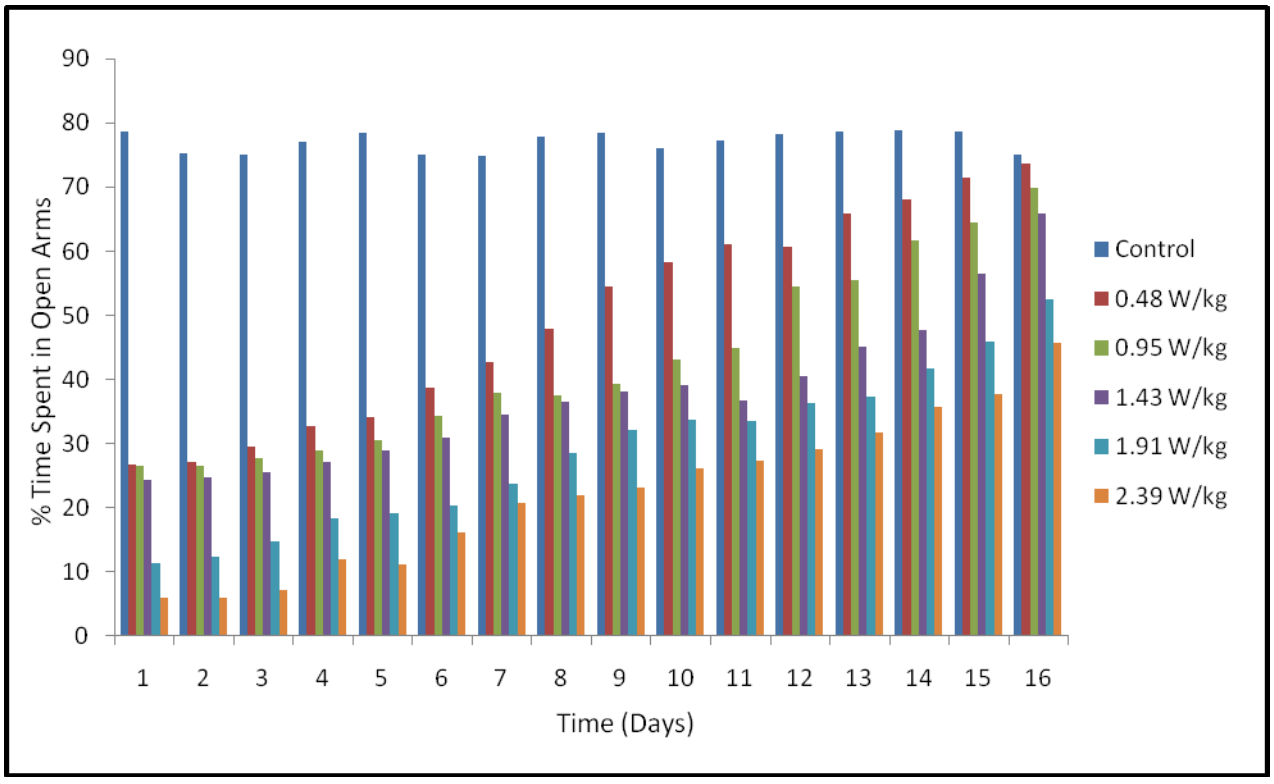


Fig. 4.9: % Time Spent in Open Arms of Y-Maze by Female Rats

4.3 Effects of Microwave Exposure on the Animal Reproductive Organs

4.3.1 Effects on the Body and Organ Weights of the Wistar Rats after Exposure to MW Radiation

The statistical analysis of data from the weights taken in rats before and during four weeks post-exposure to different SARs of microwave radiation showed a significant reduction in the weight gain with increasing SARs of exposures (Table 4.5 and 4.6) while Figure 4.10 and 4.11 displays the variation in the results obtained. The lowest weight gain within the four weeks was recorded in the group exposed to 2.38W/kg for both male and female rats. It was observed that those rats exposed to highest SAR withdrew from food as their feeding trough was not empty for 4 days. Similar result was reported by (Jensh, 1997) in which exposure of female rats to MW radiation of 6000MHz resulted in weight retardation and also a reduction in monocyte count. Also the weight of all the reproductive organs and other visceral organs taken at the termination of the experiment revealed a significant reduction in relative weight of the testis and seminal vesicles while a significant increase in the relative weight was recorded in the prostrate and the epididymis of exposed rats as the SAR was gradually increased to 2.38W/kg (Table 4.7). Similar trend was observed in female reproductive organs with significant reduction in ovary and fallopian tube relative weight at SAR 2.39 W/kg (Table 4.8). Dasdag *et al*. (2003) and de Rooij (2002) also reported a reduction in the histomorphometry and testicular with a reduction in seminiferous tubule diameter in rats exposed to 0.41 W/kg of SAR giving an indication of disruption of the internal environment of the testis engineered to provide nourishment and support to the developing spermatogonia.

The results obtained for liver weight showed a significant increase in the relative weight in exposed rats at SARs 1.91 and 2.38 W/kg (Table 4.7 and 4.8) in both sexes. Figure 4.12 and 4.13 shows the relative weight in percentage while the weight of liver was scaled by factor of 4 for good pictorial representation. This effect may not be unconnected with the increase in the generation of free radicals by a way of increased lipid peroxidation on exposure to MW reported by other authors (Bediz *et al*., 2006). The increased liver weight may also be as a result of increased activity of the liver cells in mopping up the free radicals as such liver toxicity marker enzymes as ALT, AST AND GSH have been reportedly increased on exposure to electromagnetic field range of 50Hz (Ibrahim *et al*., 2008).

4.3.2 Effects of Microwave Radiation on the Spermatozoa Parameters

Spermatozoa concentration in rats exposed to microwave radiation varying from 0.48 to 2.39 W/kg recorded a significant reduction compared with control (Table 4.9). The reduction in sperm count decreased progressively with increased SARs of the MW radiation, with the lowest sperm concentration recorded in the group exposed to 1.91 and 2.39W/kg. Figures 4.14, 4.15, 4.16 and 4.17 are the plots of the measured parameters on semen analysis.

This corroborates report by Kowalczuk *et al*., 1983, Saunders *et al*., 1991 that acute microwave exposure affects the spermatogenic epithelium, and thus male fertility, through a rise of the testicular temperature. The decrease sperm concentration in exposed groups of rats was also accompanied with a decreased in the percentage life/dead ratio there was increased death of sperm cells as large numbers of the sperm cell membranes took up the eosin-nigrosin vital stain. The major types of abnormal sperm cells observed in this study were coiled tail which gives an indication of alteration of cell membrane integrity, detached head and pyriform head and there was significant increase in the number of abnormal sperm cells (Table 4.18). Ji-Geng *et al*., (2007) have earlier reported that carrying mobile phone very close to the reproductive organs for a long time will affect adversely sperm motility which is a vital indicator of male fertility. It was reported by Naziroglu *et al*., (2004) that electromagnetic waves are able to penetrate living organism to alter the cell membrane potential and the activities of Na^+ - K^+ ATPase which is responsible for energy generation for the progressive motility of sperm cell (Behari *et al*., 1998) and protein kinase C which is important for cellular communication and response (Paulraj *et al*., 2006).

The influence of MW radiation has also been linked with increased oxidative stress damage in the cell with increased production of oxidative stress makers such as Superoxide dismutase, Catalase and glutathione peroxidase (Aweda 2003, Watambe *et al* 1997). It was not surprising therefore, as the sperm motility score results revealed a progressive decrease in motility with increasing SARs of the microwave radiation (Table 4.9). The decrease became significant right from the lowest SAR (0.48W/kg) studied.

Many literatures on the effect of microwave radiation on reproduction and development of small mammals have reported effects that were related to increased temperature of the range of 5 ^{0}C (Kowalczuk *et al* 1983, Saunders *et al* 1991). Throughout this study however, the body temperature increase recorded was 1.40 ± 0.20 ^{o}C. Thus, the various alterations recorded could not have been due to temperature changes but to the non-thermal effect of radiation.

Table 4.5: Effects of Microwave Radiation Exposure on the Average Weight of the Male Rats (g)

	Control	0.48 W/kg	0.95 W/kg	1.43 W/kg	1.91 W/kg	2.39 W/kg
Initial Weight	86.0 ± 11.22	96.0 ± 12.55	97.0 ± 10.14	109.5 ± 15.19	113.0 ± 9.88	116.0 ± 9.56
1 Week	117.6 ± 7.88	97.0 ± 5.98	99.0 ± 9.22	114.2 ± 5.23	113.1 ± 1.33	115.2 ± 2.81
2 Weeks	129.43 ± 2.30	117.70 ± 1.47	119.76 ± 8.27	128.55 ± 0.65	131.38 ± 1.77	121.33 ± 1.83
4 Weeks	136.37 ± 3.40	130.51 ± 10.48	128.81 ± 3.41	139.5 ± 2.11	140.62 ± 9.88	136.41 ± 2.21
Weight Change (%)	58.57 ± 4.13	35.95 ± 4.93	32.79 ± 3.00	27.40 ± 6.55*	24.44 ± 4.81*	17.90 ± 3.66*

Table 4.6: Effects of Microwave Radiation Exposure on the Average Weight of the Female Rats (g)

	Control	**0.48 W/kg**	**0.95 W/kg**	**1.43 W/kg**	**1.91 W/kg**	**2.39 W/kg**
Initial Weight	90.0 ± 3.54	98.0 ± 9.42	102.0 ± 12.77	110.8 ± 6.31	110.0 ± 1.55	116.5 ± 4.86
1 Week	114.33 ± 12.31	102.2 ± 2.13	107.3 ± 7.95	115.61 ± 3.64	113.3 ± 10.35	112.42 ± 1.11
2 Weeks	135.43 ± 5.16	117.33 ± 2.45	121.8 ± 3.96	126.50 ± 5.67	122.44 ± 2.56	120.88 ± 1.83
4 Weeks	140.68 ± 2.98	132.07 ± 9.15	130.05 ± 11.03	132.96 ± 1.85	130.8 ± 3.74	134.22 ± 4.56
Weight Change (%)	56.31 ± 4.31	34.77 ± 4.04	27.50 ± 3.87	20.00 ± 2.03*	18.91 ± 3.97*	15.2 ± 1.90*

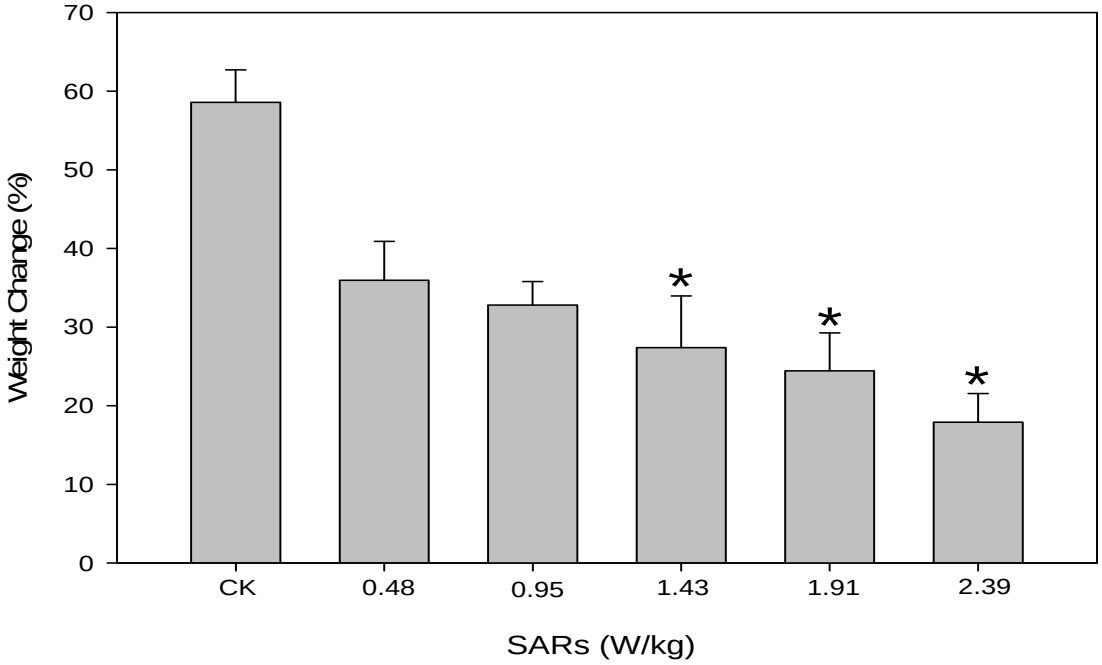


Fig. 4.10: Variation in Body weights over a period of 4 weeks (Male)

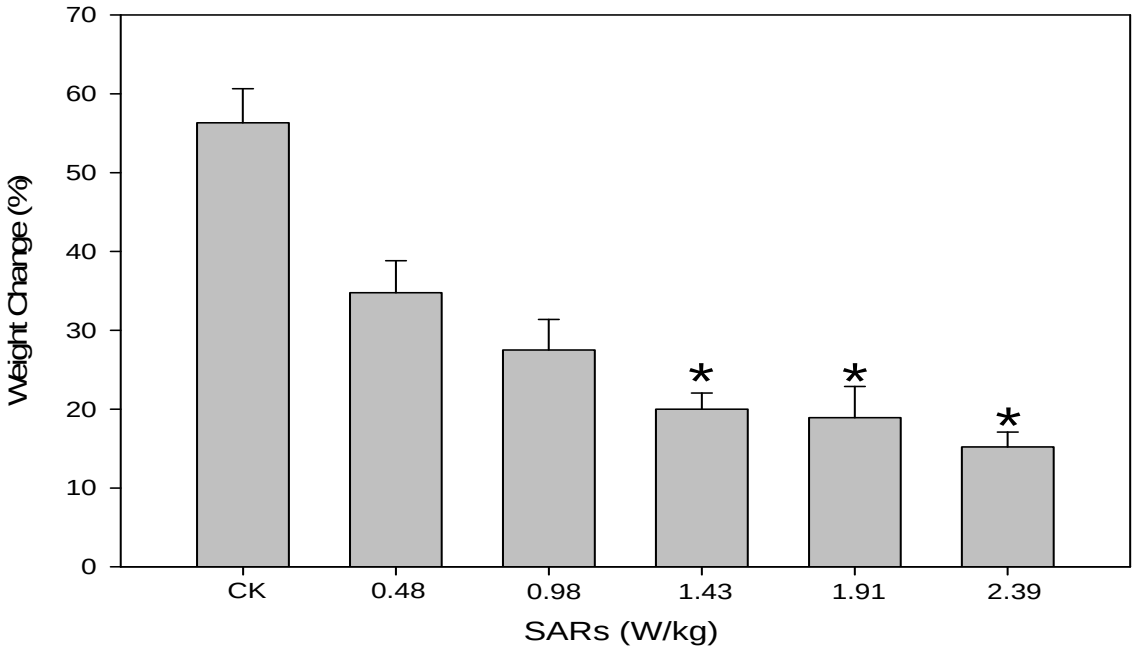


Fig. 4.11: Variation in Body weights over a period of 4 weeks (Female)

* Significantly different compared to Control ($p < 0.05$)

Table 4.7: Effect of exposure on matured male Sprague Dawley to 2.45 GHz microwave radiation after four weeks post-exposure period on reproductive organ and other visceral vital organs weight

Organs	Control	0.48 W/kg	0.95 W/kg	1.43 W/kg	1.91 W/kg	2.39 W/kg
Final Body Weight (g)	136.37±3.4	130.51±10.5	128.81±3.4	139.5±2.1	140.62±9.9	136.41±2.2
Heart (%)	0.36±0.05	0.34±0.05	0.35±0.05	0.37±0.05	0.36±0.05	0.38±0.05
Kidney (%)	0.32±0.02	0.29±0.02	0.32±0.02	0.41±0.02	0.38±0.02	0.38±0.02
Liver (%)	3.79±0.30	3.59±0.35	3.85±0.41	4.05±0.30	4.21±0.38*	4.66±0.40*
Right Testis (%)	0.75±0.03	0.56±0.02*	0.66±0.03*	0.75±0.02	0.72±0.03	0.63±0.02*
Left Testis (%)	0.76±0.03	0.68±0.02*	0.66±0.03*	0.76±0.02	0.73±0.03	0.56±0.02*
Epididymis (%)	0.01±0.01	0.01±0.00	0.03±0.01	0.14±0.02*	0.19±0.03*	0.23±0.00*
Seminal Vesicle (%)	0.44±0.02	0.26±0.02*	0.25±0.01*	0.17±0.01*	0.24±0.01*	0.36±0.02*
Prostate Gland (%)	0.08±0.01	0.06±0.01	0.07±0.00	0.11±0.01	0.10±0.01	0.10±0.01

* Significantly different compared to Control ($p < 0.05$)

Table 4.8: Effect of exposure on matured female Sprague Dawley to 2.45 GHz microwave radiation after four weeks post-exposure period on reproductive organ and other vital organ Weights

Organs	Control	0.48 W/kg	0.95 W/kg	1.43 W/kg	1.91 W/kg	2.39 W/kg
Final Body Weight (g)	138.68±3.0	132.07±9.2	130.05±11.0	132.96±1.9	130.8±3.7	134.22±4.6
Heart (%)	0.39±0.03	0.37±0.01	0.37±0.02	0.39±0.01	0.40±0.03	0.41±0.02
Kidney (%)	0.37±0.05	0.35±0.03	0.37±0.01	0.35±0.02	0.39±0.014	0.40±0.01
Liver (%)	3.56±0.10	3.61±0.23	3.57±0.61	3.59±0.15	4.41±0.26*	4.66±0.55*
Right Ovary (%)	0.05±0.01	0.04±0.01	0.035±0.02	0.037±0.02	0.025±0.03*	0.021±0.02*
Left Left (%)	0.051±0.02	0.043±0.02	0.034±0.03	0.039±0.02	0.026±0.03*	0.02±0.01*
Fallopian Tube with Uterus (%)	0.21±0.0 1	0.22±0.00	0.21±0.01	0.19±0.02	0.13±0.03*	0.11±0.01*

* Significantly different compared to Control ($p < 0.05$)

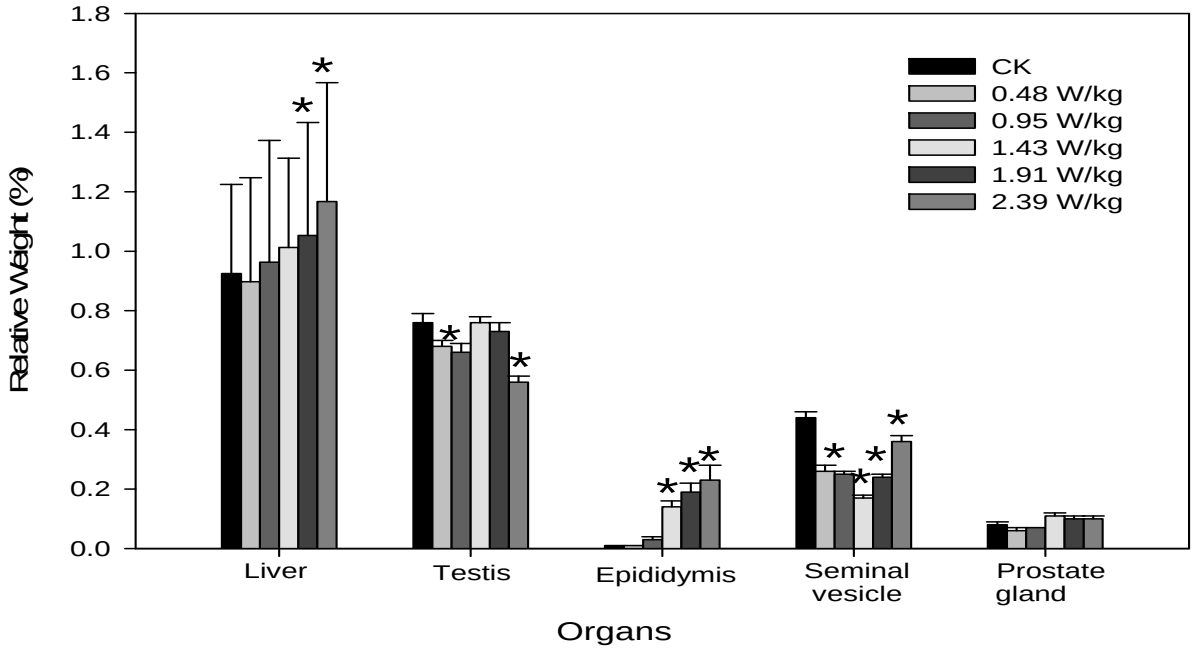


Fig. 4.12: Relative weight of vital organs four weeks post exposure (Male)

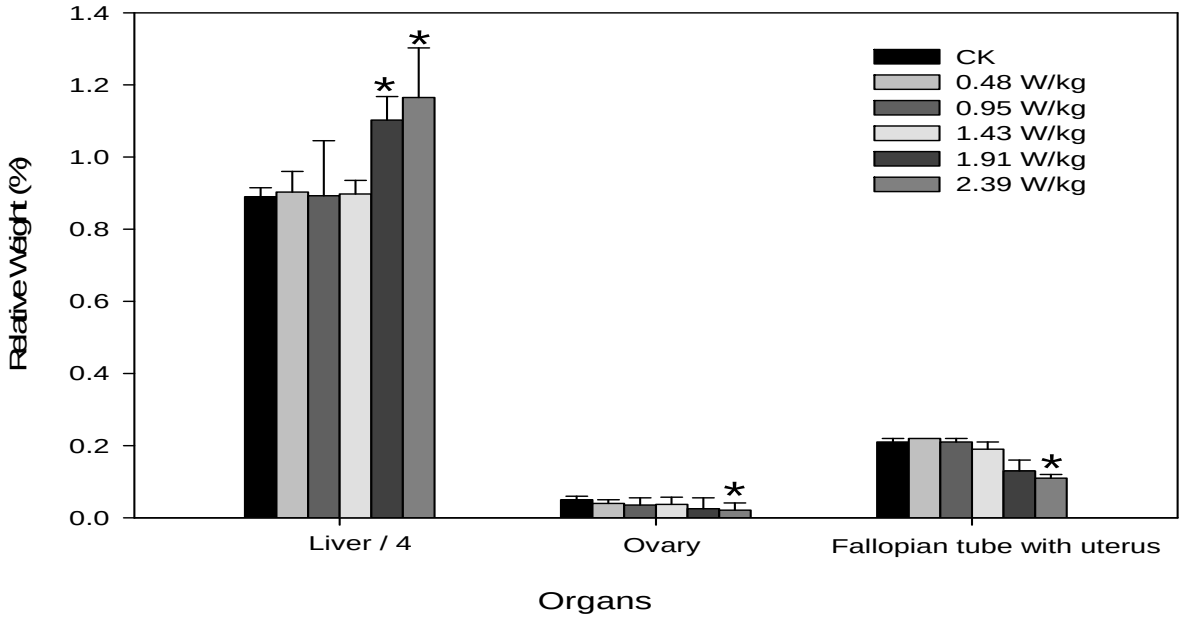


Fig. 4.13: Relative weight of vital organs four weeks post exposure (Female)

* indicates significantly difference ($p < 0.05$) compared to control

Table 4.9: Effect of exposure of matured male Sprague Dawley to 2.45 GHz microwave radiation after four weeks post-exposure period on semen parameters

Parameter	Control	0.48 W/kg	0.95 W/kg	1.43 W/kg	1.91 W/kg	2.39 W/kg
Sperm count ($x10^6$/ml)	105.00±5.05	80.00±4.05*	75.00±3.00*	74.05±2.50*	54.50±1.00*	55.50±1.00*
Progressive gross sperm motility (%)	76.40±0.17	60.00±0.29*	55.00±0.55*	55.30±1.03	55.00±0.00*	50.50±0.11*
Life sperm/ Dead sperm ratio (%)	84.00±2.00	24.00±2.00*	44.00±1.50*	54.00±1.50*	46.00±1.50*	48.00±2.00*
Percentage abnormal sperm cells in semen sample (%)	3.13±0.27	25.96±0.66*	25.32±0.12*	22.60±0.85*	43.44±0.33*	39.18±0.37*

*Significantly different from the Control unexposed group

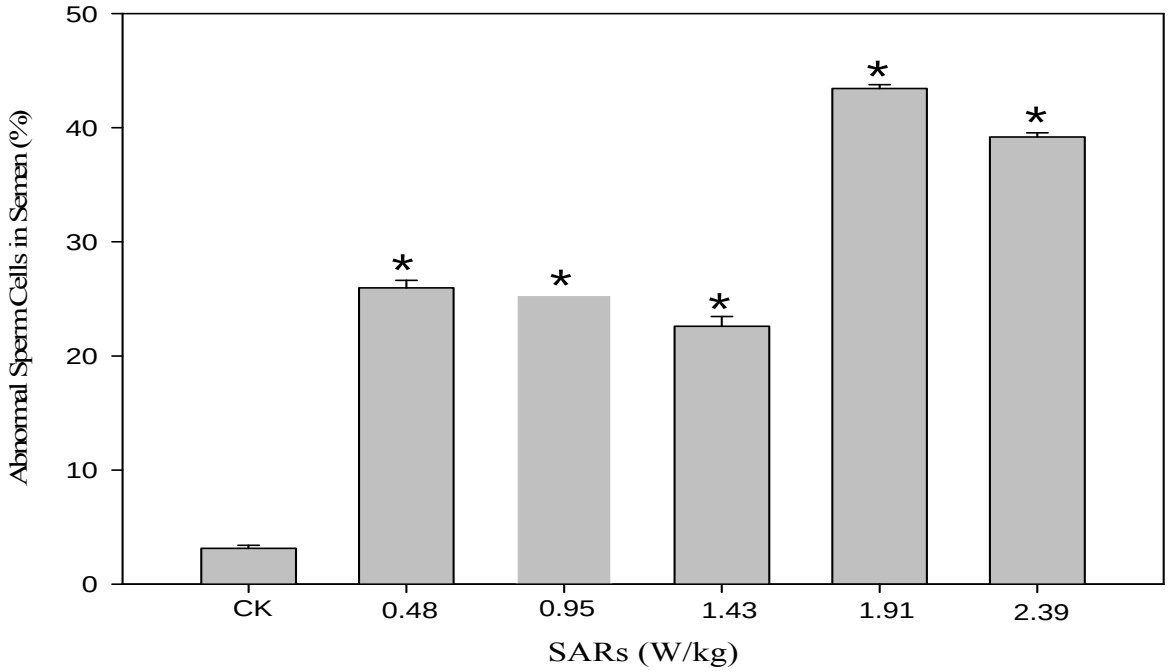


Fig. 4.14: Variations in the semen cells morphology four weeks post exposure

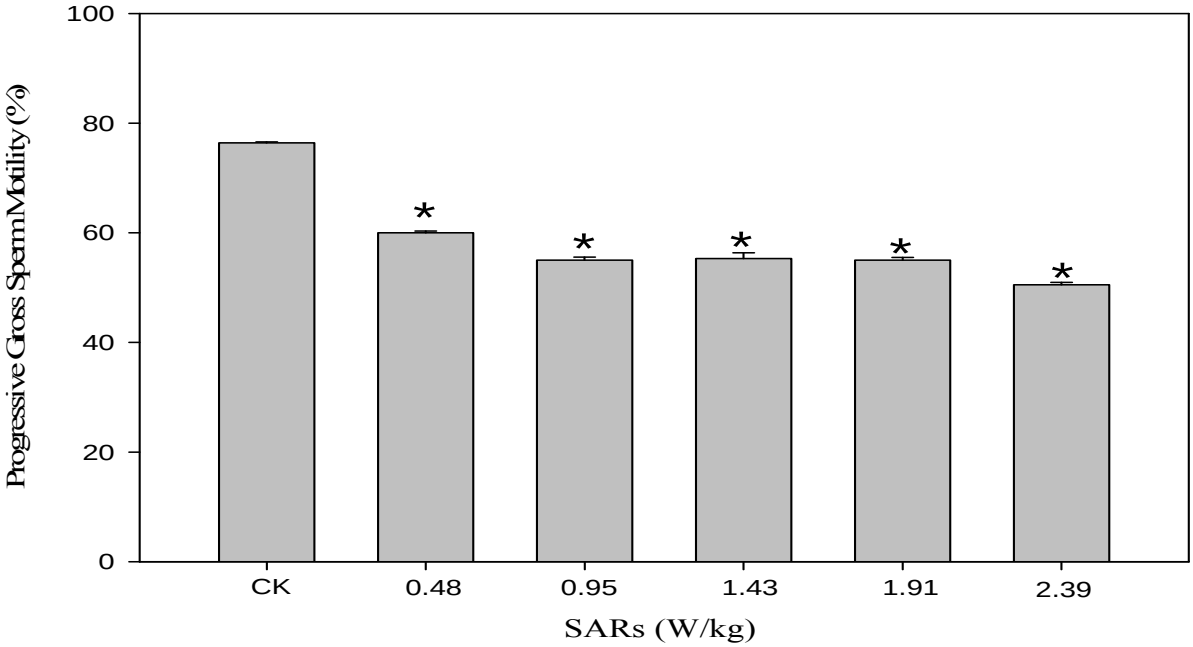


Fig. 4.15: Variations in the gross sperm motility four weeks post exposure

* indicates significantly difference ($p < 0.05$) compared to control

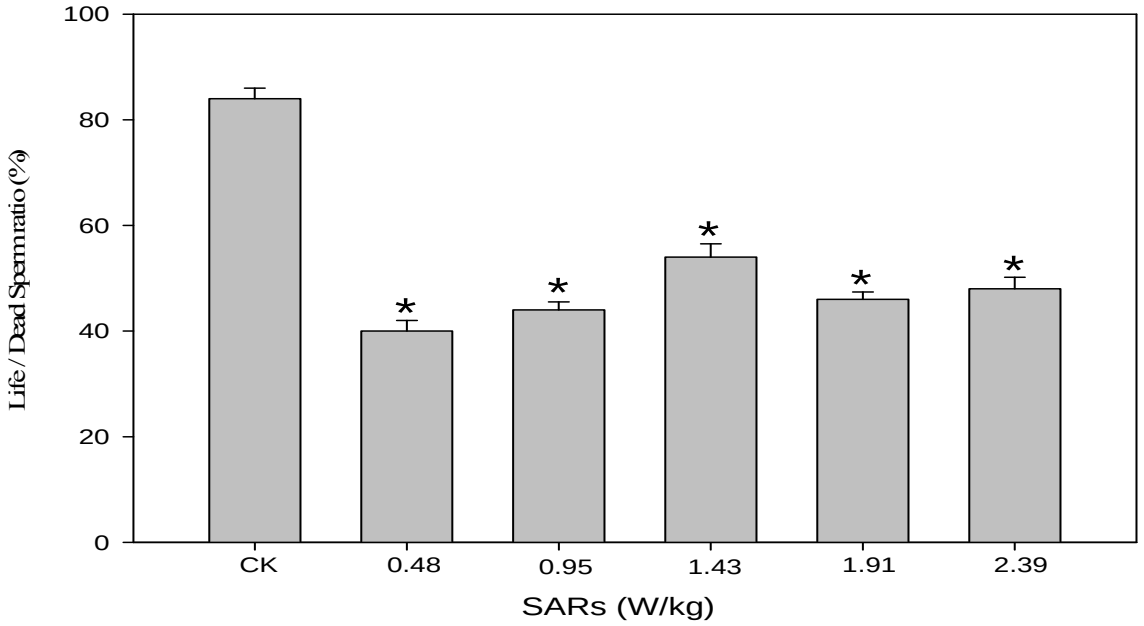


Fig. 4.16: Variations in the % Life/dead Sperm four weeks post exposure

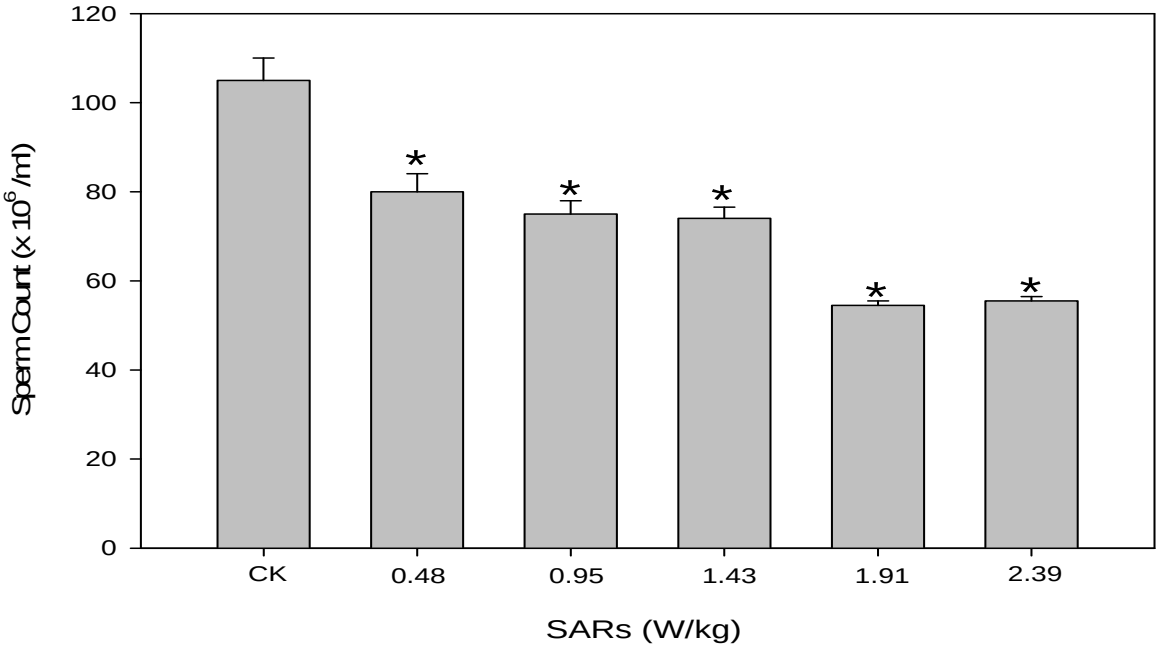


Fig. 4.17: Variations in the Sperm Counts four weeks post exposure

* indicates significantly difference ($p < 0.05$) compared to control

4.3.3 Effects MW Radiation on the Histopathology Study

No histological or macroscopic alterations were observed in the tissues of the control rats both in male and female, there was normal arrangement of the cells in all the observed tissues (Plates 4.1A, F and K for female; Plate 4.1Q and Plate 4.1V for male). The results obtained from the exposed groups are the following.

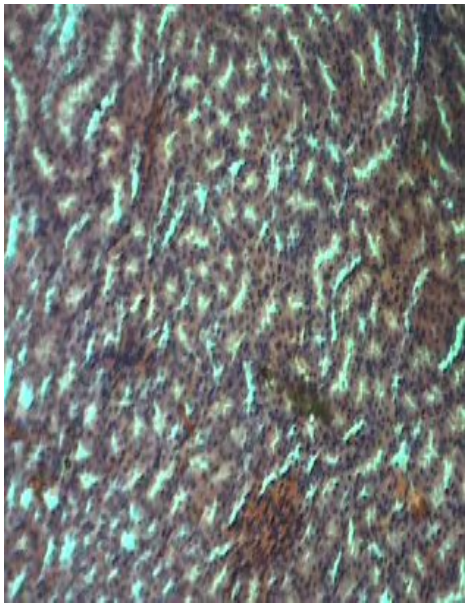
The group (Female) exposed to SAR 0.48 W/kg: The uterus, liver and heart were normal no histophatological evidence of cell injury when compared to the control, while the ovary shows thickness of sections and the kidney have evidence of foci of lymphocytic aggregate, however, no evidence of cell death (Plate 4.1L and Plate 4.1B). The group exposed to SAR 0.95 W/kg: The kidney, heart, and uterus were normal no histophatological evidence of cell injury when compared to the control, while the liver cell looked cloudy and there were some cells with oedema and the ovaries contained numerous follicles of varying sizes (Plate 4.1G and Plate 4.1M). The group exposed to SAR 1.43 W/kg: The heart shows no significant histopathology cell injury compared to the control. While the endometrial, epithelial of the uterus are not histological remarkable. The ovary follicles showed increased hyperchomasia, coarse nuclear chomatin and cellular oedema. The liver shows isolated pyknotic heptacyte, increased mitotic figures, some cells with cloudy cytoplasm and occasional cells with increased hyperchomasia. The kidney tubules epthilinear lining is cloudy; the glomeruli are congested and cellular oedema (Plate 4.1N, H and C). The group exposed to SAR 1.91 W/kg: The liver, heart and the uterus are cloudy but no remarkable cellular injury compared to control group. The ovary shows vascular congestion, hyper-chomatic granulocer cell and cellular oedema. The kidney shows presence of vascular/glomerular congestion, interstitial space hemorrhage and tubular cells are cloudy (Plate 4.1O, I and D). The group exposed to SAR 2.39 W/kg: The heart and the uterus show no remarkable cellular injury compare to control group. The ovary shows evidence of cellular oedema and hyperchomatism in some of the follicles. The liver shows area of cellular oedema and narrow sinusoids. The kidney has congestion, interstitial space hemorrhage and cellular oedema (Plate 4.1P, J and E).

The group (Male) exposed to SAR 0.48 W/kg: The heart, kidney, prostate and epididymis show no remarkable changes when compared to the control group. Mild reduction of germ cells was observed in the testis and the seminal vesicle, cellular cloudiness was observed in the liver (Plate 4.1R and Plate 4.1W). The group exposed to SAR 0.95 W/kg: The kidney, heart and prostate were normal no histophatological evidence of cell injury when compared to the control, while testis

shows numerous somniferous tubule of germ cell at various stages of maturation. However, there is mild reduction in the number of mature germ cell as compared to control group. In the epididymis, there is low secretion of germ cell but no obvious damage to cells. Mild reduction in the number of spermatozoa was observed in the seminal vesicle as compared to control group and the liver shows mild oedema with cloudiness (Plate 4.1S and Plate 4.1W)). The group exposed to SAR 1.43 W/kg: The heart, prostate and epididymis are not histologically remarkable. The testis and seminal vesicle show reduction in the number of germ cells, oedema, cloudiness and glomeruli congestion was observed in the liver and the kidney shows occasional congested vessels (Plate 4.1T and Plate 4.1W)). The group exposed to SAR 1.91 and 2.39 W/kg: The heart, kidney and prostate have no remarkable changes compared to control group. The testis, seminal vesicle and epididymis show reduction in the number of germ cells as compared to control group, cellular swelling and cloudiness occasional isolated cell necrosis was observed in the liver (Plate 4.1U and Plate 4.1X).

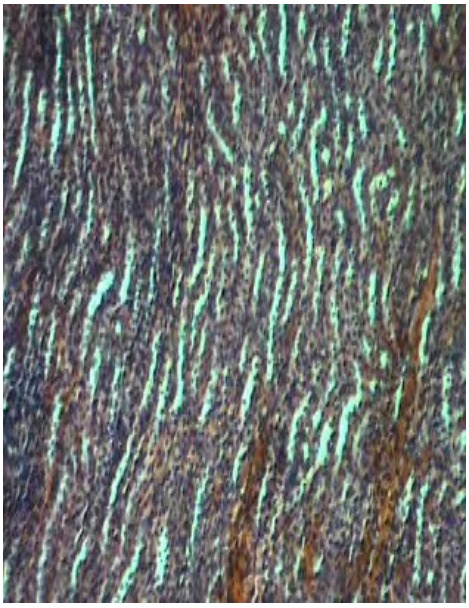
According to the results of this histology, it was evident that the kidney and ovary were most affected in the entire female exposed to MW radiation. Hyperchromasia was observed in the ovary of the animals exposed to MW radiation and this is a descriptive term referring to the hyperchomatic state of nucleus (elevated chomatin) and this state suggests malignancy. Vascular/glomerular congestion, interstitial spaces hemorrhage and tubular cells cloudiness was observed in the kidney and the chief function of the kidney is to process blood plasma and excrete urine. These functions are important because they play a vital role in the clearance and excretion of xenobiotics including drugs and drug-product, from the body. This situation also suggests possibility of renal failure if the radiation SAR is higher than the values used and if exposed for longer time. In the male animals, the testis, seminal vesicle, epididymis and liver were the most affected organs when exposed to various level of MW radiation. Oedema, cloudiness, glomeruli congestion and occasional cell necrosis was observed in the liver the severity of the pathology was SAR dependent.

A



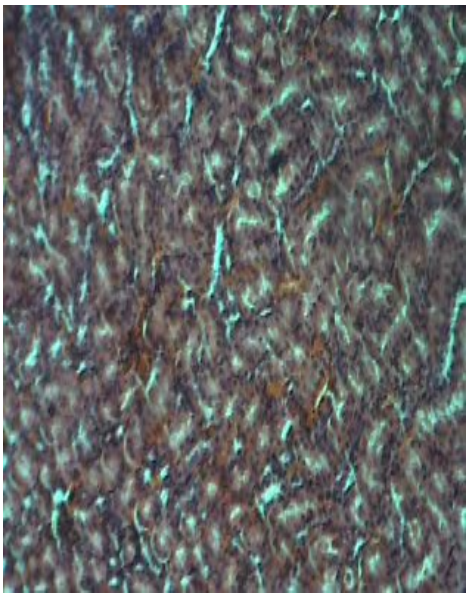
Micrograph of the kidney of female rats in the control group showing normal arrangement of cells H and E stain. Mag. x 40

B



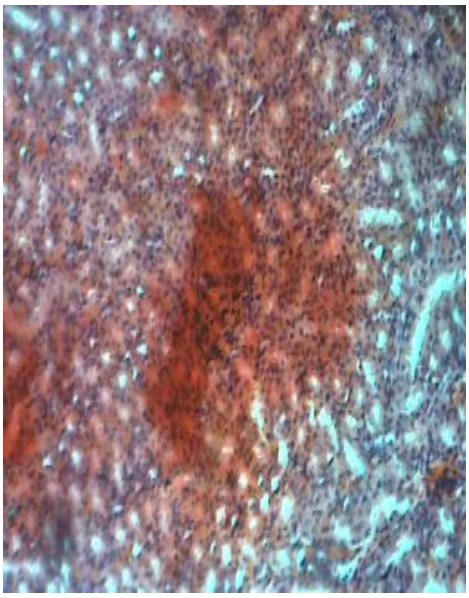
Micrograph of the kidney of female rats in the group exposed to SAR 0.48 W/kg showing foci of lymphocytic aggregate H and E stain. Mag. x 40

C



Micrograph of the kidney of female rats in the group exposed to SAR 1.43 W/kg showing cloudy vascular /tubules epthilinear lining, glomeruli congestion and cellular oedema H and E stain. Mag. x 40

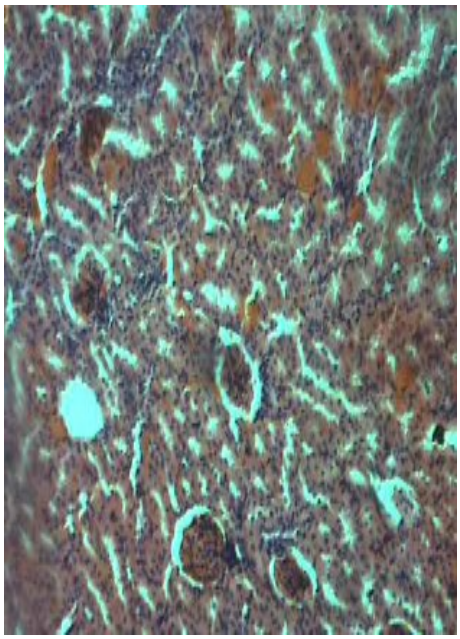
D



Micrograph of the kidney of female rats in the group exposed to SAR 1.91 W/kg showing glomeruli congestion, interstitial space heamorrage and cellular cloudiness H and E stain Mag. x 40

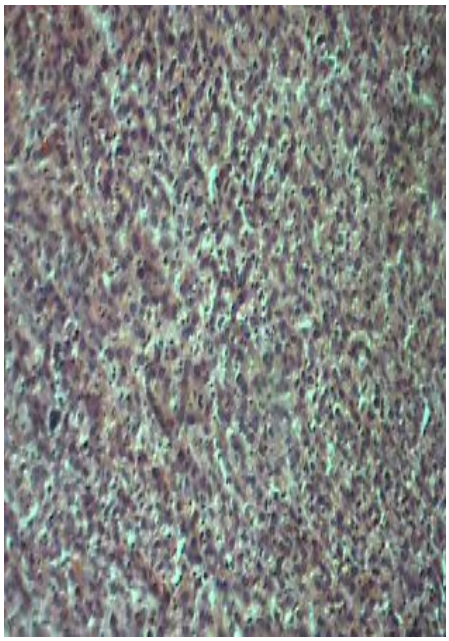
Plate 4.1A - D

E



Micrograph of the kidney of female rats in the group exposed to SAR 2.39 W/kg showing glomeruli congestion, interstitial space heamorrage and cellular cloudiness H and E stain Mag. x 40

F

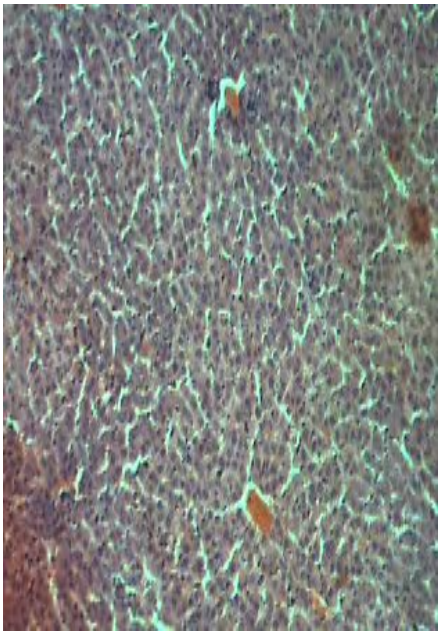


Micrograph of the liver of female rats in the control group showing normal arrangement of cells H and E stain Mag. x 40

G

Micrograph of the liver of female rats in the group exposed to SAR 0.95 W/kg showing cell isolated loudiness and oedema

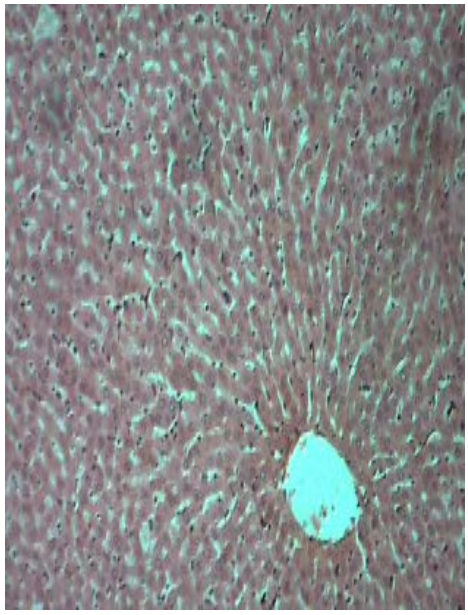
H



Micrograph of the liver of female rats in the group exposed to SAR 1.43 W/kg showing pyknotic heptacyte, increased mitotic cloudy cytoplasm and increased hyperchomasia

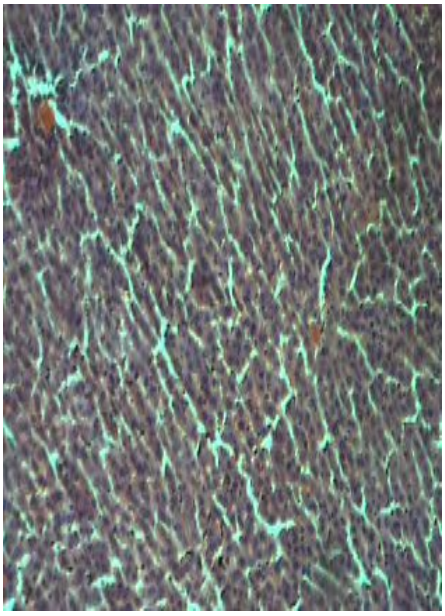
Plate 4.1E - H

I



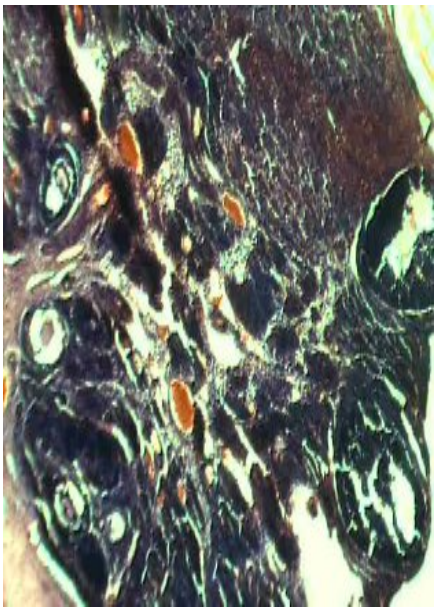
Micrograph of the liver of female rats in the group exposed to SAR 1.91 W/kg showing cloudiness

J



Micrograph of the liver of female rats in the group exposed to SAR 2.39 W/kg showing cellular oedema and narrow sinusoids

K



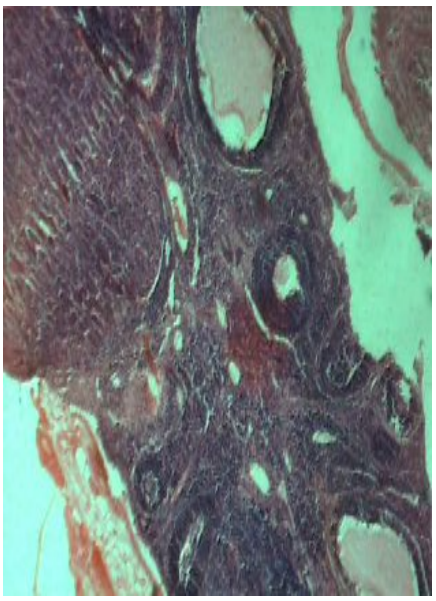
Micrograph of the ovary in the in the control group showing normal arrangement of follicles

L

Micrograph of the ovary in the in the group exposed to SAR 0.48 W/kg showing thick sectioning coarse chomatin and oedema

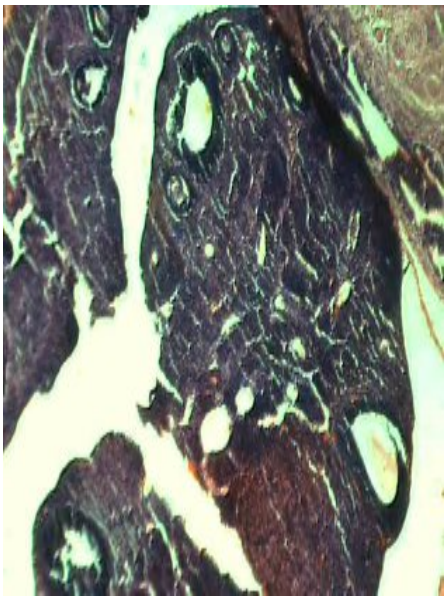
Plate 4.1I- L

M



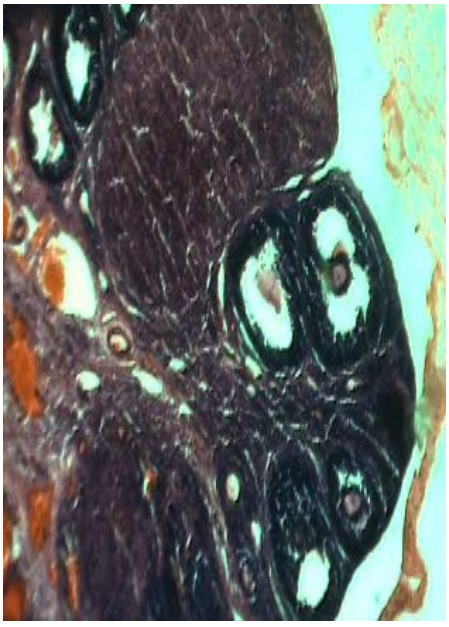
Micrograph of the ovary in the in the group exposed to SAR 0.95 W/kg containing few varying sizes

N



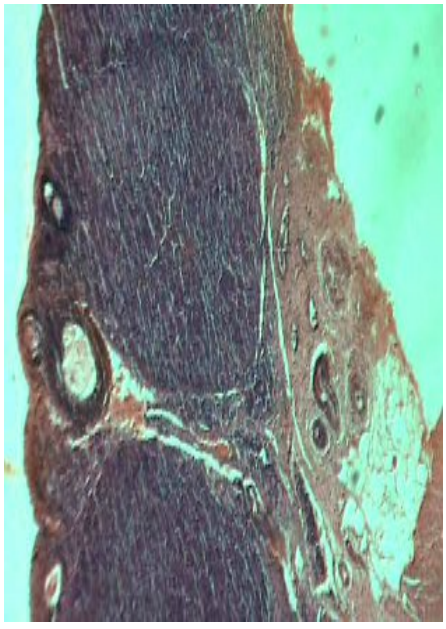
Micrograph of the ovary in the group exposed to SAR 1.43 W/kg showing increased ,follicles of hyperchomasia coarse nuclear chomatin and cellular oedema

O



Micrograph of the ovary in the group exposed to SAR 1.91 W/kg showing vascular congestion Hyperchomatic granulocer cell and cellular oedema

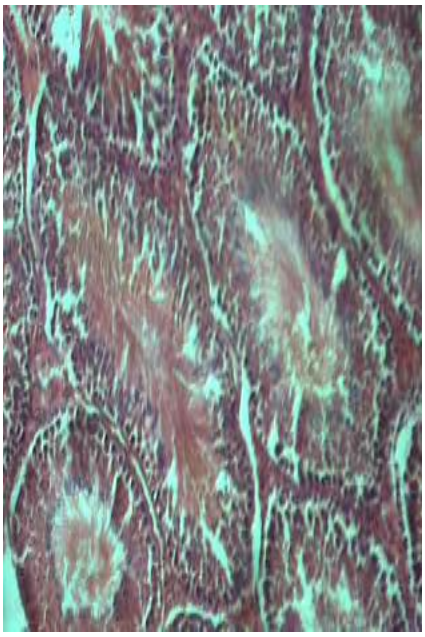
P



Micrograph of the ovary in the group exposed to exposed to SAR 2.39 W/kg showing cellular oedema and hyperchomatism in some of the follicles

Plate 4.1M - P

Q

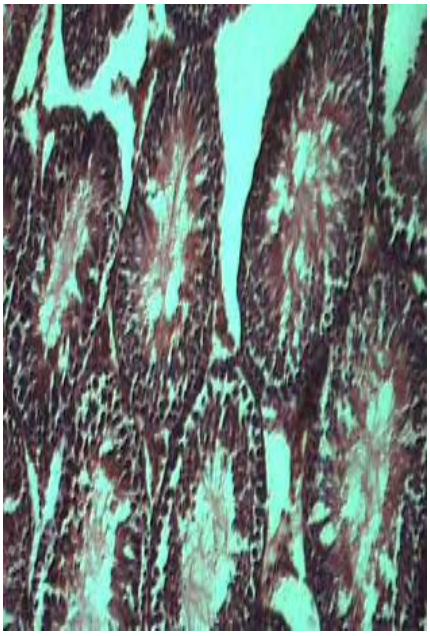


Micrograph of the testis in the in the control group showing normal arrangement of germ cells

R

Micrograph of the testis in the group exposed to SAR 0.48 W/kg showing reduction in the number of germ cells as compared to control.

S



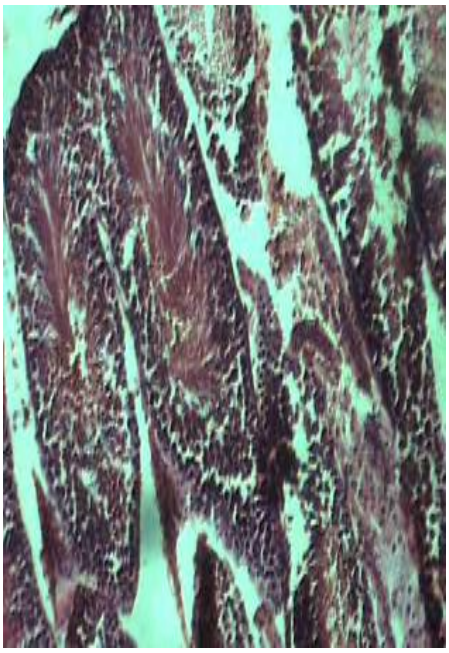
Micrograph of the testis in the group exposed to SAR 0.95 W/kg showing reduction in the number of germ cells as compared to control

T

Micrograph of the testis in the group exposed to SAR 1.43 W/kg showing mild reduction in the number of germ cells as compared to control

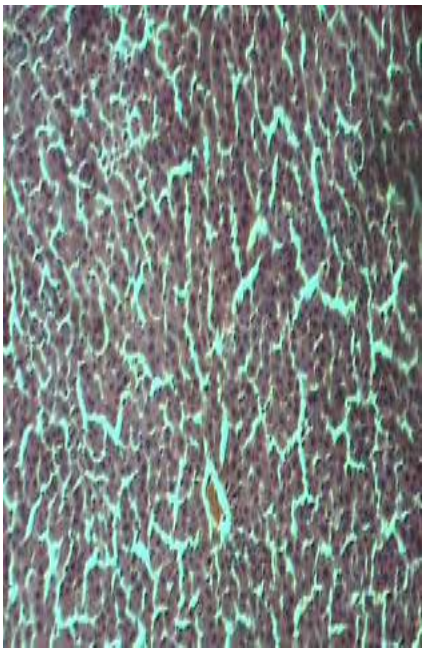
Plate 4.1Q - T

U



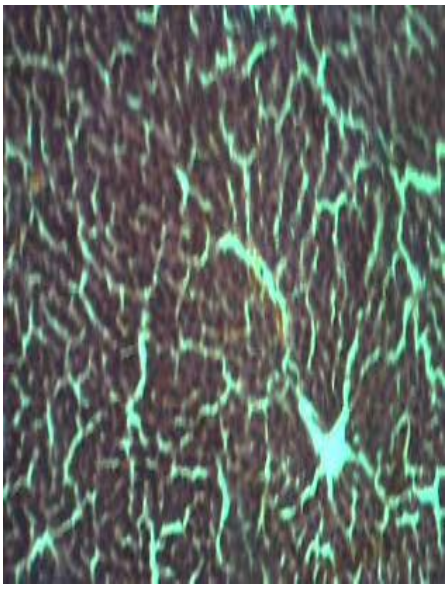
Micrograph of the testis in the group exposed to SAR 1.91 W/kg showing reduction in the number of germ cells as compared to control

V



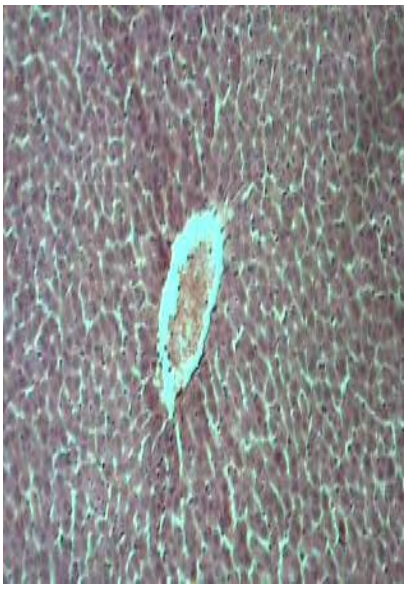
Micrograph of the liver of male rats in the control group showing normal arrangement of cells

W



Micrograph of the liver in the group exposed to SAR 0.48, 0.95 and 1.43 W/kg showing cloudiness and glomeruli congestion

X



Micrograph of the liver in the group exposed to SAR 1.91 and 2.39 W/kg showing cellular swelling, cloudiness and occasional isolated cell necrosis

Plate 4.1U - X

4.4 Effects of Microwave Radiation on the Chromosomal Study

Microwave showed the ability to induce the following structural chromosome aberrations: gaps; acentric; breaks and centric rings. The number of structural aberration obtained is presented in Figure 4.18 for male rats. Group exposed to SAR 2.39 W/kg have the highest gaps (6.45 ± 0.24); highest breaks (7.28 ± 0.08); highest acentric (8.11 ± 0.15) and centric rings (1.24± 0.21). The exposure of the animals to various SARs produced significant difference in all the exposed groups compared with the control. The effects of the exposure on the chromosomal aberration is SAR-dependent as the all lowest structural aberrations were observed in group exposed to SAR 1.0 W/kg.

The same trend was observed in female rats. Group exposed to SAR 2.5 W/kg have the highest gaps (6.45 ± 0.24); highest breaks (7.28 ± 0.08); highest acentric (8.11 ± 0.15) and centric rings (1.24± 0.21). The lowest chromosomal aberrations were consistently observed in the group exposed to SAR 0.95 W/kg. The effect of MW radiation on the chromosomal aberrations as observed in this study was not sex-dependent as there was no significant difference between the measured values for both male and female rats, there is correlation between the values obtained for both sexes (C = 0.96). The number of chromosomal aberration in female rats is shown in Figure 4.19 which displays the variations in the measured values for clear comparisons.

This finding correlates with the result of Mashevich et al. (2003) they reported a linear increase in chromosome 17 aneuploidy as a function of SAR value in HPBLs exposed for 72 h to continuous 830 MHz in the SAR range of 1.6–8.8 W/kg. Data related to the genotoxic potential of MF is an important basis for the assessment of MF-induced cancer risk. Although past investigations have suggested that ELF-MF induces genotoxicity such as strand breaks, clustered DNA damages and micronuclei formation, the identity of specific lesions responsible for these biological effects of ELF-MF remains elusive (Simko´ & Mattsson 2004). However, it is thought that MW shows its effects by increased ROS, although there is no clear information on this issue. On the other hand, it is known that unrepaired oxidatively induced DNA base modifications can lead to genomic instability, chromosomal abberations and abasic site (Yokus et al. 2005, Simko´ 2004).

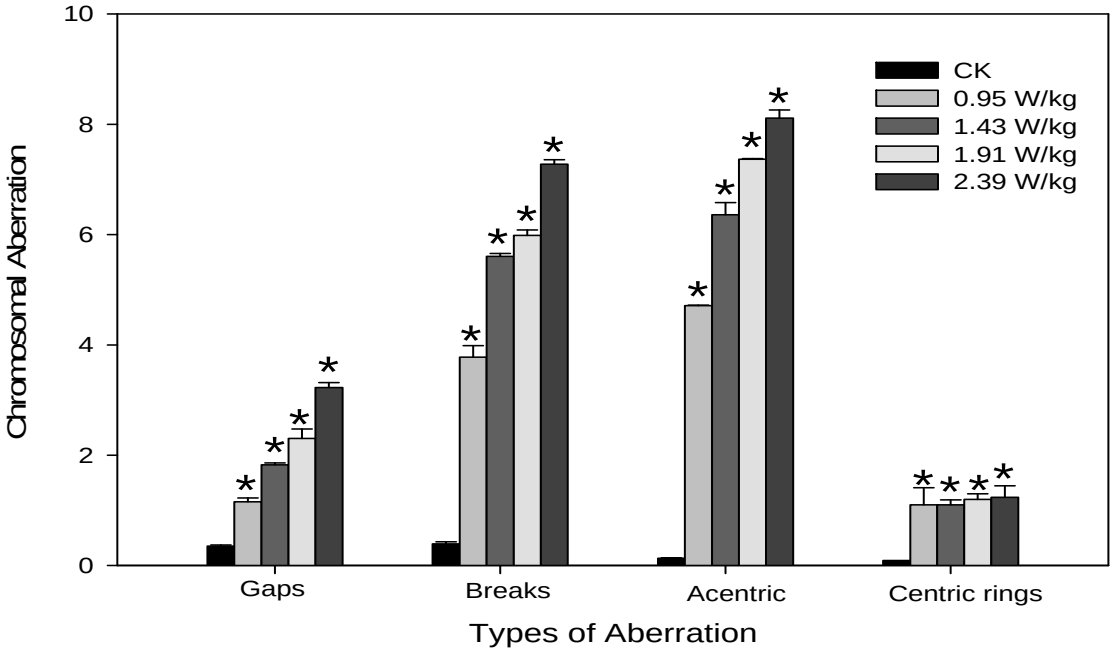


Fig. 4.18: Variations of the Chromosomal Aberrations in Male Rats

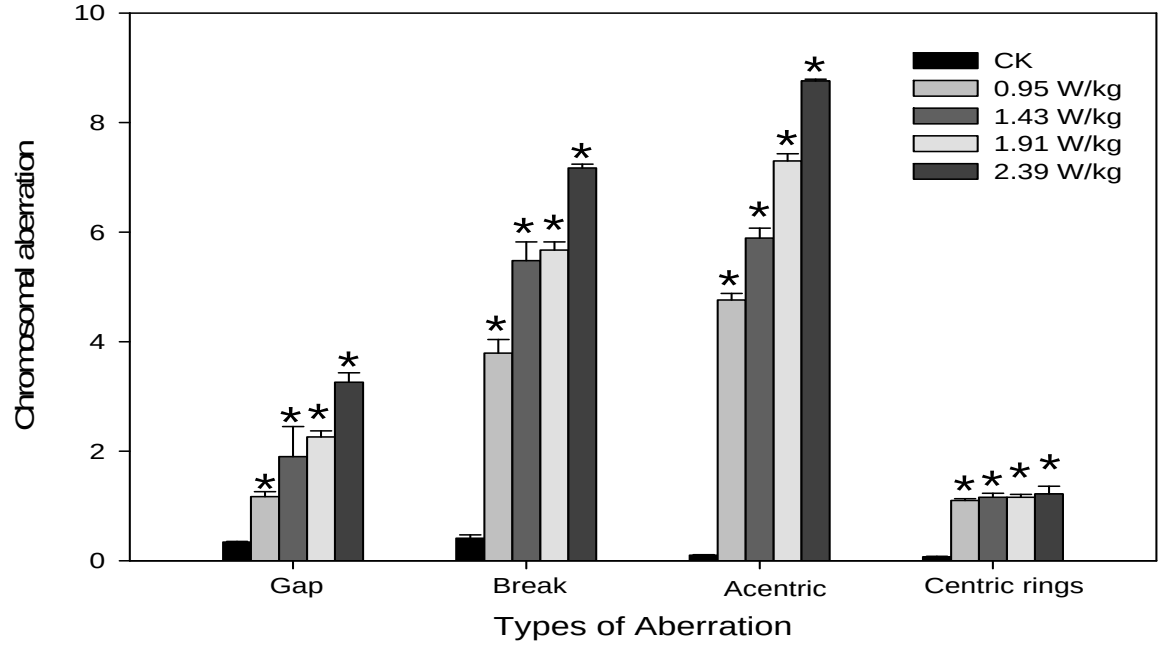


Fig. 4.19: Variations of the Chromosomal Aberrations in Female Rats

* indicates significantly different ($p < 0.05$) compared to control

4.5 Genotoxic Effects of Microwave

4.5.1 Microwave Effects on DNA Direct Amplification of Length Polymorphisms (DALP) Analysis

It was observed from the electrophoresis direct analysis of DNA from various tissues that MW is capable of inducing genotoxic effects on living tissues. Plates 4.2 to 4.11 are the photos of the electrophoresis gel and the densitometric analysis of various lane are shown in Figure 4.20 to 4.29. A multilocus monomorphic band profile was demonstrated with direct amplification of length polymorphisms (DALP) of rats DNA in both tissues studied. The hybridization profile of DNA of all tissues show a sharp bands (molecular weight 58 bp) in both the control and the exposed group of animals as shown in all the figures. However, in the exposed animals, an additional band appeared below and above this particular band (40 - 200 bp). Gel track analysis of the control (lane a) of all the figures and in the tail of the animals before exposure to MW radiation Figure 4.27 consistently have sharp peak marked 1 at the same molecular weight. While in the exposed animals there are always additional peaks either before or after this particular peak marked 2,3,4, etc. Multilocus monomorphic band profiles (Ali *et al*., 1993) are particularly useful because any loss or gain of band due to sequence rearrangement or sporadic mutation can be easily detected. In this work both control and exposed animals have similar profile with exception of additional fragment specific to all exposed animals, which is not present in any of the control animals. Since this particular fragment is not present in the control or even the same animals before exposure to MW radiation but appears after microwave exposure, it is suggested that probably in the unexposed animals, the copy number of these repeat sequences is not sufficient to form a distinct band. Microwave exposure may have led to the amplification of these tandem sequences generating more copies of sequences in this particular region. Although it is not known at present whether exposure to a mutagenic agent or a specific class of mutagens increases the mutation rate in the region of these tandem repeat units, it is known that stress induces amplification by extra replication of DNA segments in the non-coding repeat sequences (Ramel, 1989). The observed change of DNA rearrangement after exposure, in the present study, can be attributed to some sort of non-specific stress created by MW field and is not a result of indirect thermal effects. The work of Pilla (1979) indicated the existence of a mechanism of interaction of weak electromagnetic fields with biological systems with no accompanying cell heating. Ehling (1989) has suggested

that germ cell mutation in mice can be used as a standard for protecting the human genome. Integrity of genetic information is fundamental for living systems.

The results also revealed that each tissue has different sensitivity to microwave radiation as the extra peaks were distinct in some tissues even for 0.48 W/kg, while in some it was not distinct until after 1.91 W/kg. For instance, in brain, blood and liver DNA (Plates 4.3, 4.2 and 4.6) right from 0.48W/kg the extra peak was obvious while in kidney it was not seen distinctly until lane f (Plate 4.8) corresponding to 2.9 W/kg. In thyroid the extra peak was not seen until 4.5 W/kg whereas in the prostate there was total deletion of this peak at this SAR lane j (Plate 4.9B). The effects studied are SAR dependent and sex dependent. Additional peaks were observed in the female brain and liver DNA more than that of male ones (Plate 4.3 and 4.6). This may suggests that female brain and liver DNA are more sensitive to MW exposure. The differences in tissues sensitivity and different exposure conditions used may also account for the disparity in the results obtained by different researchers regarding MW effects.

4.5.2 Microwave radiation Effects on Comet Assay

This finding showed that exposure to 2.39 W/kg of 2.45 GHz MW radiation can result in single strand DNA break in brain, liver, lung, spleen and testis cells of rats. The mean ± SD of theolive moment and % tail DNA obtained were displayed in Figure 4.30 and 4.31 for clear comparison. It shows that there are significant increases in the tail of DNA and olive moment of the exposed as compared to the control animals. From the result brain has the highest DNA damage comet assay this may suggest that brain cells are more susceptible to MW radiation. Maes *et al*. (1993) observed that acute 30–120 min exposure to 2.45 GHz at SAR 75 W/kg and constant temperature, 36.1^{o} C increased dicentric and acentric chromosomal fragments and micronuclei formation in human lymphocytes. Mitchell *et al*. (1988) result showed a decrease in motor activity in rats after 7 h of exposure to CW 2450 MHz (10 mW/cm^2, average SAR 2.7 W/kg). Lai and Singh (1995, 1996) in their studies reported that acute exposure (2 h) to both pulsed and continuous wave (CW) 2.45 GHz radiation (2 mW/cm^2, SAR 1.2 W/kg) produce a significant increase in the DNA single and double strand breaks in rat brain. According to studies of Sagripanti and Swicord (1986), MW radiation may cause both single and double strand breaks in the DNA molecule and this damage can be correlated to mutagenic and cancerogenic changes (Sargentini and Smith, 1985). The present result is in agreement with these studies showing a significant difference in DNA single strand breaks in the exposed tissues. DNA damage is closely related to human health risk.

Particularly, DNA damage in brain cells could affect neurological functions and also possibly lead to neurodegenerative diseases (Lai and Singh, 1996). It is therefore vital for cells that DNA damage induced by reactive oxygen species (ROS) such as free radicals or by other mutagens is effectively recognized and repaired efficiently (Simko´, 2004). Unrepaired or inaccurately repaired DNA damage can lead to cell death as well as to genomic instability, mutations, and ultimately to cancer, aging and other diseases (Halliwell, 2002). It is obvious that MW/RF is not able to induce genotoxic effects by direct interaction with DNA, because their intrinsic (quantum) energy is too low to dislodge an electron from a molecule. Among the putative mechanisms by which MW may affect DNA is increasing free radical life span and the concentration of free radicals in cells (Grissom 1995, Lupke *et al*. 2004). Oxidative damage to DNA caused by free radicals, especially by the highly reactive hydroxyl radical, generates a multiplicity of modifications, which include modified bases and sugars, DNA–protein cross-links, base-free sites and strand breaks (Dizdaroglu 1992). Among the many modified bases generated by free radicals in DNA, 8-OH-Gua is the most investigated lesion as a marker of cellular oxidative stress relevant to mutagenesis and carcinogenesis. This is because this compound has been shown to cause G - T transversions, and because it is readily measured as its nucleoside 8-OH-dG by a method using high-performance liquid chomatography (HPLC) with electrochemical detection (ECD) (Dizdaroglu 1998). However, 8-OH-Gua is not the only product resulting from oxidative damage to DNA. There is a plethora of other products that are formed with yields comparable to that of 8-OH-Gua (Evans *et al*. 2004; Brean *et al*., 2008). 2, 6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde) are typical products in DNA resulting from hydroxyl radical reactions with purines (Evans *et al*. 2004). These lesions are often mutagenic and genotoxic, and have been implicated in the etiology of many diseases, including cancer and aging (Delaney *et al*. 2002, Wiederholt & Greenberg 2002, Kalam *et al*. 2006). Since various tissues or cell types differ in their susceptibility towards EMF exposure, hence conflicting results among the mammalian cell types are reported (REFLEX, 2004).

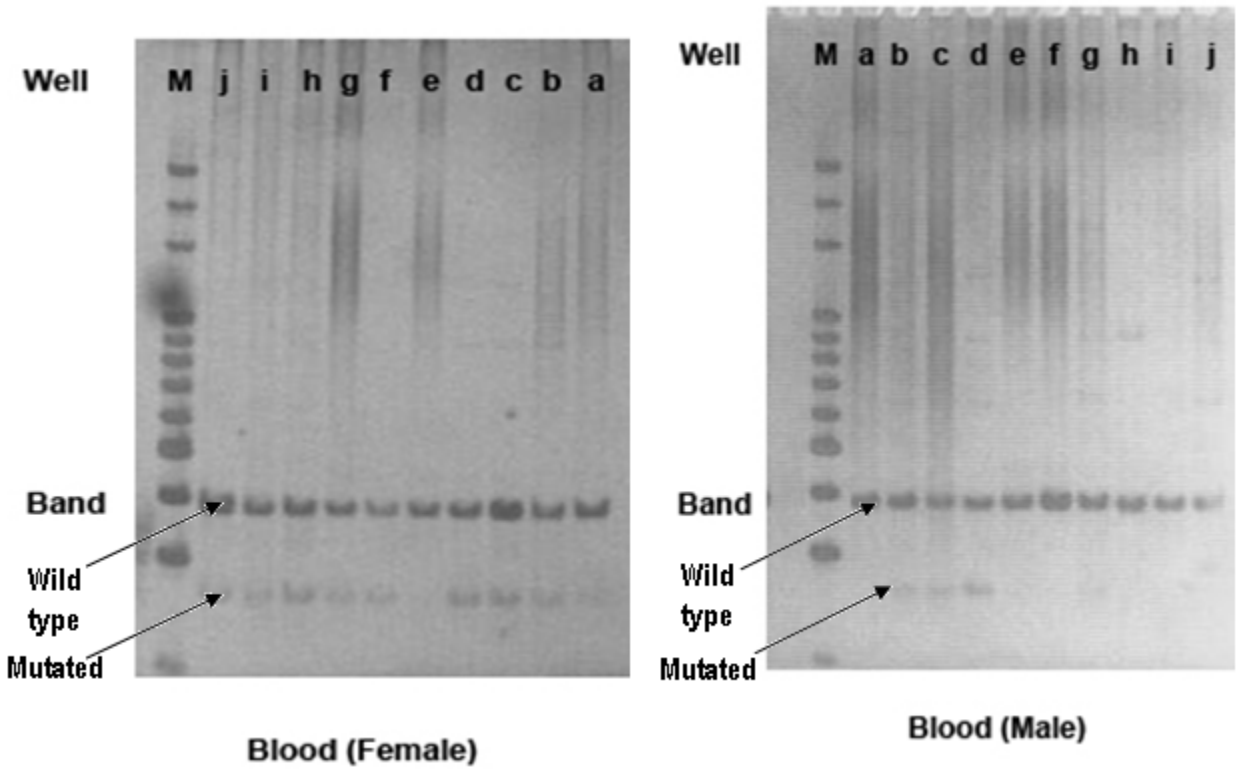


Plate 4.2: Hybridization of the blood DNA in male and female rats. Note the appearance of other bands in lane b –j (exposed animals). Lanes a-j have been tracked densitometrically as shown in Fig. 4. 20

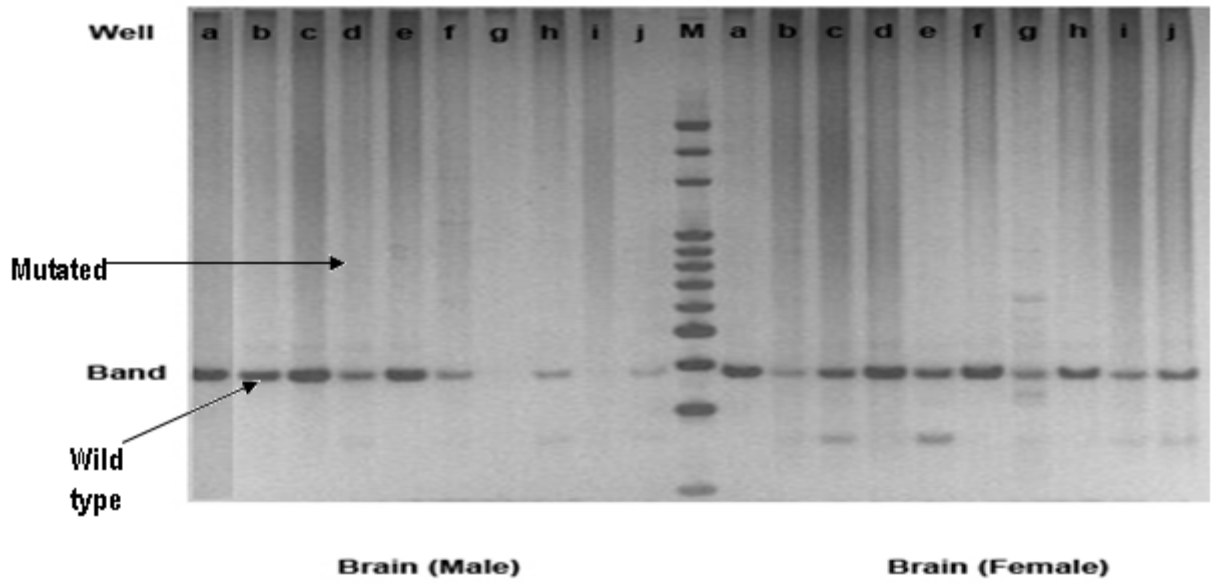


Plate 4.3: Hybridization of the brain DNA in male and female rats. Note the appearance of other bands in lane b –j (exposed animals). Lanes a-j have been tracked densitometrically as shown in Fig. 4.21

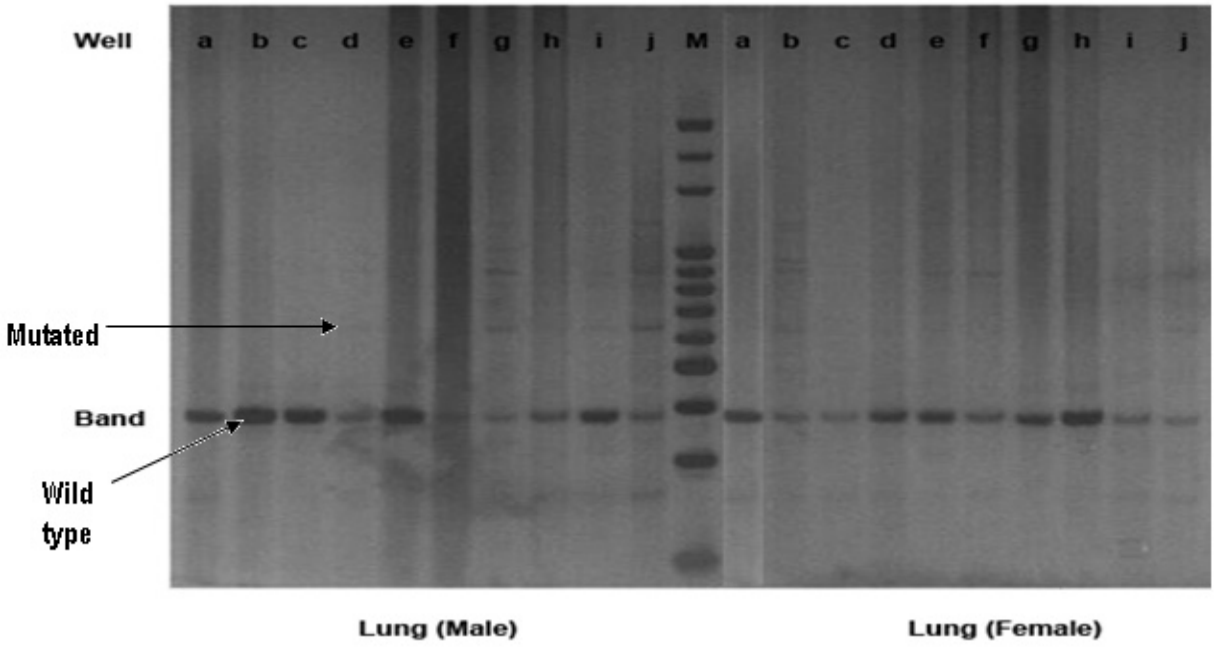


Plate 4.4: Hybridization of the lung DNA in male and female rats. Note the appearance of other bands in lane b –j (exposed animals). Lanes a-j have been tracked densitometrically as shown in Fig. 4.22

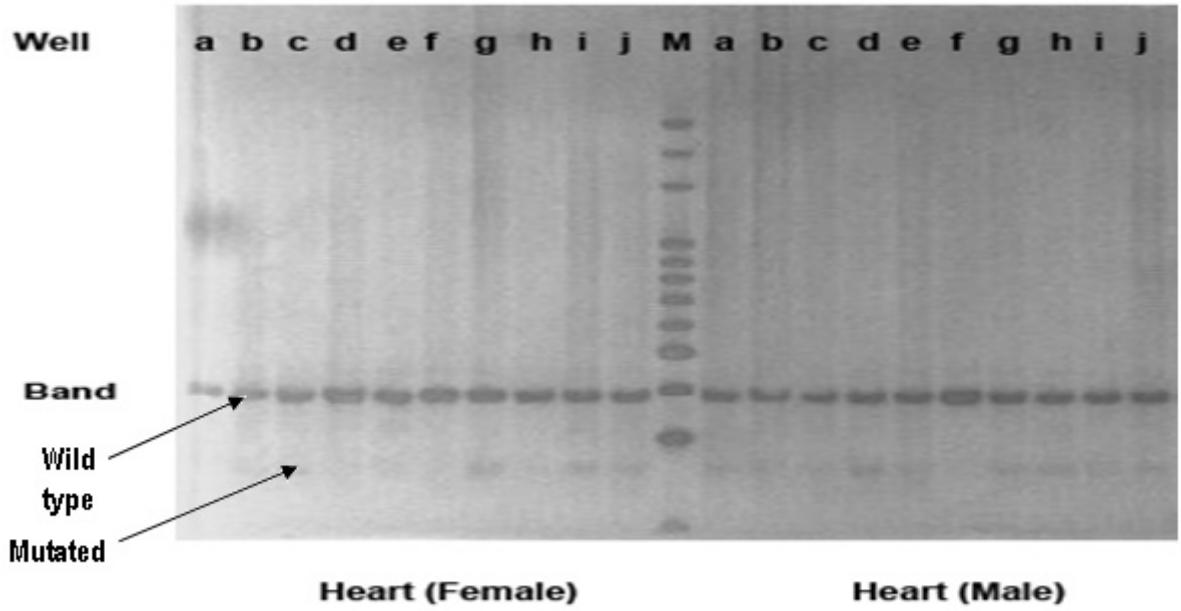


Plate 4.5: Hybridization of the heart DNA in male and female rats. Note the appearance of other bands in lane b –j (exposed animals). Lanes a-j have been tracked densitometrically as shown in Fig. 4.23

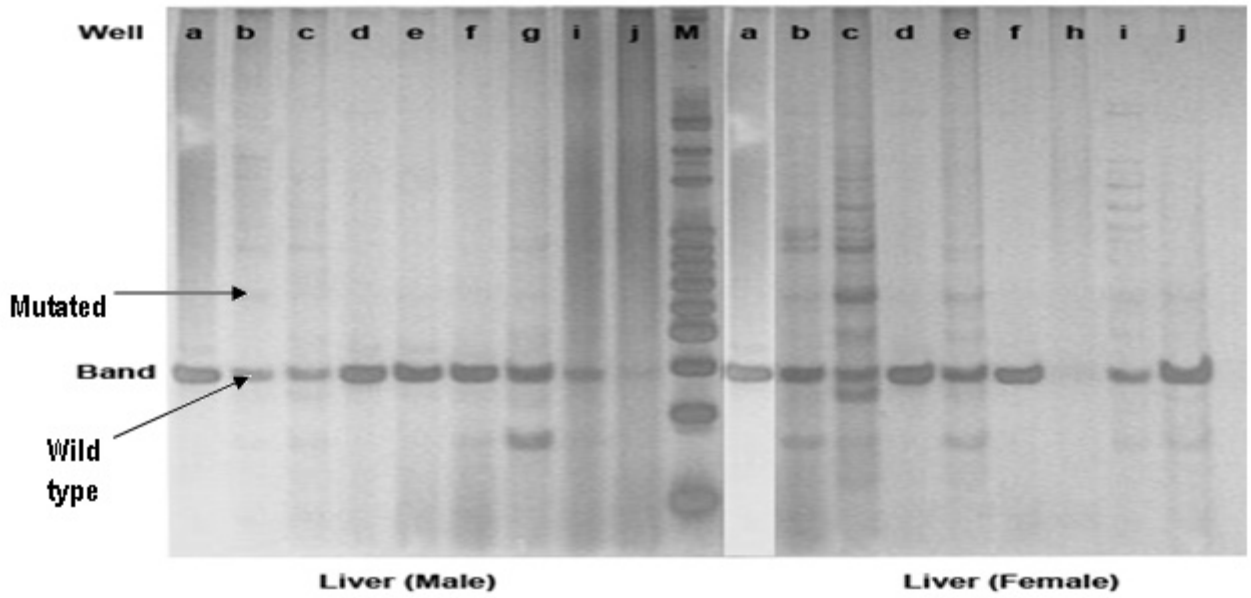


Plate 4.6: Hybridization of the liver DNA in male and female rats. Note the appearance of other bands in lane b –j (exposed animals). Lanes a-j have been tracked densitometrically as shown in Fig. 4.24

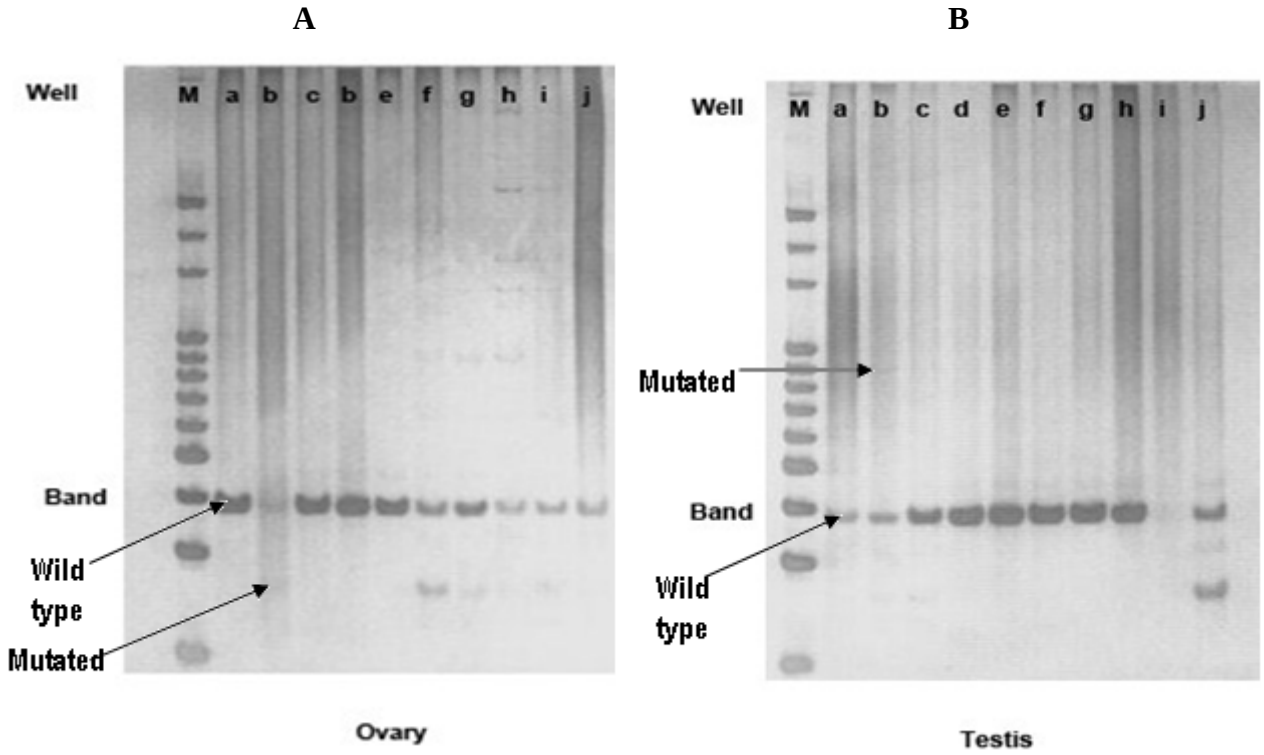


Plate 4.7: A Hybridization of the ovary DNA. B. Hybridization of the testis DNA. Note the appearance of other bands in lane b –j (exposed animals). Lanes a-j have been tracked densitometrically as shown in Fig. 4.25 A and B

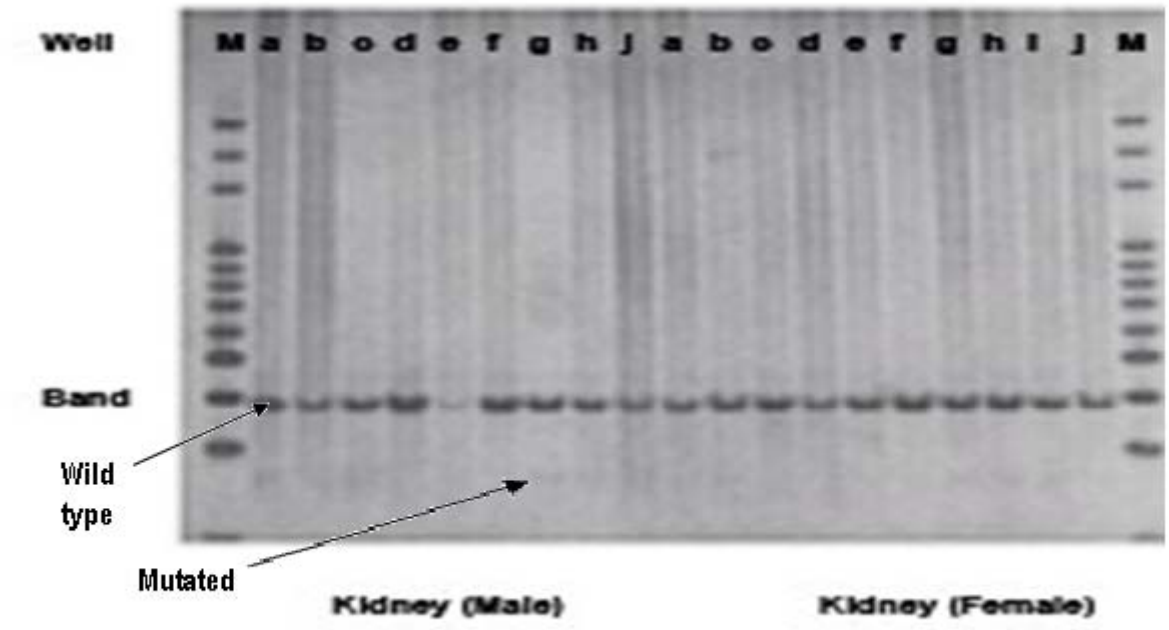


Plate 4.8: Hybridization of the kidney DNA in male and female rats. Note the appearance of other bands in lane b –j (exposed animals). Lanes a-j have been tracked densitometrically as shown in Fig. 5.26

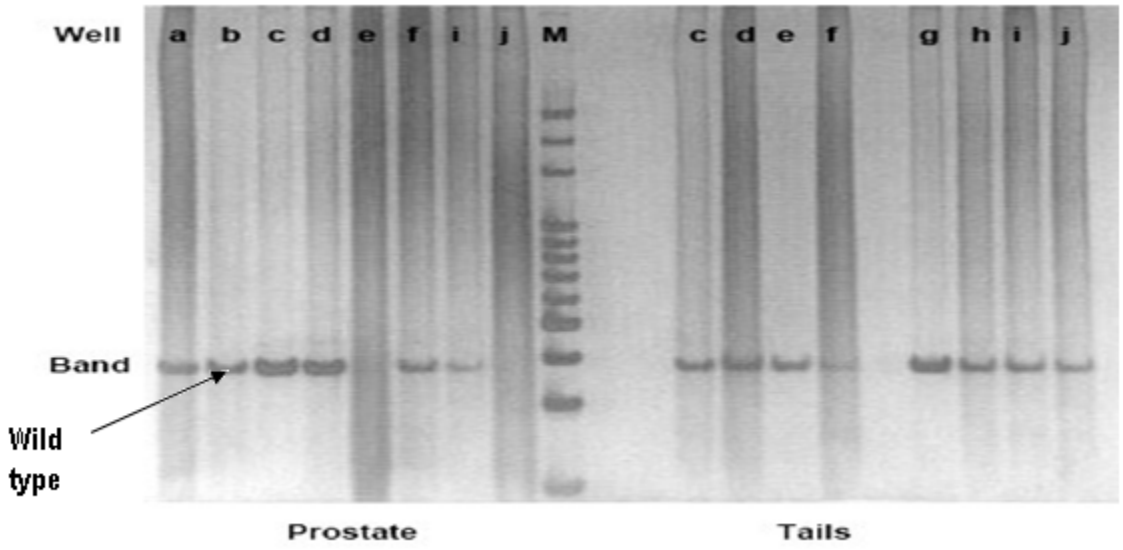


Plate 4.9: Hybridization of the prostate and control tails DNA in male and female rats. Note the appearance of other bands in lane b –j (exposed animals). Lanes a-j have been tracked densitometrically as shown in Fig. 5.27 A and B

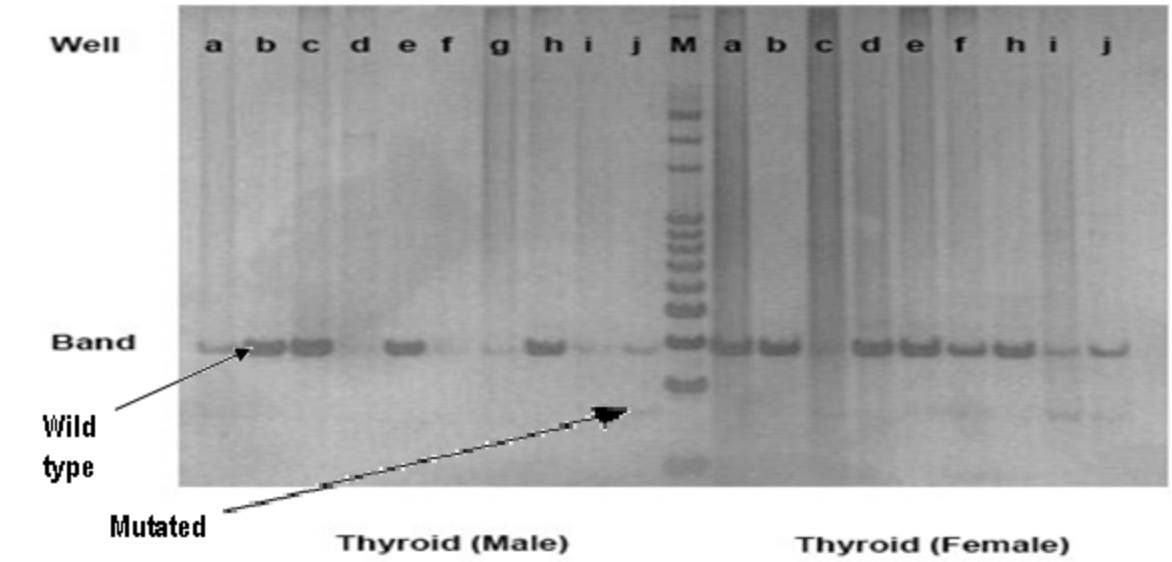


Plate 4.10: Hybridization of the Thyroid DNA in male and female rats. Note the appearance of other bands in lane d –j (exposed animals). Lanes a-j have been tracked densitometrically as shown in Fig. 4. 28

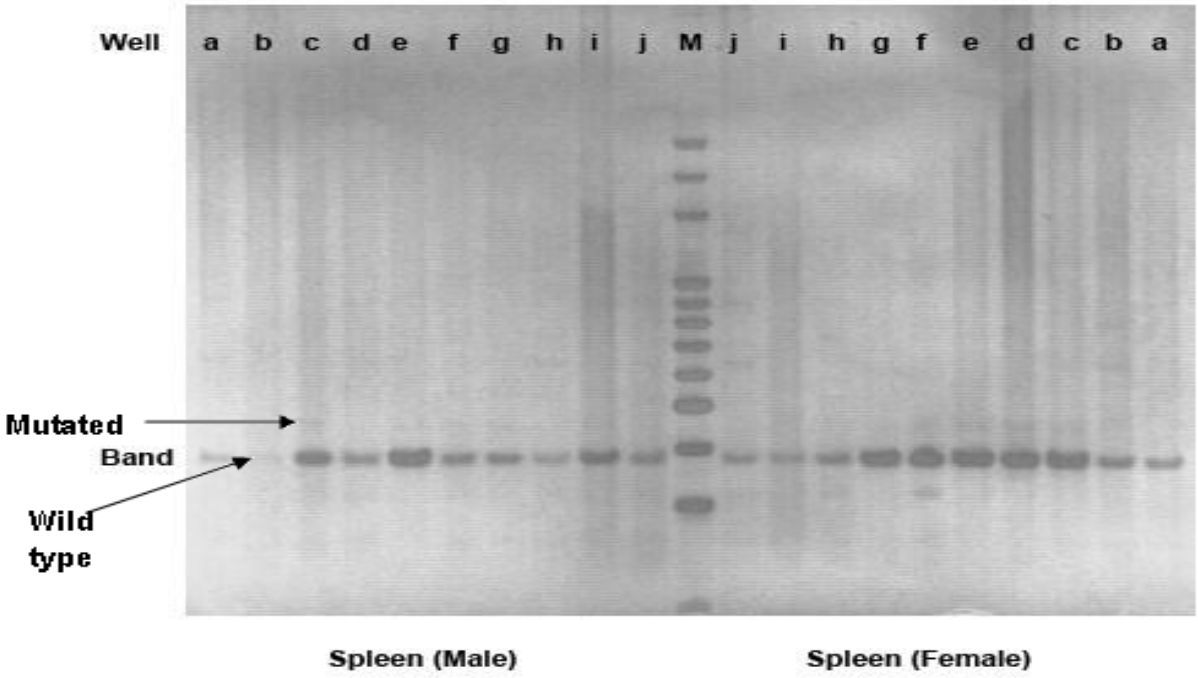
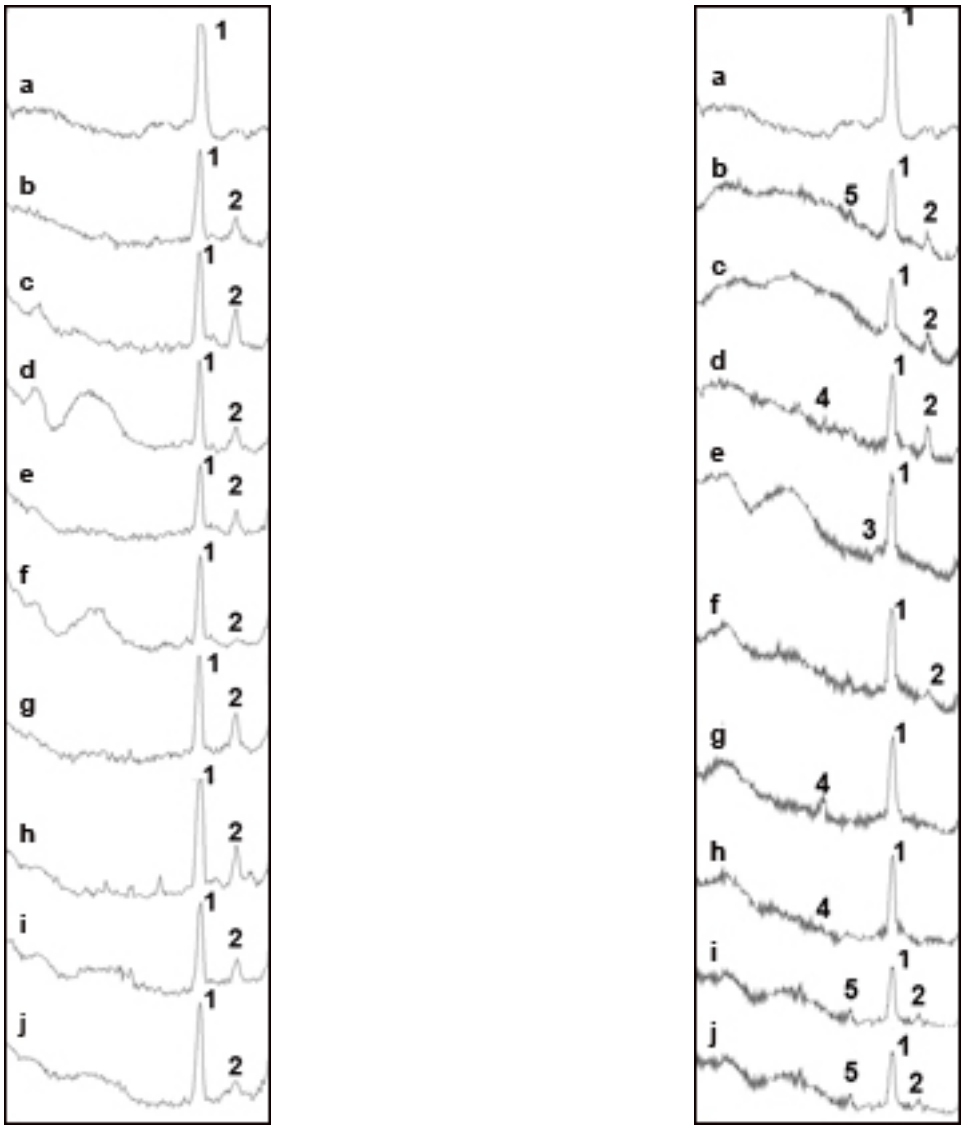
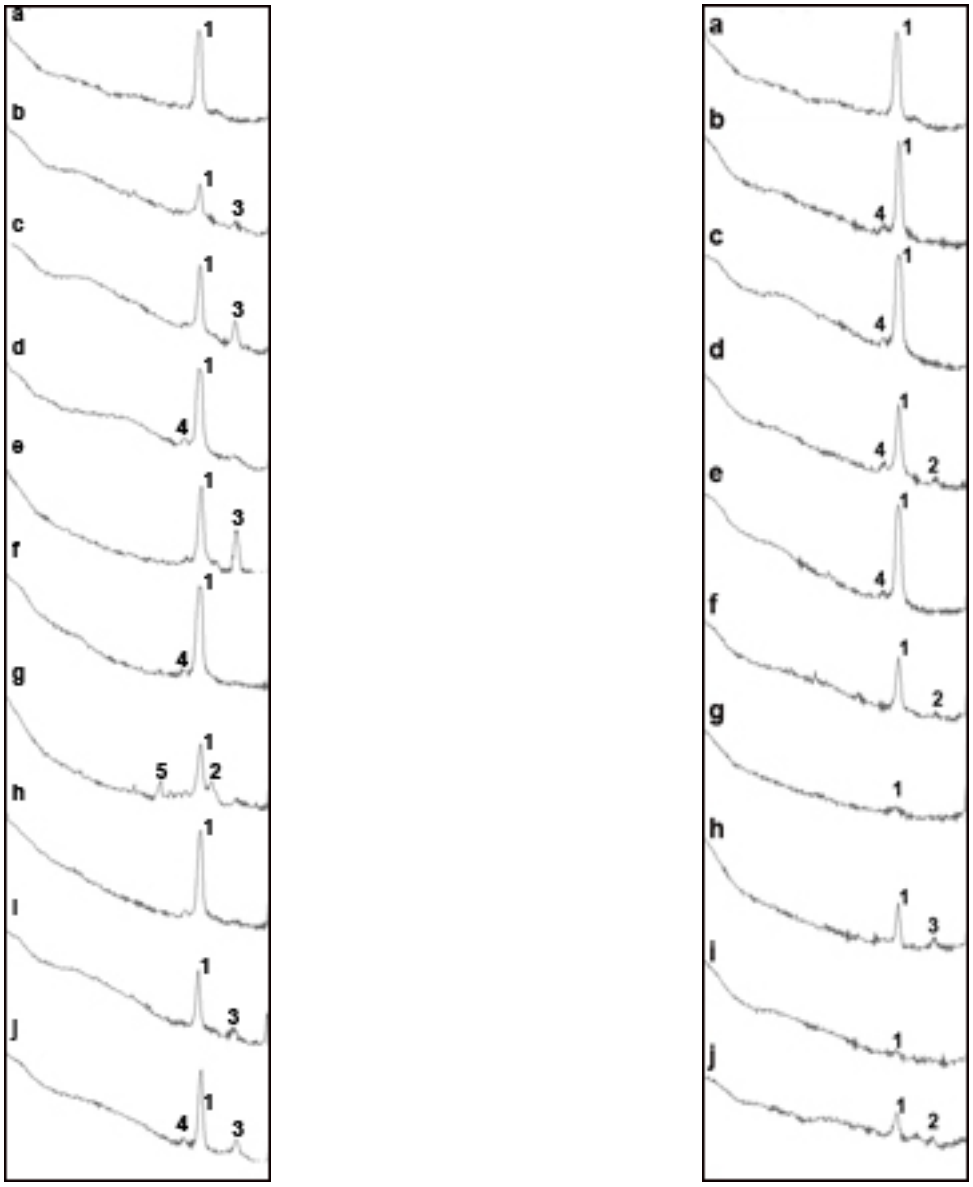


Plate 4.11: Hybridization of the spleen DNA in male and female rats. Note the appearance of other bands in lane b –j (exposed animals). Lanes a-j have been tracked densitometrically as shown in Fig. 4.29



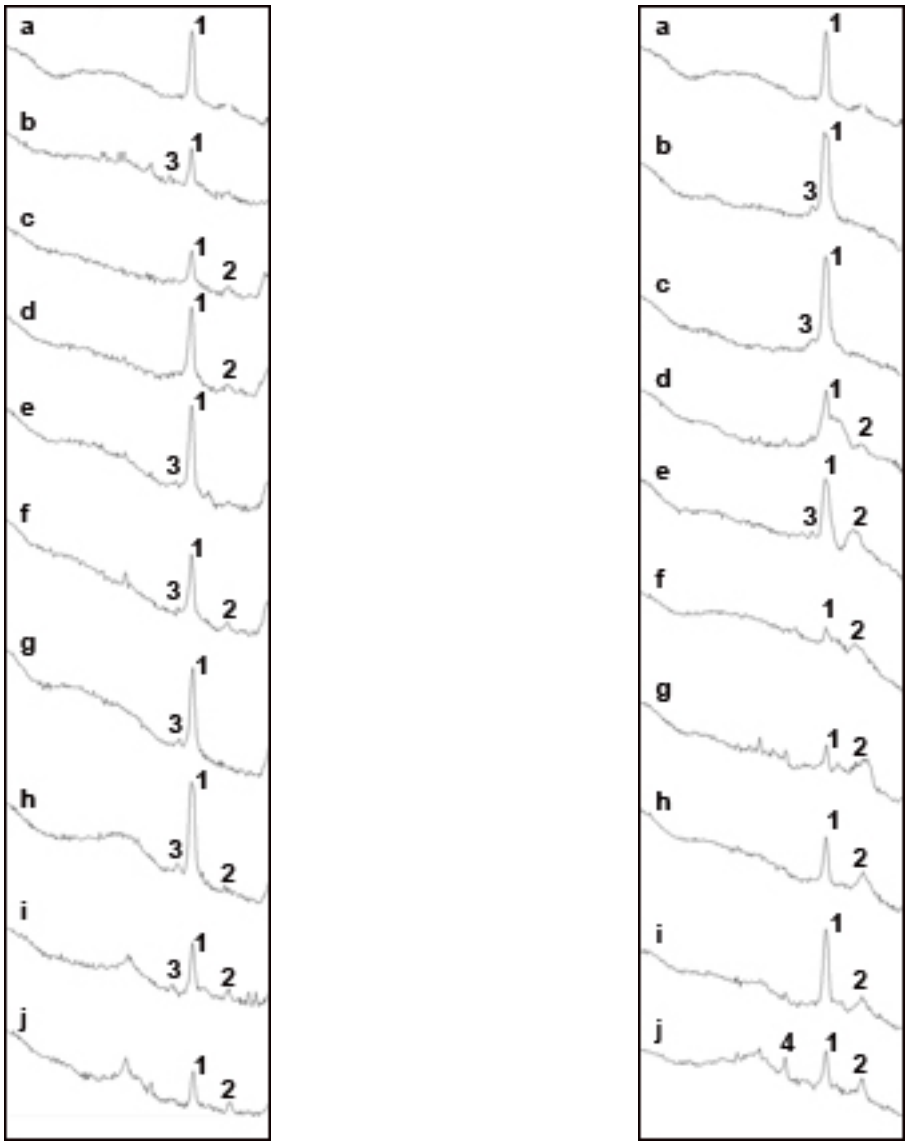
Blood female Blood male

Fig. 4.20: Densitometric track analysis of male and female blood DNA. Lane (a) is the DNA of the control; (b - j) are DNA from exposed animals. Peak 1 is present in both control and exposed while other peaks appear in all exposed animals.



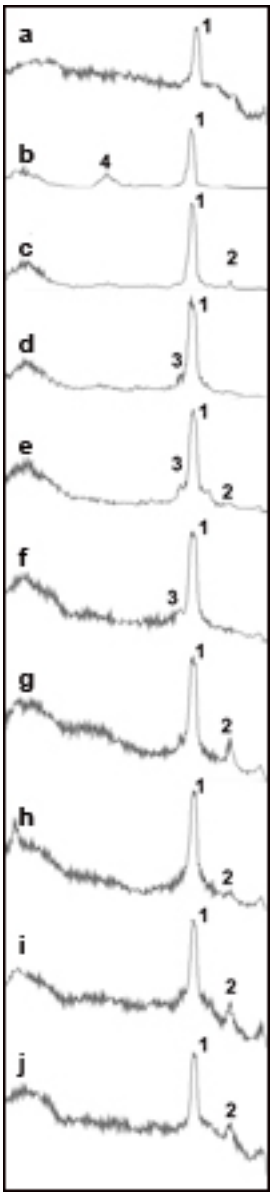
Brain Female Brain male

Fig. 4.21: Densitometric track analysis of male and female brain DNA. Lane (a) is the DNA of the control; (b - j) are DNA from exposed animals. Peak 1 is present in both control and exposed while other peaks appear in all exposed animals.

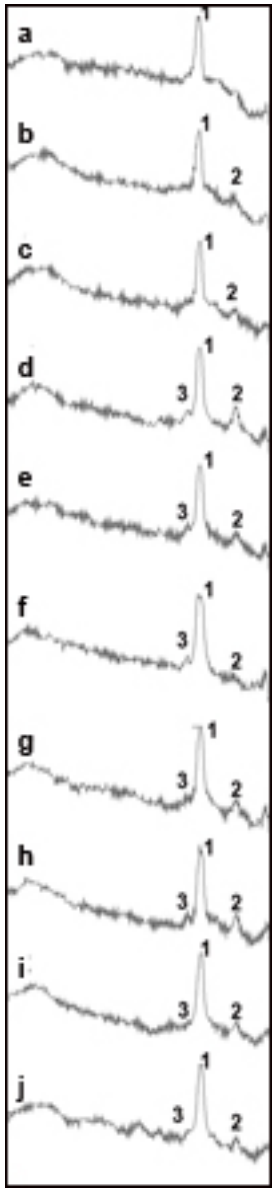


Lung Female Lung Male

Fig. 4.22: Densitometric track analysis of male and female lung DNA. Lane (a) is the DNA of the control; (b – j) are DNA from exposed animals. Peak 1 is present in both control and exposed while other peaks appear in all exposed animals.

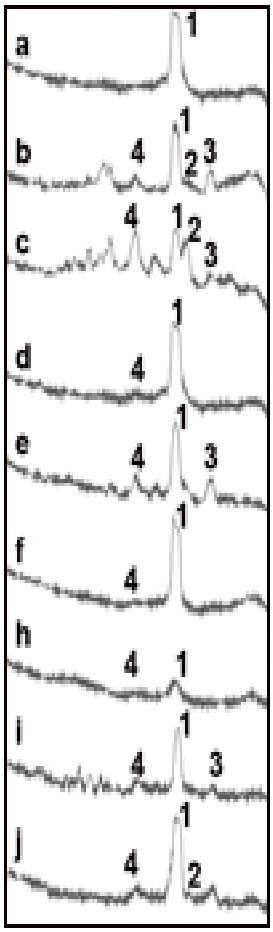


Heart female

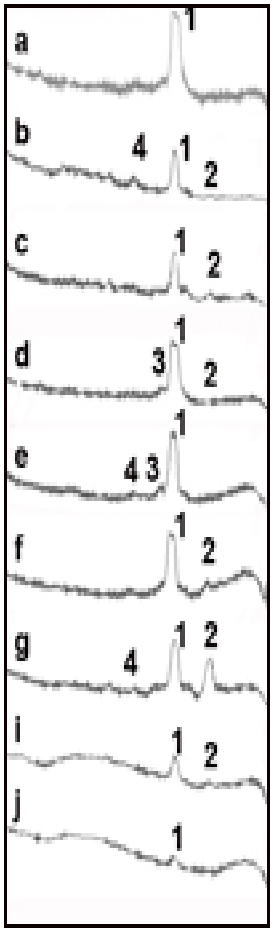


Heart male

Fig. 4.23: Densitometric track analysis of male and female heart DNA. Lane (a) is the DNA of the control; (b – j) are DNA from exposed animals. Peak 1 is present in both control and exposed while other peaks appear in all exposed animals.



Liver female



Liver male

Fig. 4.24: Densitometric track analysis of male and female liver DNA. Lane (a) is the DNA of the control; (b – j) are DNA from exposed animals. Peak 1 is present in both control and exposed while other peaks appear in all exposed animals.

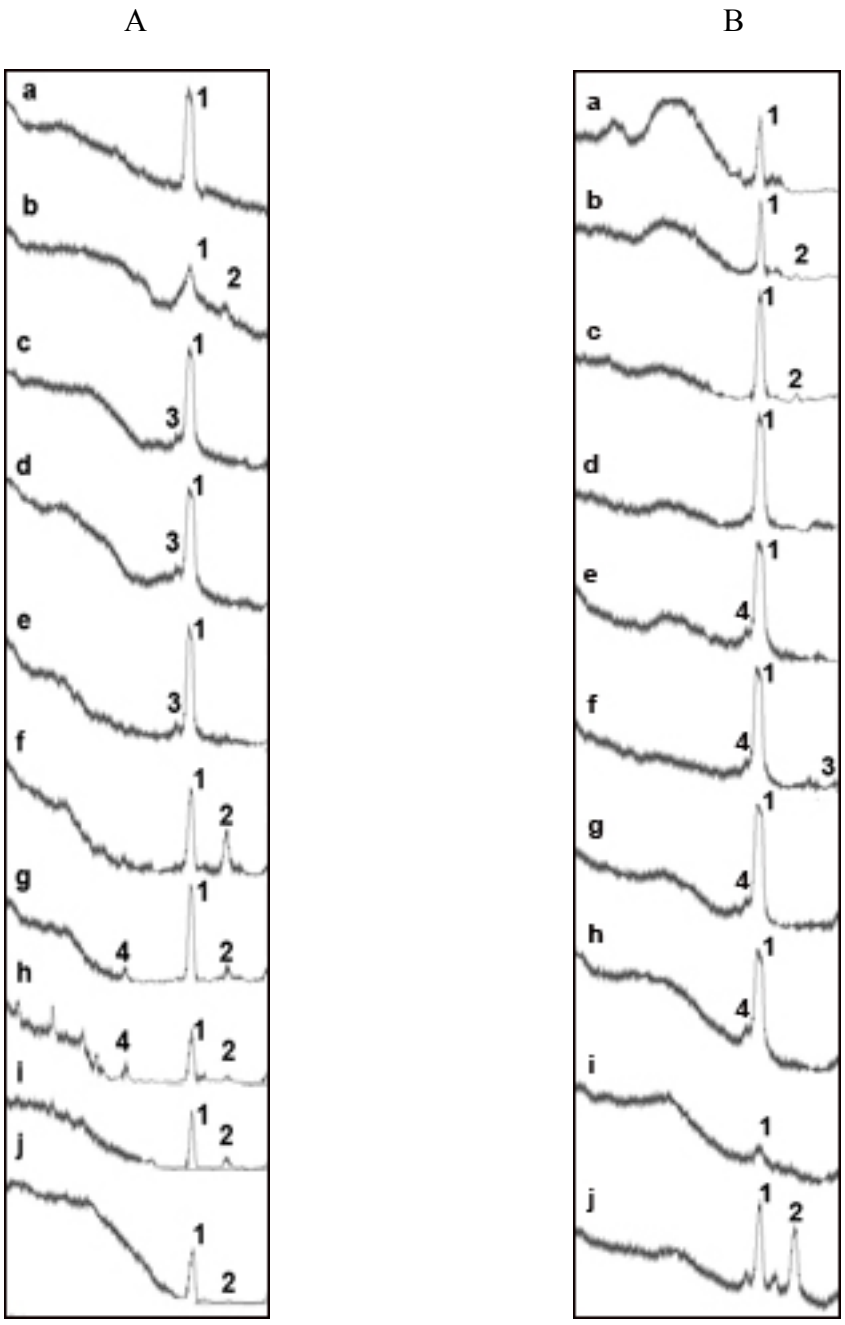
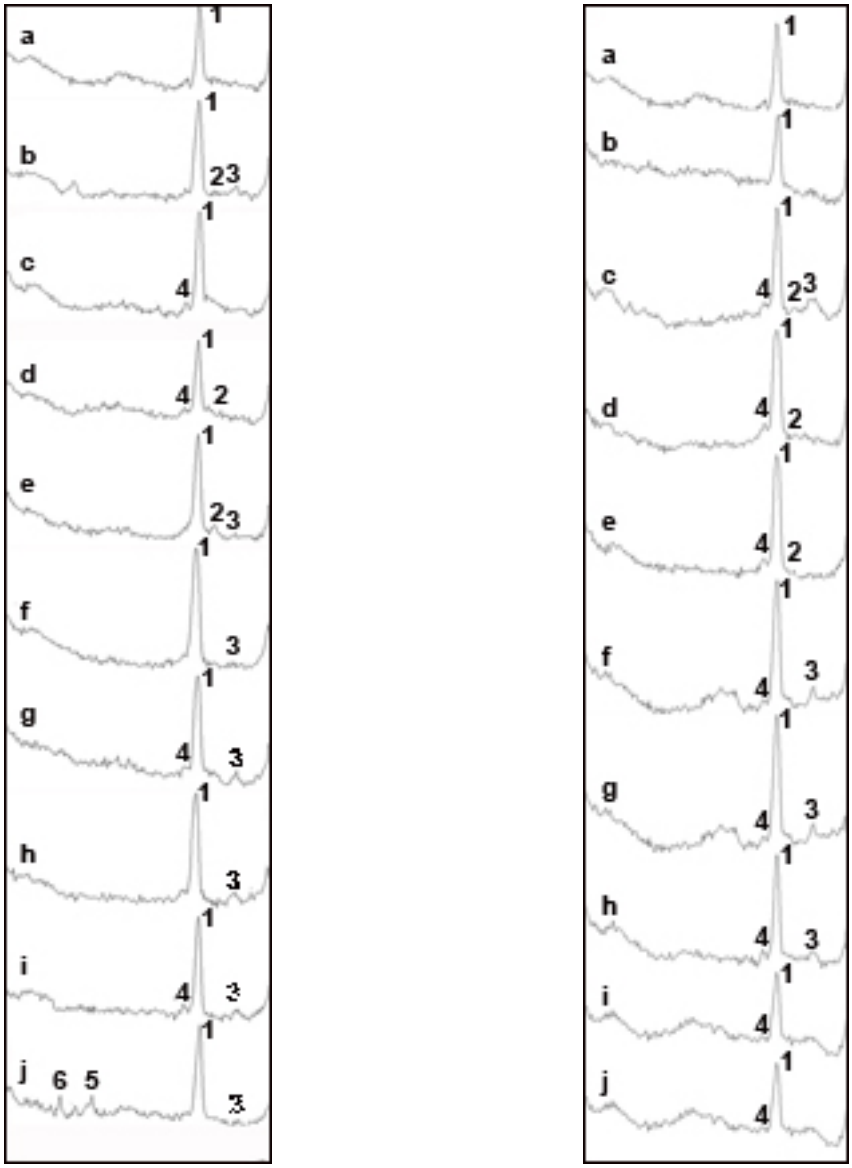


Fig. 4.25: A. Densitometric track analysis of Ovary DNA. B. Densitometric track analysis of Testis DNA. Lane (a) is the DNA of the control; (b – j) are DNA from exposed animals. Peak 1 is present in both control and exposed while other peaks appear in all exposed animals.



Kidney Female Kidney male

Fig.4. 26: Densitometric track analysis of male and female kidney DNA. Lane (a) is the DNA of the control; (b – j) are DNA from exposed animals. Peak 1 is present in both control and exposed while other peaks appear in all exposed animals.

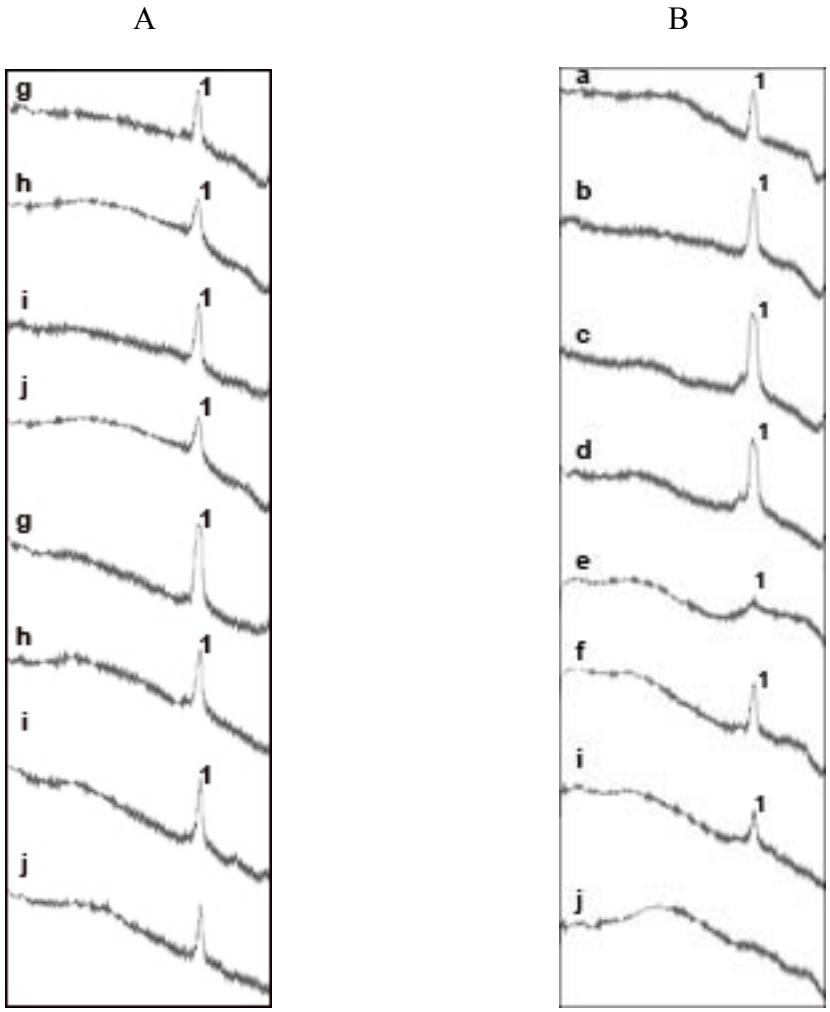
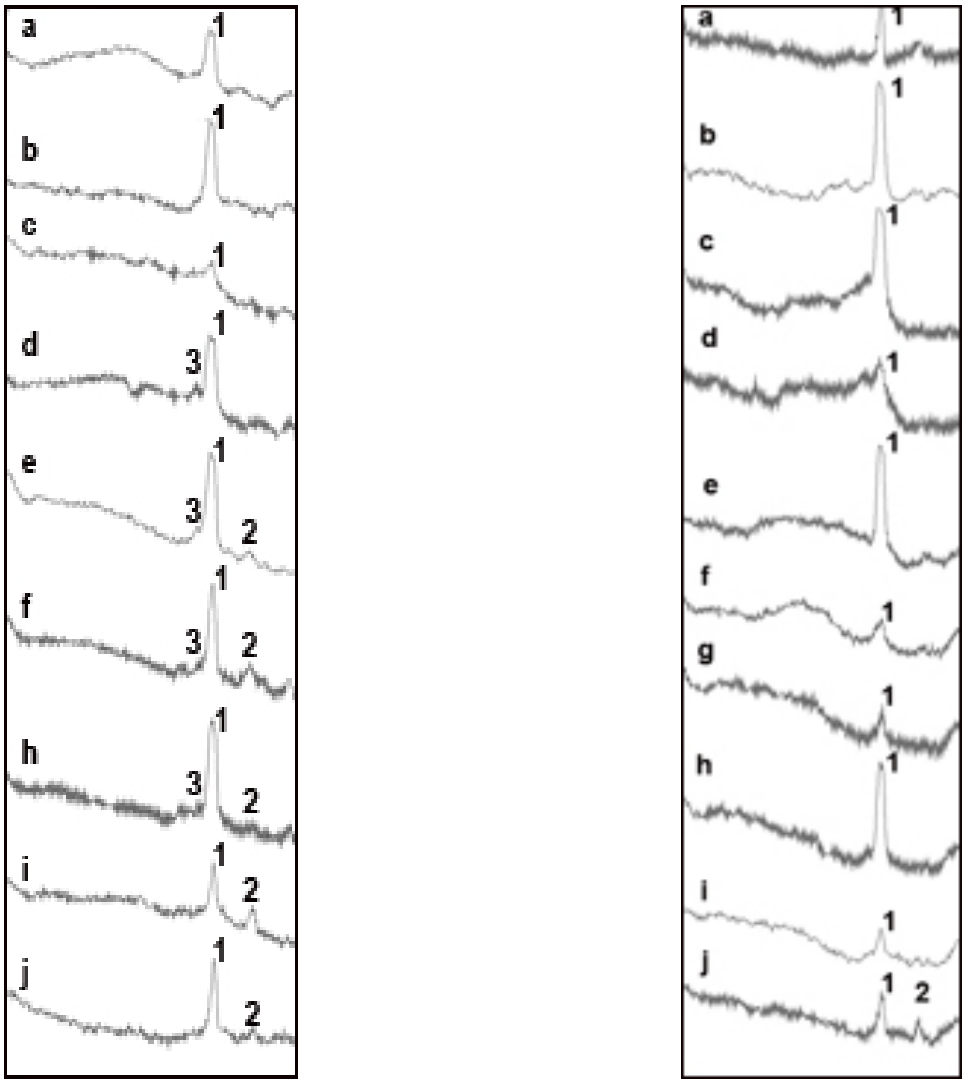
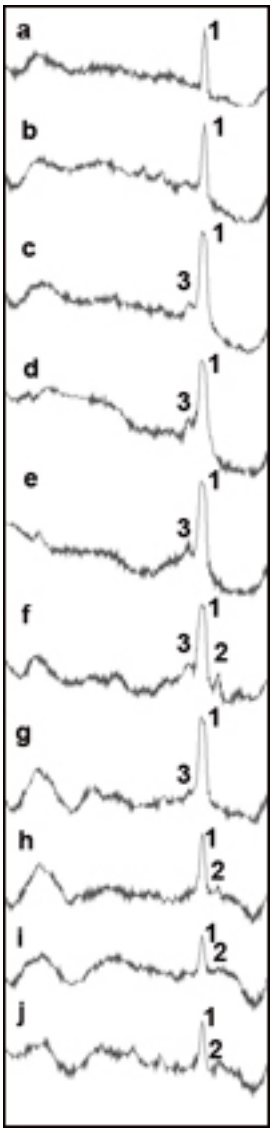


Fig. 4.27: A. Densitometric track analysis of male and female Tails DNA before exposure having peak 1 only. B. Densitometric track analysis of Prostate DNA. Lane (a) is the DNA of the control; (b - j) are DNA from exposed animals. Peak 1 is present in both control and exposed while other peaks appear in all exposed animals.

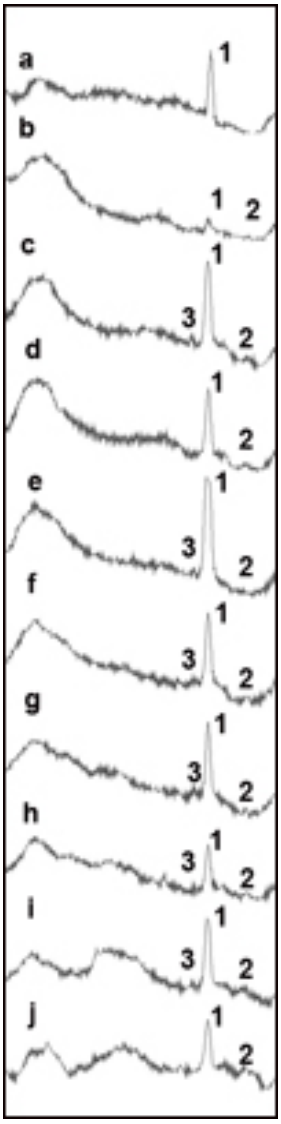


Thyroid female Thyroid Male

Fig. 4.28: Densitometric track analysis of male and female Thyroid DNA. Lane (a) is the DNA of the control; (b – j) are DNA from exposed animals. Peak 1 is present in both control and exposed while other peaks appear in all exposed animals.



Spleen Female



Spleen Male

Fig. 4.29: Densitometric track analysis of male and female Spleen DNA. Lane (a) is the DNA of the control; (b – j) are DNA from exposed animals. Peak 1 is present in both control and exposed while other peaks appear in all exposed animals.

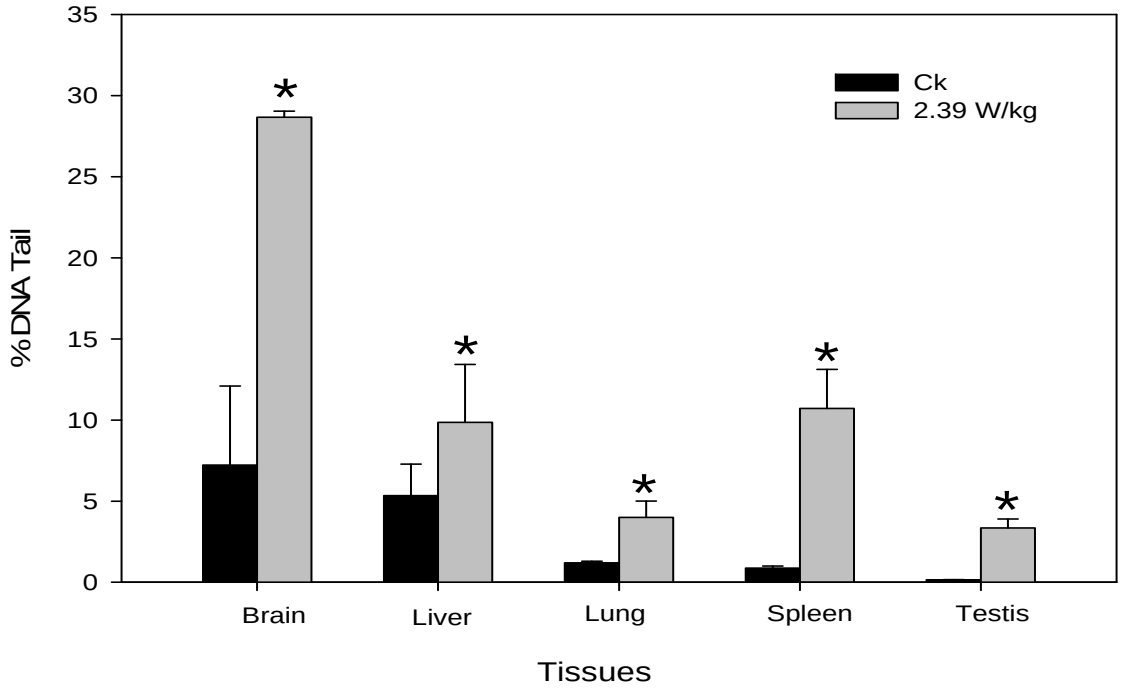


Figure 5. 31 The mean ± SD of % DNA in tail after exposure to 2.45 GHz microwave radation
* indicates statistically significant different compared with control ($p < 0.05$)

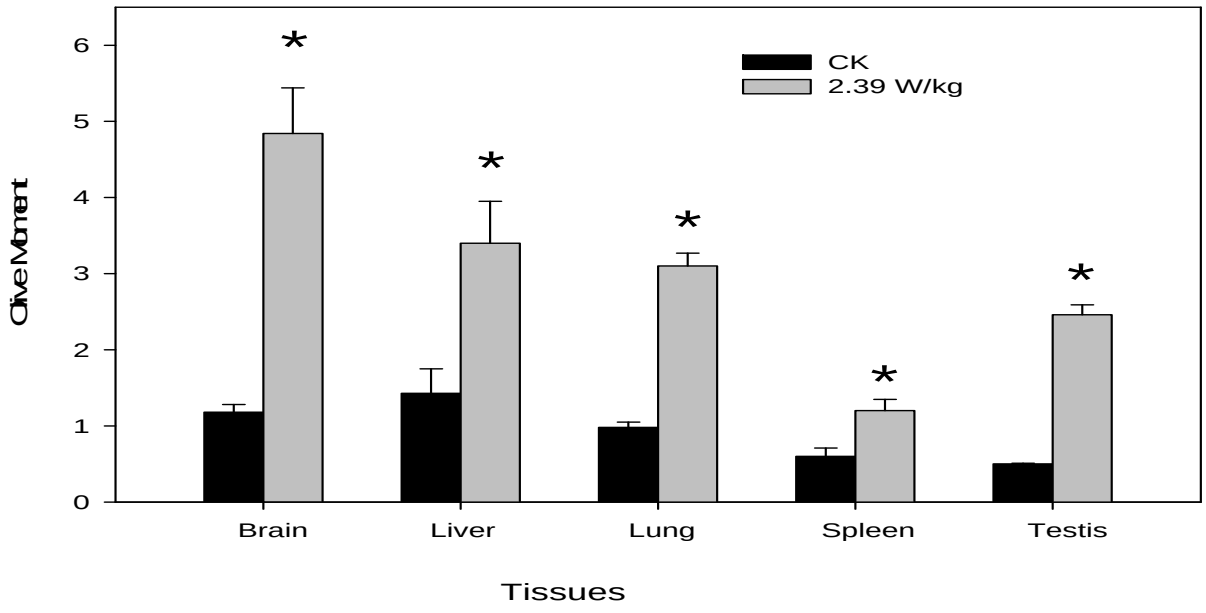


Figure 5.32: The mean ± SD of olive moment after exposure to 2.45 GHz microwave radation
* indicates statistically significant different compared with control ($p < 0.05$)

4.6 Conclusion and Recommendation

The results from this study suggest that exposure to low SAR MW radiation has harmful effects on the living systems. In all the studies in this research work, MW produces significant effects on the exposed animals compared to the control and thus, capable of inducing both somatic and genetic effects. There are modifications in tissues assessed through histopathology studies, exploratory and anxiolytic behaviour, reduction in sperm count and motility while increasing the number of abnormal sperm cells in the exposed animals compared to the control. 2.45 GHz MW radiation has have shown to have genotoxic effects on living cells as there were single strand DNA break in all the tissues studied and appearance of extra bands on the genome of exposed animals that was consistently absent in the control animals. It was observed in this study that MW effects are not sex dependent but SAR dependent, in the sense that the observed effects in both sexes follow the same trend, there is high correlation between the effects on both sexes (C = 0.98) but changes with increasing SAR. The study also revealed that blood leucocytes, brain, liver cells are more susceptible to 2.45 GHz MW radiation than other cells studied as MW produced significant effects on them even at low SAR of 0.48 W/kg. Ehling (1989) has suggested that germ cell mutation in mice can be used as a standard for protecting the human genome. Evidence of heritable mutational changes, mostly in the number of repeat units, has also been reported from the tandem repetitive hyper-variable loci in human DNA (Jeffreys *et al*., 1988). DNA damage in brain cells could affect neurological functions and also possibly lead to neurodegenerative diseases (Lai and Singh, 1996). Among the multitudinous risks to which mankind is continuously exposed, the threat of MW exposure affecting the human genome must rank very high. These findings are in agreement with some reports such as, (Maes *et al*., 1993; Halliwell, 2002; Lai and Singh, 1995) whose finding indicated that the main factor responsible for oxidative stress, cell injury and death are thermal injury and radiation. Though the complexities of the data point to the uncertainty of simple extrapolation from other species to health risk in humans, these data from the rats, mouse, and more distant species of animals and plants speak, clearly of the universality of heritable genetic damage of this radiation.

This study hereby hypothesizes that the various effects of MW exposures observed in this and other previous studies may be due to any or some of the following processes:

- MW interactions on nerve cells that may increase or decrease the amount of neurotransmitters released at the synaptic cleft which may also increase or decrease the rate of

generation of action potentials, increase the electricity conduction implies greater excitability which may be revealed in form of fear, ecstasy or increase in secretion from gland etc.

- The normal synthetic and metabolic activities of cells. Production of ROS and hence athermal effects having consequences on the nucleus; damage to organelles, DNA and chromosomes which can lead to genetic mutation and inadequate production of neurotransmitters by the Golgi apparatus.
- Deletion of receptors for the neurotransmitters on the post synaptic membrane. This event reduces rate of generation of impulses (action potential).
- If the glial cells serving as a myelin sheath, as seen in oligodendocytes – central nervous system and Schwann cells – peripheral nervous system (PNS) get affected, it may produce a degenerative effect on those lipid coatings or even lead to production of free radicals.
- If the ependima cells and menange are affected, there may be problem of cerebrospinal fluid production or excretion.
- If some tissues in the hippocampus are destroyed, similar conditions seen in Alzheimer's disease may show up.
- The heating effect of the radiation can raise or readjust the biological thermostat in the hypothalamus, thus giving the brain a higher than normal state of the body temperature. Local warming of the interior hypothalamus triggers physiological and behavioural heat loss mechanism. The animal tries to loose more heat and cool its temperature beyond the normal body temperature because the body thermostat has been readjusted. This results in hypothermia. Persistent hypothermia reduces brain metabolism.

Further work is recommended to see how these loci respond to mutagens and whether the information obtained from such unusually behaving parts of the genome is useful for risk assessment or extrapolation to conventional genes. The technical problem of detecting unambiguous, extremely rare events is enormously challenging and the novel DNA methodologies should be implemented to measure the human mutational spectrum.

We recommend that importation of fairly used microwave to this nation should be banned as they are prone to leakage which can increase the exposure of the users. It should not be switch on while the door is still open that is, it must be tightly lock before usage.

Based on the present study and the document of the Environmental Protection Agency (1984), there need for downward review of SAR safe limit currently fixed by International Commission of Non-ionizing Radiation Protection (ICNIRP) and other national regulatory bodies which reports that under certain environmental conditions, significant biological effects occur even below 1 W/kg, what seems imperative is the (re)evaluation of the mutagenic potential of MW at the prescribed safe limit for the personnel and the public members who are being exposed.

4.7 Contributions to Knowledge

1. The study established MW radiation exposures cause
    - Behavioural modifications (Explorative and Anxiolytic)
    - Deleterous effects on reproductive functions
    - Chromosomal aberration
    - DNA single strand breaks
2. Provides the bases for a downward review of the SAR safe limits currently fixed by INCRP, NCRP and other national and international regulatory bodies.

REFERENCES

Aalto, S., Haarala, C., Bruck, A., Sipila, H., Hamalainen, H. and Rinne, J.O. (2006). Mobile phone affects cerebral blood flow in humans. *J Cereb Blood Flow Metab*. **26(7):** 885-890.

Adey, W. R. (1975). Introduction: Effects of electromagnetic radiation on the nervous system. Ann. N.Y. Acad. Sci. **(247)**: 15 - 20

Airborne Instruments Laboratory (1956). An observation on the detection by the ear of microwave signals. *Proceedings of the IRE*, **(44):** 2A.

Aitken, R.J., Bennetts L.E., Sawyer D, Wiklendt A.M. and King, B.V. (2005). Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline. *Inter J Androl* **(28):**171-179.

Ali, S., Gauri, G. and Bala, S. (1993). Detection of genome specific monomorphic loci in *Bos taurus* and *Bubalus bubalis* with oligodeoxyribonucleotide probe, *Anim. Genet*., 45 - 52

Alimba, C., Bakare, A. and Latunji, C. (2006). Municipal landfill leachates induced chromosomes aberrations in rat bone marrow cells. *African Journal of Biotechnology* **5(22):** 2053-2057.

Allis, J.W. (1984). Cellular and subcellular effects. In: Elder, J.A and Cahill, D.F., Ed. *Biological effects of radiofrequency radiation*. Washington, DC, US Environmental Protection Agency, **EPA-600:** 80-83-026F.

Anne, A., Sato, M., Salati, O. M., Schwan, H. P. (1961). Relative microwave absorption cross sections of biological signilicance. In: *Biological effects of microwave radiation*, pp. 153-176. New York: Plenum Press

Armstrong, B., Theriault, G., Guenel, P., Deadman, J., Goldberg, M., and Heroux, P. (1994). Association between exposure to pulsed electromagnetic fields and cancer in electric utility workers in Quebec, Canada, and France. *A Journal of Epidemiology* **(140):** 805-820.

Arroyo M, Medina E and Ziegler H. (1990). Distribution and δ13C values of Portulacaceae species of the high Andes in Northern Chile, *Bot. Acta,* 291 – 295.

Aruoma, I., Kaur, H. and Halliwell, B. (1991). Oxygen Free Radicals and Human Diseases, *The Journal of the Royal Society for the Promotion of Health*, **111(5):** 172-177

Aweda, M.A. (2000). Photophysical processes in the clinical applications of non-ionizing radiations. *Journal of Medical Rehabilitation Therapists* **5(2):** 15-19.

Aweda, M.A., Gbenebitse, S., Meidinyo, R.O. (2003). Effects of 2.45 GHz microwave exposures on the Peroxidation status in wistar rats. *The Nigerian Postgraduate Medical Journal* **10(4)**: 243 –246.

Babior**,** B. (1990). The NADPH Oxidase of Leukocytes: The Respiratory Burst Oxidase, Cold Spring Harbor Monograph Archive

Baranski, S., and Czerski, P. (1976). Biological effects of microwave radiation. Stroudsburg, Pa.: Dowden, Hutchinson & Ross, Inc.

Barnes, F. S., and Hu, C. J. (1977). Model for some nonthermal effects of radio and microwave fields on biological membranes. *IEEE* Trans. MIT-**25:** 742 - 745

Barnes, F.S. and Hu, C.J. (1980). Nonlinear interactions of electromagnetic waves with biological materials (in P.L.E. Usilenghi, ed.) New York Academic Press. 47-80.

Barsoun, Y.H. and Pickard, W.F. (1982). The vacuolar potential of characean cell subjected to electromagnetic radiation in the range of 200-8200 MHz. *Bioelectromagnetics* **(3):** 393-400

Bawin, S. M., Medici, R. J., Adey, W. R. (1975). Effects of modulated VttF fields on the central nervous system. *Ann. N.Y. Acad. Sci.* **(247):** 74-80

Bediz, C., Baltacy, A., Mogulkoc, R. and Oztekin, E. (2006). Zinc supplementation ameliorates electromagnetic field-induced lipid peroxidation in the rat brian, *Tohuko J Exp Med,* **(208):** 133- 40.

Behari, J., Kunjilwar, K., and. Pyne S. (1998). Interaction of low level modulated RF radiation with Na+- K+ -ATPase, *Bioelectrochemistry and Bioenergetics,* **47(17):** 247-252.

Belyaev I.Y., Hillert L., Protopopova M., Tamm C., Malmgren L.O., Persson B.R., Selivanova G. and Harms-Ringdahl M. (2005). 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. *Bioelectromagnetics.* **26(3):** 173-184.

Belyaev I.Y., Koch C.B., Terenius O., Roxstrom-Lindquist K., Malmgren L.O., Sommer W., Salford L.G. and Persson B.R. (2006). Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. *Bioelectromagnetics.* **(27):** 295-306.

Belyaev I.Y., Shcheglov V.S., Alipov Y.D. and Polunin V.A. (1996). Resonance effect of millimeter waves in the power range from 10(-19) to 3 x 10(-3) W/cm2 on Escherichia coli cells at different concentrations. *Bioelectromagnetics* **17(4):**312-321.

Belyaev, I.Y., Alipov, Y.D., Shcheglov V.S. and Lystsov V.N. (1992). Resonance effect of microwaves on the genome conformational state of E. coli cells. *Z Naturforsch [C]* **47(7-8):** 621-827.

Beran, Y., Zulkuf, M., Suleyman, D., Dilek, U. and Murat, K. (2008) Extremely low frequency magnetic fields cause oxidative DNA damage in rats *Int. J. Radiat. Biol.*, **(84):** 789–795.

Bergqvist, U. (1997). Reviews of epidemiological studies, (in: N. Kuster, Q. Balzano, J.C. Lin (Eds.)) Mobile communication safety, Chapman and Hall, London, 147-170.

Berman, E. (1984). Reproductive effects. In: Elder, J.A and Cahill, D.F., Ed. *Biological effects of radiofrequency radiation*. Washington, DC, US Environmental Protection Agency, **EPA-600:** 80-83-026F.

Bernhardt, J. (1979). The direct influence electromagnetic fields on nerve and muscle cells of man within the frequency range of 1 Hz to 30 MHz. *Radiation and Environmental Biophysics* **(16):** 309-323.

Bernhardt, J.H. (1983). Hazards to human health from electromagnetic fields. Neuherberg, Dreimer Verlag, STH-Berichte Nr. 2.

Bisht, K.S., Moros, E.G., Straube, W.L., Baty, J.D. and Roti, J.L. (2002). The Effect of 835.62 MHz FDMA or 847.74 MHz CDMA Modulated Radiofrequency Radiation on the Induction of Micronuclei in C3H 10T½ Cells. *Radiation Research,* **(157):** 506–515.

Blackman, C. F., Elder, J. A., Weil, C. M., Benane, S. G., Elchinger, D. C. (1977). Two parameters affecting radiation indced calcium effiux from brain tissue. Symposium on Biological Effects of Electromagnetic waves, Airlie, Virginia, 30 Oct.

Blackman, C.F. (1980). Induction of calcium ion efflux from brain tissue by radiofrequency radiation: effects of sample number and modulation frequency on the power window. *Bioelectromagnetics* **1 (65):** 35-43.

Blackman, C.F., Weil, C.M., Benane, S.G., Eichinger, D.C. and House, D.E. (1979). Induction of calcium ion efflux from brain tissue by radiofrequency radiation: effects of modulation frequency and field strength. *Radio Science* **14(65):** 93-98.

Borbely, A.A., Huber, R., Graf, T., Fuchs, B., Gallmann, E., Achermann, P. (1999). Pulsed high-frequency electromagnetic field affects human sleep and sleep electroencephalogram. *Neurosci Lett*. **275(3):** 207-210.

Bornhausen, M. and Scheingraber, H. (2000). Prenatal exposure to 900 MHz, cell-phone electromagnetic fields had no effect on operant-behavior performances of adult rats. *Bioelectromagnetics* **21(8):**566-574.

Bowman, H.D. (1981). Heat transfer and thermal dosimetry. *Journal of Microwave Power* **16**: 121-133.

Bren, S.P.A. (1996). Historical introduction to electromagnetic field health effects. *IEEE Engineering in Medicine and Biology* (**15**):41-44.

Britigan B., Roeder, T., and Shasby, D. (1992) Insight into the nature and site of oxygen-centered free radical generation by endothelial cell monolayers using a novel spin trapping technique. *Blood,* (**79):** 699–707

Brusick, D. (1980). Bone marrow cytogenetic analysis in rats: In principles of Genetic Toxicology Plenum Press, New York and London 235-239.

Brusick, D.J. (1995). An assessment of the genotoxic activity of radiofrequency radiation, State of the Science Colloquium, Rome.

BSttcher, C. J. F., Bordwijk, P. (1978). Theory of electric polarization, Vol. **II**. Amsterdam: Elsevier Publ. Co.

BSttcher, C. J. F., Van Belle, O. C., Bordwijk, P., Rip, A. (1973). Theory of electric polarization, Vol **I**. Amsterdam: Elsevier Publ. Co.

Busljeta I., Trosic I. and Milkovic-Kraus S. (2004). Erythropoietic changes in rats after 2.45 GHz non-thermal irradiation. *Int. J. Hyg. Environ Health.* **207(6):**549-554.

Canadian Council of Animal Care (1985). Guide to the handling and use of experimental animals. Ottawa Ontario 2

Chang, S.K., Choi, J.S., Gil, H.W., Yang, J.O., Lee, E.Y., Jeon, Y.S., Lee, Z.W., Lee, M., Hong, M.Y., Ho-Son, T. and Hong, S.Y. (2005). Genotoxicity evaluation of electromagnetic fields generated by 835-MHz mobile phone frequency band. *Eur J Cancer Prev.* **14(2):** 175-179.

Chou, C.K., Guy, A.W. and Galambos, R. (1982). Auditory perception of radiofrequency electromagnetic fields, *Journal of the Accoustical Society of America,* **71:**1321-1334.

Cleary, S.F. (1980). Microwave cataractogenesis. *Proceedings of the Institute of Electrical and Electronics Engineers*, **(68):** 49-55.

Cleary, S.F., Pasternack, B.S. and Beebe, G.W. (1965). Cataract incidence in radar workers, *Archeological Environmental Health* **(11):** 179-182.

Cook, H.F. (1952). The pain threshold for microwave and infra-red radiations. *Journal of Physiology*, **(118):** 1-11.

Cotran, K., Kumar, A. and Collins, B. (1999). *Robbins Pathologic Basis of Disease*. Philadelphia: W.B. Saunders. 873 – 875

Croft, R., Chandler, J., Burgess, A., Barry, R., Williams, J. and Clarke A. (2002). Acute mobile phone operation affects neural function in humans. *Clin Neurophysiol.* **113(10):** 1623.

D'Andrea, J.A., Gandhi, O.P., Lord, J.L., Durney, C.H., Johnson, C.C. and Astle, L. (1976). Physiological and behavioural effects of exposure to 2450 MHz microwave. *Journal of Microwave Power*, **(14):** 351-362.

Dacie, J.V. and Lewis, S.M. (1991). Practical Haematology. Longmans 5th edition 37-67.

d'Ambrosio, G., Lioi, M.B., Scarfi, M.R. and Zeni, O. (1995). Genotoxic effects of amplitude-modulated microwaves on human lymphocytes exposed in vitro under controlled conditions. *Electro-magnetobiology* **(14):** 157-164.

Dasdag, M. Zulkuf A., Aksen, F.. Yilmaz, Bashan, M. Dasdag, M. and Salih, C. (2003). Whole body exposure of rats to microwaves emitted from a cell phone does not affect the testes, *Bioelectromagnetics,* **24(3):** 182-188.

Davidov, A.S. (1982). *Biology and quantum mechanics* New York, Pergamon press

D'Costa, H., Trueman, G., Tang, L., Abdel-rahman, U., Abdel-rahman, W., Ong, K. and Cosic, I. (2003). Human brain wave activity during exposure to radiofrequency field emissions from mobile phones. *Australas Phys Eng Sci Med*. **26(4):** 162-167.

de Rooij, D., Henk, J., Roelof, D., Gerard, W., Paul P. Annemarie van, D., and Johan J. (2002). Long-term effects of irradiation before adulthood on reproductive function in the male rhesus monkey. *Biology of Reproduction,* **66 (2):** 486-494

Delaney MO, Wiederholt CJ, Greenberg MM. 2002. Fapy-dA induces nucleotide misincorporation tranlesionally by a DNA polymerase. *Angewandte Chemie International Edition* **(41):** 771-775.

Dhara, A., Suba, V., Sen, T., Pal, S. and Nag-Chaudhuri, A. (2000).Preliminary studies on the anti-inflammatory and analgestic activity of the methanolic fraction of the root extract of Tragia Involucrate Linn. *Journal of Ethnopharmacology* **(72):** 265-268.

Dixon, D.M. and Polak-Wyss, A. (1991). The medically important dematiaceous fungi and their identification. *Mycoses* **(34):** 1-18.

Dizdaroglu M. (1992). Oxidative damage to DNA in mammalian chromatin. *Mutation Research* **(275):** 331–342.

Dizdaroglu M. (1998). Mechanisms of free radical damage to DNA. In: Aruoma OI, Halliwell B, editors. *DNA and free radicals: Techniques, mechanisms and applications*. Saint Lucia: OIC International. pp 1–24.

Dizdaroglu, M., Jaruga, P., Birincioglu M. and Rodriquez, H. (2002). Free radical-induced damage to DNA: Mechanisms and measurement. *Free Radical Biology and Medicine,* **(32):** 1102–1115.

Dodge, C. H., Glaser, Z. R. (1977). Trends in electromagnetic radiation bioeffects research and related occupational safety aspects. *J. Microwave Power* **(12):** 4-10

Dubreuil, D., Jay, T. and Edeline J.M. (2002). Does head-only exposure to GSM-900 electromagnetic fields affect the performance of rats in spatial learning tasks? *Behav Brain Res.* **129 (1-2):** 203-210.

Dubreuil, D., Jay, T. and Edeline J.M. (2003). Head-only exposure to GSM 900-MHz electromagnetic fields does not alter rat's memory in spatial and non-spatial tasks. *Behav Brain Res.* **145(1-2):** 51- 61.

Dumey, C.H., Iskander, M.F., Massoudi, H., Allen, S.J. and Mitchell, J.C. (1980). *Radiofrequency radiation dosimetry handbook*, 2nd ed. Brooks Air Force Base, Texas, USAF School of Aerospace Medicine, Report SAM-TR-78-22.

Dupuy, B., Govind, R., Antunes, A and Matamouros, S. (2008). *Clostridium difficile* toxin synthesis is negatively regulated by TcdC, *J Med Microbiol* **(57):** 685-689

Durney C.H. (1980). Radiofrequency radiation dosimetry handbook, 3rd edition. Brooks Air Force Base, Texas, USAF, School of Aerospace Medicine, (Report SAM-TR-78-22).

Durney, C. H., Johnson, C. C., Barber, P. W., Massoudi, H., Iskander, M. F., Lords, J. L., Ryser, D. K., Allen, S. J., Mitchell, J. C.: Radiofrequency radiation handbook, second Ed., Report SAMTR- 78-22, U.S.A.F. School of Aerospace Medicine, Brooks Air Base, Texas 78235

Edwards, G.S. (1984). Resonant microwave absorption of selected DNA molecules. *Physical Review Letters* **(53):** 1284-1287.

Ehling, U. (1989) Germ cell mutation in mice: Standards for protecting the human genome, *Mutation Research*, **(212):** 43-53.

Elder, J.A. (1984). Special senses. In: Elder, J.A. and Cahill, D.F., ed. Biological effects of radiofrequency radiation. Washington, DC, US Environmental Protection Agency, **EPA-600:** 80-83-026F.

Emery, A.F., Short, R.E., Guy, A.W., Kraning, K.K. and Lin, J.C. (1976). The numerical thermal simulation of the human body when undergoing exercise or non-ionizing electromagnetic irradiation. *Journal of Heat Transfer*, **(98):** 284-291.

Environmental Protection Agency (1984) Biological effects of radiofrequency radiation, in: J.A. Elders and D.F. Canill (Eds.), US EPS, Research Triangle Park, NC, Pub. EPA- 600/8-83-026F.

Eulitz, C., Ullsperger, P., Freude, G. and Elbert, T. (1998). Mobile phones modulate response patterns of human brain activity. *Neuroreport* **9(14):** 3229-3232.

Evans, M., Dizdaroglu, M. and Cooke, M. (2004). Oxidative DNA damage and disease: Induction, repair and significance. *Mutation Research* **(567):** 1–61.

Fairbairn, D., Olive, P. and O'NEILL, K. (1995). The comet assay: a comprehensive review, *Mutation Research*, (**339)** 37- 59.

File S and Wardrill A.(1975). Validity of head-dipping as a measure of exploration in a modified hole-board *Psychopharmacology* **44:** 53 – 59

Foster, K.R. (1987). Resonances in the dielectric absorption of DNA? *Journal of Biophysics* **52**: 421-425.

Foster, K.R. and Finch, E.D. (1974). Microwave hearing: evidence for thermo-acoustic auditory stimulation by pulsed microwaves. *Science*, **185:** 256-258.

Freude, G., Ullsperger, P., Eggert, S. and Ruppe, I. (1998). Effects of microwaves emitted by cellular phones on human slow brain potentials. *Bioelectromagnetics* **19(6):** 384-387.

Freude, G., Ullsperger, P., Eggert, S. and Ruppe, I. (2000). Microwaves emitted by cellular telephones affect human slow brain potentials. *Eur J Appl Physiol* **81(1-2):** 18-27.

Frthlich, H. (1975). Evidence for bose condensation-like excitation of coherent modes in biological systems. *Physics Letters* **(51A):** 21-22

Gabril, C. (1987). Microwave absorption in aqueous solution of DNA. *Nature* **(328):** 145-146.

Gage, M.I. (1984). Behaviour. In: Elder, J.A and Cahill, D.F., Ed. Biological effects of radiofrequency radiation. Washington, DC, US Environmental Protection Agency, **EPA-600:** 80-83-026F.

Gage, M.I. and Albert, E.N. (1984). Nervous system. In: Elder, J.A and Cahill, D.F., Ed. Biological effects of radiofrequency radiation. Washington, DC, US Environmental Protection Agency, **EPA-600:** 80-83-026F.

Gandhi, G. and Anita (2005). Genetic damage in mobile phone users: some preliminary findings. *Ind J Hum Genet* **11(2):** 99-104.

Gandhi, G. and Singh, P. (2005). Cytogenetic damage in mobile phone users: preliminary data. *Int J Hum Genet* **5(4):** 259-265.

Gandhi, O. P., Hill, D. W., Parblow, L. M., Johnson, C. C., Stensaan, L. J. (1978). Effects of millimeter waves on living tissue. *Radio Science* **(35):** 30 -39

Garaj-Vrhovac, V. (1999). Micronucleus assay and lymphocyte mitotic activity in risk assessment of occupational exposure to microwave radiation. *Chemosphere* **39(13):** 2301-2312.

Garaj-Vrhovac, V., Fucic, A. and Horvat, D. (1992). The correlation between the frequency of micronuclei and specific chromosome aberrations in human lymphocytes exposed to microwave radiation in vitro. *Mutation Research,* **281(3):**181-186.

Garaj-Vrhovac, V., Horvat, D. and Koren, Z. (1990). The effect of microwave radiation on the cell genome. *Mutation Research,* **243(2):**87-93.

Garaj-Vrhovac, V., Horvat, D., Koren, Z. (1991). The relationship between colony-forming ability, chromosome aberrations and incidence of micronuclei in V79 Chinese hamster cells exposed to microwave radiation. *Mutation Research,* **263(3):**143-149.

Gordon, C.J. (1984). Effect of RF-radiation exposure on body temperature: thermal physiology. In: Elder, J.A and Cahill, D.F., Ed. Biological effects of radiofrequency radiation. Washington, DC, US Environmental Protection Agency, **EPA-600:** 80-83-026F.

Granger, C. (1988). Some Recent Developments in a Concept of Causality, *Journal of Econometrics* **(39):** 199-211

Grant, E. H., Sheppard, R. J., South, G. P. (1978) *Dielectric behavior of biological molecules in solution*. Oxford Press

Grissom, C. (1995). Magnetic field effects in biology: A survey of possible mechanisms with emphasis on radical-pair recombination. *Chemical Reviews,* **(95):** 3–24.

Grundler, W. and Keilmann, F. (1983). Sharp resonances in yeast growth prove non-thermal sensitivity to microwaves. *Physical Review Letters*, **(51):** 1214-1216.

Guy, A.W. (1987). Dosimetry associated with exposure to non-ionization radiation of very low frequency to MW. *Health Physics* **21(6):** 569-584.

Guy, A.W., Chou, C.K., and Lin, J.C. (1975). Microwave-induced acoustic effects in mammalian auditory systems and physical material. *Annals of the New York Academy of Science*, **(247):** 194-215.

Guy, Q. W., Webb, M. D., McDougall, J. S. (1977). RF radiation absorption patterns: Human and animal modelling data. DHEW (NIOSH) Publ. No. 77-183

Hagmann, M.J., Gandhi, O.P., D'Andrea, J.A. and Chatterjee, I. (1979). Heat resonance: numerical solutions and experimental results. *IEEE Transactions on Microwave Theory and Techniques*, **MTT-27:** 809-813.

Halliwell B. 2002. Effect of diet on cancer development: Is oxidative DNA damage a biomarker? *Free Radical Biology and Medicine* **(10):** 968–974.

Hamblin, D.L., Wood, A.W., Croft, R.J. and Stough, C. (2004). Examining the effects of electromagnetic fields emitted by GSM mobile phones on human event-related potentials and performance during an auditory task. *Clin Neurophysiol*. **115(1):**171-178.

Hazlewood, C. F. (1977). Bound water in biology, *Acta Biochim. Biophys. Acad. Sci. Hung*. **12:** 263—273.

Henshaw, D.L. and Reiter R.J. (2005). Do magnetic fields cause increased risk of childhood leukemia via melatonin disruption? A Review. *Bioelectromagnetics* (**7):** S86-S97

Hill, A.B. (1971). Principles of Medical Statistics Chapter XXIV. Statistical Evidence and Inference, Oxford University Press, Oxford University, Oxford, UK, p. 309-323.

Hinrichs, H. and Heinze, H.J. (2004). Effects of GSM electromagnetic field on the MEG during an encodingretrieval task. *Neuroreport.* **15(7):** 1191-1194.

Hochstein, P. and Ernest, L. (1964). CIBA foundation symposium on cellular injury (**1):** 123-124.

Hodgkin, A. L., Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* (Lond.) **(117):** 500-544.

Hollows, F.C. and Douglas, J.B. (1984). Microwave cataract in radio linemen and controls, *Lancet* **ii:** 406-407.

Huber, R., Graf, T., Cote, K.A., Wittmann, L., Gallmann, E., Matter, D., Schuderer, J., Kuster, N., Borbely, A.A. and Achermann, P. (2000). Exposure to pulsed high-frequency electromagnetic field during waking affects human sleep EEG. *Neuroreport* **11(15):** 3321-3325.

Huber, R., Schuderer, J., Graf, T., Jutz, K., Borbely, A.A., Kuster, N. and Achermann P. (2003). Radio frequency electromagnetic field exposure in humans: Estimation of SAR distribution in the brain, effects on sleep and heart rate. *Bioelectromagnetics,* **24(4):** 262-276.

Hungerford, D.A. (1965). Leucocytes culture from small inocula of whole blood and preparation of metaphase chromosomes by treatment with KCl. *Speculations in Science and Technology* **40**: 333-335.

Ibrahim M., El-Ashry M., Ali E. 2008. The influence of 50 hz magnetic field on liver function. *Romanian J. Biophys*., **18(2):** 113–122

IEEE (2006). Std C95.1TM-2005 (Revision of IEEE Std C95.1-1991) IEEE Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields, 3 kHz to 300 GHz. IEEE 3 Park Avenue New York, NY10016-5997, USA Sponsored by the IEEE International Committee on Electromagnetic Safety (SCC39).

Illinger, K. H. (1969). Molecular mechanisms for microwave absorption in biological syslLems. Symp. Proc. BRH/DBE 70-2, Richmond, Virginia 112—116.

International Non-Ionizing Radiation Committee of the Radiation Protection Association (1985). Review of concepts, quantities, units, and terminologies for non-ionizing radiation protection. *Health Physics* **(49)**:1329-1362.

Investigation of Co-genotoxic Effects of Radiofrequency Electromagnetic Fields In Vivo. *Radiat. Res.* (**165):** 598-607.

Jeffreys, A., Royle, N., Wilson, V. and Wong, Z. (1988) Spontaneous mutation rates to new length at tandem-repetitive hypervariable loci in human DNA, *Nature*, **(332):** 278-281.

Jensh, R.P. (1997) Behavioral teratologic studies using microwave radiation: Is there an increased risk from exposure to cellular phones and microwave ovens? *Reproductive Toxicology*, **(11):** 601-611

Johnson, C. C., Durney, C. H., Massoudi, H. (1975). Long-wavelength electromagnetic power absorption in prolate spherical model of man and animals. *IEEE* Trans. MIT-**23**, 739--747

Johnson, C. C., Guy, A. W. (1972). Non-ionizing electromagnetic wave effects in biological materials and systems. Proc. *IEEE* **(60):** 692-718

Johnson, F.H., Eyring, H. and Staver, B.J. (1974). The theory of rate processes in Biology and Medicine. New York; John Wiley 68-102.

Kallen, B., Malmquist, G. and Moritz, U. (1982). Delivery outcome among physiotherapists in Sweden: is non-ionizing radiation a fetal hazard? *Archives of Environmental Health*, **(37):** 81-85.

Kowalczuk, C.I., Saunders, R.D. and Stapleton, H.R. (1983). Sperm count and sperm abnormality in mice after exposure to 2.45 GHz microwave radiation, *Mutation Research* **(122):** 155-161.

Kramarenko, A.V. and Tan, U. (2003). Effects of high-frequency electromagnetic fields on human EEG: a brain mapping study. *Int J Neurosci*. **113(7):** 1007-1019.

Krause, C.M., Bjornberg, C.H., Pesonen, M., Hulten, A., Liesivuori, T., Koivisto, M, Revonsuo, A., Laine, M. and Hamalainen, H. (2006). Mobile phone effects on children's event-related oscillatory EEG during an auditory memory task. *Int J Radiat Biol.* **82(6):** 443-450.

Krause, C.M., Haarala, C., Sillanmaki, L., Koivisto, M., Alanko, K., Revonsuo, A., Laine, M. and Hamalainen H. (2004). Effects of electromagnetic field emitted by cellular phones on the EEG during an auditory memory task: a double blind replication study. *Bioelectromagnetics*, **25(1):** 33-40.

Krause, C.M., Sillanmaki, L., Koivisto, M., Haggqvist, A., Saarela, C., Revonsuo, A., Laine, M. and Hamalainen, H. (2000). Effects of electromagnetic fields emitted by cellular phones on the

electroencephalogram during a visual working memory task. *Int J Radiat Biol.* **76(12):** 1659-1667.

Lagroye, I., Anane, R., Wettring, B.A., Moros, E.G., Straube, W.L., Laregina, M., Niehoff, M., Pickard, W.F., Baty, J. and Roti, J.L.(2004a). Measurement of DNA damage after acute exposure to pulsed-wave 2450 MHz microwaves in rat brain cells by two alkaline comet assay methods. *Int J Radiat Biol.* **80(1):**11-20.

Lagroye, I., Hook, G.J., Wettring, B.A., Baty, J.D., Moros, E.G., Straube, W.L. and Roti, J.L.(2004b). Measurements of Alkali-Labile DNA Damage and Protein-DNA Crosslinks after 2450 MHz Microwave and Low-Dose Gamma Irradiation In Vitro. *Radiat Res*. **161(2):** 201-214.

Lai, H. and Singh, N. (1995). Acute lowlevel microwave exposure increases DNA single strand breaks in rat brain cells, *Bioelectromagnetics* **(16):** 207–210.

Lai, H. and Singh, N.P. (1996). Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. *Int J Radiat Biol* **69(4):**513-521.

Lai, H. and Singh, N.P. (1997a). Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. *Bioelectromagnetics* **18(6):**446-454.

Lai, H. and Singh, N.P. (1997b). Melatonin and N-tert-butyl-alpha-phenylnitrone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells. *J Pineal Res.* **22(3):**152-162.

Lai, H. and Singh, N.P. (2004). Magnetic-field-induced DNA strand breaks in brain cells of the rat, *Environ Health Perspect.* **112(6):**687-694.

Lai, H. and Singh, N.P. (2005). Interaction of microwaves and a temporally incoherent magnetic field on single and double DNA strand breaks in rat brain cells. *Electromag Biol Med* **(24):**23-29.

Lai, H., and Singh, N. (1996). Single and double strand breaks in rats brain cells after acute exposure to radio frequency electromagnetic radiation, *Int. J. Radiat. Biol*. **(69):** 513–521.

Lai, H., Carino, M.A., Singh, N.P.(1997). Naltrexone blocks RFR-induced DNA double strand breaks in rat brain cells. *Wireless Networks* **3:**471-476.

Lary, J.M., Conover, D.L., Foley, E.D. and Hanser, P.L. (1982). Teratogenic effects of 27.12 MHz radiofrequency radiation in rats. *Teratology*, **26:** 299-309.

Lau, M.P. (1979). Induced thermal resistance in the mouse ear. The relationship between heating time and temperature. *International Journal of Radiation Biology* **35**:481-185.

Lawrence, A.F. and Adey, W.R. (1982). Nonlinear wave mechanisms in interactions between excitable tissue and electromagnetic fields. *Neurological Research*, **4:** 115-153.

Leonard, A., Berteaud, A.J. and Bruyere, A. (1983). An evaluaton of the mutagenic, carcinogenetic and teratogenetic potential of microwaves, *Mutation Research* **123**: 31-46.

Li, L., Bisht, K.S., LaGroye, I., Zhang, P., Straube, W.L., Moros, E.G. and Roti, J.L. (2001). Measurement of DNA damage in mammalian cells exposed in vitro to radiofrequency fields at SARs of 3-5 w/kg. *Radiat Res* **156:**328-332.

Liburdy, R.P. (1980). Radiofrequency radiation alters the immune system II. Modulation of in vivo lymphocyte circulation. *Radiation Research*, **83:** 66-73.

Lilienfield, A.M., Tonascia, J., Tonascia, S., Libauer, S.A. and Cauthen, G.M. (1978). Evaluation of health status of Foreign Service and other employees from selected eastern European posts. Final report to US Department of State. Baltimore, Department of Epidemiology. NTIS PB-2388163

Lin, J. C. (1977). On microwave-induced hearing sensation. *IEEE* Trans. MIT-**25**, 605-613

Lixia, S., Yao K., Kaijun, W., Deqiang, L., Huajun, H., Xiangwei, G., Baohong, W., Wei, Z., Jianling, L. and Wei, W. (2006). Effects of 1.8 GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells. *Mutation Research*, **602:** 135- 142.

Lotz, W.G. (1985). Hyperthermia in radiofrequency-exposed rhesus monkeys: a comparison of frequency and orientation effects. *Radiation Research*, **102:** 59-70.

Lovely, R.H., and Guy, A.W. (1975). Conditioned taste aversion in the rat induced by a single exposure to microwave, paper presented at the IMPI Microwave Power Symposium, University of Waterloo, Waterloo, Ontario, Canada.

Lowenthal, R., Tuck D. and Bray I. (2007). Residential exposure to electric power transmission lines and risk of lymphoproliferative and myeloproliferative disorders: a casecontrol study. *Int Med J* doi:10.1111/j.1445-5994.2007.01389.x

Lupke, M., Rollwitz, J. and Simko´ M. (2004). Cell activating capacity of 50 Hz magnetic fields to release reactive oxygen intermediates in human umbilical cord blood-derived monocytes and in mono mac 6 cells. *Free Radical Research* **(38):** 985–993.

Lyle, D. Schechter, P., Adey, W, and Lundak R. (1983). Suppression of T-lymphocyte cytotoxicity following exposure to sinusoidally amplitude-modulated fields. *Bioelectromagnetics* **4**:281-292.

Maes, A., Verschaeve, L., Arroyo, A., DeWagter, C. and Vercruyssen, L. (1993). In vitro cytogenetic effects of 2450MHz waves on human peripheral blood lymphocytes, *Bioelectromagnetics* **(14):** 495–501.

Mann, K. and Roschke, J. (1996). Effects of pulsed high-frequency electromagnetic fields on human sleep. *Neuropsychobiology* **33(1):** 41-47.

Marino, A.A., Nilsen, E. and Frilot, C. (2003). Nonlinear changes in brain electrical activity due to cell phone radiation. *Bioelectromagnetics* **24(5):** 339-346.

Mashevich M, Folkman D, Kesar A, Barbul A, Korenstein R, Jerby E, Avivi L. (2003). Exposure of human peripheral blood lymphocytes to electromagnetic fields associated with cellular phones leads to chromosomal instability. *Bioelectromagnetics* **24(2)**:82–90.

Mckelvey-Martin, V., Green, M., Schmezer, P., Pool-Zobel, B., De-M' eo M. and Collins, A. (1993). The single cell gel electrophoresis assay (comet assay): a European review. *Mutation Research*, (**288):** 47 - 63.

McRee, D.T. and Davies, H. (1984). Wholebody and local dosimetry in rats exposed to 2.45 GHz radiation. *Health Physics* **6(40):** 315.

Michaelson, S. M. (1977). Microwave and radiofrequeney radiation. World Health Organization, Document ICP/CEP 803.

Milham, S. (1988). Increased mortality in amateur radio operators due to lymphatic and hematopoitic malignancies. *American Journal of Epidemiology* **127:** 50-54.

Mitchell, C., McRee, D., Peterson, N. and Tilson, H. (1988). Some behavioral effects of short-term exposure of rats to 2.45-GHz microwave radiation, *Bioelectromagnetics* **(9):** 259–268.

Moncada. S., Palmer R. and Higgs, E. (1991) Nitric oxide: physiology, pathology, and pharmacology. *Pharmacol Rev*. **(43):**109-142.

Nathan C. and Xie Q. (1994) Regulation of biosynthesis of nitric oxide. *J. Biol. Chem.* **(269):**13725–13728.

Nathan, E. (1997). Contact lenses and corneal physiology. *Biological Sciences Review*, **9(4):** 29-31.

National Council for Radiation Protection and Measurements (1981). Radiofrequency electromagnetic fields, properties, quantities and units, biophysical interaction and measurements. NCRP report No. **67**.

Naziroglu, M., Karaoglu, A. and Aksoy, A. (2004). Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats, *Toxicology,* **(195):** 221–230.

Novoselva, E.G., Fresenko, E.E., Maker, V.R. and Sadovnikov, V.B. (1999). Microwaves and cellular immunity. Immunostimulatory effects of microwaves and naturally occurring antioxidants nutrients. *Bioelectrochemistry and Bioenergetics* **49:** 37-41.

Oncley, J. L. (1942). The investigation of proteins by dielectric measurements. *Chem. Rev.* **30**, 443—450

Oyeyemi, M., Akusu, M. and Ola-Davies, O. (2000). Effect of successive ejaculatios on the spermiogram of West Africa dwarf goals (coprahirus L). *Veter. Archiu*. **70(4):** 215-221.

Papageorgiou, C.C., Nanou, E.D., Tsiafakis, V.G., Capsalis, C.N. and Rabavilas, A.D. (2004). Gender related differences on the EEG during a simulated mobile phone signal. *Neuroreport.* **15(16):** 2557-2560.

Papageorgiou, C.C., Nanou, E.D., Tsiafakis, V.G., Kapareliotis, E., Kontoangelos, K.A., Capsalis, C.N. and Rabavilas, A.D. (2006). Soldatos CR. Acute mobile phone effects on pre-attentive operation. *Neurosci Lett.* **397:** 99-103.

Paulraj, R. and Behari, J. (2006). Single strand DNA breaks in rat brain cells exposed to microwave radiation, *Mutation Research*, **596**: 76-80.

Pickard, W.F. and Rosenbaum, F.J. (1978). Biological effects of microwaves at the membrane levels. Two possible athermal electro physiological mechanisms and a proposed experimental test. *Mathematical Biosciences* **39:**235-253.

Pilla, A. (1979) Low frequency electromagnetic induction of electrochemical information at living cell membranes: a new tool for the study and modulation of the kinetics of cell function, in: National Radio Science Meeting, Bioelectromagnetics Symposium, Seattle, WA, 18-22 July, p. 393.

Poswillo, D., Nunnerly, H., Sopher, D. and Keith, J. (1974). Hyperthermia as a teratogenic agent. *Ann. R. Soc. Coll. Surgeon* **55:**171-174.

Presman, A. S. (1968). Electromagnetic fields and life. Tzdatelstvo Nauka, Moscow, Transl. New York: Plenum Press

Ramel, C. (1989) The nature of spontaneous mutations, *Mutation Research,* **(212):** 33-42.

Rao, R.G.V., Cain, C.A., Lockwood, V.,Tompkins, W.A.F. (1983). Effects of MW in card function. *Bioelectromagnetics* **4**:141-145.

REFLEX, (2004). Risk Evaluation of Potential Environmental Hazards from Low Frequency Electromagnetic Field Exposure Using Sensitive *in vitro* Methods.

Risk evaluation of potential environmental hazards from low energy electromagnetic field exposure using sensitive in vitro methods (REFLEX), final report, 2004, pp. 183–242.

Rothman, K.J., Luoghlin, J.E., and Funch, D.P. (1996). Overall mortality of cellular telephone customers, *Epidemiology* **7:** 303-305.

Rowbotton, M.S.C. (1984). Electricity and medicine. History of their interaction. San Francisco Press 300

Rudnev, M., Bokina, A., Eksler, N. and Navakatikyan, M. (1978). The use of evoked potential and behavioral measures in the assessment of environmental insult *in*: "Multidisciplinary Perspectives in Event-Related Brain Potential Research," D.A. Otto, ed., EPA-600/9-77-043, U.S. Environmental Protection Agency, Research Triangle Park, NC.

Rundev, M.I. and Goncher, N.M. (1985). Changes in morphofunctional and cytochemical indices of blood leukocytes after exposure to microwave radiation of low intensities. *Radiobiology*, **5:** 645-649.

Sagripanti, J., and Swicord, M. (1986). DNA structural changes caused by microwave radiation, *Int. J. Radiat. Biol.*, **(50):** 47-50.

Salati, O. M., Anne, S., Schwan, H. P. (1962) Radiofrequency radiation hazards. In: Electronic Industries, pp. 96-101.

Sapareto, S.A. and Devey, W.C. (1984). Thermal dose determination cancer therapy. *International Journal of Radiation*: oncology-biology-physics **10:** 787-800.

Sargentini, N., and Smith, K. (1985) Spontaneous mutagenesis; the role of DNA repair, replication and recombination, *Mutation Res.*, **(154):** 1-27.

Sarimov, R., Malmgren, L.O.G., Markova, E., Persson, B.R.R. and Belyaev, I.Y. (2004). Nonthermal GSM microwaves affect chromatin conformation in human lymphocytes similar to heat shock. *IEEE Trans Plasma Sci* **32:**1600-1608.

Saunders, R.D. Kowalczuk, C.I., and Sienkiewicz, Z.J. (1991). Biological effects of exposure to non-ionizing electromagnetic fields and radiation: III. Radiofrequency and microwave radiation, National Radiological Protection Board, Chilton, UK, NRPB-R240.

Scheck, **M.,** Grahn, **T.,** Petts, **A.,** Gómez Hornillos**, A.**, Görgen **S.**, Greenlees, **P.,** Helariutta, **K.,** Jolie, **J.,** Juutinen**, S.** (1992)**.** Lifetimes of odd-spin yeasty states in ^{182}Hg, Journal *of Physical Review,* **(81):** 310-319

Schwan, H. (1963). Electric characteristics of tissues, a survey. *Biophysik* **1**, 198

Schwan, H. (1971). Interaction of microwave and radiofrequency radiation with biological systems. *IEEE* Trans. MIT-**19**, 146-152,

Schwan, H. (1972). Microwave radiation: Biophysical considerations and standard criteria. *IEEE* BME-**19**, 302--312

Schwan, H. (1973). Non-ionizing radiation hazards. *J. Franklin Institute* **296:** 485-497

Schwan, H. (1973). Principles of interaction of microwave at cellular and molecular level. *Proe. Int. Syrup*. Warsaw 152-159.

Schwan, H. and Sher, I. (1969). Alternating current field-induced forces and their biological implications. *J. Electrochem. Society* **(116):** 22C-26C

Schwan, H.P. and Foster, K.R. (1980). RF-field interactions with biological systems: electrical properties and biophysical mechanisms. Proceedings of the *Institute of Electrical and Electronics Engineers*, **(68):** 104-113.

Seaman, R.L. and Wachtel, H. (1978). Slow and rapid responses to CW and pulsed microwave radiation by individual aplysia pacemakers. *Journal of Microwave Power* **13:** 77-86

Shandala, M.A. (1977). Patterns of change in behavioural reactions to low power densities of microwave. Abstracts, International symposium on the Biological Effects of Electromagnetic waves (URSI), Airlie, VA.

Shapiro, A. R., Lutomirski, R. F., Yura, H. T. (1971). Induced fields and heating within the cranial structure irradiated by an EM place wave. *IEEE* Trans. MIT-**19**, 187-197

Sienkiewicz, Z.J., Blackwell, R.P., Haylock, R.G., Saunders, R.D. and Cobb, B.L. (2000). Low-level exposure to pulsed 900 MHz microwave radiation does not cause deficits in the performance of a spatial learning task in mice. *Bioelectromagnetics* **21(3):**151-158.

Sigler, A.T., Lilienfeld, A.M., Cohen, B.H. and Westlake, J.E. (1965). Radiation exposure in parents of children with mongolism (Down's syndrome). *Bulletin of the Johns Hopins Hospital*, **(117):** 374.

Simko´, M. (2004). Induction of cell activation processes by low frequency electromagnetic fields. *Scientific World Journal,* **(4):** 4–22.

Singh, N.P., Lai, H., Khan, A., 1995. Ethanol-induced single-strand DNA breaks in rat brain cells. *Mutation Research,* **(345):** 191–196

Spiegel, R. (1982). The thermal response of a human in the near zone of a resonant thin-wire antenna. *IEEE* transactions on microwave theory and techniques, **MTT-30:** 177-185.

Spiegel, R. (1984). A review of numerical models for predicting the energy deposition and resultant thermal response of humans exposed to electromagnetic fields. *IEEE* transactions on microwave theory and techniques, **MTT-32:** 730-746.

Stronati, L., Testa, A., Moquet, J., Edwards, A., Cordelli, E., Villani, P., Marino, C., Fresegna, A.M., Appolloni, M. and Lloyd, D. (2006). 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes. *Int J Radiat Biol.* **82(5):** 339-346.

Stuchly, M. A. (1979). Interaction of radiofrequency and microwave radiation with living systems. A review of mechanisms, *Radiation and Environmental Biophysics* **16:**1 –14.

Swicord, M. and Poscow, E. (1986). Handbook of biological effect of electromagnetic radiation. Boca Raton, Fl, CRC Press.

Swicord, M.L., Edwards, G.S., Saqripanti, J.L. and Davis, C.C. (1983). Chain-length-dependent microwave absorption of DNA. *Biopolymers*, **22:** 2513-2516.

Szmigielski, S. (1997). Cancer morbidity in subjects occupationally exposed to high-frequency electromagnetic radiation. *Science Total Environ*ment **180:** 9-17.

Szwarnowski, S., Sheppard, R. J. (1977). *J. Phys. E.: Sci. Instrum*. **10**, 1163-1173

Takashima, S. (1979). Effects of modulated RF energy on the EEG of mammalian brains. *Radiation and Environmental Biophysics* **16:**15-27

Takashima, S. and Minakata, A. (1973). Dielectric behavior of biological macromolecules, digest dielectric literature **(37):** 602--653

Tanchev, S., Geogieva, S., Georgiev, P. and Popov, B. (2005). Genetic effects in somatic and germ cells in rabbits following external gamma radiation. *Trakia Journal of Sciences,* **(3):** 49-52.

Tice, R.R., Hook, G.G., Donner, M., McRee, D.I. and Guy A.W. (2002). Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. *Bioelectromagnetics* (**23):** 113-126.

Turner, T., and Giles, R. (1982). Sperm motility inhibiting factor in rat epididymis. *American Journal of Physiology* **(242):** R199-R203.

United States National Institutes of health (NIH) publication, **(85):** 23

Urban, P., Lukas, E. and Roth, Z. (1998). Does acute exposure to the electromagnetic field emitted by a mobile phone influence visual evoked potentials? A pilot study. *Cent Eur J Public Health* **6(4):** 288-290.

Verschaeve, L. (1995). Can non-ionizing radiation induce cancer? *Cancer Journal* **8:** 237-249.

Verschaeve, L. and Maes, A. (1997). Genetic, carcinogenic and teratogenic effects of radiofrequency fields. *Mutation Research* **410:** 141-165.

Verschaeve, L., Heikkinen, P., Verheyen, G., Van Gorp, U., Boonen, F., Vander Plaetse, F., Maes, A., Kumlin, T., Maki-Paakkanen, J., Puranen, L. and Juutilainen, J. (2006). Review of effects of radiofrequency fields, *Mutation Research* **424:** 127-134

Veyret, B. (1991). Antibody response in mice to low power microwaves under combined pulsed and amplitude modulation. *Bioelectromagnetics* **87:** 375-380.

Vijayalaxmi, M, Leal, B.Z., Szilagyi, M., Prihoda, T.J. and Meltz, M.L. (2000). Primary DNA Damage in Human Blood Lymphocytes Exposed In Vitro to 2450 MHz *Radiat Res* **153(3):**334-338

Vijayalaxmi, M., Bisht, K.S., Pickard, W.F., Meltz, M.L. and Roti, J.L. Moros EG. (2001c). Chromosome damage and micronucleus formation in human blood lymphocytes exposed in vitro to radiofrequency radiation at a cellular telephone frequency (847.74 MHz, CDMA). *Radiat Res* **156(4):**430-432.

Vijayalaxmi, M., Frei, M.R,, Dusch, S.J., Guel, V, Meltz, M.L. and Jauchem, J.R. (1997b). Frequency of micronuclei in the peripheral blood and bone marrow of cancer-prone mice chronically exposed to 2450 MHz radiofrequency radiation. *Radiat Res* **147(4):**495-500.

Vijayalaxmi, M., Leal, B.Z., Meltz, M.L., Pickard, W.F., Bisht. K.S., Roti J.L. and Straube, W.L. and Moros E.G.(2001b). Cytogenetic Studies in Human Blood Lymphocytes Exposed In Vitro to Radiofrequency Radiation at a Cellular Telephone Frequency (835.62 MHz, FDMA). *Radiat Res* **155(1):**113-121.

Vijayalaxmi, M., Meltz, M.L. and Wittler, M.A. (1997a). Proliferation and cytogenetic studies in human blood lymphocytes exposed in vitro to 2450 MHz radiofrequency radiation. *Int J Radiat Biol* **72(6):**751-757.

Vijayalaxmi, M., Sasser, L.B., Morris, J.E., Wilson, B.W. and Anderson, L.E.(2003). Genotoxic Potential of 1.6 GHz Wireless Communication Signal: In Vivo Two-Year Bioassay. *Radiat Res* **159(4):**558-564.

Vijayalaxmi, M., Seaman, R.L., Belt, M.L., Doyle, J.M., Mathur, S.P. and Prihoda, T.J. (1999). Frequency of micronuclei in the blood and bone marrow cells of mice exposed to ultra-wideband electromagnetic radiation. *Int J Radiat Biol.* **75(1):**115-120.

Von Klitzing, L. (1995). Low-frequency pulsed electromagnetic fields influence EEG of man. *Phys. Medica* **11:** 77-80.

Wagner, P., Roschke, J., Mann, K., Fell, J., Hiller, W. and Frank C. (2000). Grozinger M, Human sleep EEG under the influence of pulsed radio frequency electromagnetic fields. results from polysomnographies using submaximal high power flux densities. *Neuropsychobiology*, **42(4):** 207-212.

Wagner, P., Roschke, J., Mann, K., Hiller, W. and Frank, C. (1998). Human sleep under the influence of pulsed radiofrequency electromagnetic fields: a polysomnographic study using standardized conditions. *Bioelectromagnetics* **19(3):** 199-202.

Watanabe, Y., Nakagawa, M. and Miyakoshi, Y. (1997). Enhancement of lipid peroxidation in the liver of mice exposed to magnetic fields," *Ind. Health,* **(35):** 285–290.

WHO - World Health Organization (2007). Extremely low frequency fields. Environmental Health Criteria, **238** Geneva, Switzerland.

WHO, (1993). Electromagnetic fields (300 Hz-300 GHz), environmental health criteria, WHO, Geneva **137:** 290.

World Health Organization (1989). Non-ionizing radiation protection, **25:** 176-182.

World Health Organization, (2007). ELF Health Criteria Monograph. Neurodegenerative

Yamaguchi, H., Tsurita, G., Ueno, S., Watanabe, S., Wake, K., Taki, M. and Nagawa, H. (2003). 1439 MHz pulsed TDMA fields affect performance of rats in a T-maze task only when body temperature is elevated. *Bioelectromagnetics* **24(4):** 223-230.

Yariv, A. (1985). Optical Electronics. Holt, Rinehart and Wiston, New York.

Yemitan, O. and Adeyemi, O. (2003). Anxiolytic and Muscle-Relaxant Activities of DALBERGIA SAXATILIS. West *African Journal of Pharmacology and Drug Research* **19(1, 2):** 42-46.

Yu, B. (1994). Rates of convergence for empirical processes of stationary mixing sequences. Ann. Probab**. (22):** 94-116

Yuasa, K., Arai, N., Okabe, S., Tarusawa, Y., Nojima, T., Hanajima, R., Terao, Y. and Ugawa, Y. (2006). Effects of thirty minutes mobile phone use on the human sensory cortex. *Clin Neurophysiol.,* **(117):** 900-905.

Yuriko, K. (1999). DNA analysis by microfabricated capillary electrophoresis device. *Nucleic Acids Symposium Series* **42:** 57-58.

Zaret, M.M. (1974). Cataracts following use of microwave oven. *New York State Journal of Medicine*, **74:** 2032-2048.

Zeni, O., Romano, M., Perrotta, A., Lioi, M.B., Barbieri, R., d'Ambrosio, G., Massa, R. and Scarfi, M.R. (2005). Evaluation of genotoxic effects in human peripheral blood leukocytes following an acute in vitro exposure to 900 MHz radiofrequency fields. *Bioelectromagnetics* **26(4):** 258-265.

Zeni, O., Schiavoni, A. S., Sannino, A., Antolini, A., Forigo, D., Bersani, F. and Scarfi, M. R. (2003). Lack of Genotoxic Effects (Micronucleus Induction) in Human Lymphocytes Exposed In Vitro to 900 MHz Electromagnetic Fields. *Radiat. Res.* **(160):** 152-158.

Appendix

Polymerase Chain Reaction (PCR) of DNA Sample

Gel picture

DNA Quantification for Blood Sample (Male)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CM	3.0	1.83	1.55	0.033	0.050	0.027	0.000
M1	8.0	1.75	1.44	0.112	0.160	0.093	0.003
M2	5.0	1.67	1.05	0.091	0.095	0.058	0.002
M3	3.0	1.69	1.13	0.047	0.053	0.031	0.000
M4	3.0	1.83	1.29	0.038	0.049	0.027	0.000
M5	4.0	1.82	1.54	0.054	0.083	0.045	0.000
M6	2.0	1.77	1.06	0.041	0.043	0.025	0.001
M7	1.0	1.56	0.74	0.036	0.027	0.018	0.002
M8	2.0	1.65	0.91	0.041	0.037	0.022	0.000
M9	4.0	1.80	1.26	0.064	0.079	0.046	0.003

DNA Quantification for Blood Sample (Female)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CF	6.0	1.86	1.47	0.083	0.118	0.066	0.006
F1	5.0	1.68	1.63	0.223	0.159	0.115	0.050
F2	4.0	1.53	0.72	0.129	0.101	0.077	0.031
F3	2.0	1.81	0.92	0.060	0.056	0.033	0.006
F4	1.0	1.77	0.062	0.048	0.03	0.019	0.003
F5	4.0	1.84	1.08	0.076	0.082	0.046	0.004
F6	7.0	1.90	1.46	0.116	0.153	0.085	0.006
F7	1.0	1.87	0.58	0.048	0.029	0.018	0.002
F8	3.0	1.91	0.93	0.085	0.080	0.051	0.018
F9	3.0	1.91	0.86	0.080	0.083	0.056	0.022

DNA Quantification for Spleen Sample (Male)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CM	68	1.91	2.02	0.721	1.406	0.762	0.053
M1							
M2	20	1.88	1.81	0.225	0.399	0.217	0.009
M3	25	1.90	2.07	0.249	0.509	0.272	0.007
M4	24	1.92	2.15	0.229	0.490	0.257	0.003
M5	24	1.89	2.03	0.253	0.503	0.271	0.011
M6	15	1.90	2.02	0.160	0.314	0.172	0.010
M7	3.0	1.81	1.51	0.041	0.062	0.034	0.000
M8	62	1.93	2.17	0.583	1.255	0.657	0.011
M9	116	1.89	2.11	1.133	2.350	1.259	0.038

DNA Quantification for Spleen Sample (Female)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CF	23	1.90	2.10	0.218	0.454	0.241	0.004
F1	89	1.91	2.09	0.882	1.808	0.965	0.035
F2	22	1.87	1.95	0.258	0.476	0.269	0.030
F3	61	1.93	2.08	0.612	1.250	0.659	0.022
F4	3.0	1.78	1.23	0.052	0.064	0.036	0.001
F5	64	1.92	2.13	0.624	1.300	0.689	0.024
F6	46	1.90	2.17	0.426	0.922	0.483	0.003
F7	5.0	1.79	1.42	0.069	0.097	0.055	0.001
F8	60	1.88	1.98	0.694	1.291	0.727	0.082
F9	105	1.90	2.06	1.055	2.134	1.144	0.039

DNA Quantification for Heart Sample (Male)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CM	25	1.90	2.30	0.221	0.508	0.267	0.000
M1	6	1.88	2.25	0.051	0.116	0.062	0.000
M2	4.0	1.80	1.87	0.039	0.074	0.041	0.000
M3	7.0	1.89	2.15	0.067	0.144	0.076	0.000
M4	10	1.90	1.95	0.101	0.197	0.104	0.000
M5	30	1.96	2.10	0.286	0.597	0.306	0.002
M6	1.0	1.22	0.48	0.023	0.011	0.009	0.000
M7	1.0	1.81	1.64	0.019	0.031	0.017	0.000
M8	47	1.92	2.23	0.419	0.934	0.487	0.000
M9	10	1.89	2.09	0.099	0.207	0.109	0.000

DNA Quantification for Heart Sample (Female)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CF	8.0	1.86	2.05	0.073	0.149	0.081	0.000
F1	5.0	1.77	1.69	0.054	0.091	0.052	0.000
F2	2.0	1.75	1.44	0.027	0.039	0.022	0.000
F3	19	1.89	2.02	0.186	0.374	0.199	0.002
F4	1.0	1.47	0.67	0.022	0.015	0.010	0.000
F5	6.0	1.87	1.92	0.060	0.116	0.062	0.000
F6	1.0	1.70	1.15	0.019	0.021	0.012	0.000
F7	1.0	1.52	0.74	0.016	0.012	0.008	0.000
F8	9.0	1.84	1.99	0.091	0.181	0.099	0.000
F9	19	1.88	2.12	0.184	0.389	0.206	0.000

DNA Quantification for Brain Sample (Male)

Sample	Density (µg/ul)	A260/280	260/230	A230	260	280	320
CM	12	1.94	1.96	0.132	0.257	0.135	0.002
M1	2.0	1.89	1.73	0.027	0.046	0.024	0.000
M2	12	1.85	1.91	0.128	0.244	0.133	0.001
M3	6.0	1.83	1.82	0.072	0.130	0.072	0.002
M4	11	1.90	2.00	0.114	0.227	0.119	0.000
M5	17	1.91	1.66	0.222	0.354	0.195	0.022
M6	4.0	1.79	1.47	0.057	0.082	0.047	0.003
M7	4.0	1.85	1.66	0.052	0.084	0.046	0.002
M8	17	1.91	1.94	0.177	0.343	0.180	0.001
M9	32	1.64	1.75	0.373	0.651	0.399	0.003

DNA Quantification for Brain Sample (Female)

Sample	Density (µg/ul)	A260/280	260/230	A230	260	280	320
CF	5.0	1.87	1.53	0.062	0.093	0.052	0.001
F1	5.0	1.82	1.60	0.067	0.105	0.060	0.004
F2	8.0	1.81	1.74	0.090	0.156	0.086	0.001
F3	20	1.93	1.75	0.228	0.396	0.207	0.003
F4	7.0	1.80	1.50	0.094	0.140	0.075	0.002
F5	3.0	1.78	1.47	0.046	0.066	0.038	0.002
F6	4.0	1.81	1.53	0.053	0.080	0.045	0.002
F7	2.0	1.67	1.11	0.033	0.037	0.027	0.002
F8	20	1.91	1.69	0.257	0.422	0.229	0.018
F9	11	1.89	1.81	0.129	0.227	0.124	0.009

DNA Quantification for Kidney Sample (Male)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CM	33	1.90	2.14	0.318	0.667	0.358	0.013
M1	6.0	1.82	1.91	0.062	0.117	0.065	0.001
M2	4.0	1.75	1.56	0.050	0.076	0.045	0.004
M3	6.0	1.79	1.57	0.081	0.122	0.072	0.009
M4	18	1.93	1.93	0.205	0.378	0.205	0.019
M5	18	1.90	2.00	0.188	0.208	0.112	0.002
M6	1.0	1.73	1.19	0.019	0.023	0.013	0.000
M7	23	1.89	2.08	0.334	0.675	0.365	0.018
M9	20	1.88	1.87	0.223	0.407	0.221	0.011

DNA Quantification for Kidney Sample (Female)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CF	18	1.88	1.96	0.193	0.365	0.201	0.014
F1	11	1.83	1.88	0.122	0.222	0.125	0.009
F2	14	1.87	1.79	0.179	0.306	0.173	0.019
F3	28	1.88	1.54	0.414	0.616	0.353	0.053
F4	2.0	1.64	1.26	0.029	0.037	0.023	0.001
F5	6.0	1.85	1.78	0.074	0.128	0.071	0.004
F6	3.0	1.74	1.48	0.041	0.059	0.035	0.003
F7	3.0	1.74	1.30	0.045	0.057	0.035	0.006
F8	20	1.85	1.85	0.231	0.413	0.232	0.018
F9	26	1.86	2.02	0.278	0.545	0.302	0.017

DNA Quantification for Lung Sample (Male)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CM	57	1.91	2.12	0.604	1.272	0.669	0.009
M1	20	1.93	2.16	0.192	0.405	0.214	0.009
M2	19	1.89	1.96	0.194	0.376	0.202	0.005
M3	10	1.89	1.84	0.108	0.197	0.105	0.001
M4	31	1.96	2.12	0.297	0.627	0.321	0.003
M5	28	1.93	2.17	0.260	0.565	0.293	0.001
M6	6.0	1.95	2.000.068	0.129	0.070	0.186	0.002
M7	6.0	1.89	2.00	0.057	0.115	0.061	0.000
M8	31	1.89	2.22	0.284	0.629	0.334	0.002
M9	30	1.91	2.08	0.287	0.594	0.312	0.003

DNA Quantification for Lung Sample (Female)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CF	12	1.89	2.18	0.107	0.232	0.123	0.001
F1	19	1.90	2.13	1.343	0.638	0.718	0.033
F2	54	1.93	2.07	0.593	1.148	0.673	0.072
F3	47	1.90	2.30	0.414	0.942	0.500	0.008
F4	4.0	1.90	1.82	0.044	0.081	0.042	0.001
F5	5.0	1.86	2.09	0.052	0.108	0.058	0.000
F6	5.0	1.83	1.92	0.052	0.100	0.055	0.000
F7	6.0	1.84	1.94	0.073	0.133	0.077	0.010
F8	15	1.96	1.89	0.177	0.323	0.171	0.014
F9	66	1.89	2.21	0.616	1.344	0.719	0.017

DNA Quantification for Testis Sample (Male)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CM	82	1.97	2.24	0.740	1.643	0.837	0.010
M1	7.0	1.89	2.07	0.072	0.149	0.079	0.000
M2	20	1.91	1.97	0.219	0.416	0.225	0.015
M3	22	1.96	2.07	0.235	0.470	0.249	0.016
M4	26	1.98	2.18	0.243	0.526	0.267	0.005
M5	69	2.03	2.16	0.648	1.392	0.689	0.009
M6	5.0	1.95	1.83	0.065	0.112	0.062	0.009
M7	3.0	1.88	1.92	0.040	0.071	0.040	0.005
M8	4.0	1.89	1.64	0.052	0.083	0.046	0.004
M9	1.0	2.05	1.39	0.021	0.025	0.016	0.003

DNA Quantification for Ovary Sample (Female)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CF	18	1.96	2.21	0.172	0.373	0.194	0.006
F1	25	1.94	2.06	0.252	0.508	0.267	0.011
F2	49	1.95	2.17	0.476	1.001	0.526	0.028
F3	24	1.96	2.13	0.233	0.489	0.253	0.008
F4	2.0	1.95	1.56	0.041	0.059	0.034	0.009
F5	13	1.94	2.13	0.127	0.261	0.139	0.009
F6	4.0	1.93	1.63	0.110	0.145	0.101	0.055
F7	5.0	1.93	1.79	0.068	0.113	0.064	0.010
F8	27	1.91	2.19	0.260	0.556	0.296	0.011
F9	17	1.88	2.21	0.162	0.351	0.190	0.007

DNA Quantification for Liver Sample (Male)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CM	14	1.94	2.05	0.135	0.275	0.142	0.001
M1	5.0	2.05	1.95	0.050	0.098	0.048	0.000
M2	25	2.00	1.96	0.260	0.505	0.256	0.006
M3	7.0	2.03	1.82	0.080	0.145	0.072	0.000
M4	47	2.07	1.95	0.495	0.954	0.465	0.009
M5	32	2.02	2.04	0.314	0.639	0.317	0.001
M6	3.0	2.03	1.62	0.042	0.067	0.033	0.000
M7	2.0	2.14	1.49	0.030	0.044	0.021	0.000
M8	45	2.02	2.15	0.438	0.924	0.465	0.016
M9	49	2.00	2.13	0.470	0.989	0.501	0.011

DNA Quantification for Liver Sample (Female)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CF	69	2.05	1.80	0.799	1.410	0.706	0.035
F1	14	1.97	1.78	0.154	0.275	0.140	0.000
F2	18	1.97	1.90	0.205	0.376	0.198	0.014
F3	35	2.02	2.02	0.356	0.713	0.354	0.004
F4	12	1.99	1.93	0.128	0.247	0.124	0.000
F5	63	2.05	1.96	0.657	1.274	0.631	0.018
F6	1.0	2.08	1.08	0.025	0.027	0.013	0.000
F7	27	1.99	2.08	0.267	0.546	0.278	0.008
F8	52	1.99	1.97	0.537	1.048	0.531	0.009
F9	38	1.97	2.05	0.371	0.760	0.386	0.001

DNA Quantification for Thyroid Sample (Male)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CM	37	1.97	1.91	0.393	0.746	0.382	0.005
M1	13	1.96	1.71	0.156	0.261	0.137	0.001
M2	6.0	1.88	1.53	0.082	0.124	0.067	0.002
M3	5.0	1.92	1.53	0.075	0.111	0.061	0.006
M4	18	1.99	1.74	0.212	0.368	0.186	0.003
M5	38	1.97	2.09	0.367	0.760	0.390	0.008
M6	100	1.93	2.22	0.906	2.012	1.041	0.002
M7	3.0	1.91	1.28	0.047	0.060	0.032	0.001
M8	87	2.01	1.90	0.937	1.767	0.890	0.017
M9	19	1.98	1.36	0.287	0.389	0.199	0.006

DNA Quantification for Thyroid Sample (Female)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CF	42	1.94	2.09	0.404	0.839	0.436	0.005
F1	34	2.07	1.68	0.412	0.684	0.336	0.011
F2	12	1.91	1.94	0.131	0.248	0.133	0.006
F3	48	1.96	1.71	0.573	0.974	0.503	0.013
F4	4.0	1.85	1.40	0.065	0.088	0.051	0.007
F5	9.0	1.96	1.68	0.106	0.175	0.092	0.005
F6	3.0	1.82	1.16	0.048	0.055	0.031	0.002
F7	84	1.92	2.16	0.799	1.706	0.894	0.016
F8	64	1.95	1.88	0.703	1.302	0.679	0.021
F9	53	2.02	1.88	0.570	1.061	0.532	0.011

DNA Quantification for Prostate Sample (Male)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
CM	63	1.99	2.06	0.623	1.273	0.646	0.011
M1	7.0	1.83	1.27	0.134	0.165	0.098	0.017
M2	14	1.95	1.77	0.165	0.283	0.150	0.011
M3	12	1.86	1.45	0.196	0.269	0.162	0.038
M4	13	1.94	1.67	0.161	0.266	0.144	0.009
M5	10	1.95	1.65	0.133	0.210	0.114	0.014
M6	2.0	1.88	0.98	0.053	0.053	0.036	0.018
M7	1.0	1.72	0.80	0.044	0.037	0.024	0.007
M8	7.0	1.88	1.72	0.087	0.145	0.080	0.006
M9	53	1.98	2.07	0.533	1.085	0.556	0.016

DNA Quantification for Tail Sample (Male and Female)

Sample	Density (μg/ul)	A260/280	260/230	A230	260	280	320
M6	10	1.85	1.67	0.137	0.216	0.125	0.019
M7	12	1.82	1.39	0.223	0.288	0.184	0.056
M8	22	1.55	1.48	0.302	0.433	0.288	0.009
M9	11	1.93	1.81	0.140	0.243	0.132	0.013
F6	5.0	1.83	1.41	0.056	0.115	0.069	0.014
F7	9.0	1.85	1.46	0.142	0.198	0.116	0.021
F8	18	1.92	1.89	0.206	0.377	0.203	0.013
F9	14	1.89	1.83	0.161	0.284	0.156	0.012

CF –F9 represent the female and CM-M9 is for male rats

Effect of Microwave Radiation on the Exploratory Behaviour of Male Rats; The number of head dips corresponding to different SARs used.

Time (Days)	0.48 W/kg	0.95 W/kg	1.43 W/kg	1.91 W/kg	2.39 W/kg	CONTROL
IH	8.5 ± 0.58	6.8 ± 1.64	6.25 ± 0.5	3.4 ± 0.55	2.8 ± 0.45	15.6 ± 4.88
1	8.3 ± 0.34	6.3 ± 0.22	5.8 ± 0.34	3.2 ± 0.14	2.3 ± 0.26	15.5 ± 0.56
2	7.5 ± 0.24	5.8 ± 0.14	5.3 ± 0.23	2.7 ± 0.11	2.1 ± 0.13	15.5 ± 0.44
3	7.0 ± 0.13	5.2 ± 0.23	4.8 ± 0.05	2.5 ± 0.22	1.8 ± 0.11	15.7 ± 1.88
4	7.1 ± 0.26	5.3 ± 0.27	4.6 ± 0.34	2.45 ± 0.12	1.8 ± 0.33	15.8 ± 0.53
5	7.3 ± 0.44	5.6 ± 0.18	5.1 ± 0.54	2.6 ± 0.33	2.0 ± 0.16	15.6 ± 0.74
6	7.3 ± 0.34	5.6 ± 0.44	5.1 ± 0.22	2.6 ± 0.45	2.0 ± 0.19	15.7 ± 1.34
7	7.4 ± 0.62	5.8 ± 0.19	5.5 ± 0.87	2.9 ± 0.32	2.4 ± 0.25	15.6 ± 0.69
8	7.9 ± 0.21	5.9 ± 0.25	5.8 ± 0.45	3.0 ± 0.42	2.6 ± 0.77	15.7 ± 1.22
9	8.1 ± 0.31	6.0 ± 0.16	5.8 ± 0.27	3.0 ± 0.12	2.6 ± 0.54	15.5 ± 2.30
10	8.4 ± 0.51	6.1 ± 0.28	5.9 ± 0.15	3.0 ± 0.11	2.6 ± 0.17	15.6 ± 0.45
11	8.5 ± 0.25	6.1 ± 0.07	5.9 ± 0.33	3.1 ± 0.41	2.7 ± 0.15	15.7 ± 1.21
12	10.7 ± 0.47	7.4 ± 0.21	6.3 ± 0.11	3.7 ± 0.13	3.3 ± 0.05	16.2 ± 0.48
13	11.2 ± 0.22	7.8 ± 0.51	6.6 ± 0.22	4.2 ± 0.15	3.5 ± 0.09	15.6 ± 1.01
14	12.5 ± 0.61	8.1 ± 0.66	7.1 ± 0.81	5.3 ± 0.44	4.2 ± 0.14	15.8 ± 1.10
15	13.7 ± 1.23	9.5 ± 0.71	9.1 ± 0.17	6.6 ± 0.84	6.1 ± 0.27	15.9 ± 0.92
3 Weeks	15.4 ± 0.44	15.1 ± 0.43	14.9 ± 1.03	14.6 ± 0.55	13.6 ± 0.54	16.1 ± 0.23

Effects of Microwave Radiation on the Exploratory Behaviour of Female Rats; The number of head dips corresponding to different SARs used

Time (Days)	0.48 W/kg	0.95 W/kg	1.43 W/kg	1.91 W/kg	2.39 W/kg	CONTROL
IH	8.3 ± 0.44	5.9 ± 0.95	5.7 ± 0.35	3.2 ± 1.07	2.9 ± 0.12	14.8 ± 1.51
1	8.1 ± 0.15	5.6 ± 0.09	4.6 ± 0.29	2.1 ± 0.48	2.0 ± 0.10	14.6 ± 0.14
2	7.90 ± 0.23	5.2 ± 0.87	4.3 ± 0.02	2.0 ± 0.43	2.0 ± 0.54	14.9 ± 0.98
3	7.5 ± 0.47	5.1 ± 0.18	4.1 ± 1.08	1.7 ± 0.13	1.5 ± 0.01	14.5 ± 1.17
4	7.20 ± 0.10	4.7 ± 1.03	4.0 ± 0.31	1.5 ± 0.10	1.3 ± 0.19	14.8 ± 0.12
5	7.5 ± 0.26	5.3 ± 0.10	4.0 ± 0.26	1.7 ± 0.10	1.3 ± 0.17	14.9 ± 0.18
6	7.8 ± 0.71	5.3 ± 1.14	4.1 ± 0.17	1.7 ± 0.26	1.1 ± 0.00	15.1 ± 0.21
7	7.8 ± 0.33	5.4 ± 0.12	4.8 ± 0.78	1.8 ± 0.57	1.6 ± 0.07	15.3 ± 1.06
8	8.0 ± 0.06	5.9 ± 0.83	5.1 ± 0.36	2.4 ± 0.15	1.9 ± 0.76	15.1 ± 0.91
9	8.1 ± 0.00	6.2 ± 0.13	5.8 ± 0.18	2.8 ± 1.04	2.1 ± 0.83	14.7 ± 0.10
10	8.35 ± 0.17	7.1 ± 1.27	6.3 ± 0.29	3.4 ± 0.55	2.7 ± 0.66	14.7 ± 1.01
11	8.5 ± 1.16	7.9 ± 0.99	6.5 ± 0.22	3.9 ± 0.37	2.9 ± 0.42	14.9 ± 0.44
12	10.5 ± 1.08	8.2 ± 1.44	7.1 ± 1.25	4.2 ± 0.21	3.5 ± 0.71	15.2 ± 0.51
13	12.7 ± 0.29	8.85 ± 1.09	7.7 ± 0.73	5.7 ± 0.19	4.3 ± 0.13	15.3 ± 0.26
14	12.9 ± 1.72	9.20 ± 0.34	8.2 ± 0.46	6.1 ± 0.17	4.8 ± 1.03	14.7 ± 0.31
15	14.0 ± 0.48	12.2 ± 1.64	8.6 ± 0.52	7.2 ± 1.11	5.4 ± 0.65	14.8 ± 0.48
3 Weeks	14.7 ± 1.90	13.7 ± 0.93	13.4 ± 0.27	13.4 ±1.06	13.1 ± 0.11	15.1 ± 0.12

Chromosomal Aberrations in Bone Marrow Cells of Male Rats Exposed to Various SARs of Microwave Radiation

Chromosomal Aberrations	No. of Metaphase Analyzed	Control	0.95 W/kg	1.43 W/kg	1.91 W/kg	2.39 W/kg
Gaps	200	0.35 ± 0.02	2.31 ± 0.07*	3.65 ± 0.41*	4.61 ± 0.17*	6.45 ± 0.24*
Breaks	200	0.39 ± 0.04	3.76 ± 0.21*	5.66 ± 0.05*	5.98± 0.10*	7.28 ± 0.08*
Acentrics	200	0.13 ± 0.01	4.71 ± 0.01*	6.36 ± 0.22*	7.37 ± 0.01*	8.11 ± 0.15*
Centric rings	200	0.09 ± 0.0	1.10 ± 0.31*	1.12 ± 0.09*	1.20 ± 0.10*	1.24± 0.21*

Chromosomal Aberrations in Bone Marrow Cells of Female Rats Exposed to Various SARs of Microwave Radiation

Chromosomal Aberrations	No. of Metaphase Analyzed	Control	0.95 W/kg	1.43 W/kg	1.91 W/kg	2.39 W/kg
Gaps	200	0.34 ± 0.01	1.17 ± 0.09*	1.90 ± 0.55*	2.26 ± 0.11*	3.26 ± 0.17*
Breaks	200	0.41 ± 0.06	3.79 ± 0.25*	5.48 ± 0.34*	5.67 ± 0.15*	7.17 ± 0.07*
Acentrics	200	0.1 ± 0.01	4.76 ± 0.12*	5.89 ± 0.18*	7.30 ± 0.13*	8.76 ± 0.03*
Centric rings	200	0.07 ± 0.0	1.10 ± 0.03*	1.16 ± 0.07*	1.16 ± 0.05*	1.22 ± 0.14*

DNA Single Strand Break following Exposure to 2.45 GHz Microwave Radiation at 2.39 W/kg

		% DNA Tail	Olive Moment
Brain	Control	7.22 ± 4.88	1.18 ± 0.10
	2.39 W/kg	28.67 ± 0.37*	4.84 ± 0.60*
Liver	Control	5.34 ± 1.94	1.43 ± 0.32
	2.39 W/Kg	9.86 ± 3.57*	3.40 ± 0.55*
Lung	Control	1.20 ± 0.10	0.98 ± 0.07
	2.39 W/kg	4.00 ± 1.01*	3.10 ± 0.17*
Spleen	Control	0.87 ± 0.13	0.60 ± 0.11
	2.39 W/kg	10.72 ± 2.40*	1.20 ± 0.15*
Testis	Control	0.15 ± 0.01	0.50 ± 0.01
	2.39 W/kg	3.35 ± 0.55*	2.46 ± 0.13*

* indicates significantly difference ($p < 0.05$) compared to control