EFFECTS OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS ON HUMAN CHROMOSOMES.

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Genetics

at the

Institute of Molecular BioSciences
Massey University, Palmerston North,
New Zealand

by

MOHAMMED ABDUL WAHAB

2005

Abstract.

Electromagnetic fields (EMFs) have been associated with increased incidences of cancer as suggested by epidemiological studies. The *in vitro* sister chromatid exchange (SCE) technique, radiation-induced micronucleus assay (MN assay), COMET assay, and fluorescence *in situ* hybridization (FISH) were used in the present study to test the carcinogenic potentiality of extremely low frequency (ELF) EMFs on human peripheral blood lymphocytes. All experiments were performed single blind and used lymphocytes taken from 6 age-matched donors. The SCE experiments were conducted twice: round 1 (R1) and round 2 (R2), in order to determine whether or not the results obtained could be duplicated.

Detailed analysis of the SCE results showed that there was a significant increase in the number of SCEs/cell in the grouped experimental conditions compared to the controls in both rounds. Similarly, in the MN assay, a significant increase of *mean number of micronucleated CB cells/100 CB cells* (M_a) and *mean number of micronuclei/100 CB cells* (M_b) was observed in the grouped experimental conditions compared to the controls. Moreover, the highest SCE frequency in R1 was 10.03 for a *square continuous* field, and the SCE frequency of 10.39 for a *square continuous* field in R2 (albeit a different strength) was the second highest in this latter round. But in the MN assay a *square pulsed* field with increasing EMF strength showed the greatest effect on the DNA repair system. The COMET assay also showed that both a *1mT square* field *(continuous or pulsed)* resulted in significant fragmentation of the DNA. On the other hand, a FISH analysis failed to show any translocations.

In the field of EMF research, perhaps the most outstanding question that remains to be answered with certainty is how weak EMFs exert their effects at the molecular level. Various mechanisms are reviewed and evaluated in this thesis. From the results of the research performed in the current study which concentrated on testing and discovering genetic effects, a model is postulated that weak EMFs stimulate the production of free radicals which result in genetic damage. Further extensive research should be conducted to test this hypothesis.

Acknowledgments.

I wish to extend my sincere gratitude to my supervisors, Dr. R.E. Rowland, and Dr. J.V. Podd, without whom I would not have had the wonderful opportunity to tackle this piece of work. Their constant invaluable guidance, advice, encouragement and supervision kept me on track and from both of them I have learnt a lot.

I would like to express my sincere thanks to:

- **Elizabeth Nickless-**who was an endless help in providing the invaluable advice throughout my research on methods and equipment.
- **Charlotte James -** who was my friend in need, and endless helper during my study.
- **Bruce Rapley -** for his invaluable contribution in building the special incubator to generate accurate ELF EMFs and assistance during the study.
- **Chris Kendrik -** his assistance with blood collection was invaluable and greatly appreciated. Chris s smiling face continually inspired me.
- **Chad Johnson** - who taught me how to perform the COMET assay.
- **Ted Drawneek -** is a nice person, who assisted me with all the statistical analysis.
- **Paul Hauquard, Michael Wilson** - who were two of my blood donors and gave me endless assistance and made my IMBS life much easier.
- **Amira Pearson** - Auckland University Medical School, who is a person with infinite patience from whom I learned the MN assay technique.
- **Colin Dicks and his oncology staff in the Palmerston North Hospital -** for their endless friendship and co-operation during this study.
- **Rissa Ota, Matthew Phillips, Leon Perrie, Kerryn Slack, Tim White, and Andrew Clarke -** who generously offered their computer expertise.

To my family, especially my wife **Kamrunnahar Begum**, and my many friends and colleagues who have given me endless support and space to complete this work, I give my hearfelt thanks. Their help and encouragement are sincerely appreciated.

Finally, I would like to express my sincere thanks to Massey University for providing me with the Massey Doctoral Scholarship and Palmerston North Medical Research Foundation for providing me with a research grant.

DEDICATED

TO

MY BELOVED WIFE

KAMRUNNAHAR BEGUM (Hira)

IN APPRECIATION OF HER
CONTINUOUS HELP,
SUPPORT AND
LOVE.

Table of Contents.

Chapter One: Introduction.

Chapter Two: Review of Literature.

Chapter Three: Materials and Methods.

Chapter Four: Results.

Chapter Five: Discussion.

Chapter Six: Summary and Limitations.

List of Tables.

List of Figures.

Chapter One

Introduction.

The use of electrical energy has been one of the most important conditions for the development of our present-day society. Electrical energy provides benefits and conveniences that society now deems necessities. As a consequence this has meant that technological development in the modern industrial world has lead to a steady increase in the production of artificial alternating current (AC) electromagnetic fields (EMFs). Almost all living organisms, including man, are exposed to a potpourri of these EMFs, but little is known about the biological effects of this kind of exposure, especially in the extremely low frequency (ELF) range as emitted by power appliances. This was the rationale for pursuing the current study in an attempt to answer the question: do weak EMFs exert an effect on human genetic material?

EMFs are invisible lines of force, which consist of an electric and magnetic component (Figure 1.1). The movement of current creates magnetic fields (MFs) and their strength

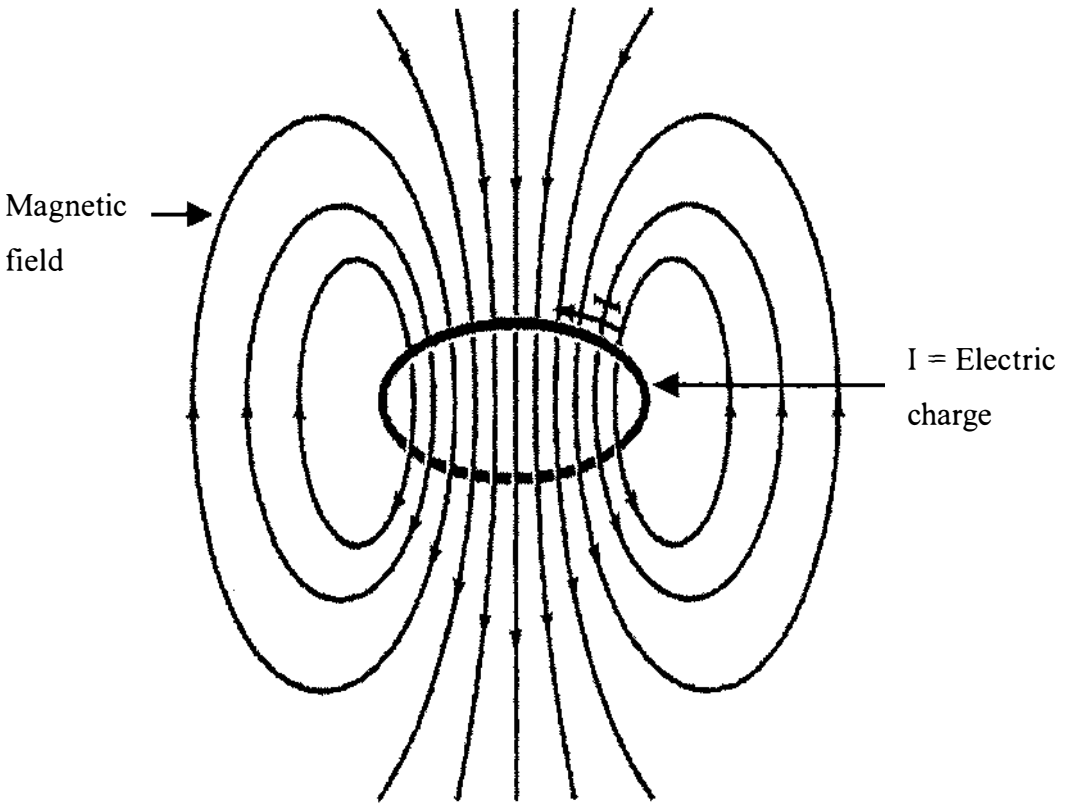


Figure 1.1: Showing the electric charge and magnetic field (Giancoli, 2000).

increases with the strength of current. NIOSH (National Institute for occupational safety and health) (1995) has drawn a comparison with water pressure in order to illustrate in simple terms the flow of electricity and the generation of MFs (Appendix 1a and 1b). Appendix 1c shows approximate MF strengths recorded from around a photocopier by EPA (Environmental Protection Agency, U.S.A.) (1992), and illustrates that the strength of the field decreases with increasing distance from the source. The harmful effects of short wavelengths in the electromagnetic spectrum, such as gamma rays, X-rays and ultraviolet (UV) light, are well known. These short wavelengths possess sufficient energy to break a chemical bond and can thereby destabilize an atom or molecule. Hence the term ionizing radiation . Longer wavelengths in the microwave range (10^9-10^{11} Hz), however, possess insufficient energy to disrupt chemical bonds (non-ionizing radiation), but do cause rapid heating of tissue as energy is absorbed. Even longer frequencies such as those used in the conductance of electricity (50-60 Hz) exert negligible thermal effects (Weaver and Astumian, 1990; Adair 1991; Tenforde, 1996). Nevertheless, evidence that these weak fields can exert a biological effect is steadily growing.

In recent years, interest has focused on the 50-60 Hz power-frequency range, largely because epidemiological studies have shown an increased risk of leukemia in children living close to electric power distribution lines (Blank, 1995). A great deal of attention has been generated by epidemiological studies associating weak EMFs with small increases in the odds ratios for various types of cancers (Werheimer and Leeper, 1979; Savitz *et al.* 1988; London *et al.* 1991; Feychting and Ahlbom, 1993). Washburn *et al.* (1994), in a meta-analysis of 13 published studies, also found statistically significant elevation in the risk of childhood leukemia and nervous tissue tumours among subjects living in close proximity to electricity transmission and distribution equipment.

Trans Power , the company which controls the distribution of electricity in New Zealand, has acknowledged some research findings which show a possible link between adverse health effects and EMFs (Power Line, 1994). Elevated risk of breast cancer among electrical workers was also reported in some studies (Matanoski *et al.* 1991; Demers *et al.* 1991; Tynes *et al.* 1992; Loomis *et al.* 1994). But no association between residential exposure and cancer risk among adults has been observed in several independent studies (National Radiological Protection Board, NRPB, 1992). Blackman

et al. (1985a, b) reported that, in some experiments, 'windows' of frequency or intensity have been observed, that is, effects were visible at one frequency and/or intensity that disappeared for both higher and lower values of the parameters.

On the other hand, electric devices based on ELF pulsed electromagnetic fields (PEMFs) have been approved for therapeutic use to accelerate healing of bone fractures (Andrew and Bassett, 1995; Fontanesi *et al.* 1986). Low and high frequency time-varying MFs are used in medical diagnostic procedures such as magnetic resonance imaging (MRI) (Brandon *et al.* 1996). High frequency EMFs also have a number of well-known medical uses ranging from diathermic warming of painful tissues to inducing hyperthermia in tumors.

Obviously, the need for sound scientific information has increased with growing public awareness of EMFs in the environment. In view of the above, the present study was conducted to evaluate the possible genetic effects in human peripheral blood lymphocytes (HPBLs) of ELF EMFs (50 Hz). Using a specially designed incubator that was built by members of our research team, cultured lymphocytes were exposed to all combinations of two different flux densities, one strong (1mT)[a] and one weak (1μT)[b], two different Waves (sine or square) and two different Forms (continuous or pulsed). In addition, a separate one-off experiment was conducted whereby a peripheral blood lymphocyte (PBL) culture was subjected to a complex field as emitted from the back of a computer, in order to detect whether or not this type of exposure can result in genetic effects.

The following four methodologies were applied in this study to investigate possible genetic effects on PBLs which were cultured for 3 days in the presence of certain ELF EMFs:

1) Sister Chromatid Exchange (SCE),
2) The Micronucleus Assay (MN assay),
3) The COMET Assay, and
4) Fluorescence *in situ* Hybridization (FISH).

(a) **1mT:** one milli-Tesla, (b) **1μT:** one micro-Tesla.

1.1 Sister Chromatid Exchange (SCE)

SCE is a widely acknowledged procedure for detecting genetic damage. The usefulness of this technique in determining DNA damage exploits the instability of the DNA helix during replication at S phase in a dividing population of cells. Many clastogenic[c] factors are known to interfere with DNA replication and cause the helix to twist. This exerts a tension on the newly replicating half-strand which switches across to the opposite backbone. In the presence of bromodeoxyuridine, a halogenic analogue of uridine, this can be detected in c-metaphase complements as an SCE (Figure 2.3). It is well documented that any increase in the frequency of SCE in an individual is linked to ill health (Sandberg *et al.* 1984).

In a normal healthy human, the background SCE frequency per cell varies with the conditions of the experiment, for example, lymphocyte concentration. Nevertheless, it is widely accepted that in a controlled experiment, any increase in the frequency of SCE is interpreted as evidence of clastogenic activity (Perry and Evans, 1975; Popescu *et al.* 1977).

In the present study, the frequency of SCE from two sets of experiments was analyzed in peripheral blood lymphocytes of six (3+3) healthy, non-smoking male blood donors. Participants were selected with similar age, lifestyles, occupational history, medical history and diet history in each round in order to identify any of the factors that may confound the analysis of SCEs. The background information of each individual in this study was obtained through a questionnaire (Appendix 2). All experiments were performed blind in order to eliminate bias in the analysis of data which have the potential to be highly controversial. It is important to mention here that the aim of this study was to compare cellular effects not to compare individual effects. The whole study was repeated blind under the same strict conditions.

(c) **Clastogen:** any substance or environmental agent that causes chromosomal abnormalities.

1.2 Micronucleus Assay (MN assay)

In our daily life we are unknowingly exposed to different environmental agents that damage our genetic material. But fortunately our body possesses molecular mechanisms that repair this damage. If these biological processes start to malfunction due to old age, ill health or as a consequence of clastogenic activity, then it is not unusual to find fragments or damaged chromosomes in dividing lymphocytes, which fail to attach to the mitotic spindle during cytokinesis. Chromosomes, which have lost their centromeres, or small acentric fragments, are excluded from nuclei and form what are called micronuclei (Figure 3.9).

The MN assay is a sophisticated cytomolecular technique, developed over recent years, that measures the degree of malfunctioning of a person's DNA repair system in the G_1 phase of the cell cycle. The technique has been extensively used for the biological monitoring of populations exposed to mutagenic and carcinogenic[d] agents in various environmental and occupational settings. Many cancer laboratories apply the technique when following the effects of radiotherapy and chemotherapy on patients (Catena *et al.* 1993). Some researchers speculate that in a few years the method may even be used as an alert signal in individuals prior to the clinical manifestation of cancer (Scott *et al.* 1996).

In essence, the MN assay involves counting numbers of micronuclei per hundred binucleated cells. An increase in the frequency of micronuclei in a targeted group compared to the controls indicates a breakdown of DNA repair. This technique was employed in the current study to determine whether or not ELF EMFs affect the DNA repair system in humans to any degree.

1.3 The COMET Assay

The COMET assay is also known as microgel electrophoresis. The COMET assay takes its name from the fact that highly fractionated lymphocytes look like comets under a microscope. They have a head and a tail, with the head being the stained remains of the

---

(d) **Carcinogenic:** A cancer-causing substance or agent.

lymphocyte nucleus and the tail being fractionated DNA, which has tailed out along the gel when electrophoresis is conducted. When DNA is broken, it breaks into little fractions, thus fractionation. The more fractionation, the more damage. The COMET assay measures the amount of DNA fractionation in any particular person studied. Caution is necessary when applying this assay: it is very sensitive and thus open to various interpretations as to what may be causing any fractionation observed. Nevertheless, it is a test that is widely conducted when searching for any genetic damage.

In the COMET assay, fresh lymphocytes are mixed on an agarose gel and mounted on a special microscope slide. The lymphocytes are lysed so that the nuclear membrane is disrupted. With the aid of various chemicals all of the non-DNA materials are removed. The slide is then placed into a buffer solution and electrophoresis performed. After a certain length of time the lymphocytes are stained with a fluorescent dye and observed under a fluorescent microscope. The background is naturally dark and the residual lymphocytes look like bright yellow comets with short tails in a sky (Figure 4.28b). In the current study a computer software package was used to analyse the length and morphology of these tails, but in essence, the longer the tail, the greater the degree of fractionation and thus the greater the genetic damage.

1.4 Fluorescence *in situ* Hybridization (FISH)

It is well known that one of the effects of radiation damage in humans is an increase in chromosome translocations (Bauchinger *et al.* 2001; Kodama *et al.* 2001; Lambert *et al.* 2001; Nakano *et al.* 2001; and Vorobtsova *et al.* 2001) which have the potential to lead to ill health, particularly a range of cancers, or inherited defects which may be passed on to offspring. To search for possible translocations as a consequence of EMF exposure, another technique was employed in this current study (FISH: fluorescent *in situ* hybridization) to selectively paint the chromosomes. By annealing a DNA probe from whole chromosomes 2, 3 and 5, these chromosomes were selectively stained with a green fluorescent dye (FITC) and the rest of the chromosomes were stained with a blue fluorescent dye (DAPI) (Figure 4.30). With this technique it is possible to detect any translocations (shift of any green across to a blue chromosome) that may occur as a consequence of exposure to a certain EMF during the 3-day period of peripheral

lymphocyte culture. In addition, hybridization of all the chromosomes with a pan-centromeric DNA probe labelled with a pink fluorescent dye was done in order to search for dicentric or multicentromeric chromosomes, which is common in radiation studies (Figure 4.30).

1.5 Aim, Objective and Prediction

The aim of this thesis was to test the null hypothesis that 50 Hz, ELF EMFs, as used in electric appliances, do not exert an effect on the human genetic material and thereby will not manifest clastogenic or mutagenic potentiality.

The objective of this thesis was to test the hypothesis using four cytomolecular techniques:

1) Sister Chromatid Exchange (SCE),
2) The Micronucleus Assay (MN assay),
3) The COMET Assay, and
4) Fluorescent *in situ* Hybridization (FISH).

Following the empirical method for scientific investigation, a prediction was made for each technique that if the null hypothesis were true, then no significant statistical difference should be observed between lymphocytes exposed to a weak EMF and sham-exposed controls.

Chapter Two

Review of Literature

2.1 ELF EMFs and Living Organisms

Electric power is a modern convenience that is irreplaceable. It benefits our daily lives and fuels appliances and machinery that we use frequently. Conversely, electric power lines, household wiring and electronic devices can cause serious injury from electric shock if handled improperly. To avoid danger, however, electric power comes with certain precautions that are well known to the user.

Appliances that operate either with batteries or which are connected to a household electric outlet are usually equipped with an AC/DC switch. If switched to alternating current (AC), the appliance uses electric power that flows back and forth or "alternates" at a (NZ) rate of 50 cycles per second (50 hertz, or Hz). If direct current (DC) is chosen, current flows in one direction from the battery to the appliance. AC fields induce weak electric currents in conducting objects, including humans; DC fields do not, unless the DC field changes in space or time relative to the person in the field. Induced currents from AC fields have been a focus for research on how EMFs could affect human health.

Power lines, electrical wiring and appliances all produce electric fields and MFs. EMFs are invisible lines of force that surround any electrical device while operating. Electric fields and MFs have different properties and possibly different ways of causing biological effects. It is worth mentioning here that while electric fields are easily shielded or weakened by conducting objects (e.g., trees, buildings, and human skin), MFs are not. However, both electric fields and MFs weaken with increasing distance from the source (Appendix 1c).

There is increasing concern that the household and environmental exposures to power frequency (50-60 Hz) EMFs may cause adverse health effects in humans. Interest in the possible health effects of EMFs grew with the publication of epidemiological studies that have shown an increased risk of cancer in children living close to electric power distribution lines (Wertheimer and Leeper, 1979). Two similar studies (Savitz *et al.*

1988; London *et al.* 1991) also found associations between residential wiring configuration and the incidence of childhood cancer. The study by Savitz *et al.* (1988) measured fields within the home in addition to classification by wiring configuration, and found an increase in risk correlated with MFs. However, the study by London *et al.* (1991) observed no correlation between measured MFs within the home and the incidence of cancer.

The first evidence of co-carcinogenic effects of ELF EMF exposure came from epidemiological studies by Savitz and Ahlbom (1994). DNA damage that accumulates in cells over a period of time could be the cause of slow-onset diseases, such as cancer. DNA strand breaks may affect cellular functions, which could lead to carcinogenesis and cell death and possibly to the onset of neuro-degenerative diseases.

2.1.1 Human Exposure to ELF EMFs

Most human exposure to ELF EMFs comes from electrical appliances, household wiring, AC transmission, and distribution lines. Below overhead transmission lines, electric field strengths may be as high as 12 kilovolts per metre (kV/m), depending on the line voltage and the distance between the conductor and the ground. Around electricity generating stations, electric fields up to 16 kV/m may be encountered (Simon, 1992). Electric fields around appliances can be of the order of 0.5 kV/m. Naturally occurring 50-60 Hz electric fields are of the order of 0.1 millivolts per metre (mV/m), and accompanying MFs are approximately 0.01 nano-Tesla (nT). In residences and most work places, the average 50-60 Hz MFs are between 0.1 and 0.3 μT (National Research Council, NRC, USA, 1996). Many household appliances produce MF, which range from 50-150 μT; the fields decrease rapidly with increased distance from the source. Below overhead transmission lines, the average magnetic flux density can be up to 30 μT for multi-conductor 765 kV lines and 10 μT for 380 kV lines. Around power generating stations, average fields may be as high as 40 μT, and peak fields of 270 μT may be encountered. Industrial processes can be responsible for occupational exposures in welders as high as the 130 mT peak fields (Simon, 1992).

2.1.2 Electromagnetic Field Mechanism(s)

Embarking on any investigation into whether ELF EMFs have an effect on living systems undoubtedly draws controversy and sometimes outright skepticism. Despite these sometimes strongly held opinions, the researcher of the present thesis has followed strict scientific procedures in formulating a null hypothesis (that ELF EMFs have no effect on human genetic material), followed by the construction of an empirical experiment to test the hypothesis (namely the application of established cytomolecular techniques that are known to detect clastogenicity), with the intention of attempting to determine with the technology available whether or not any effect can be observed.

Many researchers have published in reputable international journals claiming to have observed cellular effects (Nordenson *et al.* 1984; 1988; 1994; Rosenthal and Obe, 1989; Khalil and Qassem, 1991; Garcia-Sagredo and Monteagudo, 1991; Cohen *et al. 1*986a and 1986b; Lai and Singh, 1997a, 1997b and 1997c) and indeed whole organism effects with ELF EMFs (Milham, 1982; NRPB, UK.1992; Theriault *et al.* 1994), which inevitably gives rise to the question, "what is the underlying mechanism whereby ELF EMFs exert their effect?" This is where many biologists become "unstuck" because many of them do not possess sufficient depth of knowledge in physics to offer a plausible mechanism. The present author is one such researcher; he readily admits that his knowledge of physics is not strong; he is first and foremost a cytogeneticist, but this does not invalidate the observations made either by him or any other non-physicist in the stated area of study. Nevertheless, it is beholden upon any person studying EMFs to possess at least a basic understanding of the various ideas and models that have been advanced to explain those effects that have been observed. The author will attempt here to present his understanding of the literature as follows.

One of the main areas of controversy involves "thermal" vs "non-thermal" effects. All living systems possess thermal kinetic energy (in joules, *J*), known as kT, (where k is the Boltzmann constant and T is the absolute temperature in kelvin, *k*), which is the background energy from the movement of molecules inside cells that are involved in life processes. It is universally accepted that the kT inside a cell is below that of ELF EMFs, which, if an effect were observed, would probably rule out any thermal model as an explanation for any biological effect. This means one has to search for a "non-

thermal" model, which is where problems and considerable controversies arise. Most of the models are untestable empirically, which contravenes accepted scientific practice. Nevertheless, Copernicus faced the same difficulty in his day, which shows that science does not always progress with full understanding when an idea is in its infancy.

The models most frequently advanced to explain observed biological effects of ELF EMFs embrace a variety of views as outlined below.

A number of innovative models have been proposed over the course of the last few decades. One class of models that has received particular attention is resonance models.

Ion cyclotron resonance (ICR) model of Liboff

The term 'cyclotron' itself derives from the so-called cyclotron accelerator - a familiar tool in atomic physics since the 1930s - in which charged particles move inside an evacuated chamber mounted between the poles of a magnet. Under the influence of the MF, the particles spiral round at a fixed frequency (the cyclotron frequency) while they receive regular accelerating pulses from an electric field applied at the same point in each orbit so that their energy increases in a resonant fashion (Male, 1992).

The resonance model involves the combined action of an ELF MF and the static geomagnetic fields (GMFs). Liboff (1985) first proposed the ICR model as a mechanism that could facilitate the movement of ions such as Ca^{++} through membrane channels in the presence of the GMF and a weak ELF MF tuned to ICR frequency. The resonance condition is formally expressed by the relationship $f_c = QB/2\pi m$. where f_c = ICR frequency, Q = ion charge, B = a DC MF, and m = ion mass. For the typical value of the GMF over the surface of the earth (50 μT), the resonant frequencies of many biologically important ions such as Na^+, K^+, and Ca^{++} fall within the ELF range. This has given the basis to assume that a cyclotron resonance could underlie the observed phenomena. Many authors developed the theme of such resonance in magnetobiology (Liboff *et. al.* 1987; Smith *et al.* 1987; Parkinson and Hanks, 1989; Liboff and Parkinson, 1991; Durney *et al.* 1992; Galt *et al.* 1993; and Coulton *et al.* 1993). Nonetheless, it has not received recognition due to the lack of correct physical

substantiation. At the same time, these experiments showed the essential role of ions, especially Ca^{++}, in magnetobiology.

Quantum beats or parametric resonance model

Lednev (1991) proposed this model, in which combined static and ELF fields affect vibrational energy levels and transition probabilities of bound ions (e.g., Ca^{++} ions bound to calmodulin). Shuvalova *et al.* (1991) have obtained data on the effect of combined fields on the rate of calmodulin-dependent phosphorylation of myosin that appear to support the predictions of the Lednev model. Another study, however, in which an optical technique was used to study Ca^{++} binding to calmodulin and to metallochromic dyes, failed to find any effects of combined static and time-varying fields under the resonance conditions predicted by Lednev's model (Bruckner-Lea *et al.* 1992). In a separate analysis Blanchard & Blackman (1994) claimed that the original Lednev model contained mathematical errors that led to an incorrect prediction for the ion transition probability between energy levels. Using their revision of the Lednev model, these investigators obtained a reasonably good fit to data on combined static and EMF field inhibitory effects on neurite outgrowth in cultured PC-12 cells stimulated by nerve growth factor (Blackman *et al.* 1994). In view of the significant differences in the original and revised Lednev's model, there is a clear need for further investigations on both a theoretical basis and biological implications of this resonance model for the action of combined static and ELF MFs. However, regardless of the details of the model, Adair (1992a) has pointed out that the Lednev model of resonant field effects is improbable because of the long lifetime of the excited vibrational states (approximately 8 sec).

Ionic cyclotron resonance model of Zhadin & Fesenko

Zhadin & Fesenko (1990) proposed this model in which the rotational energy levels of bound ions are pumped by combined static and ELF MFs. Similar arguments, which have been presented against the Lednev model, might be raised against the Zhadin & Fesenko (1990) model. In addition, the rate of transition of a bound ion to an excited state that is predicted by Zhadin & Fesenko's model is so low that a transition would occur only once in several months at typical environmental MF levels.

Stochastic resonance model

The concept of stochastic resonance first put forward in the seminal paper by Benzi *et al.* (1981) has been proposed as a possible mechanism through which weak EMFs could be detected in biological systems (Kruglikov and Dertinger, 1994). Stochastic resonance is the phenomenon whereby the addition of an optimal level of noise to a weak information-carrying input to certain non-linear systems can enhance the information content at their outputs. For example, in some systems, such as radio receivers, turning up the volume in order to hear a faint signal amidst much noise usually only results in turning up the noise as well. However, in other systems, increasing the amount of ambient noise actually enhances (up to a certain point) the signal-to-noise ratio through a complicated non-linear cooperation between the system and detector. This effect, known to operate in neurons, and lasers, is called stochastic resonance. The noise fluctuations might be stochastic (meaning totally random) but the detection of a desired signal can be maximised by tuning the noise.

Preliminary experiments with mechanoreceptor hair cells from crayfish have given evidence for the enhanced detection of weak stimuli in the presence of random noise (Wiesenfeld and Moss, 1995). It has been suggested that stochastic resonance is the basis for this effect, and perhaps underlies many other examples of weak signal detection by sensory systems (Douglas *et al.* 1993). Arguments have also been made that stochastic resonance is a plausible mechanism for the detection of periodic EMFs under conditions in which the signal-to-noise ratio is less than one (Kruglikov and Dertinger, 1994). This hypothesis clearly merits further evaluation and experimental testing in biological systems that exhibit reproducible responses to weak ELF fields.

Biogenic magnetite

Biogenic magnetites are natural microscopic crystals of magnetite, within the body of some animals and bacteria, which are capable of being magnetized. An external MF influences such crystals so that they tend to change their orientation. Consequently, the crystals exert pressure on the adjacent tissue and cause a biological response. In a number of cases, it is possible to explain by this manner the biological reception of a weak static MF. A series of chains of uniformly sized single domain magnetite particles

embedded in the ethmoid tissue in the front of the cranium of Chinook Salmon fish (*Oncorhynchus tshawytscha*) is used for accurate navigation (Kirschvink *et al.* 1992). Microscopic crystals of magnetite or greigite (Fe_3O_4) were found in the brain of some birds, which are known to navigate well in the GMF (Kirschvink *et al.* 2001). Traces of magnetite are also found in magnetotactic bacteria (Blakemore, 1982 and Torres de Araujo, 1986).

Magnetic contamination

Ferromagnetic contamination is one of the ideas in the area of magnetobiology proposed by Kobayashi *et al.* (1995). The authors suggested that small polluting magnetic particles are present not only in the air dust but also deposited on the surface of laboratory devices, and penetrate into plastics, glasses, chemicals and even into purified water. Their mean size is about 10^{-5} cm. The particles consist of ferro- and ferrimagnetics, which possess spontaneous magnetization. The authors have shown that routine laboratory procedures like pouring or/and rinsing give rise to enrichment of the cell cultures by pollutants. The number of particles in cultures could be 10 times more than that of cells. The energy of such a particle is approximately three orders of magnitude greater than kT. In this research the authors postulated that the magnetic particle absorbed by a cell membrane can transfer its energy to the adjacent biophysical structures, for example, mechanically activated ion channels.

These mechanisms of magnetobiological effects stand apart and do not solve the basic problem of magnetobiology. Indeed, many unicellular organisms, wherein magnetite is absent, are capable of reacting to the MF. The reaction in many cases is of a complex non-linear, character depending on the MF parameters. The basic problem of magnetobiology is exactly in the explanation of this phenomenon, which is a paradoxical one from the viewpoint of orthodox physics.

Free radicals

Very recently, Simko *et al.* 2004 have also proposed a mechanism that genotoxicity after exposure to EMF is due to secondary induced mechanisms, such as the production of free radicals, which must be induced by different signal transduction pathways. Free radicals are important intermediates in natural processes and are released during natural cell metabolism. These intermediates arise in mitochondrial respiration and are also a key feature of the phagocytic process. These authors speculate on the basis of experiments they have conducted in rats that EMFs might be a stimulus to induce an "activated state" of the cell such as phagocytosis, which then enhances the release of free radicals, in turn leading to genotoxic events. They envisage that EMF exposure increases free radical levels which can cause both acute and chronic effects on different cellular processes. The pathways they propose are as follows: (1) Direct activation of macrophages (or other cells) by short-term exposure to EMF leads to phagocytosis (or other cell specific responses) and consequently, free radical production. (2) EMF-induced macrophage (cell) activation which includes direct stimulation of free radical production. (3) An increase in the lifetime of free radicals by EMF which leads to persistently elevated free radical concentrations. (4) Long-term EMF exposure leads to a chronically increased level of free radicals, subsequently causing an inhibition of the effects of the pineal gland hormone melatonin. Taken together, they advance the hypothesis that these EMF-induced reactions could lead to a higher incidence of DNA damage and therefore, to an increased risk of tumour development (see Figure 2.0).

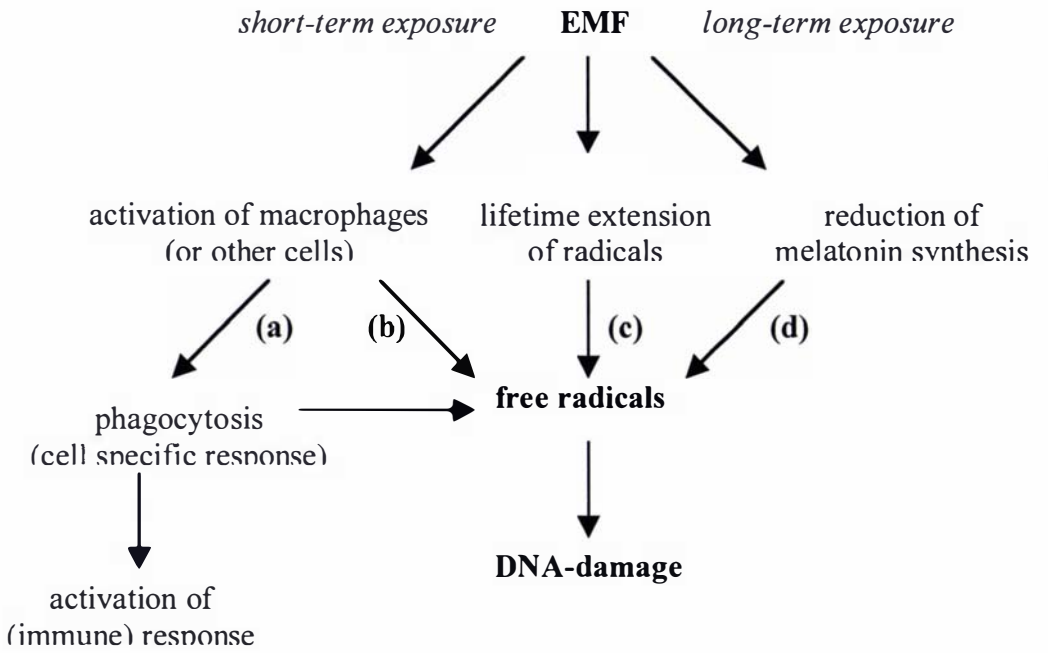


Figure 2.0: Pathways of reactive oxygen species (ROS) involvement in cellular reactions subjected to short- and long-term EMF-exposure. Stimulation of the immune system through macrophage activation is a favourable response to short-term EMF- exposure **(a)**. Free radical production may arise directly from macrophage or other cell specific activation **(b)** or radical lifetime extension **(c)**. Similarly, increase of ROS has been attributed to the inhibiting capacity of EMF on the availability of the pineal gland hormone melatonin which is a known scavenger for free radicals **(d)** after long-term EMF-exposure (Simko *et al.* 2004).

Other proposed interaction mechanisms

Current evidence suggests that cell processes can be influenced by weak EMFs (Nair *et al.* 1989; Adey, 1990; Anderson, 1991; Tenforde, 1992). It would also be true to say that the effects represent a weak perturbation of the homeostatic state, that EMFs appear to be a stress that a cell can adapt to in most cases without catastrophic consequences. But there is no question that a change in the physiological state of the plasma membrane in particular, representing the boundary between the external environment and the organism, is measurable and significant in response to certain weak EMFs (Azanza and Del Moral, 1993; Goodman *et al.* 1995). Lawrence and Adey (1982) have demonstrated this in their cell membrane studies. Strands of protein are strategically located on the surface of cells in tissue, where they act as detectors of electrical and chemical messages arriving at cell surfaces, transducing them and transmitting them to the cell interior. Through the protein strands, cell membranes perform a triple role in signal detection, signal amplification, and signal transduction to the cell interior.

The most important issue in understanding the pathways by which ELF EMF signals might influence membrane and cellular functions is the elucidation of mechanisms by which these fields are transduced within the cell membrane. One approach that has been taken in addressing this question is to consider the intricate biochemical pathways that have evolved as a mechanism by which a living cell communicates with its extracellular environment. As illustrated in Figure 2.1A by Tenforde (1993), the binding of a single molecule of a messenger substance to a specific receptor within the membrane triggers a cascade of events that involve conformational shifts in membrane-associated proteins. These events in turn lead to signal transduction and amplify the production of cytoplasmic second messengers and internal effectors such as free Ca^{++} and protein phosphorylases (kinases) that regulate DNA transcription and protein biosynthesis (Alkon & Rasmussen, 1988; Luben 1991). The end result of a single messenger binding event at the membrane surface is thus a cytoplasmic signal that is amplified to a level that can produce robust effects on macromolecular synthesis and cellular responses involving significant changes in functional states of the cell.

Similarly, the interaction of EMF fields with biological membranes might in principle lead to alterations in each component of the signaling process that occurs in living cells.

A useful working hypothesis (Figure 2.1B), which was advanced by Tenforde (1993), is that the particular fields and currents induced by an applied EMF initiate

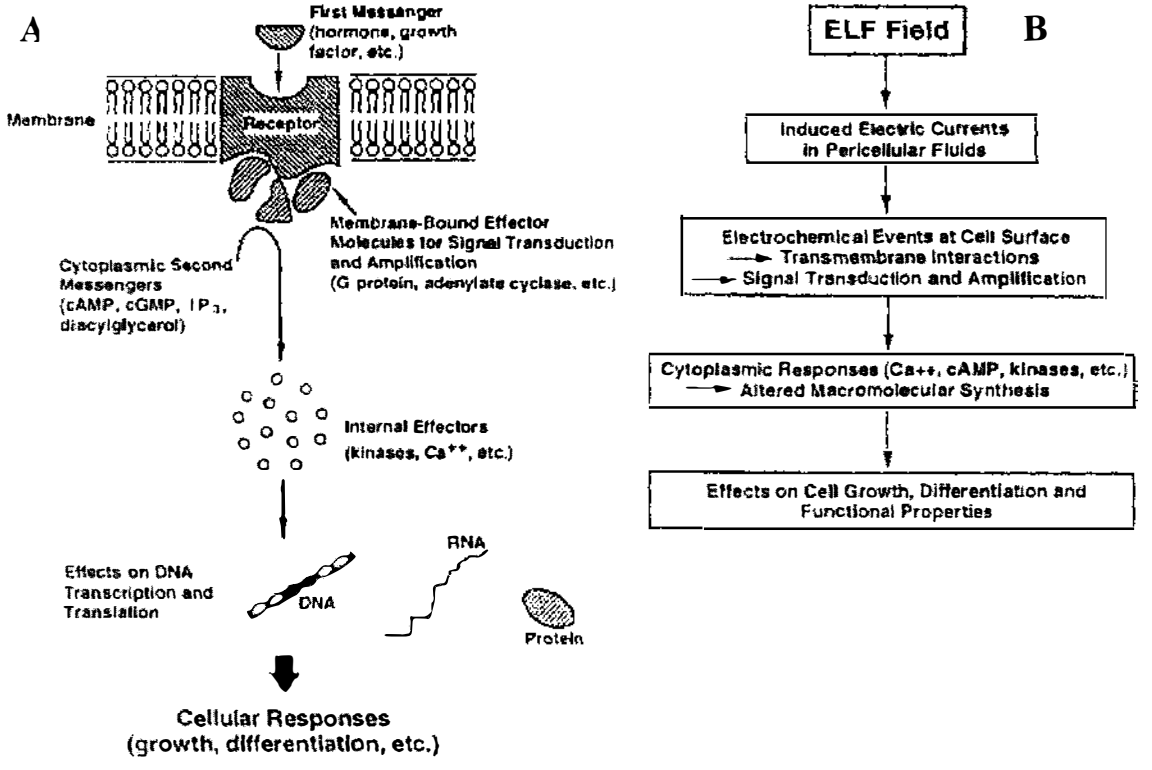


Figure 2.1: (A) Biological signal transduction pathways involving first- and second-messenger systems. Abbreviations- cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate; IP_3: triphosphoinositol (which releases Ca^{++} from intracellular stores). **(B)** Hypothesized sequence of membrane-mediated events leading to cellular and tissue responses to applied ELF fields (Tenforde, 1993).

electrochemical events within the cell membrane that are important elements of the primary signal transduction and amplification process. These biochemically-mediated events then produce cytoplasmic second messenger responses that trigger changes in the biosynthesis of macromolecules and perhaps alterations in cellular growth, differentiation, and functional properties.

In examining the *in vivo* and *in vitro* response of cells to low frequency EMFs there is a growing consensus among investigators that the sequence of events leading to EMF signal transduction and amplification at the cellular level does occur. For example, Luben *et al.* (1982) have demonstrated that pulsed electromagnetic fields (PEMF) with EMF repetition frequencies inhibit the production of cyclic adenosine monophosphate (cAMP) by bone cells in response to the binding of parathyroid hormone (PTH) to

surface receptors. Moreover, studies by Cain *et al.* (1987) have shown that PEMF action can lead to the inability of the PTH-receptor complex to activate the alpha subunit of the G protein, thereby interfering with the sequence of events that activates adenylate

cyclase at the cytoplasmic membrane interface. Byus *et al.* (1984) in a separate study using human lymphocytes has shown that exposure to microwave fields with amplitude modulation at ELF frequencies leads to the inhibition of non-cAMP-dependant histone kinases.

Some other interaction models has been advanced to explain a possible interaction between EMFs and biological systems, but none have gained prominence as an acceptable working model and are thus not highlighted in this treatise. They include: (1) Eddy electric currents, induced by alternating MF (Lerchl *et al.* 1990; Schimmelpfeng *et al.* 1997); (2) Interference of quantum states of bound ions and electrons (Binhi, 1997, 1997a); (3) Coherent quantum excitations (Frohlich & Kremer, 1983); and 4) Magnetosensitive free-radical reactions and other "spin" mechanisms (Grundler *et al.* 1992).

2.1.3 EMFs and Cancer

The debate over a possible link between cancer and ELF EMFs began first with a study by Wertheimer and Leeper (1979) that showed an increased risk of cancer in children living close to electric power distribution lines. Subsequently, a number of other epidemiological studies have also reported that residential and occupational exposure of ELF (50-60 Hz) EMFs is related to different types of cancers (Davies *et al.* 1992; Sagan, 1992; Stevens *et al.* 1992; Savitz and Ahlbom, 1994). However, the National Research Council, National Academy of Sciences, Washington, D.C. (1996) reported that the results from such epidemiological studies have been inconsistent and contradictory. In reference to the ambiguous results of these epidemiological studies on association between ELF EMFs exposure and risk of cancer, laboratory studies are necessary to determine a link, if any, between such fields and carcinogenesis.

The development of cancer is a multistage process. During normal development, immature cells undergo a process called differentiation in which they become highly

specialized and are less able to continue proliferating. In the first stage of cancer, termed initiation, a cell's DNA is damaged through mutation, causing a differentiated cell to resemble an immature one, in effect reversing the process of differentiation. In the second stage, promotion, normal cellular controls are disrupted and the mutated cell multiplies.

The majority of *in vitro* studies have indicated that ELF-EMFs have no mutagenic effects and do not initiate cancer. It has been suggested, however, that they could play a role as a cancer promoter (McCann *et al.* 1993 and Murphy *et al.* 1993) through epigenetic mechanisms and may induce cancer in cells that have already mutated. Many cellular studies support this view as ELF EMFs are observed to influence enzyme action, signal transduction, calcium ions (Ca^{2+}), protein synthesis, gene expression, ornithine decarboxylase (ODC), cell proliferation, and melatonin.

Tumour promotion is often accompanied by an increase in the activity of the enzyme ODC. ODC is a key enzyme in the biosynthesis of polyamines, which have roles in cell proliferation and control of gene expression. Changes in ODC have been reported from 50 Hz exposures *in vivo* (Mevissen *et al.* 1995). In this study, female rats were exposed to a 50 μT EMF to observe ODC activity in different tissues, including mammary tissue. Rats were exposed for a period of six weeks either with or without oral administration of the chemical carcinogen 7-12-dimethylbenz(a)anthracene (DMBA) and all data were compared with those from sham-exposed controls. MF exposure resulted in an approximate doubling of ODC in mammary tissue. A significant ODC increase was also seen in the spleen, but not in the liver, small intestine, bone marrow, and ear skin. The ODC increase produced by EMF exposure in the mammary tissues was of a similar magnitude as that observed after treatment with DMBA. Combined treatment with EMF and DMBA was not as effective in increasing ODC as treatment with DMBA alone, except in liver tissue. These results on *in vivo* increases of ODC by EMF exposure strengthen the hypothesis that weak 50-Hz EMFs affect ODC activity and may thus function as a tumor-promoting or co-promoting agent.

A tumour promoting effect of the EMFs was also observed in three different studies of mammary cancer development in rodents exposed to ELF MFs following tumour initiation with a chemical initiator. In the first study, rats were injected intravenously

with a 50 mg/kg dose of nitrosomethylurea (NMU), which produced mammary tumours in 54% of the rats (Beniashvili *et al.* 1991). An increased percentage (86%) of rats with mammary tumours were observed when a group of rats were injected with NMU followed by an exposure of 0.2 mT MF (50 Hz), 3 h / day for 5 weeks. In the second study, mammary tumours were initiated through the administration of oral doses of DMBA (20 mg total) followed by 23 weeks exposure to a 30 mT MF (50 Hz) (Mevissen *et al.* 1993). A significantly increased number of tumors were observed per tumor-bearing rat. A third study, which was conducted by Löscher *et al.* (1993), used the same protocol as the second study described above, except that a considerably smaller MF level of 0.1 mT was used. In this study, 50% higher mammary tumour incidence was observed in the MF exposed rats than the sham-exposed controls.

In a separate study, Kumlin *et al.* (1998) investigated the possible influence of MFs and simulated solar radiation (SSR) on ODC and polyamines in mouse epidermis. Chronic exposure to combined EMF and SSR, however, did not cause persistent effects on ODC activity or polyamines compared to the animals exposed only to SSR, although the same EMF treatment was previously found to accelerate skin tumor development.

Signal transduction (ST) is one of the major, fundamental pathways for cell communication and it is a general process in multi-cellular organisms in which a ligand molecule binds to its receptor site on the cell surface, which in turn triggers a cascade of biochemical events associated with the cell membrane. Liburdy *et al.* (1993) investigated receptor-ligand binding in human lymphocytes exposed to EMF. Human peripheral blood lymphocytes pre-labelled with fluorescently modified antibody markers against CD20 (pan B-cell surface marker) or against the CD3 (T-cell receptor) determinant were exposed to 22 μT EMF (60-Hz sinusoidal) at 37C for an hour. Following field exposures, T-lymphocytes were reported to show more anti-CD3 antibody released from cells compared to sham exposed controls, whereas EMF-treated B-lymphocytes showed only a slight increase in CD20 release compared to controls. This suggests that EMFs have the potential to alter receptor binding involving the CD3 receptor in T-lymphocytes. The CD3 molecule is physically integrated with the T-cell receptor in the lymphocyte cell membrane and is the major complex responsible for T-cell activation and triggering of the ST cascade in this cell.

The series of events in the ST cascade that follows receptor-ligand binding is the activation of membrane bound enzymes. Many researchers have studied different membrane bound enzymes during EMF exposures. Protein kinase C (PKC) is a key enzyme in the ST cascade and is believed to be the membrane receptor for phorbol esters, which are a potent class of tumour promoters (Kikkawa *et al.* 1989). Therefore, if the binding of this class of tumour promoters to PKC is influenced by EMF then altered cancer promotion would be expected. Monti *et al.* (1991) showed that HL-60 lymphocytes exposed to a 50 Hz, 8 mT EMF have increased binding of the PKC-specific phorbol ester, suggesting that EMFs may modify the cellular response to tumour promoters. PKC activity was also seen to elevate in human pre-B leukemia cells exposed to 60 Hz, 0.1 mT EMFs. Moreover, activation of PKC was dependent on the activation of *lyn* kinase, a tyrosine kinase of the *src* family which is known to be involved in proliferation of leukemia clones (Uckun *et al.* 1995).

The final endpoint in the process of carcinogenesis is an obvious loss of control of cell proliferation. This is a complex process, which is under the control of cellular signal transduction (ST) pathways. In one study, Rosenthal and Obe (1989) exposed human peripheral lymphocytes to a 5 mT (50 Hz) EMF and showed a 10-15% increase in cell-cycle progression. Antonopoulos *et al.* (1995) independently confirmed the effects of a 5 mT (50 Hz) field reported by Rosenthal and Obe (1989). West (1994) demonstrated increased colony growth in anchorage-independent JB6 cells after 10-14 days exposure to a 1.1 mT (60 Hz) EMF. On the other hand, Hiroaki *et al.* (2002), whilst not refuting that EMFs have an effect on cell proliferation, reported no effect of MFs specifically upon the rate of DNA synthesis. This was measured by determining the rate of [^{3}H]thymidine incorporation into DNA when cell lines derived from human tumours were exposed to either a 50 Hz or 60 Hz field for 3 days.

Cridland *et al.* (1999a) used human fibroblasts to investigate the effects of power frequency magnetic fields on the kinetics of cell cycle progression using human fibroblasts. In this study, normal human fibroblasts were synchronised in the G_0 phase of the cell cycle and exposed to 50 Hz magnetic fields at a range of flux densities. Progression through the cycle was monitored by examining the timing of entry into S phase, as characterised by the onset of DNA synthesis. Simultaneous positive controls were exposed to human recombinant fibroblast growth factor to demonstrate that the

system was responsive to external stimuli. Exposure to magnetic fields at 20 and 200 μT induced a small but significant increase in the length of the G_1 phase of the cell cycle. Exposure at higher flux densities of 2 and 20 mT had no significant effect.

Chen *et al.* (2000) used a Friend erythroleukemia cell line, which can be chemically induced to differentiate, to determine whether ELF EMF could alter proliferation and differentiation in these cells in a manner similar to that of a chemical tumor promoter. Exposure of this cell line to 60 Hz ELF EMF resulted in a dose-dependent inhibition of differentiation, with maximal inhibition peaking at 40% at 4 μT. ELF EMF at 1μT and 2.5 μT inhibited differentiation at 0 and 20%, respectively. ELF EMF at 100 μT and 1mT stimulated cell proliferation 50% above the sham-treated cells. The activity of telomerase, a marker of undifferentiated cells, decreased 100-fold when the cells were induced to differentiate under sham conditions, but when the cells were exposed to 50 μT there was only a 10-fold decrease. They concluded that ELF EMF can partially block the differentiation of Friend erythroleukemia cells, and this results in a larger population of cells remaining in the undifferentiated, proliferative state, which is similar to the published results of Friend erythroleukemia cells treated with chemical-tumor promoters.

In a well-designed study, Mattei *et al.* (1999) exposed cultured bone cells to a pulsed EMF (PEMF) for different times to find the minimal exposure time necessary to stimulate an increase of DNA synthesis. These researchers used human osteosarcoma cell lines and normal human osteoblast cells and observed that the proliferative responses of normal human osteoblast cell (NHOC) and human osteosarcoma cell lines to PEMF exposure are quite different. Moreover, NHOC required minimal exposure times to PEMF to increase their cell proliferation, similar to that needed to stimulate bone formation *in vivo*.

In summarizing this section, it is evident from all the studies described above that ELF EMF whilst perhaps not directly initiating cancer, do appear to play an important role in carcinogenesis by influencing enzyme action, signal transduction, cell-cycle progression, protein synthesis, ODC, and cell proliferation. However, further research is indeed warranted to substantiate this far-reaching claim.

2.1.4 EMFs and Transcription

Several key studies seem to indicate that ELF-EMFs can influence the rate of mRNA transcription. In an elegant study, Goodman and Henderson (1991) observed an increase in mRNA transcription levels for selected chromosomes of salivary gland cells of *Drosophila* and *Sciara* after brief exposures of up to 60 min at 37C, to 0.5-1.1 mT EMFs (sinusoidal, 72 and 60 Hz). Some chromosomes showed loci with an increase in transcription from 10 to 1000-fold over sham exposed control cells. Exposure of human HL-60 cell line to 0.57-570 μT EMFs (sinusoidal, 60 Hz) also induced an increase in mRNA transcription (Goodman and Henderson, 1992).

In an earlier study, Goodman *et al.* (1983) worked with two types of pulsed fields: repetitive single pulse and repetitive pulse train. These were used to test the hypothesis that weak pulsed EMFs can modify biological processes. They observed that a single pulse increased the specific activity of messenger RNA after 15 min and 45 min of exposure. Pulse train, however, increased specific activity only after 45 min of exposure.

In a similar study, Litovitz *et al.* (1990) exposed cells to relatively low-intensity, pulsed, ELF EMFs and observed transient augmentation of RNA synthesis. They also observed that under certain conditions of irradiation, the augmentation of RNA synthesis enhanced with the increase in EMF strength.

In a separate study, transcriptional rates in HL-60 cells exposed to sinusoidal 60 Hz EMFs were investigated by Greene *et al.* (1991). In their study, transcriptional rates were measured by labeling the cells with [^{3}H]uridine. A maximum of 50-60% increase in overall transcriptional rates was reported in HL-60 cells exposed to a 1mT, sinusoidal 60 Hz EMF. This increase was time dependent and transient with maximum enhancement occurring at 30-120 min of exposure with a decline to basal levels by 18 h. The increase in transcription activity in HL-60 cells and the time-dependence are in general agreement with the findings of Goodman and Henderson (1992).

Human HL-60 cells were also exposed for 20 minutes to an EMF at frequencies ranging from 0.2-2.3 mT in a similar study by Lin-Xiang *et al.* (1990). Following each

exposure, quantitative levels of *c-myc* and histone H2B transcription were determined by dot-blot hybridization analysis and compared with sham exposed control samples. A four-fold increase in transcription was observed after each exposure at 50 Hz compared with sham exposed controls.

Similarly, with the help of transcription autoradiographic methods, Goodman *et al.* (1992) also observed changes in transcriptional activity at 13 defined regions of the right arm of chromosome 3 (3R) in *Drosophila* following 20 min exposures of salivary glands to five different intensities of ELF EMFs. Other observations that were made included changes in translational patterns in ELF EMFs exposed cells, and an increase in over-all polypeptide synthesis.

All these above studies are milestone papers linking weak EMF exposure to gene activity. Since these pioneer studies were performed, several papers have been published supporting these data to varying degrees. Noteworthy research, relevant to the current study, is reviewed as follows:

Ding *et al.* (2002) investigated the distribution and expression of growth associated protein-43 (GAP-43) in human glioma cells (MO54) after exposure to a 60 Hz, 5 mT MF with or without initial X-ionizing radiation (2 Gy). Immunocytochemistry and the reverse transcription polymerase chain reaction (RT-PCR) were used to determine the EMF-effect. GAP-43 was present in the cytoplasm, accumulating in the perinuclear area. An increase in GAP-43 expression was observed with a peak at 10 h in mRNA levels and at 12 h in protein levels, after exposure to the EMF. The increased level of GAP-43 protein returned to a normal level within 24 h of exposure to a 5 mT EMF. The kinetic pattern of GAP-43 expression induced by X-ionizing radiation was very similar to that induced by the EMF. The investigators suggest that the stimulation of GAP-43 expression could occur by a similar mechanism following exposure to X-rays or EMFs.

The effects of a 50 Hz sinusoidal ELF EMF on the expression of genes relating to cytokine receptors were studied in HL60 cells by Zhou *et al.* (2002). Transcription levels of tumor necrosis factor receptor (TNFR) p55 and p75, interleukin-6 receptor (IL-6R) and transforming growth factor-receptor 1 (TGFR1) were quantified in cells exposed to an intensity of 0.1 or 0.8 mT for periods ranging from 30 min to 72 h. The

results showed that MF exposure at 0.1 and 0.8 mT for 72 h increased TNFR p75 and IL-6R mRNA expression in HL60 cells. No significant change in gene expression levels of TNFR p55 and TGFR1 was observed under any of the exposure conditions.

Ghadire & Mahnaz (2002) studied the biological effect of alternating EMFs on developmental stages of *Drosophila melanogaster*. In this study, *D. melanogaster* eggs, first, second and third instar larvae were exposed to an 11 mT 50 Hz field produced from a pair of Helmholtz coils. Eggs and each of the larval stages were exposed to EMFs for 2, 4, 6 and 8 h. A significant number of adult flies derived from larvae with varying degrees of exposure was observed with morphological changes in head thorax and abdomen. Whereas, no significant increase in abnormal morphology was observed in the group arising from EMF-exposed eggs. They also observed a dose-dependent increase in the number of abnormal flies compared to the control.

In a very recent *in vitro* study that used pluripotent embryonic stem (ES) cells, Czyz *et al.* (2004) studied the non-thermal effects of 50 Hz, ELF EMF. In their study, wild-type (wt) and p53-deficient ES cells were exposed either continuously or pulsed (various on/off cycles) to power line frequency MF with different magnetic flux densities (0.1 mT, 1.0 mT or 2.3 mT) for 6 h or 48 h. Transcript levels of regulatory genes, such as egr-1, p21, c-jun, c-myc, hsp70 and bcl-2, were analysed by semi-quantitative RT-PCR immediately after exposure or after a recovery time of 18 h. Pulsed EMF exposure to 5 min on/30 min off cycles at a flux density of 2.3 mT for 6 h resulted in a significant up-regulation of c-jun, p21 and egr-1 mRNA levels in p53-deficient, but not in wild-type cells. No significant EMF effects were observed in both cell systems at lower flux densities, longer exposure time or after 18 h recovery time. They also reported that 5 min on/30 min off pulsed MF exposure is capable of causing non-thermal responses in ES cells.

The effect of ELF EMFs on biochemical properties of human oral keratinocytes (HOK) was studied by Manni *et al.* (2004). Cells exposed to a 2 mT, 50 Hz EMF showed no changes in gene expression but also modification in shape and morphology by scanning electron microscopy (SEM). These modifications were also associated with different actin distribution, revealed by phalloidin fluorescence analysis. Moreover, exposed cells had a smaller clonogenic capacity, and decreased cellular growth. Indirect

immunofluorescence with fluorescent antibodies against involucrin and β-catenin, both differentiation and adhesion markers, revealed an increase in involucrin and β-catenin expression. The advance in differentiation was confirmed by a decrease of expression of epidermal growth factor (EGF) receptor in exposed cells, supporting the idea that exposure to EMF carries keratinocytes to higher differentiation level. These observations support the hypothesis that 50 Hz EMFs may not only affect gene expression, but also modify cell morphology and interfere in differentiation and cellular adhesion of normal keratinocytes.

Shin-ichi *et al.* (2001) exposed HeLa cells to 60 Hz, AC, 0.25 to 0.5 T MFs to investigate the biological effects of EMFs on DNA synthesis, transcription or repair, using *in vitro* model systems with defined sequences. In contrast to most studies, these researchers observed no statistically significant change in the rate and fidelity of DNA polymerase catalyzed DNA synthesis, nor of RNA polymerase catalyzed RNA synthesis. Similarly, Cridland *et al.* (1999b) also showed a null effect of EMFs on RNA synthesis in human fibroblasts when exposed to a 50 Hz field at a range of flux densities between 2 μT and 20 mT.

Most of the research to date on EMFs and gene expression appears to have established a link between weak EMF exposure and gene activity. The biological ramifications of such a conclusion are enormous when one considers the level of exposure living organisms are now subjected to in the modern world. These are fields to which living organisms have never been exposed in their long evolutionary history prior to the generation of man-made electricity. Research is only just beginning to discover the possible genetic consequences of human technological progress.

2.1.5 EMF and Enhancement of the Intracellular Ca^{2+} Response

Calcium plays an important role for many cellular functions, especially for transmission of extracellular signals, the regulation of intracellular transport of compounds, the release of secretion products, bone metabolism, and muscle contraction. Therefore, maintaining an optimal cellular calcium concentration is of key importance. This is obtained by a number of very precise active ion-transport mechanisms through cell

membranes. Many investigations, mostly *in vitro*, have shown that ELF EMFs influence the release of calcium from the cell (Blackman *et al.* 1988).

Persuing this line of study, Lindstrom *et al.* (1993) exposed Jurkat cells to 50 Hz, 0.1 mT EMFs and examined free intracellular Ca^{2+} in individual cells, using Fura-2. Within 15-200 sec after the Helmholtz coils were energized, intracellular Ca^{2+} increased to 200-400 nM from baseline levels of 50-100 nM. When similar experiments were performed with mitogen-activated peripheral lymphocytes, a similar enhancement in intracellular Ca^{2+} response was observed. However, only 10% of the lymphocytes tested responded to the EMFs, whereas about 85% of the Jurkat cells showed enhanced intracellular Ca^{2+} in response to an applied EMF. EMFs failed to enhance intracellular Ca^{2+} levels in non-dividing endocrine pancreatic cells from mice or rats or when Ca^{2+} was absent from the cell perfusion medium. These studies have an additional unique feature in that single cells were observed continuously before and after EMFs were activated and the change in Ca^{2+} level was perfectly correlated with EMFs.

2.1.6 EMF and Plasma Membrane

The majority of theories addressing the pathways of interaction between biological systems and EMFs suggest that the primary site of interaction is the plasma membrane. However, direct experimental data showing EMF-induced membrane perturbations are sparse. Grandolfo *et al.* (1991) exposed chick myoblasts to a 50 Hz sinusoidal MF at intensities between 1 and 10 mT. No field effects were observed on the fusion of myoblasts although decreases in the conductivity and permittivity of the cells were observed. The conductivity changes were interpreted as evidence for a decrease in transport of various ions, but the site of inhibition was not determined. The decrease in permittivity was interpreted as a change in surface charge owing to alteration in lipids, proteins or both. An interesting aspect of this study was the observation that the maximum response of the cells was found at 5 mT rather than at the highest applied intensity of 10 mT, suggesting that a linear dose response might not be involved. It is noteworthy that Goodman and Henderson (1991) also reported that the lower intensity fields in the μT range are more effective in inducing bioeffects than more intense fields.

Fisher *et al.* (1986) examined the effect of PEMFs on [^{3}H]glucosamine incorporation in plasma membranes of human fibroblasts and rat sarcoma cells. About a 100% increase in labeled glucosamine incorporation into existing cell surface glycoproteins following a 48 h exposure to PEMFs was observed. Chromatographic data indicated that anionic residues (N-acetylglucosamine, N-acetylgalactosamine) were also being synthesized and incorporated on the cell surface.

The effects of ELF EMFs (50-60 Hz) in the form of alteration of membrane transport processes were observed by Stange *et al.* (2002) in *Vicia faba* root tips. In their study, *Vicia faba* seedlings were exposed to a 10 μT MF (50 Hz, square wave) for 40 min together with a radioactive pulse, and they observed a marked increase in amino acid uptake into intact roots. A more modest increase was observed with a 100 μT (50 Hz, square wave). Moreover, an increase in media conductivity at low field intensities from 10 μT (50 Hz square wave), 100 μT (50 Hz sine wave), and 100 μT (60 Hz square wave) fields, indicated an alteration in the movement of ions across the plasma membrane, most likely due to an increase in net outflow of ions from the root cells. Similarly, a marked elevation in media pH, indicating increased alkalinity, was observed at 10 and 100 μT for both square and sine waves at both 50 and 60 Hz.

2.1.7 Melatonin and ELF EMFs

Recent studies have indicated that certain ELF EMFs can influence melatonin levels in humans, and that this in turn may have a bearing on genetic events (Stevens, 1987; Pfluger and Minder, 1996; Griefahn *et al.* 2002; Wood *et al.* 1998). Thus it is important that the current status of research conducted in this area should be addressed.

Circadian rhythms in humans and animals are driven by the day/night cycle and are synchronised with natural GMFs. The major control gland over this natural cycle is the pea-sized pineal gland (Cherry, 1997), weighing approximately 50-150 mg in man and 1 mg in the rat. It is located in the centre of the human brain. In recent years, the pineal gland has been recognized as a highly important endocrine organ. It has been found to be sensitive to light levels and is located on the top of the brain of many early vertebrates. For most animals, including humans, it is located in one of the innermost, and protected, areas of the head. The pineal gland is a very active organ, having the

second highest blood flow after the kidneys, and is equal in volume to the pituitary gland. Although it is located in the centre of the brain, it is actually outside the blood-brain barrier.

A main function of the pineal gland is the synthesis of the hormone, melatonin. The ability of the pineal gland to produce melatonin is directly related to the amount of visible electromagnetic radiation (i.e., light) that is detected by the eyes. At night, the absence of light due to sleep stimulates the pineal gland to produce melatonin, which is derived from the amino acid, tryptophan. After its uptake by the endocrine cells of the pineal gland, known as pinealocytes, tryptophan is converted to serotonin (5-hydroxytryptamine) and eventually converted to melatonin (N-acetyl-5-methoxytrypta mine), which is an indole (Jan *et al.* 1994). During the day, light, which falls on the eye's retina, produces signals that are biochemically amplified to stimulate the pineal gland to reduce its melatonin production (Omura *et al.* 1993).

Wilson *et al.* (1981) first reported the *in vitro* evidence for EMF-dependent depression of melatonin in animals. They reported that 60 Hz electric field exposure depressed nocturnal melatonin levels in rats. It has also been reported that 50 Hz circular polarized MFs, but not linear 50 Hz MFs (Kato *et al.* 1994), depress melatonin in rats (Kato *et al.* 1993). Yellon (1994) reported that 60 Hz MFs depress melatonin in hamsters, and Selmaoui and Touitou (1995) reported depression of pineal serotonin N-acetyl transferase and serum melatonin in rats.

It is important to mention here that melatonin is a most efficient natural cell protection and anti-carcinogenic agent in our bodies because of its ability to eliminate free radicals (Reiter *et al.* 1993 & 2001; Omura *et al.* 1993). Production of melatonin floods our bodies particularly at night, eliminating the build up of free radicals that are being produced, allowing DNA synthesis and cell division to occur with a much lower chance of damage and hence producing more healthy cells. Melatonin also dampens the release of estrogen, prolonged exposure to which may increase the risk of breast cancer (Cherry, 1997).

Based on a number of studies it has been reported in the field of chemistry that ELF EMFs increase the yields of some types of free radicals (Scaiano *et al.* 1994a, b;

Canfield *et al.* 1994; Grissom, 1995). Free radicals are known to be an important factor in cell proliferation and hence to be known as cancer promoters. The production of free radicals (.OH) is a consequence of the utilization of oxygen by all organisms. About 1-2% of inspired oxygen ends up as toxic free radicals which can damage macromolecules such as DNA, proteins and lipids. This damage is referred to as oxidative stress. Melatonin is an efficient neutraliser of these free radicals. To illustrate this, safrole, which is a known chemical carcinogen, damages DNA by inducing the production of large numbers of oxygen free radicals. But Tan *et al.* (1993) showed that the hormone melatonin inhibits DNA damage induced by safrole *in vivo*. When they injected rats with safrole alone, they observed extensive DNA damage after only 24 hours. However, when melatonin was injected along with safrole the DNA damage was reduced by 99%.

Since melatonin is a known natural cell protector and possesses anti-carcinogenic properties (Reiter, 2000; and Liburdy *et al.* 1993), it is believed that any factors that suppress its secretion may contribute to an increased cancer risk. There are a number of reports on human and animals suggesting that synthesis of melatonin and its precursors are altered in the presence of an EMF. For example, Stevens (1987) first put forward the hypothesis that the use of electric power may increase the risk of breast cancer. This hypothesis was based on a number of experimental reports indicating an effect of light at night (LAN) and powerline-frequency (50 or 60 Hz) EMFs on pineal melatonin production, and on the relationship of melatonin to mammary (breast) carcinogenesis.

Referring to the Stevens's hypothesis, EMF and LAN are said to reduce circulating levels of melatonin. This in turn would allow estrogen levels to rise and stimulate the turnover of breast epithelial stem cells and increase the risk for malignant transformation. A follow-up study of the Stevens' hypothesis was performed by Graham *et al.* (2001b). In their three laboratory-based studies a total of 53 healthy young women were exposed at night to EMF or to LAN under controlled exposure conditions to determine whether such exposures reduced melatonin and altered estrogen levels. But their experiment failed to show any effect of industrial-strength EMF-exposure (28.3 μT, 60 Hz,) on the blood levels of melatonin or estradiol. However, nocturnal melatonin levels were profoundly suppressed, and the time of peak concentration was significantly delayed in women exposed to LAN, regardless of whether they were in the follicular or luteal phase of the menstrual cycle.

In a study of seven healthy men (16-22 years), Griefahn *et al.* (2002) reported that light suppressed melatonin production and delayed night-time rectal temperature and heart rate. During the control session, subjects were kept at complete bed rest for 24 h at less than 30 lux at 18C. Experimental subjects were exposed to either light (at 1500 lux), 0.2 mT MF (16.7 Hz), or infrared radiation at 65C from 5 pm to 1 am. Salivary melatonin level was determined hourly, but rectal temperature and heart rate were recorded continuously. Melatonin synthesis was completely suppressed by light but resumed thereafter with darkness. The night-time rectal temperature and heart rate were delayed. The MF had no effect. Infrared radiation elevated rectal temperature and heart rate. Only bright light affected the circadian rhythms of melatonin synthesis, rectal temperature, and heart rate.

In another study, Wood *et al.* (1998) studied the effects of power-frequency MFs on night-time plasma melatonin in a group of 30 adult male human subjects. The time of onset of rise in melatonin concentration for each sham-exposed subject was predicted from a previous screening night. Then the subjects were exposed to 20 μT (50 Hz) at certain times and the time of onset of rise in melatonin concentration for a particular individual was recorded. Response to this exposure was compared to sham-exposure (in random order). When exposure preceded onset of rise, a significant delay in onset time relative to sham-exposure of approximately half an hour was observed, with indications of a reduction in maximum melatonin level. MFs generated by square-wave currents produce more marked reductions in the maximum level when compared to sinusoidal waveforms, but there was no significant difference in onset time.

Levallois *et al.* (2001) studied the effect of residential exposure to electric fields and MFs from high-power lines on female urinary excretion of 6-hydroxymelatonin sulfate (6-OHMS). A sample of 221 women living near a 735-kV line was compared with 195 women the same age living away from any power lines. Participants provided morning urine samples on two consecutive days and wore a magnetic dosimeter for 36 consecutive hours to measure personal magnetic exposure. The indoor electric field was assessed by spot measurements. After adjustment for other factors associated with low melatonin secretion, such as medication use or light exposure, night-time concentration of 6-OHMS was similar in the two groups. When either 24 h or sleep-time exposure to MF or electric field measurements was used, no exposure-effect relation was evident.

However, the trend of decreasing 6-OHMS concentration with age was more pronounced for women living near the lines, as was a lower 6-OHMS concentration in women with high body mass index. Chronic residential exposure to MFs from high-power lines may accentuate the decrease in melatonin secretion observed in some vulnerable subgroups of the population.

In a similar study, Pfluger and Minder (1996) investigated the effects of EMF exposure (16.7 Hz) on pineal melatonin production in healthy humans. The study was based on comparing urinary 6-OHMS levels of 108 male railway workers. A *repeated* measure design was used, which means each volunteer served as his own control. The exposure ranged between 1μT to 20 μT. Melatonin level was measured by sampling urinary 6-OHMS both in the morning and the early evening. Their results suggested that MFs do alter 6-OHMS excretion in humans exposed to 16.7 Hz MFs.

In another study, Davis *et al.* (2001) also studied the reduction of melatonin levels by night-time residential EMF exposure (60 Hz) in humans. The researchers investigated the association between night-time residential EMF exposure with lower nocturnal urinary concentration of 6-OHMS in 203 women (20-74y) with no history of breast cancer. They observed reduction in nocturnal melatonin production.

In a similar study, Burch *et al.* (2000) investigated the melatonin metabolite in electric utility workers. In this study, personal exposure to MFs and ambient light, and excretion of the melatonin metabolite, 6-OHMS, were measured over 3 consecutive workdays in electric utility workers. There was a MF-dependent reduction in adjusted mean nocturnal and post-work 6-OHMS levels among men working more than 2 hours per day in substation and 3-phase environments and no effect among those working 2 hours or less. No changes were observed among men working in 1-phase environments. The results suggest that circular or elliptical MF polarization, or another factor linked to substations and 3-phase electricity, is associated with MF-induced melatonin suppression in humans.

To study the secretory activity of the pineal gland, Welker *et al.* (1983) investigated the effects of artificial MFs on pineal serotonin-N-acetyltransferase (NAT) activity and melatonin content in male Sprague-Dawley rats. Experimental inversion of the

horizontal component of the natural MF, performed at night-time, led to a significant decrease of both parameters investigated. During day-time, this effect was less conspicuous. During night-time, inversion of the horizontal component was followed by a reduced pineal secretory activity for about 2 h. After a 24 h exposure to the inverted horizontal component, return to the natural condition was followed by a renewed clear depression of pineal NAT activity and melatonin content, indicating that the main stimulus is not the inverted MF itself but rather its change. Changing the inclination of the local MF from 63 degrees to 58 degrees, 68 degrees or 78 degrees, respectively also decreased the secretory activity of the rat pineal gland.

In an interesting study of rats, Olcese *et al.* (1985) investigated whether MF effects on mammalian pineal function are direct or indirect via retinal disturbances. In this study, acutely blinded and intact male rats were exposed to a single nocturnal magnetic stimulus. Then pineal N-acetyltransferase activity and melatonin content were assayed. Only in intact animals did the magnetic stimulus significantly reduce pineal activity, i.e., no effects were detected in blinded animals. These data point to a retinal magnetosensitivity, which may serve to modulate pineal gland function.

In persons with intact vision, pineal secretion is highest early in the morning and reduces following exposures to light. Therefore, increased exposures to LAN may decrease nocturnal secretion and thus contribute to the observed increases in breast cancer incidence rates. It has been reasoned that women who are profoundly blind, and therefore not susceptible to LAN, should have a reduced risk of breast cancer compared with those with intact vision. In one study, Verkasalo *et al.* (1999) studied the cancer risk patterns in persons with visual impairments to explore the hypothesis that women with visual impairment might have decreased breast cancer risk. A total of 10,935 women with visual impairment were identified from the Finnish register of visual impairment and followed up for cancer through the Finnish cancer registry for the years 1983-1996. The authors observed a statistically significant decreased breast cancer risk with increased visual impairment, which suggests a dose-response relationship between visible light and breast cancer risk.

A series of studies has shown that low exposure to EMFs can inhibit melatonin's cancer-preventive activity. For example, Harland and Liburdy (1996) reported that a 1.2 μT EMF could significantly reduce the growth-inhibitory action of melatonin and tamoxifen on breast cancer cell MCF-7 growth. Blackman (1996) found that melatonin can inhibit the growth of MCF-7 cells in culture, and that a 1.2 μT, 60 Hz MF can completely block this growth-inhibitory action. Harland and Liburdy (1996) observed that 1.2 μT computer monitor MFs also inhibit the ability of melatonin to suppress breast cancer cells *in vitro*. Finally, Liburdy and Afzal (1996) suggested that 1.2 μT 60 Hz exposure of a Video Display Terminal (VDT) MF inhibits the cancer-preventive action of melatonin *in vitro*.

A number of other studies in animals where responses have been observed suggest that specific aspects of the exposure might be critical (Rogers *et al.* 1997). For example, Yellon (1996) suggested that *repeated* MF exposures result in a loss of the detection of the melatonin effect in hamsters, i.e., where the melatonin depression is seen only after a single exposure but not with continued 3-week exposures.

Yellon (1991) had previously shown that the nocturnal production of pineal melatonin in Djungarian hamsters is extremely sensitive to a brief 15 min exposure to a sinusoidal 60-Hz, 0.1 mT MF. When the field was applied 2 h before the onset of darkness, the night-time rise in pineal melatonin was heavily suppressed and the peak production of this hormone was phase shifted by approximately 2 h to a later part of the dark period. Acute exposures of short-day animals produced similar results (Yellon, 1994). By contrast, daily exposures of the same type for as long as 3-weeks had no effect.

Reiter (1994) proposes that melatonin is more rapidly taken up by tissues during the ELF EMF exposure. He noted that if EMFs result in higher levels of free radicals, then an antioxidant like melatonin would disappear from the blood more quickly than is normal because it would be required for the scavenging of free radicals. Reduction of melatonin at night, by any means, increases the cell's vulnerability to alteration by carcinogenic agents. Thus, if in fact artificial EMF exposure increases the incidence of cancer in humans, a plausible mechanism could involve a reduction in melatonin that is a consequence of such exposures.

Referring to the above discussion, it is the view of the current author that although there have been a number of negative experimental findings concerning the biological effects of EMF on melatonin production, there exists a persistent pattern of evidence, both *in vitro* and *in vivo*, showing that fields can produce significant biological responses in humans even at low levels. The responses are as follows: 1) suppression of melatonin production; (2) increase of estrogen levels; (3) reduction in the growth-inhibitory action of melatonin and tamoxifen on breast cancer cell MCF-7 growth; (4) inhibition of cancer-preventive action of melatonin; and (5) MFs generated by square-wave currents produce more marked reductions in the maximum level night-time plasma melatonin when compared to sinusoidal waveforms.

2.1.8 Geomagnetic Field (GMF)

The current study was performed to determine the possible effects of 1μT or 1mT (50 Hz, AC) ELF EMFs on human chromosomes. Although the earth's geomagnetic field (GMF) is DC and is quite different to artificially produced AC fields, it is nevertheless a weak MF to which living organisms respond in a wide variety of circumstances. It is therefore appropriate to review the discoveries that have been made during the last few decades concerning the ability of living organisms, including humans, to respond to the GMF.

The Earth acts like a great spherical magnet, and is therefore surrounded by a MF. The Earth's MF resembles, in general, the field generated by a dipole magnet (i.e, a straight magnet with a North and South Pole) located at the centre of the Earth. The axis of the dipole is offset from the axis of the Earth's rotation by approximately 11.5 degrees. This means that the north and south geographic poles and the north and south magnetic poles are not located in the same place (Figure 2.2). The GMF originates from electric current flow in the Earth's core and Dubrov (1978) published the first extensive review of the biological effects of the GMF. The Eearth's MF is irregular, and at the surface of the earth has a steady component of approximately 50 μT.

Sensitivity of living organisms to the Earth's MF has been one of the most controversial topics in the behavioural and natural sciences for many years. Biologists criticized the

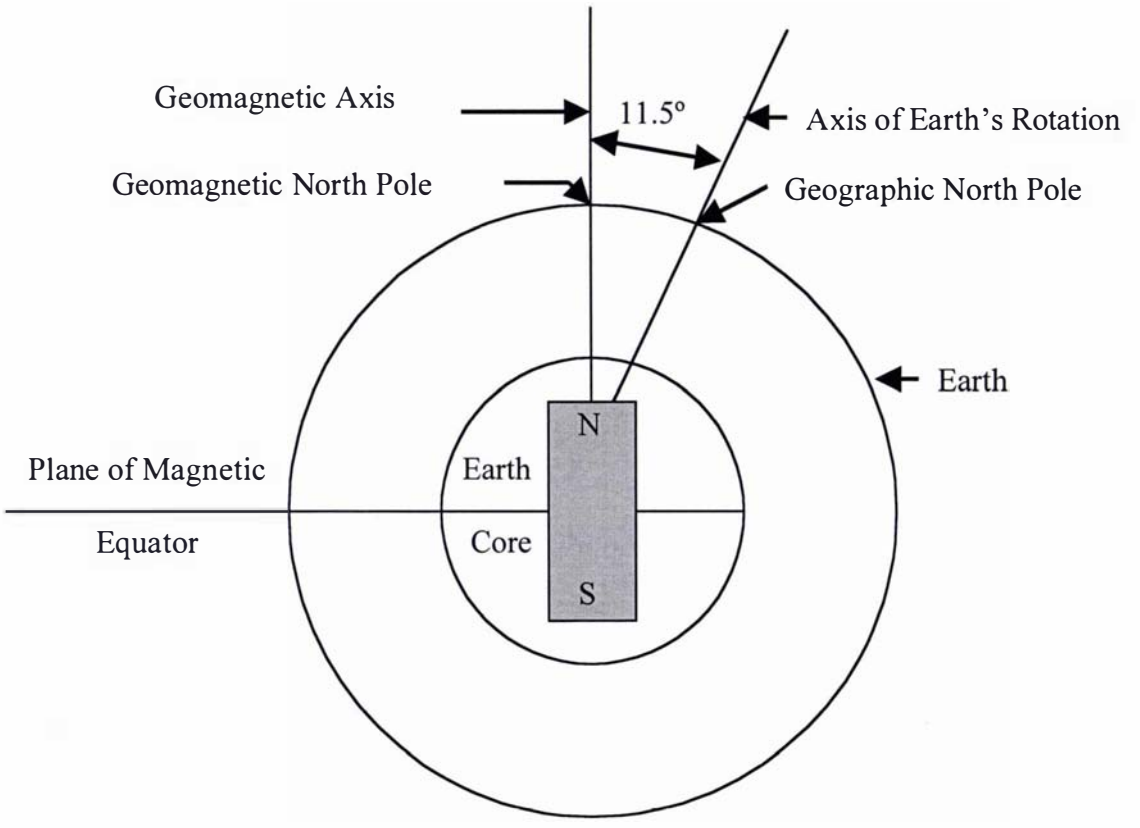


Figure 2.2: Showing geographic and geomagnetic poles.

earlier reports of magnetic effects on animals because they were unable to reproduce the effects, and physicists could not determine a genuine biophysical mechanism by which animals could detect the weak MF of the Earth (Griffin, 1944, 1952).

Radical changes occurred, however, during the last two decades in three distinct areas: 1) Discovery of many highly reproducible magnetic effects on behaviour, which includes the magnetotactic bacteria and protozoans, and magnetic effects on homing and navigational behaviour by migrating animals. It has been revealed that apparently two separate magnetic sensory systems exist in higher animals: a directional compass and a magnetointensity sense. 2) At least two plausible biophysical mechanisms are now known through which the Earth's MF can be transduced to the nervous system. These mechanisms include use of the ferromagnetic mineral magnetite (Fe_3O_4), which is a biochemical precipitate in virtually all groups of higher organisms, including humans. It forms chainlike structures in several groups of vertebrates ideally suited for responding to MFs (Kirschvink *et al.* 1985; Mann *et al.* 1988; Beason and Brennan, 1986). Another potential transduction mechanism is "Optical pumping" (Leask, 1977;

Schulten, 1982; Schulten and Windemeuth, 1986) by which Earth MFs could influence charge-transfer reactions in organic molecules. 3) A group of researchers obtained clear records of magnetically influenced signals in single nerve units connecting magnetite-bearing tissues with the brain, and these signals suggest strongly that the magnetite-bearing tissues of the ethmoid sinus contain a magnetoreceptor.

The next section is a brief review of these three developments discussed above.

Magnetoreception

Kirschvink *et al.* (2001) reported that orientation, navigation, and homing were critical traits expressed by organisms ranging from bacteria through to higher vertebrates. Sensory systems that aid such behavior have provided key selective advantages to these groups over the past 4 billion years, and are highly evolved; magnetoreception is no exception. Across many species and groups of organisms, compelling evidence exists that the physical basis of this response is tiny crystals of single-domain magnetite. Kirschvink postulated that all MF sensitivity in living organisms, including elasmobranch fishes, is the result of a highly evolved, finely-tuned sensory system based on single-domain, ferromagnetic crystals.

Blakemore (1982) and Torres de Araujo *et al.* (1986) reported a good example of directional compass response in magnetotactic bacteria. Microscopic observation shows that living cells swim in straight lines parallel to a MF, and their swimming direction can be changed instantly by using a small hand magnet. The cells of these organisms contain 0.5–2.0 % magnetite or greigite by weight, and the arrangement of crystals into chains provides each cell with magnetic moments large enough to align the organism passively like a compass needle in the GMF.

Soon after the discovery of magnetotactic bacteria, Blakemore (1975) proposed the theory that all magnetotactic bacteria are microaerophilic and indigenous in sediments. Frankel *et al.* (1997) clearly showed that these bacteria passively align and actively swim along the inclined GMF-lines as a result of their magnetic dipole moment. Blakemore called this behavior magnetotaxis, which helps to guide the cells down to less oxygenated regions of aquatic habitats at the surface of sediments. Once cells have

reached their preferred microhabitat they would presumably stop swimming and adhere to sediment particles until conditions changed, as for example, when additional oxygen was introduced. This theory is supported by the predominant occurrence of magnetotactic bacteria that are north-seeking (i.e., swim in the direction indicated by the north-seeking pole of a magnetic compass needle) under oxic conditions in the northern hemisphere whereas bacteria are predominately south-seeking in the Southern Hemisphere. Due to the negative and positive sign of the GMF-inclination in the Northern and Southern Hemispheres, respectively, magnetotactic bacteria in both Hemispheres therefore swim downward toward the sediments (Blakemore, 1982).

A possible connection between magnetic material and MF effects was found in honeybee behavior, and these behavioural effects have been reviewed by von Frisch (1967), Martin and Lindauer (1977), Martin *et al.* (1989), Gould (1980), Hsu and Chia-wei (1994). Worker honeybees communicate the location of a food source by means of a "waggle dance" to the other worker bees in a hive on a vertical honeycomb. The angle between the direction of the dance and the vertical direction indicates the angle between the food source and the Sun. Consistent errors in the dance angle occur when the MF in the hive is eliminated by means of external coils. In anomalous situations where bees are made to dance on horizontal surfaces, after an initial period of disorientation they dance eight magnetic, compass directions (N, NE, E, SE, etc.). If the GMF is eliminated the dances become disoriented. Martin and Lindauer (1977) suggested that bees could also use the diurnal variations in the GMF to set their circadian rhythms.

Phillips and Sayeed (1993) reported a directional compass response in male fruit flies, (*Drosophila melanogaster*). This discovery is an exciting development, as numerous genetic mutants exist for *Drosophila* that could be used as an aid in the localization of the receptor cells, as well as aid in the understanding of the transduction mechanism. The experimental protocol followed appears to be straightforward and simple. It has been suggested that the fruit fly could be used to replace the honeybee as the organism of choice in magnetic studies, if the results prove to be reproducible.

Lohmann *et al.* (1991) also observed a directional compass response in marine mollusks, (*Tritonia diomeda*). Lohmann *et al.* (1991) used intracellular electrophysiological recording techniques and observed that the large cells of the left and right

pedal (lPe5 and rPe5) alter their firing patterns slowly but consistently in response to a rotation of the MF direction. The ability to identify these distinct, magnetically sensitive cells offers hope that neurological staining techniques may eventually locate the actual receptor cells and help to clarify the transduction mechanism.

A directional compass response was also found in sockeye salmon (fish) *O. nerka* by Quinn & Brannon (1982), male eastern red-spotted newts (amphibians) *Notophthalmus viridescens* by Phillips (1986), and in loggerhead sea turtle (reptiles) *Caretta caretta* L. by Lohmann (1991).

Magnetointensity perception

A study by Walcott *et al.* (1979) on pigeon homing suggested that MF information could be used for orientation. The ability of pigeons to sense MFs may be associated with a small, unilateral structure between the brain and the skull that contains a magnet, and appears to be a single domain.

In a separate study, however, Walcott (1991) concluded that the primary navigation of homing pigeons is by the position of the sun but when the sun is obscured by cloud then they use the GMF for navigation. Similarly, migratory birds are also able to use the GMF as a compass to find and to maintain direction. Migratory sparrows (*Passerculus sandwichensis*), for example, navigate by the GMF (Able and Able, 1995).

Kirschvink *et al.* (1986) tested the hypothesis that cetaceans use weak anomalies in the GMFs as cues for orientation, navigation and/or piloting. Using the positions of 212 stranding events of live animals in the Smithsonian compilation which fall within the boundaries of the East-Coast Aeromagnetic Survey (USGS), they found that there are highly significant tendencies for cetaceans to beach themselves near coastal locations with local magnetic minima. Monte-Carlo simulations confirm the significance of these effects. These results suggest that cetaceans have a magnetic sensory system comparable to that in other migratory and homing animals, and predict that the magnetic topography and in particular the marine magnetic lineations may play an important role in guiding long-distance migration. The 'map' sense of migratory animals may therefore be largely based on a simple strategy of following paths of local magnetic

minima and avoiding magnetic gradients. A central problem, however, in the study of magnetic sensitivity in animals has been the lack of behavioural techniques sufficiently powerful for the systematic psychophysical work required for an understanding of magnetosensory capacity and of the transduction mechanism.

In the past, it has been shown repeatedly that free-flying honeybees can be trained in discrimination experiments to respond to local magnetic anomalies (Walker and Bitterman, 1989, 1989a, b; Kirschvink and Kobayashi-Kirschvink, 1991, Kirschvink *et al.* 1992a). Therefore, honeybees are one of the best experimental animals available for testing biophysical hypotheses concerning the sensory transduction of the GMF in animals. Walker and Bitterman (1989b) developed an experimental protocol in which individual honeybees were trained to discriminate between the presence or absence of a small static (DC) magnetic anomaly in one of two targets mounted vertically. Kirschvink *et al.* (1992a) reported the replication of this model and used it to measure the range of frequencies, of constant amplitude, to which the honeybee magnetoreceptor system was capable of responding. Honeybees demonstrated an ability to detect AC fields of 2.2 mT peak amplitude from (DC) frequencies up to 60 Hz. Above 60 Hz the behaviour approached randomness. This result was broadly consistent with the biophysical prediction for a magnetite-based magnetoreceptor (Kirschvink *et al.* 1992a).

Walker and Bitterman (1989) reported a remarkable measurement of the threshold level of static magnetic intensity perception in the honeybee. Such was the ingenuity of their experimental design, it is worth detailing their procedure. In their two-choice threshold conditioning experiments, free-flying honeybees were conditioned to discriminate the presence of localized magnetic dipole anomalies superimposed on the uniform background field of the earth. In the beginning they developed a two-choice training paradigm using two sucrose-water feeder assemblies mounted on a vertical window frame. Each assembly had a pair of double-wrapped coils that could either produce a sharply focused magnetic anomaly or a matching null-field anomaly, but with the same thermal effects. Individual foraging bees were trained via a reward-punishment scheme to feed preferentially from the feeder paired with the magnetic anomaly. Usually within 10-20 repeat visits they would learn to land at the feeder with the magnetic anomaly and avoid the non-magnetic one. By starting with a moderately strong anomaly (3 mT) and by reducing the amplitude of the anomaly in small exponential steps, the point at which

the bees were no longer able to discriminate correctly could determine the threshold sensitivity. Of nine bees run through the procedure, the median was 250 nT in the presence of the Earth's field. The best bee lost the ability to discriminate in fields below 25 nT (0.06% of background).

Kirschvink *et al.* (1997) used a modified Walker and Bitterman (1989b) protocol to place relative constraints on the threshold sensitivity of honeybees to AC MFs at frequencies of 10 and 60 Hz to complement their results for static magnetic anomalies. Their results indicate that the sensitivity of the honeybee magnetoreception system decreases rapidly with increasing frequency. At 60 Hz, alternating field strengths above 100 μT are required to show discrimination. In other words, the magnetic sense of the honeybee has its maximum sensitivity below 10 Hz, which is consistent with the evolution of this system within the known geomagnetic frequency spectrum.

Biophysics of magnetoreception

The materials found in living organisms are normally either nonmagnetic (e.g., water, fatty substances) or paramagnetic (e.g., deoxyhaemoglobin in red blood cells). The direct physical influence of the Earth's MF is very weak on these types of materials, with the energy of magnetic interaction being many orders of magnitude below that of the background thermal energy, kT (where, k = Boltzmann constant and T = absolute temperature). However, another category of materials, known as ferromagnetic, interacts very strongly with the Earth's MF. Magnetotactic bacteria are guided by the direct interaction of the field with particles of ferromagnetic material secreted within their cytoplasm (Tenforde, 1989). In the marine diatom, *Soffeaeformis*, movement is enhanced by calcium uptake in MFs (Male, 1992).

As mentioned previously, many higher organisms including mammals, birds and insects are thought to be able to detect and use the GMF for navigation and homing purposes, although the nature of the primary interaction mechanisms that may be involved is not well understood. In mammals, the pineal gland is a light-sensitive time-keeping organ in which the cell activity is affected by MF pulses of the order of the strength of the GMF (Semm *et al.* 1980).

Natural magnetoreception

Kisliuk and Islay (1977) have observed foetal effects on pupae of hornets when a horizontal MF was laid down over a hive. Upon emerging from pupae, young hornets were sluggish for a few days and then they built abnormally oriented combs.

A series of chains of uniformly sized single domain magnetite particles embedded in the ethmoid tissue in the front of the cranium of Chinook Salmon fish (*Oncorhynchus tshawytscha*) is used for accurate navigation (Kirschvink *et al.* 1985). Salamanders (*Eurycea lucifuga*) were trained to choose which corridors to move along in response to the angle of the EMF surrounding the corridor (Phillips, 1977).

The apparently synchronous turning and wheeling of flocks of several bird species, particularly starlings (*Sternus vulgaris*) has long been a phenomenon of curiosity and interest (Frank and Haffner, 1974). Based on several reports the authors suggested that birds may be able to detect the earth's MF and the turning movements in bird flocks may be coordinated by geomagnetic means. Such a signal would be instantaneous, could reach all birds in the flock regardless of their position relative to the signal source, and could carry sufficient information for turning movements. Analysis of films of turning flocks of birds and experimental studies of startle reaction times in birds have suggested that the geomagnetic radiation model could provide a parsimonious explanation for the observed behaviour of the birds.

In a seminal paper, Walker *et al.* (1997) described the key components of a magnetic sense underpinning this navigational ability in the rainbow trout fish (*Oncorhynchus mykiss*). The authors identified an area in the nose of the trout fish where candidate magnetoreceptor cells are located and reported the behavioural and electrophysiological responses to MFs. They have tracked the sensory pathway from these newly identified candidate magnetoreceptor cells to the brain and associated the system with a learned response to MFs.

They have identified single neurons in the superficial ophthalmic ramus (ros V) of the trigeminal nerve which do respond to varying magnetic intensity but do not respond to the direction of an imposed MF. They have also identified the candidate magnetite-

based magnetoreceptor cells in the nose of the trout with the aid of new imaging and confocal microscopic techniques. The cells contain crystals that are iron-rich and almost identical in form to single-domain magnetite crystals previously extracted from sockeye salmon (Mann *et al.* 1988). They have also traced fine branches of the ros V nerve to the same cellular layer (the lamina propria within the olfactory lamellae) as the candidate magnetoreceptor cells. Identification of the critical components of the magnetic sense brings a new perspective to the study of long-distance orientation in a variety of vertebrate groups.

It appears from the above discussion that living organisms do respond to the minute static GMFs. These responses of living organisms to GMFs are in the form of 1) a directional compass - used by magnetotactic bacteria, honey bee, drosophila, marine mollusks, sockeye salmon, male eastern red-spotted newts, and loggerhead sea turtle; 2) magnetointensity perception - used in homing, navigation and orientation by pigeon, migratory sparrows, and cetaceans; 3) In mammals, the pineal gland is a light-sensitive time-keeping organ in which the cell activity is affected by MF pulses of the order of the strength of the GMF.

2.1.9 DNA Damage, Chromosomal Aberration and ELF EMFs

It is generally accepted that ELF EMFs do not transfer energy to cells in sufficient amounts to directly damage DNA. It is possible, however, that certain cellular processes altered by exposure to ELF EMF, such as free radicals, indirectly affect the structure of DNA. Most researchers have looked for strand breaks and other chromosomal aberrations (CAs), including SCE, formation of micronuclei, and/or effects on DNA repair. In a number of studies conducted to assess the effects of ELF EMFs, human peripheral blood lymphocytes (HPBLs) have been exposed to different magnetic flux densities.

In one study, EMFs were found to induce CAs in human lymphocytes (Khalil and Qassem, 1991). Exposure of human lymphocytes to a pulsed field with a peak magnetic flux density of 1.05 mT for 24-72 h induced a statistically significant, 2-fold increase in the frequency of CAs and simultaneous lowering of the mitotic index.

Garcia-Sagredo *et al.* (1991) studied the effects of ELF EMFs on HPBLs *in vitro*. An increase of CAs was observed after exposure to PEMFs of amplitudes ranging from 1mT to 4 mT. The treatment effect at 4 mT was statistically significant. This result led them to conclude that PEMFs may have a clastogenic effect on humans.

In a PEMF study by Vijaylaxmi *et al.* (1997), HPBLs were exposed to radio-frequency radiation (RFR) at 2.45 gigahertz (GHz) for 30 min on-30 min off over 90 min. Lymphocytes were cultured immediately after exposure for 48 h and 72 h. An analysis showed no significant difference between RFR-exposed and sham-exposed lymphocytes with respect to i) mitotic index, ii) chromosome damage, iii) exchange aberrations, and iv) number of micronuclei.

Scientific studies have also focussed on the question of whether radiation emitted from mobile phones causes any damage to human health. With this aim, Mashevich *et al.* (2003) exposed HPBLs to continuous 830 MHz EMFs and observed losses and gains of chromosomes (aneuploidy), a major "somatic mutation" leading to genomic instability and thereby to cancer.

A linear increase in chromosome 17 aneuploidy was observed as a function of the specific absorption rate (SAR) value, demonstrating that this radiation has a genotoxic effect. The SAR-dependent aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in SAR-dependent genetic toxicity. Control experiments (i.e., without any RF radiation) carried out in the temperature range of 34.5-38.5C showed that elevated temperature is not associated with either the genetic or epigenetic alterations observed following RFR, the increased levels of aneuploidy, and the modification in replication of the centromeric DNA arrays. These findings indicate that the genotoxic effect of the electromagnetic radiation is elicited via a non-thermal pathway. One important aspect of the above study not noted by the authors, but observed in passing by the writer of this thesis is that chromosome 17 houses one of the most important tumour suppressor genes in humans: p53. Loss of this chromosome in a cell line by aneuploidy could have far-reaching effects on the proliferation of that line.

Cytogenetic investigations were performed by Maes *et al.* (2000) in HPBLs following exposure to 50 Hz MFs alone or in combination with the chemical mutagen, mitomycin C, or with X-rays. No significant effects of EMFs up to 2,500 μT on the chromosome aberration and SCE frequency were observed.

Nordenson *et al.* (1994) observed a significant increase in the frequency of chromosomal aberrations in human amniotic cells after exposure to a sinusoidal 50 Hz, 30 μT, pulsed MF. Amniotic cells from eight different foetuses were exposed to a 50 Hz, 30 μT MF in a 15 sec on and 15 sec off fashion for 72 h. Among exposed cells, aberration frequency was 4% compared to 2% in sham-exposed cells. A similar increase in CA was observed in another series of eight experiments in a 2 sec on and 2 sec off fashion. On the other hand, continuous exposure of cells to 50 Hz, 300 μT for 72 h did not increase the frequency of chromosomal aberrations.

In a study by Rapley *et al.* (1998) seedlings of *Vicia faba* were exposed to different MFs continuously for 3 days: 0 Hz (DC) at 5 mT, 50 Hz at 1.5 mT, 60 Hz at 1.5 mT, and 75 Hz at 1.5 mT. The length of all the mitotic phases differed from the controls in all treatments using alternating MFs and for prophase and metaphase in the DC condition. In all treatments, the length of the prophase increased significantly in meristematic root-tip cells compared with the controls. The length of the prophase, however, did not vary significantly between the treatments. Moreover, exposed seedlings had a lower frequency of chromosome breakage than the controls.

The effects of acute exposure to 60 Hz MFs on DNA strand breaks, a common form of DNA damage, in the brain cells of rat were observed by Lai and Singh (1997a). The 'Comet-assay' (DNA microgel electrophoresis) was used in their study to detect DNA fragmentation in single cells. Rats were exposed for 2 h to a 60 Hz MF (flux densities 0.1, 0.25 and 0.5 mT) and a dose-dependent increase in DNA strand breaks in the brain cells at 4 h post-exposure was observed. An increase in single-strand DNA breaks were also observed in all three parameters of exposure (0.1, 0.25 and 0.5 mT) but double-strand DNA breaks were observed only in higher parameters of exposure (0.25 and 0.5 mT).

Setlow and Setlow (1972) reported that several types of electromagnetic radiation might cause damage to a cell's DNA. Strong energy radiation is effective at breaking DNA and promoting chemical modification in the form of gamma-endonuclease sites (Patterson *et al.* 1976) or protein-DNA crosslinks (Fornace *et al.* 1980). It is well known that ultraviolet light produces pyrimidine dimers, as well as causing numerous other alterations in DNA (Setlow and Setlow, 1972), while irradiation of BrdU substituted DNA with near-ultraviolet light produces a different set of changes, most notably single-strand DNA breaks (Hutchinson, 1973).

In a study by Fiorani *et al.* (1992), a human tumor cell line (k562) was grown in suspension culture and exposed to 50 Hz, 0.2, 20, 100, or 200 μT MFs for 4, 6, 12, or 24 h in an alkaline elution assay and analysed for DNA strand breaks. The induced electric field calculated from the geometry of the exposure system reported by Dacha *et al.* (1993) was 0-0.4, 0-4, 0-40, 0-80, or 0-200 μV/m, respectively. No increase in strand breaks was observed in cultures exposed to the MF as compared to controls.

An overall summary of all the studies reviewed above shows a mix of positive and negative findings with respect to the possible effects of ELF EMFs on the genetic apparatus. On balance, however, it is the current author's opinion that the weight of evidence at this stage would favour the view that some genetic effects can occur under certain circumstances, the nature of which remains to be clarified.

2.2 Sister Chromatid Exchange (SCE)

The SCE technique is one of the most widely used specialized techniques that has been applied to investigate clastogenicity. It is a highly sensitive technique that can be used to determine the levels of genetic damage and was applied in the current study in two complete rounds of experiments. It is therefore important to review the SCE technique and its utilization in detail.

2.2.1 Introduction

A sister chromatid exchange (SCE) may be defined as a reciprocal exchange between sister chromatids of the same replicating chromosome. This interchange is the product of DNA breakage and rejoining at apparently homologous loci and is evidenced by differences in chromatin staining intensity in metaphase chromosome preparations (Figure 2.3).

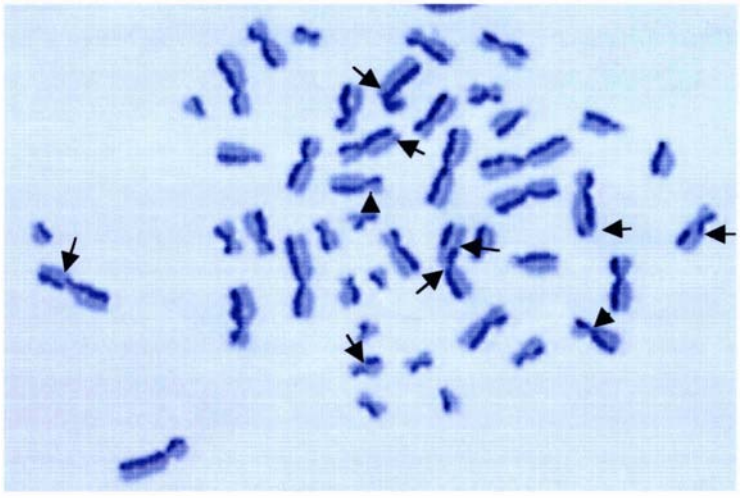


Figure 2.3: A c-metaphase chromosome spread of a dividing peripheral blood lymphocyte that has undergone two rounds of DNA replication in the presence of BrdU, showing 10 SCEs (arrows).

Taylor *et al.* (1957), who used ^{3}H-thymidine to differentially label the DNA of replicating cells and autoradiography to distinguish the silver grain pattern on the two sister chromatids, first visualized SCEs. This method was eventually replaced by cytomolecular methods for differentiating sister chromatids. Latt (1973) demonstrated that when the halogenated thymidine analogue, bromodeoxyuridine (BrdU) was incorporated into DNA, it could quench the fluorescence of the fluorochrome Hoechst 33258. Perry and Wolff (1974) found that incorporated BrdU also diminished the uptake of Giemsa stain into the chromatin.

DNA replicates semiconservatively and each chromosome acts as if it is composed of a single DNA duplex. Chromosomes that have replicated in the presence of BrdU for two cell cycles each contain one unifilarly substituted and one bifilarly substituted chromatid (Figure 2.4) and one chromatid will stain more intensely than the other in second-generation metaphase cells. If cells are allowed three consecutive generations in the presence of BrdU, only one out of four chromatids will stain more intensely.

If it is agreed that these exchanges arise by a breakage and reunion between corresponding sites of a duplex DNA in each chromatid, then some predictions can be made about the structure of the chromatid and the nature of recombination. The SCE might take place after the first round of replication in BrdU or after the second round. If cells are treated with colchicine, chromosome segregation after the first round is prevented and both pairs of sister chromatids are retained in the same cell. When an exchange occurs after the second round of replication it will affect only one of the two original sister chromatids (Figure 2.4). However, if it occurs after the first round, it will be perpetuated by replication, so that it is seen in both pairs in tetraploid cells (Figure 2.5). Since there are twice as many chromosomes in the second round, there should be twice as many single exchanges as twin exchanges.

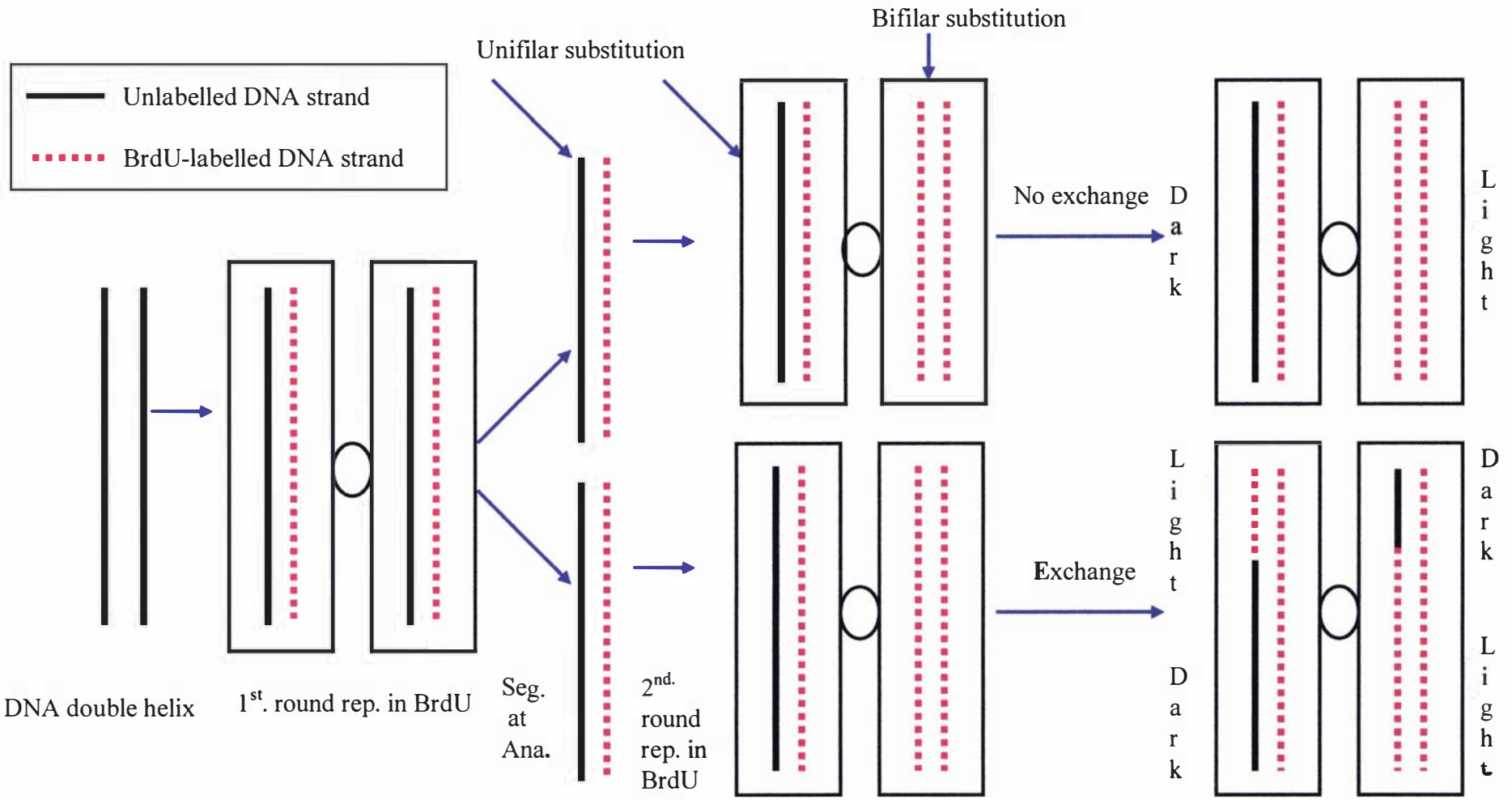


Figure 2.4: Showing how SCE staining method produces differentially stained chromatids by the incorporation of BrdU.

This assumes that the frequency of exchange is the same in both division cycles. This cannot be accepted a *priori*, as bifilarly substituted DNA might be more active in exchange than unifilarly substituted DNA. However, a study by Mazrimas and Stetka (1978) indicates that there is no difference in the frequency of SCE between the two cycles. Attempts to measure the ratio between single and twin exchanges with autoradiography were not successful but with the BrdU technique a ratio of very close to 2:1 has been measured (Wolff and Perry, 1975). This suggests that a single unineme duplex of DNA constitute the only unit that can be exchanged between chromatids. Any exchange of a half strand of DNA would generate different patterns (Kihlman, 1975; Shafer *et al.* 1976 and Taylor, 1958). If this were so, after the first round of replication, totally substituted and totally unsubstituted DNA could be produced (Figure 2.6).

SCEs have been found in all organisms in which they have been sought: in a variety of mammals, including humans (Khalil and Qassem 1991; Bender *et al.* 1992a; Atalay *et al.* 2000), in birds (Bloom, 1978; Bloom and Hsu, 1975), in fishes (Kligerman, 1979; Kligerman and Bloom, 1976; Kligerman *et al.* 1975), in insects (Gatti *et al.* 1979), in plants (Schubert *et al.* 1980) and in marine worms (Pesch and Pesch, 1979). Most SCE analyses have been performed on cultured cells, but *in vivo* studies have been performed on mammalian bone marrow, spleen, regenerating liver, and spermatogonia (Kanda, 1982). In principle, SCEs can be observed in any cell that has completed two replication cycles in the presence of BrdU. The biological significance of SCEs is unclear, since the genetic makeup of a cell is not altered if a SCE takes place at identical sites on the sister chromatids. However, if the breakpoints in the two chromatids are different, the resultant unequal (ectopic) SCE leads to a duplication of the intervening segment on one chromatid and its deletion from the other chromatid. For instance, the variation of the heterochromatic segment on the Y chromosome obviously has come about through unequal SCE (Eeva and Millard, 1992).

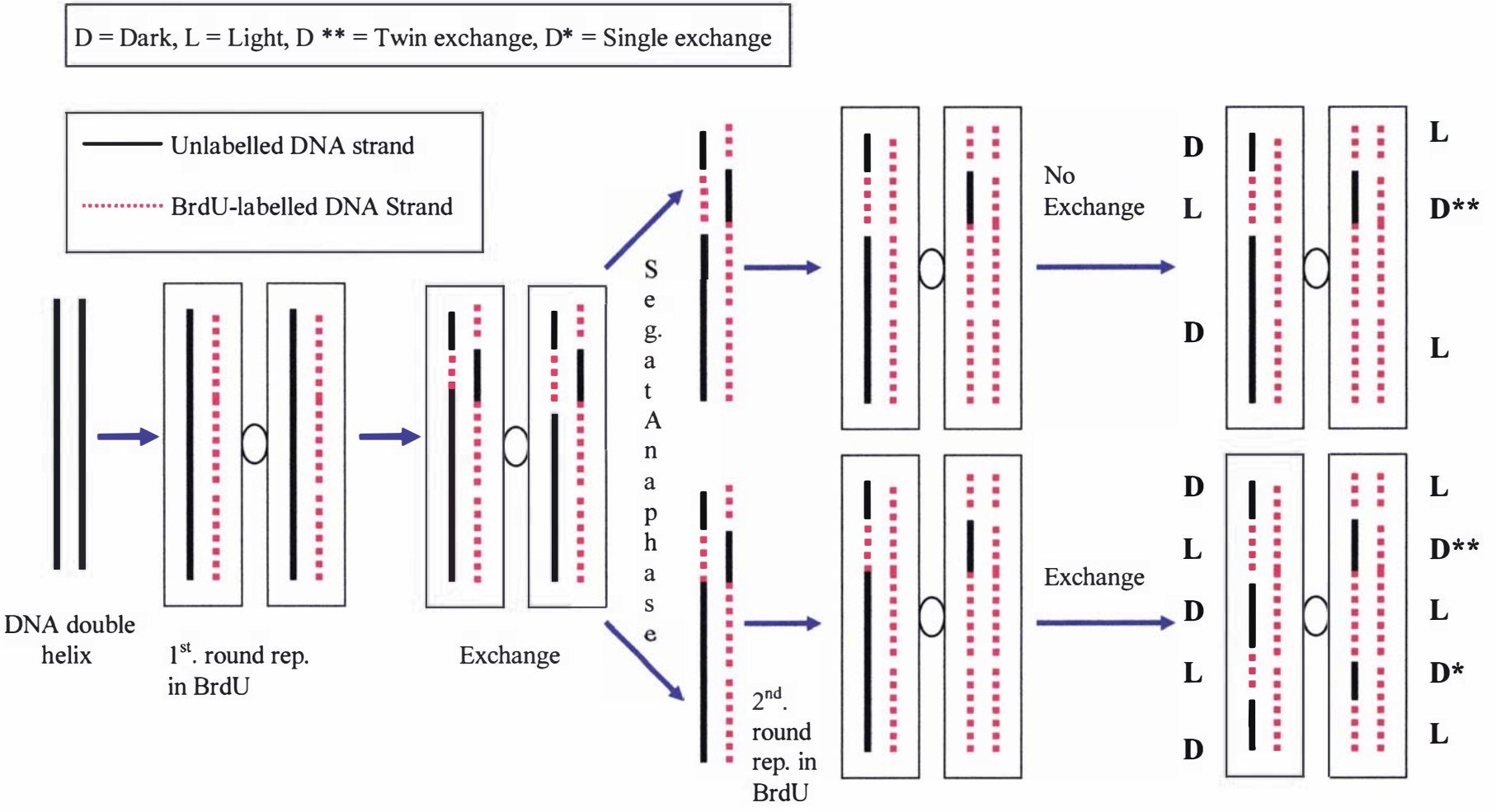


Figure 2.5: Showing "single" and "twin" sister chromatid exchanges. Sister chromatid exchange might take place after the first round of replication in BrdU or after the second round. If an exchange occurs after the first round, it will be perpetuated in both pairs of cells, such exchanges are called twin exchanges (D**).When an exchange occurs after the second round of replication, it will affect only one of the two original sister chromatids, these are called single exchanges (D*).

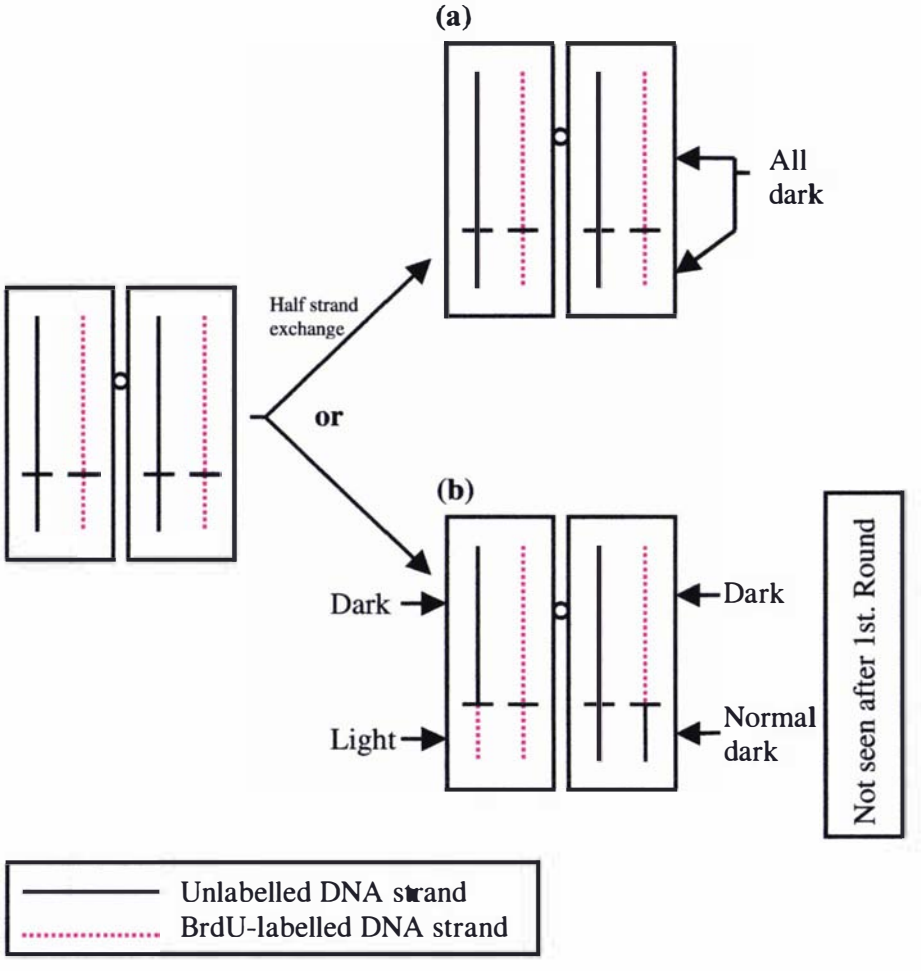


Figure 2.6: Showing the single stranded (unineme) nature of chromosomes, i.e., a single unineme duplex of DNA constitutes the only unit that can be exchanged between chromatids. Any exchange of a half strand of DNA would generate different patterns (**a** or **b**). If this were so, after the first round of replication, totally substituted (normal dark) and totally unsubstituted (light) DNA could be produced. It is important to know that this pattern has not been observed after the first round of replication in BrdU.

2.2.2 Genetic Basis of SCE

If the present study shows elevated SCE frequencies as a consequence of exposure to ELF EMFs, then it is imperative that one should have a sound understanding of how SCEs are thought to be generated, and how EMFs might exert their effect. Therefore, the following section covers the various models that have been advanced to explain how SCEs arise at the molecular level.

The measurement of SCE, first described by Taylor (1958) using autoradiography, has become much more accurate since the introduction of dye-photometric techniques (Latt, 1973) and in particular, the Fluorescence-Plus-Giemsa (FPG) technique of Wolff and Perry (1974). Once it became possible to rapidly assess SCE frequencies with this new technique by substitution of 5-bromodeoxyuridine (BrdU) in thymidine sites in DNA, the SCE assay has become extensively used as a sensitive test for agents with mutagenic or carcinogenic potential (Chaganti *et al.* 1974; Dutrillaux *et al.* 1974; German *et al.* 1965, 1974; Kato, 1974a; Latt, 1974a, b; Perry and Evans, 1975; Perry and Wolff, 1974).

In humans, the most common system for ascertaining the possible mutagenic or carcinogenic potential of substances, either chemical or physical, is based on observations of peripheral blood lymphocytes. Human blood cultures can be treated with a particular agent and lymphocytes can be grown in the presence of BrdU. The number of SCEs is then scored. An increase in SCE rate is widely considered to be an indication of the mutagenic and carcinogenic potential of an agent (Perry and Evans, 1975; Nichols *et al.* 1978; Wolff, 1979; Carrano *et al.* 1979; Husum *et al.* 1981; Sandberg, 1982; Popescu *et al.* 1977; Popescu and Dipaolo, 1982; Kram, 1982; Wolff, 1983; Sandberg *et al.* 1984; Latt *et al.* 1984; Perera *et al.* 1992).

Whether SCEs are actually associated with or causally linked to mutagenic or carcinogenic alterations is still unclear. To resolve this issue, an understanding of SCE mechanisms at the molecular level is necessary. Taylor s (1958) early SCE studies suggested two major assumptions about eukaryotic chromosome structure which are fundamental to describing the model: i) that eukaryotic chromosomes have a unineme organization with one DNA duplex molecule in each chromatid, and ii) that the rejoining of DNA strands is restricted by the structural polarity of each duplex strand. While only autoradiographic methods were available, these conclusions remained in doubt primarily because the observation of small isolabeling segments suggested the possibility of a bineme or polyneme organization (Gatti *et al.* 1974). These issues have been clarified by the new high resolution BrdU/staining methods which show that isolabeling is an artifact of grain scatter (Wolff and Perry, 1974; Kato, 1974c) and that an equal exchange of chromatid material occurs even with an extremely high SCE frequency. Other studies also confirm structural polarity (Wolff and Perry, 1975; Tice *et*

al. 1975) and establish that polarity extends through the centromere (Lin and Davidson, 1974). Therefore, chromosome organization may be simply described as a single DNA duplex in the G_1 stage before replication, and as a pair of daughter duplexes in the G_2 and metaphase stages after replication. A unineme organization would, of course, make cells highly vulnerable to genetic damage particularly in the G_1 stage.

Formation of SCE is tightly coupled to DNA synthesis; i.e., cells need to pass through S-phase before SCE induction can be observed (Wolff *et al.* 1974). Kato (1974a) observed maximum SCE induction during the DNA synthetic period while DNA synthetic activity of Chinese hamster cells was determined autoradiographically in BrdU-substituted duplicate cultures by labeling the cells with ^{3}H-thymidine during the period of visible illumination. DNA synthetic activity was expressed as the % labeled metaphases. Observations were also made in synchronized cells that maximum induction occurs at the start of S-phase and decreases progressively throughout the rest of S-phase (Latt and Loveday, 1978).

The phenomenon of SCEs is now widely accepted as intrachromosomal exchanges of DNA replication products, presumably at homologous sites on each chromatid and involve DNA breakage and reunion (Latt *et al.* 1981). Although the precise nature and mechanism(s) involved in the formation of SCE are not clear, SCE analysis has come into use as a most sensitive means of detecting DNA damage (Perry and Evans, 1975; Abe and Sasaki, 1977, 1982; Wolff and Carrano, 1979; Tucker *et al.* 1986). Nevertheless, it is widely accepted that in a controlled experiment, any increase in the frequency of SCE is interpreted as evidence of clastogenic activity.

Although SCEs are readily observed, the molecular mechanism(s) responsible for the exchange are not fully known. Several mechanisms have been proposed to explain SCE formation, which are consistent with uninemic chromosome structure. These mechanisms belong to two basic groups. One group is assumes that the replicating point is the site of SCE formation (Kato, 1974a; Shafer, 1977; Schvartzman and Gutierrez, 1980; Ishii and Bender, 1980; Kato, 1980). The other group assumes that the SCE may be a post-replicational event closely linked to S-phase (Kato, 1977; Schvartzman *et al.* 1979; Painter, 1980; Maria *et al.* 1989).

In the former category, Shafer (1977) proposed a model in which SCE results from completion of DNA replication at the site of an unrepaired DNA crosslink. The model was subsequently generalized to apply to other lesions (Shafer, 1982) and he discussed the possible mechanism of the alternative replication bypass model. There are different alternative replication bypass mechanisms, all of which are based on the hypothesis that different SCE mechanisms may be required for different DNA lesion states and conditions, the principle being that SCEs occur as ongoing replication processes that encounter intact or partially repaired DNA lesions. Kato (1974a) postulated that in BrdU-substituted chromosomes, fluorescent light induces the formation of SCEs but only if irradiation coincides with the time of DNA replication. The replication detour model of Ishii and Bender, (1980), based on Kato s work (Kato, 1974a), suggests that SCE occurs at the replicating fork of DNA.

Schvartzman and Gutierrez (1980) studied the effectiveness of a given dosage of visible light in inducing increased yields of SCE in *Allium cepa* L. meristems. Cells were grown for 1 cycle time in the presence of BrdU and then irradiated at different times throughout the second cell cycle. The effectiveness of this treatment in provoking the formation of SCE increases the closer the irradiation time is to the beginning of the S phase, and then decreases rapidly as cells progress through the S period. The largest increase in SCE is obtained when irradiation coincides with early S phase. SCE may arise at the time of DNA replication due to the presence of unrepaired lesions. Since repair appears to be a time-dependent process, the shorter the interval between damage induction and DNA replication, the greater the number of lesions that remain un-repaired, and as a consequence, the higher the effectiveness of the damaging treatment in provoking the formation of SCE.

In contrast, homologous displacement involving topoisomerase II alone provides a mechanism for the strand switching required in the models of Kato (1977) and Cleaver (1981) in which SCEs occur between replicated double strands. Pommier *et al.* (1985) suggested that SCE results from exchange of topoisomerase II subunits. Homologous displacement , an alternative mechanism, is proposed in which strand switching occurs during removal of parental helical turns by topoisomerases.

Painter s (1980) SCE model is based on the idea that double-strand breaks are generated at junctions between a completely duplicated replicon cluster. Most models generally provide a rationale for strand switches relative to the replication fork, but vary in exact details.

2.2.3 SCE Models

If low frequency EMFs can affect SCE frequency, then it is essential to understand the current models advanced to explain how SCEs arise. Only by examining the different models in detail at the molecular level, is it possible to interpret ELF EMF effects should they occur. The following sections, which examine SCE formation are rather long for a Literature Review, but inclusion of these models, and criticisms thereof, was thought necessary by the author towards providing a meaningful mechanism if an effect is observed.

SCE model of Kato

Ben-Hur and Elkind (1972) reported that fluorescent light (FL) induces strand breaks only in bromodeoxyuridine (BrdU)-substituted DNA, and that these breaks eventually lead to the formation of SCEs (Ikushima and Wolff, 1974; Kato, 1974a; Wolff and Perry, 1974). Kato (1977) studied the response of SCEs to Fluorescent light (FL) in Chinese hamster chromosomes in which, out of four DNA strands, BrdU-substitution had occurred either in one or three strands. The FL-induced SCE frequency did not differ greatly between these two types of chromosomes. However, when they were submitted to caffeine treatment, a drastic increase in the frequency was detected in the trifilarly-substituted (*sic*) chromosomes while a significant decrease occurred in the unifilarly-substituted (*sic*) chromosomes.

Based on these above results, Kato (1977) hypothesized that at least two different mechanisms are involved in the induction of SCEs (Figure 2.7). One operates at replication forks probably utilizing the machinery of DNA replication, and the other is considered to act only in the post-replicational DNA portion.

To test the above hypothesis, Kato (1977) in his experimental system controlled the labeling conditions and obtained chromatids with a known number of BrdU-labeled DNA strands and thus a known number of break-bearing strands following photolysis by FL. The labeling patterns of DNA in the chromosome at the second S phase are shown in Figure 2.7 A and B, respectively. Chromosome A is referred to as a pulse-labeled (PL) which has undergone one round of replication in the presence of BrdU and followed by a subsequent round in its absence. On the other hand, chromosome B is referred to as a continuous-labeled (CL), which has undergone two rounds of replication in the presence of BrdU.

According to the author, the first pathway (Figure 2.7: I, II) needs only one FL-induced single-strand break for its initiation. One of the possibilities may be that a free end of a single-strand break could somehow accomplish exchange with the discontinuously replicating DNA strand, probably with the aid of the machinery of DNA replication. Temporal detachment of histones from the replicating point may facilitate such an interaction. Thus, strictly, this pathway would require two breaks for its initiation, one being induced by FL and the other occurring spontaneously, both residing in co-parallel strands. Therefore, if the exchange site is restricted to the replicating point as shown in Figure 2.7: I, II, A & B, the exchange frequency would be the same for both types of chromosomes.

The second type of pathway (Figure 2.7: III a or b) would become available only when normal repair of FL-induced DNA damage is disturbed somehow, and function only in the post-replicational DNA regions, probably in a similar fashion to that assumed in models of meiosis or gene conversion (Holliday, 1964; Whitehouse, 1963). It requires the presence of two single-strand breaks at juxtaposition in two sister chromatids, and eventual displacement of a free end of a single strand at the break point, thereby enabling the interaction of two DNA molecules. If the exchange site were located in the duplicated DNA region, the exchange frequency in the CL-chromosomes would be higher than in the PL-chromosomes.

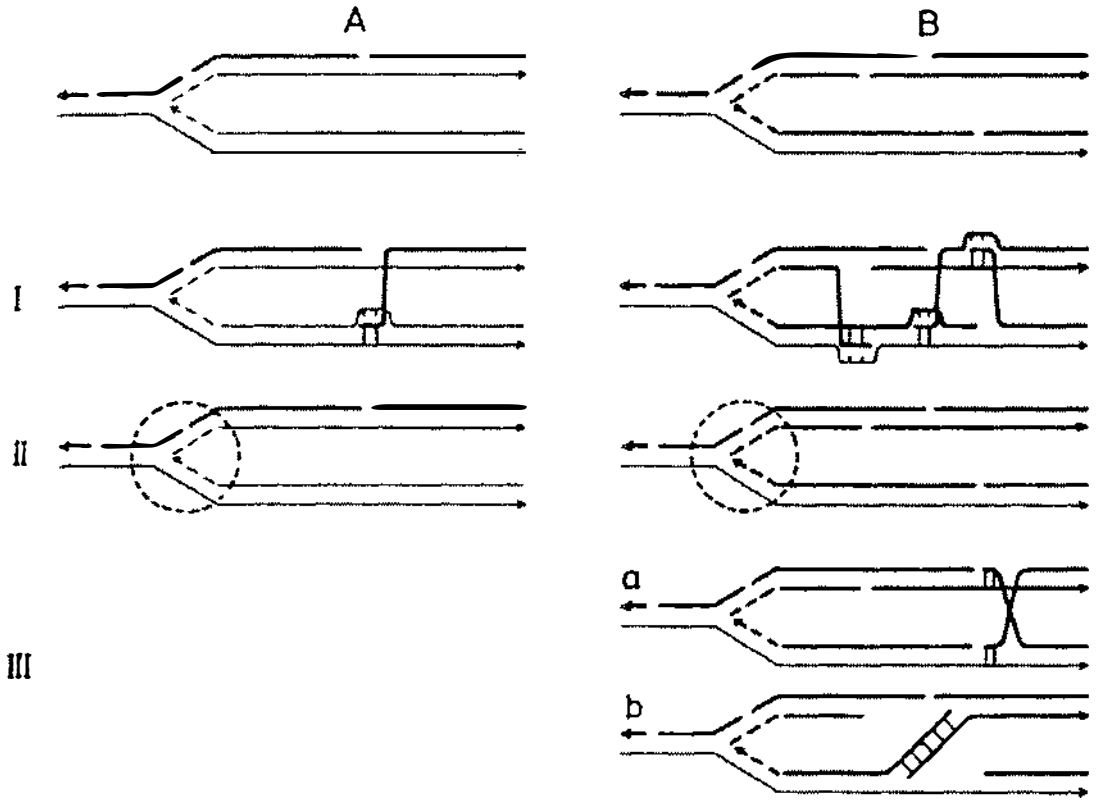


Figure 2.7: A and **B**. Schematic illustration of the labeling of chromosomes at the second post-labeling S phase and possible steps (**I, II** and **III**) of the initiation of the formation of sister chromatid exchange following introduction of strand breaks by photolysis in BrdU-substituted DNA. A and B are tentatively designated as pulse-labeled (PL) and continuous-labeled (CL) chromosomes, respectively. Thick lines indicate BrdU-substituted DNA strands and thin lines unsubstituted strands. Discontinuities in thick lines show the sites of photolysis. In IIIa, the site of a break in the BrdU-substituted parental strand is shown, for convenience sake, at juxtaposition with another break in the co-parallel nascent strand. Encircled regions in step II are considered to be the site of the exchange initiation, though the exact process remains to be elucidated (Kato 1977).

The replication bypass model of SCE

There were only a few important clues to an SCE mechanism before the rapid influx of BrdU-incorporation SCE-studies. Taylor (1958) reported that a double-strand exchange was involved in SCEs. Wolff *et al.* (1974) pointed out that lesions induced in DNA had to pass through an S phase before they could result in SCEs, and Kato (1974a) likewise noted that SCE induction was maximized during the period of DNA synthesis. Considering the above findings, the replication bypass model for SCE was originally proposed in 1975 to explain DNA crosslink-induced SCEs, since it appeared that i) crosslinking agents were the most potent inducers of SCEs (Perry and Evans, 1975;

Latt, 1974a); ii) Crosslinks would inhibit or prevent the normal separation of DNA strands as required for bidirectional replication (Geiduschek, 1961); and iii) that crosslink repair required an undamaged parallel DNA duplex in order to complete a recombinational exchange (Cole, 1973 and 1974).

On the basis of the above clues, Shafer (1977) proposed a model in which SCEs result from completion of DNA replication at the site of an unrepaired DNA crosslink. The model was subsequently generalized to apply to other lesions (Figure 2.8) (Shafer, 1982), and he discussed the possible mechanism of the alternative replication bypass model. The most important characteristic of this proposed model was that SCEs could result from a series of sequential events, which might occur as bidirectional replication encountered a crosslink. These events may be summarized as follows: a) the advance of a leading strand of nascent DNA toward a crosslink site would create distortional stress near the crosslink resulting in a parental-strand break or incision; b) the leading nascent strands would approach the crosslink along opposite parental strands; c) two-resultant breaks or incisions would occur contralaterally on opposite parental strands above and below the crosslink (Figure 2.9, D). Due to convergent unwinding processes, the free ends produced in the first stage become terminally aligned irrespective of their opposite polarity. Completion of an SCE could then be accomplished by either of two rejoining processes-terminal ligation of the free ends via nascent Okazaki pieces or heteroduplex complementation by overlapping of the free ends (Figure 2.9 & 2.10).

This bypass mechanism would allow replication to continue past the crosslink, leaving it intact, but would also result in a switching of parental strands and their attached incomplete nascent strands above and below the crosslink site, producing an apparent exchange between sister chromatids.

Evidence and criticism of Shafer s replication bypass model.

The replication bypass model for SCE proposed by Shafer is examined in detail by Stetka (1979). Although it was acknowledged that alternate replication bypass mechanisms for single-strand lesions were suggested, this evaluation criticized the model for its limitations solely as a crosslink bypass mechanism and, on this basis, found it inadequate for explaining SCEs induced by other lesions. Secondly, an assumption was made, without justification, that the model predicted that crosslinks

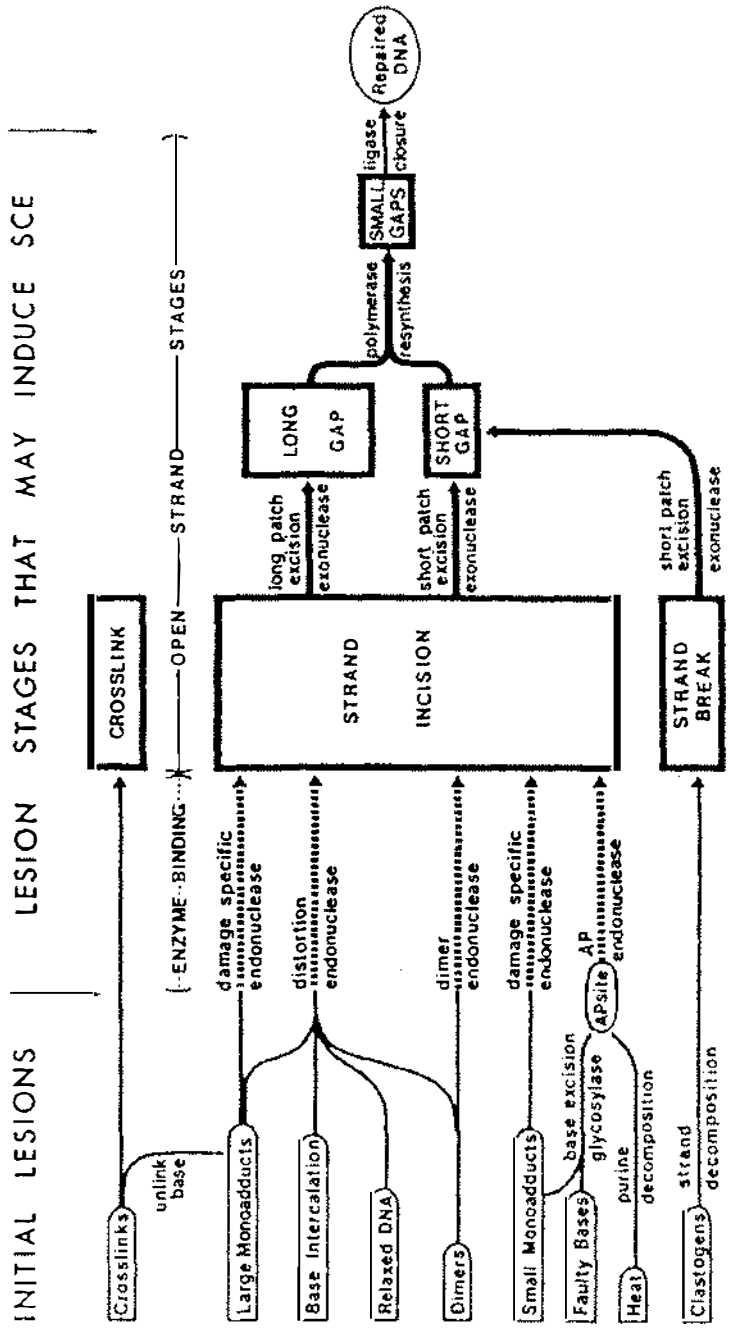


Figure 2.8: Multiple lesion pathways leading to a small number of SCE-inducing lesion states. Some lesions such as crosslinks and strand breaks may cause SCE directly, though the latter lesion type has only a short time span. Most other lesions are converted by a variety of repair processes to a similar strand incision stage and subsequent repair intermediate stages that may induce SCE by a single or several SCE mechanisms. Prior to completion of repair any one or more of these open-strand stages may induce SCEs. Alternatively, SCE induction with such lesions may be caused if replication encounters a lesion in the process of endonuclease binding (Shafer, 1982).

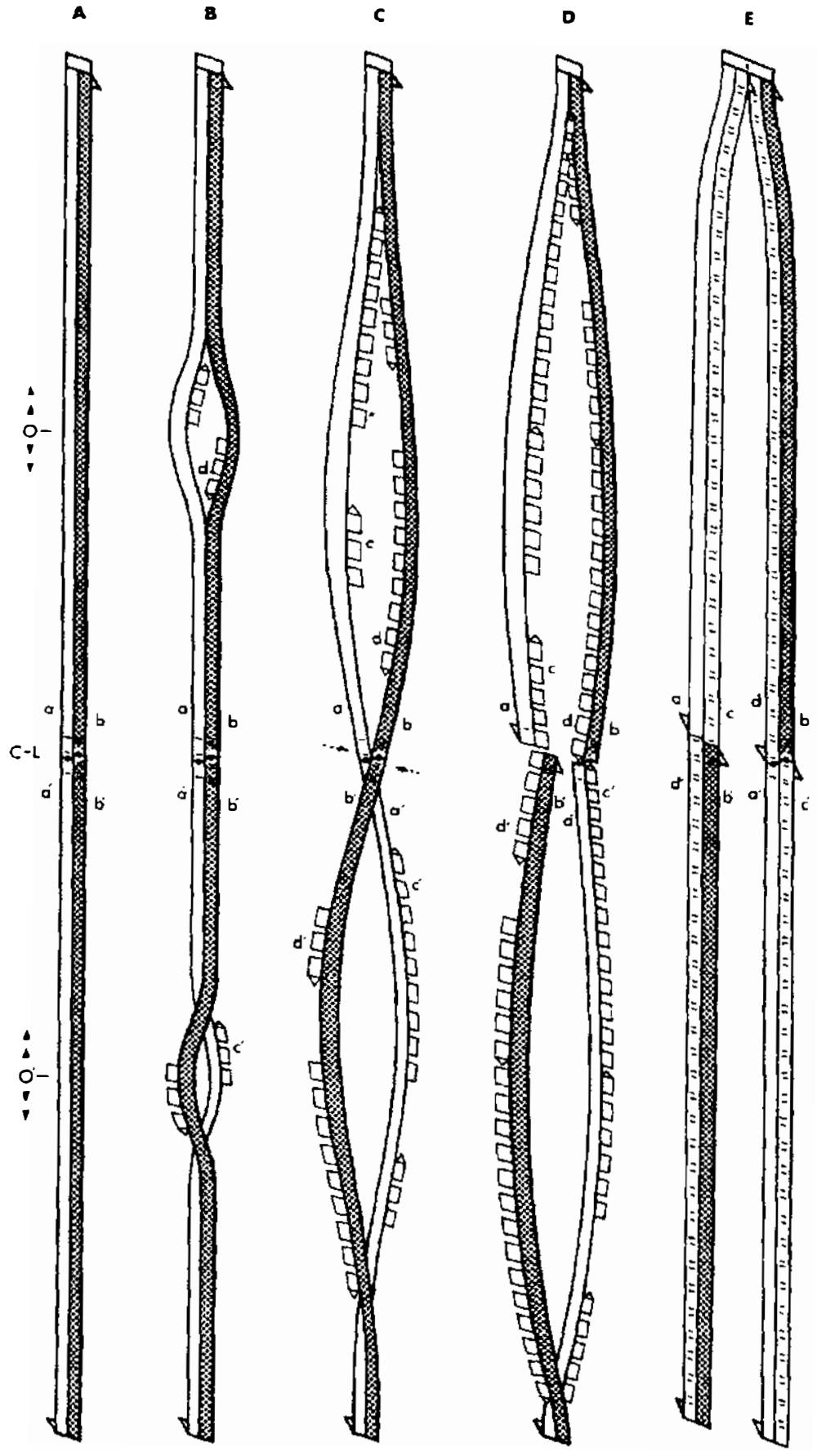


Figure 2.9 of Shafer, 1977: (For caption, see next page).

Figure 2.9 from Shafer, 1977: Replication crosslink bypass model of the SCE mechanism. A) The interphase G_1 chromosome is depicted as a single DNA duplex with structural polarity. Based on the BrdU/ SCE staining methods, the shaded strand b-b' indicates the distinctly staining thymidine-retaining strand and the unshaded strand a-a' indicates the BrdU substituted strand. An interstrand crosslink is indicated at C-L. The crosslink bonding angle is based specially on the covalent linkages which occur between alternate bases on their 5' side with agents such as mitomycin-C, nitrogen mustard, and psoralen, plus light. For graphic simplicity only two replicons and their origins 0 and 0' are represented. B) Bidirectional replication is initiated and the nascent strands d and c' which advance towards the crosslink are on opposite parental strands b and a'. C) As the advancing strands d and c' proceed along the growing fork, the complementary regions on the opposite parental strands are back-filled with Okazaki pieces represented by the nascent strands c and d'. When the advancing strands d and c' approach the crosslink, they will come into parallel competition with the parental strands a and b' at the stress points (arrows). At this stage normal replication should be prevented by the crosslink. The proposed SCE mechanism would occur by a two step displacement/rejoining event which may involve unique incision and/or ligation enzyme systems. The stress points (arrows) indicate where the displacement will occur when the advancing strands d and c' reach the crosslink. The SCE mechanism is completed in D. After the chain breaks are affected, the advancing strands d and c' terminate replication at the crosslink; and new Okazaki pieces c and d' are initiated which backfill from the break point. Visualized three-dimensionally, the break sites are in contralateral proximity such that they may line up in correct polarity across the break via the new Okazaki pieces. Ligation of the breaks may occur by chemical processes similar to the normal means for linking completed Okazaki pieces during replication. E) After replication the daughter duplexes form the two chromatids. Above and below the crosslink the shaded strand segments b and b' containing Thymidine are now separated into different chromatids and results in the SCE staining effect. This SCE mechanism occurs during replication and does not involve recombination as the incomplete nascent complementary strands remain bound to their original parental strand during the exchange. The effect observed at metaphase appears equivalent to a post-replication double strand exchange. The crosslink remaining in one chromatid may now be removed by crosslink repair. These diagrams only depict a hypothetically perfect SCE mechanism in which no bases are lost in the exchange (see Fig. 2.10). Enzymatic incision may not, in fact, be possible nearer than two bases from the crosslink (Shafer, 1977).

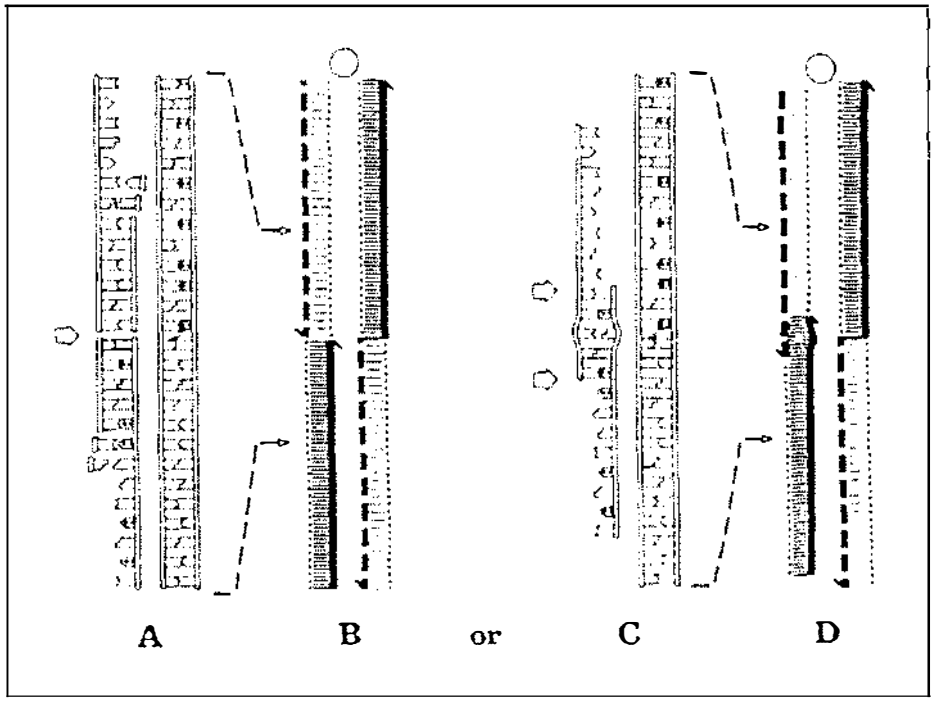


Figure 2.10: Alternate rejoining processes for the replication bypass SCE mechanism. A represents the structural detail of stage D in Figure 2.9. In this hypothetically perfect process, opposite parental strands may be rejoined by terminal ligation (at open arrow) via the formation of nascent Okazaki pieces. The shaded bases represent the normal thymidine bases retained by semi conservative replication. These bases allow the chromatid portions carrying them to stain darkly at metaphase (B). C represents the structural details of rejoining by aberrant complementation (between open arrows). Overlapping parental strands may rejoin in regions of similar or repetitive sequences producing heteroduplex segments. While this process may occur quite readily, the resulting metaphase structure (D) would lead to base loss and/or mutational error in the daughter cells (Shafer, 1977).

could not be repaired prior to a second cell cycle. Stetka concluded that, according to Shafer s model, the retention of crosslinks through two S phases would cancel the observation of twin SCEs in induced tetraploid cells. Finally, secondary speculations about Bloom syndrome (BS) and Fanconi anemia (FA) were evaluated as if they could be considered experimental tests of the model. Stetka also suggested that Shafer s model was unsupported because one such hypothesis stimulated by the model, that high SCEs in BS may result from deficient crosslink repair, was in conflict with evidence that BS cells had normal repair capacity.

Involvement of replication and repair in SCE.

SCE studies of other types have also confirmed a close relation between SCE induction and DNA replication. By comparing SCE effects to chromosome replication banding patterns, Latt *et al.* (1977) demonstrated that the location of PUVA-induced [8Methoxypsoralen (8MOP) plus near UV=PUVA] SCEs correlated with those regions undergoing DNA replication at the time of induction. Hoo and Parslow (1979) reported similar replication-related differences in the location of SCEs using a method allowing simultaneous observation of SCEs and chromosome replication bands. Kato s (1980) study clearly pinpointed a precise relation of SCE induction to the replication fork, where cells were grown in fluorodeoxyuridine (FudR) to preferentially accumulate replication forks and then SCE induction was evaluated with blue fluorescent light. Using equipotent light treatments, this study demonstrated that the relative frequency of SCEs increased as the number of growing points increased and, thus, implied that the site of exchange was confined to the replication forks.

The post replication SCE model of Painter

This model is based on the idea that double-strand breaks occur frequently at the junctions of adjacent replicon clusters during their replication. Painter (1980) hypothesized that the specific supercoiled subunits of the chromosome somehow allow DNA at a junction to be susceptible to double-strand break formation during replication of adjoining clusters.

DNA damage in replicon clusters that are at various stages of completion may present a condition where a delay in the progression of the replication fork in a replicating cluster may extend the time when replicated clusters adjoin unreplicated ones. Painter (1980) suggests that DNA double strand breakage at these junctions (mediated perhaps by topoisomerase-II) and subsequent joining of the daughter strands of the replicated replicon cluster to the strands of the unreplicated cluster may be responsible for the formation of an SCE (Figure 2.11).

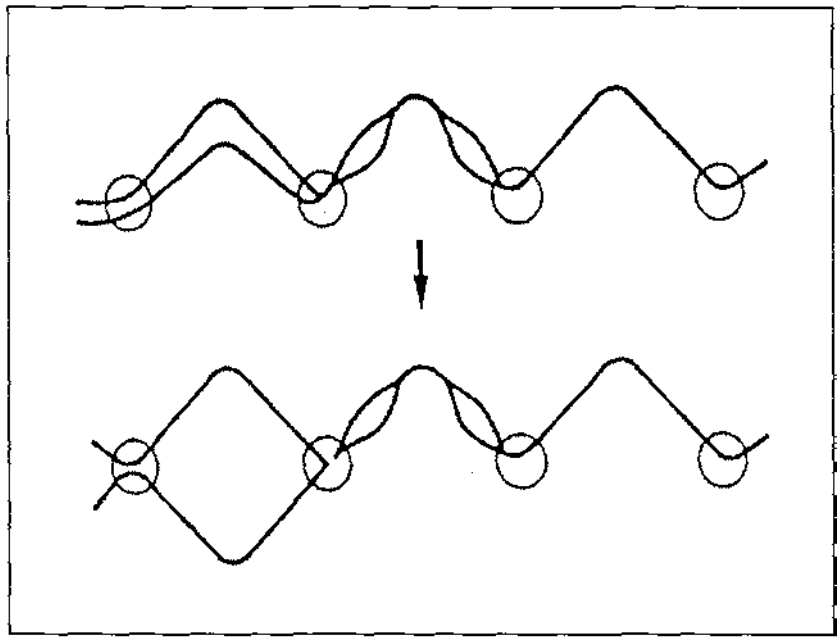


Figure 2.11: Model for possible effects of DNA-damaging agents on cluster replication and segregation. An array of these clusters is depicted; the one on the left is completely replicated and the one on the right is unreplicated. In the center, damage has blocked fork progression so that the cluster is only partially replicated. Lines represent the super-supercoiled Watson-Crick DNA double helices. Junctions where RNA and/or proteins maintain the separate domains of clusters are indicated by circles. In the lower half of the diagram, segregation in the completely replicated cluster is accompanied by a double strand-break at the junction between this cluster and the partially replicated cluster (Painter, 1980).

If this happens, both a daughter and parental strand of each polarity would be available for ligation with the DNA of the unreplicated adjacent cluster (Figure 2.12), and there would be a definite probability that the daughter strands, rather than the parental ones, will join with the unreplicated strands. When replication of the other strand finishes, the normal ligation process at the junction will complete the exchange.

This exchange requires only one double-strand break in the parental strands, which is consistent with data showing that the production of SCEs is a linear function of dose (Carrano *et al.*, 1978; Perry and Evans, 1975). That is, SCE is a product of a single hit, even though two double-stranded molecules participate in each exchange. Agents that block chain elongation will often cause DNA in clusters to remain partially replicated for long times.

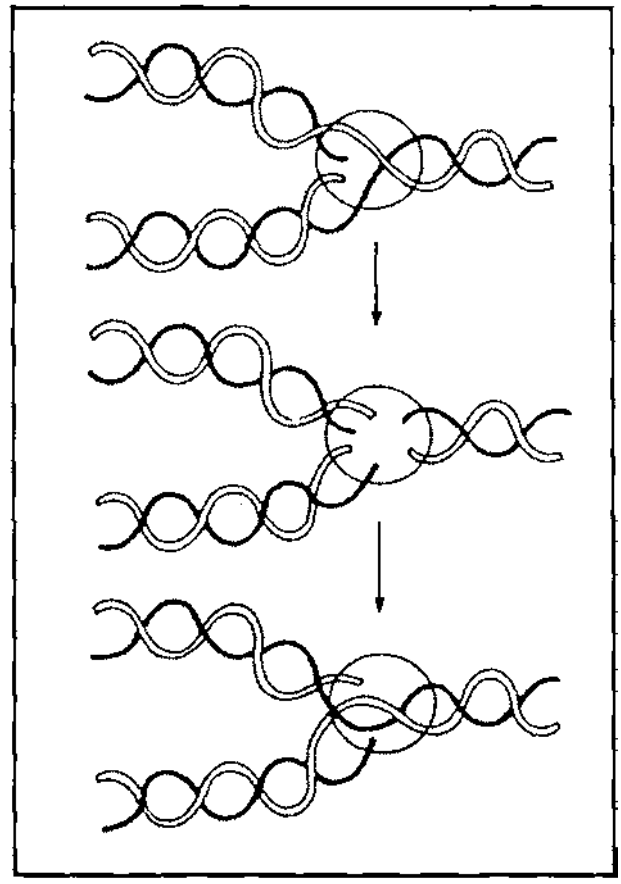


Figure 2.12: Double-strand recombination at the junction between replicon clusters. The exchange shown is between the newly formed strands of the replicated cluster and the parental strands of the unreplicated cluster. The system responsible for joining DNA at the junction will later complete the SCE (Painter, 1980).

This hypothesis may also explain the high number of spontaneous SCEs observed in cells from individuals with Bloom s syndrome (Chaganti *et al.*, 1974). Hand and German (1975) have shown that the average rate of DNA-fork displacement is about 30% slower in these cells than in normal cells. An examination of their data shows that this average decrease is due to a large fraction of replicons whose fork-displacement rate is about two-thirds that of normal cells and a smaller fraction whose average fork-displacement rate is the same as in normal cells. In Bloom s syndrome patient s cells, a partially defective polymerase or other component of DNA replication may exist in a fraction of the clusters that are in operation at any one time; the rate of chain elongation in these clusters would be slower than in nearby clusters containing normal components. The slowly replicating clusters would not finish their duplication at the same time as adjacent clusters, resulting in the same effects seen in mutagen-damaged cells.

The proposed hypothesis of Painter does not implicate any repair process in the formation of SCEs; the only role for repair is to reduce the number of lesions before the damaged DNA replicates and thereby to lower the frequency of SCEs. ELF EMFs might be one of the agents which blocks the chain elongation which cause the DNA in clusters to remain partially replicated for a long time and as a result double strand breaks take place in replicating cluster junctions. Then both newly synthesized strands may join with the parental strands and when synthesis resumes from these free ends of the two remaining parental strands, SCE is completed.

Cleaver s SCE model

Baseline SCE increases as a function of average replicon size in a variety of human and Chinese hamster cell lines (Wolff *et al.* 1975, 1977 and Takehisa and Wolff, 1978). This observation is the basis for this model in which SCEs are generated by errors in the unraveling of daughter double helices by topoisomerases. According to Cleaver (1981), these errors cause exchanges behind the replication fork, not at the fork as several current models assume. This model also provides an explanation for SCEs induced when residual DNA damage that was replicated interferes with the normal processes of unraveling the daughter strands.

In Cleaver s model the newly replicated DNA (Figure 2.13, C) is considered to be initially in an entwined form because of the Watson-Crick helical structure of the parental DNA strands before replication, and because of the multiple orders of coiling involved in the packaging of eukaryotic DNA (Cook and Brazell, 1976). The two entwined replicated DNA double helices may be unwound and unknotted by the action of enzymes, such as topoisomerases I and II to produce separate daughter helices (Baldi *et al.* 1980; Hsieh and Brutlag, 1980; Kreuzer and Cozzarelli, 1980). Cells with small replicons would presumably have less demand for topoisomerases to unravel the replicated DNA than would cells with large replicons. Therefore, there would be less opportunity for errors to generate SCE in cells that replicate DNA in small units as compared to large. This principle is consistent with the report of Blumenthal *et al.* (1974) that the DNA replicon size in Drosophila cells is very small and which may lead to low frequency baseline SCE (Gatti *et al.* 1979). Similarly, unrepaired damage remaining in double stranded, replicated daughter DNA could interfere with the

recognition and function of topoisomerases such that homologous chromatids are rejoined incorrectly and generate SCEs.

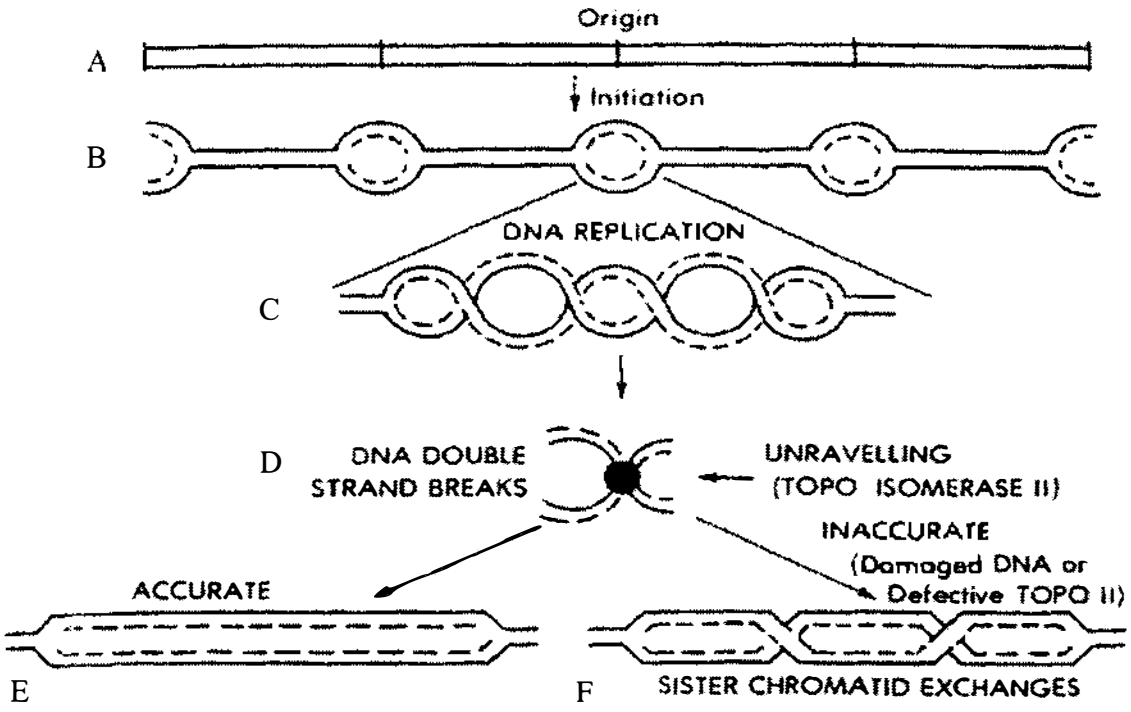


Figure 2.13: Showing the possible mechanism of SCE formation. Replication past damaged sites is presumed to leave damage in parental strands in intact daughter double helices without gaps or other abnormal structures. The normal entwined configuration of newly replicated DNA is postulated and unravelled by double strand breakage and reunion through the action of DNA-topoisomerases. Errors introduced by DNA damage or a low frequency of spontaneous errors will cause faulty recognition, crossing and rejoining by topoisomerases and ultimately cause SCEs. Large origin-to-origin distances, i.e., large replicons, will require more action of topoisomerases for unravelling and consequently a higher possibility for error. The length of DNA is indicated as having multiple origins (**A** & **B**). An individual replicon is in an entwined configuration soon after initiation (**C**), and subsequent action of topoisomerases allows accurate or inaccurate unravelling of the strand (**D**). SCEs are produced by inaccurate rejoining (**F**) (Cleaver, 1981).

According to this model for SCE formation (Figure 2.13), excision-defective Xeroderma pigmentosum (XP) cells show high UV-induced SCE formation because of the high frequency of damage sites remaining in replicated DNA (de Weerd-Kastelein *et al.* 1977). This model can also be used to explain the high baseline SCE frequency in Bloom s syndrome (Chaganti *et al.* 1974) in terms of a possible defect in the enzymes involved in unravelling newly replicated DNA.

Topoisomerase II subunit exchange model of SCE

That SCEs occur at or near the replication fork is generally accepted, based on the observation that in synchronized cells the ability of a DNA-damaging agent to induce SCEs decreases with the amount of the genome remaining to be replicated (Latt and Loveday, 1978). Schvartzman and Gutierrez (1980) also suggested that the SCEs arise at the time of DNA replication due to the presence of unrepaired lesions. They argued that the DNA repair system is a time-dependent process, the shorter the interval between damage induction and DNA replication, the greater the number of lesions that remain unrepaired, and as a consequence, the higher the effectiveness of the damaging treatment in provoking the formation of SCEs. The exact locations at which the strand switches occur relative to a replication fork differ among other proposed models. In the topoisomerase II subunit exchange model of Pommier *et al.* (1985) all 4 strand switches occur in a concerted action. The property of topoisomerases of forming DNA covalent linkages after DNA incision is a central factor in this model.

DNA-topoisomerase complexes and homologous displacement

The Homologous displacement model (Pommier *et al.* 1985) proposes that strand switching occurs during removal of parental helical turns by topoisomerases. It has been shown (Hsiang *et al.* 1985 and Chen *et al.* 1984) that both topoisomerase I and II form covalent linkages to DNA in the process of DNA incision (Figure 2.14, A and B). These covalent linkages are broken in the process of topoisomerase-mediated ligation. A single-strand incision is created by topoisomerase I and the enzyme covalently binds to the 3' end of the incision. The strand on the other side of the incision with free 5' may rotate around the intact complemetary strand prior to ligation. Topoisomerase II, which is a dimer, causes double strand incision. On each side of the double-strand incision, the 5' strand is covalently linked to one of the topoisomerase II monomers while the 3' strand remains free. Another intact DNA double-strand duplex can pass through the double-strand incision. The covalently-linked complexes between topoisomerases and DNA have been called cleavable complexes .

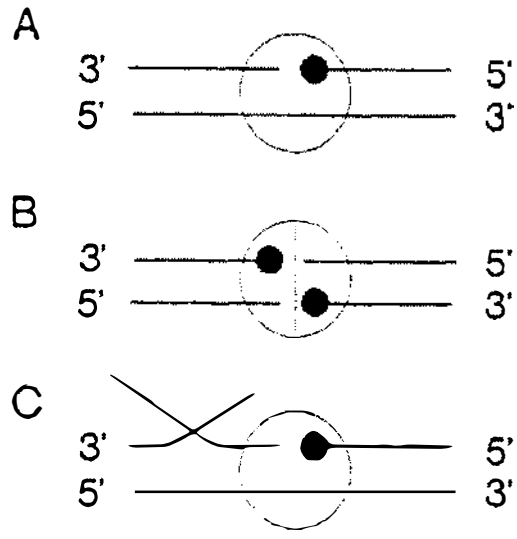


Figure 2.14: (**A**) Topoisomerase I cleavable complex; single-strand incision with topoisomerase I covalently bound to the 5' end. (**B**) Topoisomerase II cleavable complex; double-strand incision with each topoisomerase II monomer covalently bound to a 5' end. (**C**) Homologous displacement; a homologous strand has displaced the non-covalently bound strand in a topoisomerase I cleavable complex (Dillehay *et al.* 1989).

A strand switch could conceivably occur if a nearby homologous strand of DNA not involved in a cleavable complex displaced, through complementary binding, a non-covalently-bound strand from a cleavable complex. A topoisomerase I cleavable complex is illustrated in (Figure 2.14, C). True matching of the displaced and displacing strands could result in ligation of the latter to the covalently-bound strand. The authors speculate that such a homologous displacement strand-switching mechanism may be involved in SCE. Therefore, it is necessary to fulfill two conditions, the means and the opportunity, to make homologous displacement occur. First a cleavable complex must form at the proper location and then a homologous strand with a true-matching free end must come in close proximity.

Replication detour model of Ishii and Bender

Ishii and Bender s (1980) replication detour model of SCE is shown in Figure 2.15. In this model it has been assumed that replication on the template strand containing a lesion has stopped, but that synthesis on the undamaged template strand continues. This

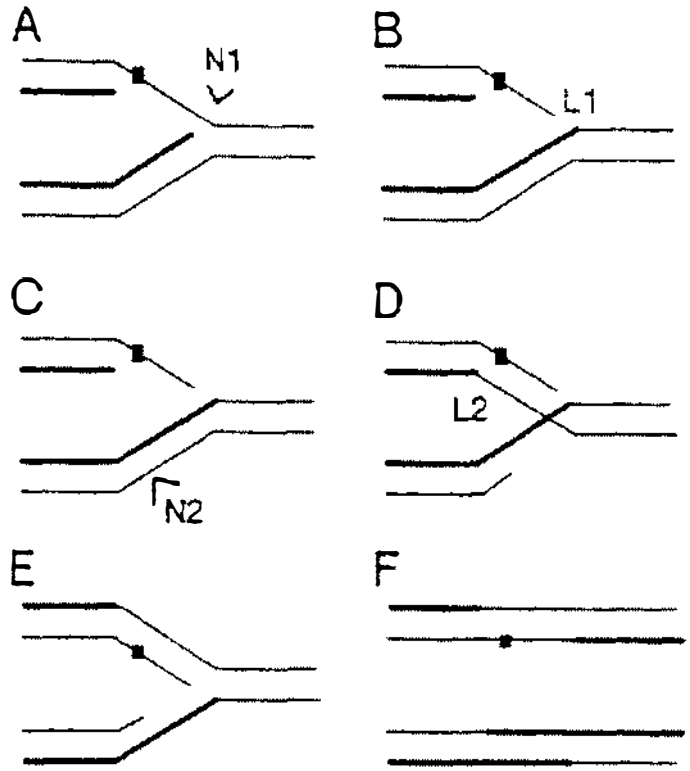


Figure 2.15: Replication detour SCE model of Ishii and Bender. Newly synthesized DNA strands are represented by the heavier lines. (**A**) A lesion (square) has blocked replication of the upper template strand. Replication of the other template strand has progressed farther downstream. N1 marks the site at which a nick (incision) occurs in the template strand downstream from the lesion. (**B**) Improper ligation (L1) has joined the downstream end of the nick in the template strand to the end of the newly synthesized strand. (**C**) N2 marks the site at which a nick occurs in the other template strand. (**D**) The downstream end of this nick has ligated (L2) to the end of the blocked newly replicated strand. (**E**) Replication resumes from the new free ends. (**F**) SCE following replication completion (Dillehay *et al.* 1989).

model states that the first step towards the formation of SCE is nicking (incision) of the lesion-containing template strand downstream from the lesion (Figure 2.15, A). After incision, the end of one of the newly replicated strands displaces the template strand (the two strands being homologous) on the upstream side of the incision and is then ligated to the template strand on the downstream side of the incision (Figure 2.15, B). Following this event, the model then requires a second incision to be made in the other template strand at the position opposite the point at which synthesis has stopped on the lesion-containing strand (Figure 2.15, C). The blocked strand then displaces its homologue on one side of the incision and is ligated to the other side (Figure 2.15, D). Two of the four strand switches required for an SCE accomplished by the above steps. Following these steps, there remain two free ends of two original template s DNA

strands at the replication fork. Ishii and Bender postulated that the replication fork could reorient (Figure 2.15, E) and synthesis resume from these free ends, accomplishing the last two joinings of template to daughter strands to complete the SCE (Figure 2.15, F).

To prove whether homologous displacement accomplishes any of the steps in this model, they first consider the case in which the lesion is blocking synthesis of the new strand, which grows in the 5' to 3' direction. The model assumes that atopoisomerase I-cleavable complex has arisen downstream from the replication fork, with the incision in the template strand containing the lesion. If replication continued off the other template strand, as suggested in the model, then the configuration shown in Figure 2.16, A would result when the replication fork reached the topoisomerase I complex. Consequently, if homologous displacement and ligation then occurred, the first two steps of the model would be accomplished.

On completion of the first two steps, could homologous displacement involving topoisomerase I accomplish the next two steps? The formation of a topoisomerase I-cleavable complex exactly opposite the blocked newly replicated strand is shown in Figure 2.16, C. Homologous displacement would first require that the newly replicated strand come sufficiently close to displace the template strand. Such a displacement appears impossible since the strand, which would have to be displaced, is covalently linked to the topoisomerase I. However, homologous displacement at a topoisomerase II-cleavable complex appears possible. If a topoisomerase II-cleavable complex were present at the site shown in Figure 2.16, D, the template strand to be displaced would not be covalently linked. The necessity for proximity of the homologous strand might be achieved if the DNA containing the lesion were being passed through the double-strand break at the topoisomerase II-cleavable complex.

The first two steps for the case of blocking the lesion in the other template strand is considered in Figure 2.16, B. Homologous displacement at a topoisomerase I-cleavable complex could not occur, again because of protein-DNA covalent attachment at the strand to be displaced. By the same reasoning used above, homologous displacement at a topoisomerase II-cleavable complex would be required for the first two steps and at a

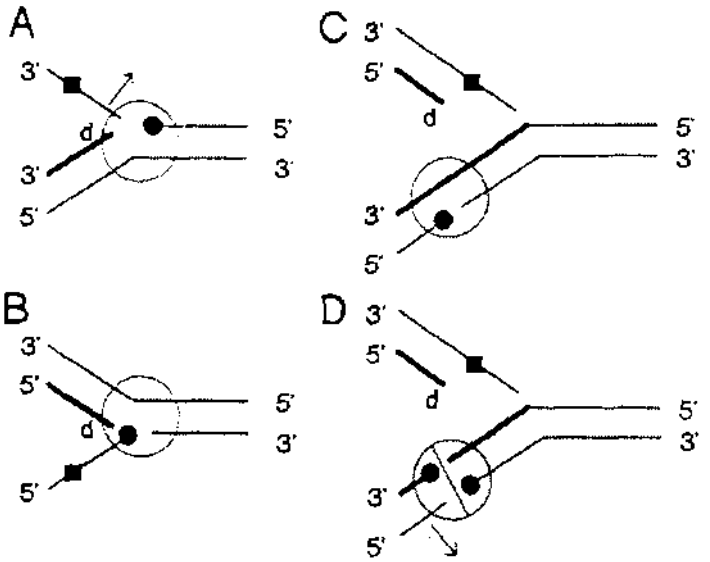
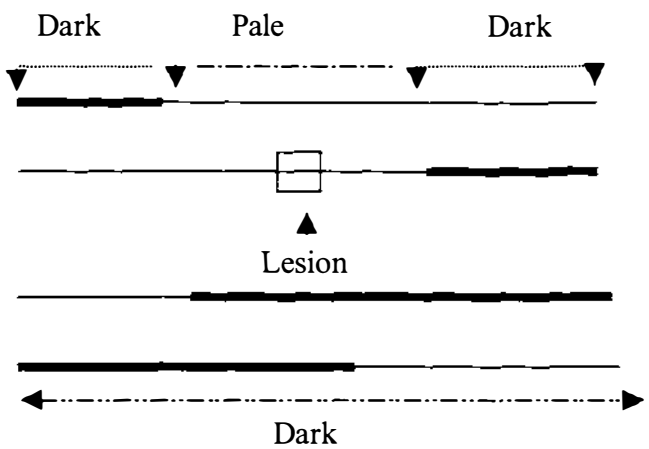


Figure 2.16: Strand switching by homologous displacement at blocked replication forks. (**A**) Replication fork near a lesion (square) on the template strand whose daughter strand replicates continuously (5' to 3'). Topoisomerase I (large open circle) has nicked this strand and is covalently bound (dark circle within the open circle) to the 3' (downstream) end of the nick. This corresponds to step A in Fig. 2.15. Homologous displacement of the strand on the 5' end of the nick by the newly synthesized strand of the same polarity would accomplish step B in Fig. 2.15. In this figure an arrow indicates the movement of the displaced strand. (**B**) Same situation as A except the lesion is on the template strand which is replicated discontinuously (3' to 5'). When topoisomerase I nicks this strand it is bound covalently to the 3' (upstream) end of the incision, which cannot be displaced by the 3' end of the newly synthesized strand. (**C**) Situation if topoisomerase I performs steps A and B in Fig. 2.15 and topoisomerase I nicks the other template strand (Step C in Fig. 2.15). Since topoisomerase I is bound to the 3' end of the incision, homologous displacement could not occur. (**D**) If topoisomerase II performed the second incision (Step C in Fig. 2.15), displacement of the free 3' end and ligation (Step D in Fig. 2.15) would be possible (Dillehay *et al.* 1989).

topoisomerase I cleavable complex for the next two steps. However, in this case, some additional mechanisms would be necessary to bring the blocked free end in to proximity with the topoisomerase I cleavable complex. Thus, homologous displacement appears to be a plausible mechanism by which SCEs could occur within the Ishii and Bender s model; both topisomerase I and II would be active in each SCE and only lesions on the continuously-replicated strand would lead to SCEs.

Criticism of Ishii and Bender s model.

Ishii and Bender in their model (Figure 2.15, F) claimed the completion of SCE when replication resumes from the new free ends, but it is very clear from the figure that in the upper DNA duplex there is a dark-pale-dark region and only dark on the lower one. This is quite difficult for the SCE model to explain.



From figure 2.15, F

Does homologous displacement occur in the Replication Cluster Junction model of Painter?

Painter (1980) proposed that an SCE could occur when a replication fork reaches a replicon cluster junction before the oppositely-moving replication fork of the adjacent replicon. Although Painter suggested that topoisomerases could be involved, no detailed description of the strand switching was given and there is no evidence that topoisomerases are located at these sites. However, if SCEs occurred at these sites by homologous displacement, both a topoisomerase I and topoisomerase II-mediated strand switch would be required.

Does homologous displacement appear to be a plausible mechanism for the Shafer s Converging Replication Forks model?

In Shafer s (1977) proposed model, an SCE results from completion of DNA replication at the site of an unrepaired DNA crosslink. The model was eventually generalized to apply to other lesions (Shafer, 1982). The model states that SCEs occur when bidirectionally-moving replication forks meet a crosslink that prevents them from merging. As in the two formerly discussed models, this model also needs an incision in each of the two template strands and ligation of one side of the break to a newly-synthesized strand.

The two strand switches involve DNA of the same polarity, so if performed by topoisomerases, then both switches would have to be done by topoisomerase I or both by topoisomerase II. A topoisomease I-cleavable complex formed at the site of one of the template incisions needed in the model is shown in (Figure 2.17, A). The structure, which results after the first ligation step in the model, is shown in (Figure 2.17, B). To get from the structure in Fig. 2.17, A to that in Figure 2.17, B, the newly-synthesized strand that must do the displacing from the topoisomerase is not homologous to the strand to be displaced. Thus, homologous displacement does not appear to be a plausible mechanism for the strand switches required in this model.

Does SCE occur by homologous displacement between replicated strands?

Cleaver (1981) proposed that SCE occurred during the removal of parental turns (Figure 2.13) like other replication fork model discussed before. However, he suggested that the exchange occurred between replicated double strands and resulted from an unknown topoisomerase action. Only topoisomerase II would be expected to be able to remove parental turns in replicated DNA as described in Figure 2.18. Kato (1977) also suggested a model (Figure 2.7) in which SCEs occur between replicated strands. He postulated that a free end of a single-strand break in one of the chromatids would pair with the complimentary sequence on the sister chromatid and induce a break in the displaced strand. Two such nearby events would be required to produce an SCE. How homologous displacement could produce these events is shown in Figure 2.18, A-D. Each strand switch would be initiated by a single-strand break in one of the two double strands. Homologous displacement could then occur when this double strand was being passed through a topoisomerase II-cleavable complex on the opposite strand. The

structure shown in Figure 2.18, C would be the result of two homologous displacements at nearby sites. Melting and reannealing could then produce the structure shown in Figure 2.18, D, and the ligation of the two remaining strands breaks would complete the SCE.

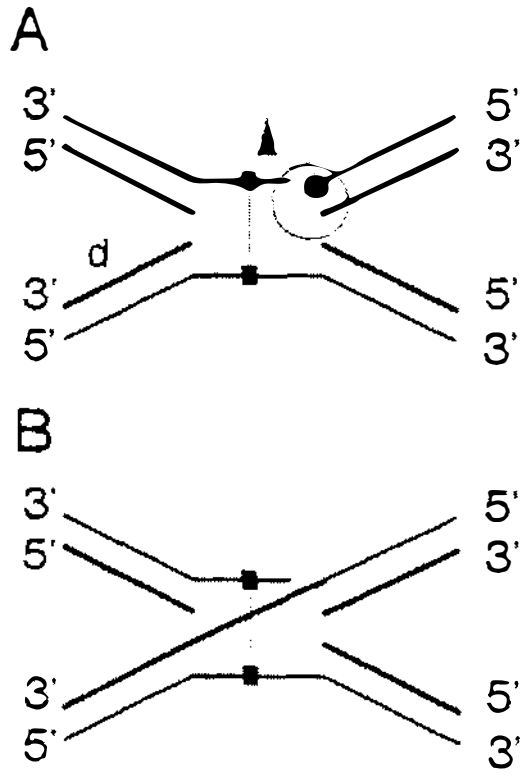


Figure 2.17: Strand switching in the model of Shafer. In (**A**), two replication forks have converged at an interstrand crosslink (two linked squares) which prevents them from merging. Shafer proposed that a break occurs in a template strand on one side of the crosslink, followed by ligation of one end of the break to a newly synthesized strand on the opposite side of the crosslink. (**B**) If the process is repeated, DNA synthesis could be completed and an SCE would have occurred. In A, the incision is shown as a topoisomerase I-cleavable complex. However, since the displacing and displaced strands are not homologous, homologous displacement does not appear a plausible mechanism for the required strand switching (Dillehay et al. 1989).

Cleaver postulated that DNA-damaging agents increase SCE because unrepaired damage remaining in the replicated DNA stimulates faulty topoisomerase action. For the homologous displacement mechanism, the strand break needed in the template strand (Figure 2.18, A) might be generated by repair incision at the site of unrepaired damage. However, this mechanism also needed a strand break in the newly replicated

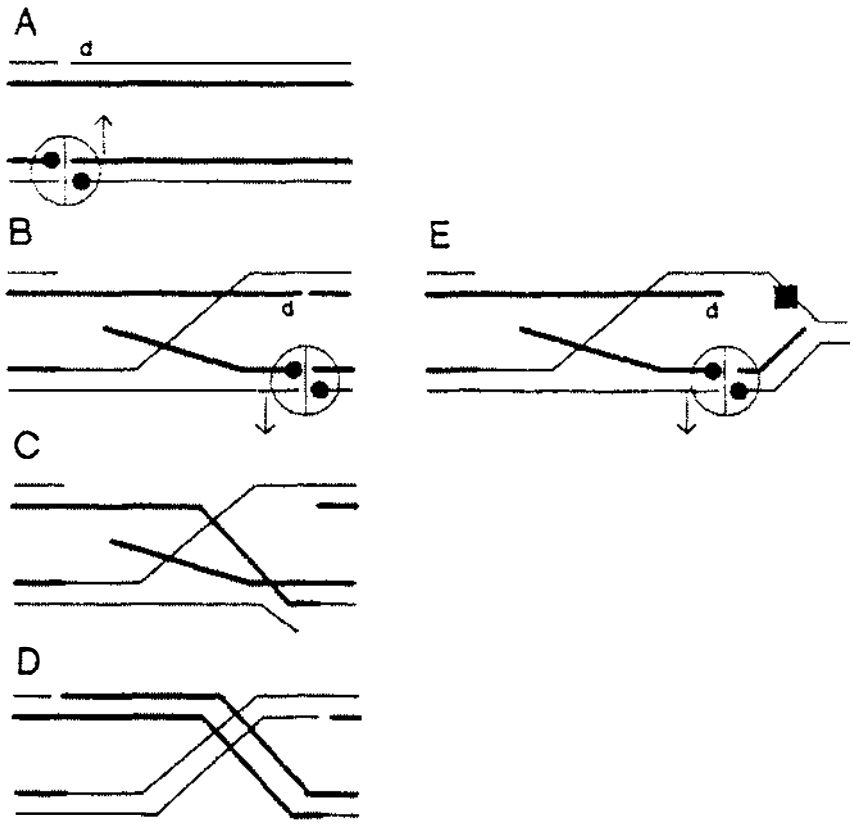


Figure 2.18: SCE between replicated strands by two topoisomerase II-mediated strand switches. (A) The first strand switch would be initiated by a single-strand break in the upper double strand opposite a topoisomerase II-cleavable complex on the lower double strand. This strand switch would result in the configuration on the left side of B. The second strand switch would be initiated by a second strand break opposite topoisomerase II shown on the right side of B. The configuration that results after the second strand switch is shown in C. Reannealing of the displaced strands results in D. Ligation of the two remaining strand breaks would complete the SCE. Alternatively, as shown in E, the second strand switch might be initiated by a free end at a replication fork, as in Fig. 3.16, D (Dillehay *et al.* 1989).

(intact) strand for the second strand switch. By exposing cells to light Kato (1977) produced breaks in BrdU-substituted DNA. Based on the SCEs induced with different substitution patterns, he concluded that not all SCEs could be occurring completely in the replicated DNA; for some of the SCEs one or more strand switches would have to be initiated at a nearby replication fork. In Figure 2.18, E it has been shown how the free end required for a displacing strand in the second switch could be supplied at a blocked replication fork. The second switch would then be exactly the same process as the second switch in the Ishii and Bender model (Figure 2.16, D).

Thus it appears that this model can reasonably account for the increased induction of SCEs by DNA-damaging agents. However, this model is different from that of Ishii and Bender, and it is not clear what mechanism would ensure that both strand switches take place and complete the SCE.

2.2.4 SCE Induction

SCE analysis has been used most extensively to assess the impact of clastogens on chromosomes. A wide variety of agents that cause chromosome breaks have also been found to induce SCE s. These exchanges are now known to occur as a normal event of cell division in mammalian tissues (Tice *et al.* 1976; Latt *et al.* 1981; Schubert and Rieger, 1981; Sandberg, 1982; Tucker *et al.* 1986). SCEs are efficiently induced by substances that form covalent adducts to the DNA or that otherwise interfere with DNA metabolism or repair (Perry and Evans, 1975; Wolff, 1977; Carrano and Thompson, 1982). The four-strand DNA exchange that results in the SCE occurs during DNA replication (Wolff *et al.* 1974) and it has been demonstrated that the polarity of DNA is maintained in this process (Taylor, 1958; Wolff and Perry, 1975). Many of the agents initially used to induce SCE are also well known mutagens (Carrano *et al.* 1978; Perry and Evans, 1975); hence, it has been suggested that SCE analysis could be used to assay and monitor mutagens and carcinogens.

Attempts to correlate SCE induction with known DNA repair have failed. de Weerd-Kastelein *et al.* (1977) measured the induction of SCEs by ultraviolet light in normal and *Xeroderma pigmentosum* cells and found no systematic correlation between the ability of the cell to perform excision repair and the production of SCEs. They also observed similar SCE frequencies in both normal and *Xeroderma pigmentosum* variant cells, which presumably have a defect in post replication repair . Thus, although there is obviously a role for DNA damage in the production of SCEs, the known mammalian DNA repair processes do not seem to be active participants.

One of the more striking features of SCEs is that their yield is many times lower than the number of lesions in DNA; for instance, the number of SCEs is about 1/20,000 the number of ultraviolet-induced dimers in the DNA (Reynolds *et al.* 1979).

Another feature of SCE is the relatively high frequency found after damage by agents which cause DNA lesions that retard the progression of the replication fork during DNA replication, and the relatively low frequency induced by agents that cause damage resulting in inhibition of the initiation of replicons. In the former category are agents such as Mitomycin C (MMC) that cause crosslinks. By their nature, crosslinks must be strong blocks to chain elongation. Another example is ultraviolet light, which forms dimers that act as either temporary or permanent blocks to fork progression but causes very few strand breaks. In contrast, X-radiation, which is the best example of a DNA-strand—breaking agent, causes either very few or no SCEs (Littlefield *et al.* 1979; Wolff *et al.* 1974). Strand breaks seem to be the most important DNA lesions involved in inhibition of replicon initiation (Povirk and Painter, 1976).

Chemicals capable of alkylating DNA are among the most effective and extensively studied agents that induce SCEs. A clear relationship has not yet emerged between the specificity with which particular agents react with DNA, or the type of DNA repair stimulated by these reactions, and the relative potential with which SCEs are induced (Latt, 1981b). SCE formation can be increased both by alkylating agents that are very sensitive to target nucleophilicity and by those that are not. The former, which include methylmethanesulfonate (MMS) and dimethylsulfate (DMS), react relatively cleanly with DNA, forming primarily N^7 guanine or N^3 adenine adducts, while the latter, which include methylnitrosourea (MNUA) and N-methyl-N -nitro-N-nitrosoguanidine (MNNG), react at many different sites, forming among other products, highly mutagenic O^6 guanine substituents (Lawley, 1974).

After three rounds of DNA replication in the presence of BrdU third-division metaphase cells can be scored for the frequencies of SCE that occurred during cycles one and two, and also for the frequency of SCE that occurred during cycle three. This procedure was used to resolve the issue of SCE induction by replication of BrdU-substituted DNA templates vs. induction by BrdU incorporation into nascent DNA (Stetka and Spahn, 1984). Third-cycle SCE frequencies in Chinese hamster ovary (CHO) cells are dependent upon the amount of BrdU that was present during cycles one and two and are not dependent on the BrdU concentration during the third cycle. BrdU serving as a template, rather than BrdU being incorporated in the nascent DNA will initiate the SCE event.

Induction of SCEs in human chromosomes is greatly increased by alkylating agents, but ionizing radiation is not very effective. A dose-dependent increase in SCEs in CHO cells with X-irradiation was reported by Perry and Evans (1975), but only if the irradiation was done during the G_1 or S phase prior to harvest. Such cells had already undergone at least one cycle of BrdU incorporation, and damage produced at sites of BrdU incorporation might well have caused the SCE induction. X-irradiated human lymphocytes during the preharvest G_1 phase induce SCEs (Solomon and Bobrow 1975, Abramovsky *et al.* 1978) is subject to a similar explanation. On the other hand, ionizing radiation breaks chromosomes effectively, but increases SCE only slightly. A dose of X-rays that increases chromosome breaks 20-fold only doubles the SCE rate (Kato, 1977). Cells from patients with the disease Xeroderma pigmentosum are hypersensitive to the induction of SCEs by ultraviolet light and alkylating agents (Wolff *et al.* 1975, 1977).

In a comparative study by Carrano *et al.* (1979), CHO cells were treated with DNA-crosslinking chemicals, MMC and porfiromycin (POR), and their monofunctional derivative, decarbamoyl MMC. After exposure, the cells were studied for the induction of SCEs and mutations at the hypoxanthine phosphoribosyltransferase and adenine phosphoribosyltransferase loci. All three compounds were potent inducers of SCEs, but only weakly mutagenic. All three chemicals by concentration were approximately equally effective in inducing SCEs or mutations. The results gained in this study indicate that the DNA interstrand crosslink is not the major lesion responsible for the induction of SCE or mutation by these compounds.

The highest incidence of SCEs has been achieved with bifunctional alkylating agents. The most effective SCE inducers include methylmethanesulfonate, MMC, dimethylsulfate, ethylmethane-sulfonate, and the mustards (Latt, 1981a). Another powerful inducer of SCE is ultraviolet light and is well known to produce pyrimidine dimers, as well as numerous other alterations in DNA (Setlow and Setlow, 1972).

2.2.5 SCE and Disease

The frequency of spontaneous, and MMC-induced SCE was investigated by Yokota *et al.* (1987) in PBLs of 31 untreated patients with cervical cancer, as well as of seven untreated patients with myoma uteri and 16 healthy women. Factors that might have affected SCE frequency were also investigated, including age and smoking, and were not found to influence the SCE frequency. However, a family history of cancer significantly increased MMC-induced SCE frequency in the cancer group. The MMC-induced SCE frequency was also higher in the cancer group. Spontaneous and MMC-induced SCE frequency gradually increased with the progression of cervical cancer. All chromosome groups equally contributed to the increased SCE frequency in the cancer group. These results suggested that DNA of all chromosomes were damaged in the patients with cervical cancer.

The frequencies of spontaneous and MMC-induced SCEs were investigated by Sou *et al.* (1986) in PBLs of women in different stages (O-III) of cervical cancer. The spontaneous SCE frequency was found to be significantly higher in the cancer group than that of the control group, and the SCE frequency gradually increased with the progression of the cervical cancer. The SCE frequency with carcinoma *in situ* (CIS), the lowest stage of cervical cancer, was significantly higher than that of the controls. The frequency of MMC-induced SCE was higher than that of spontaneous SCE in all groups and the difference in frequency between MMC-induced and spontaneous SCEs of cervical cancer group of the stages I-III was significantly different from that of the control group. The average generation time of the cervical cancer group was shorter than that of the control group, but the difference was not statistically significant.

Cortes-Gutierrez *et al.* (2000) reported that the frequency of SCE in peripheral lymphocytes is significantly higher in cervical cancer patients than those in normal individuals. The mean number of SCEs per metaphase in women with carcinoma of the cervix uteri (7.80 – 1.05) was higher than the control group (6.98 – 1.13).

SCEs were studied in 20 patients with breast cancer (stage II) before surgery, one month after surgery, and after three years as a follow-up study. Data from 50 age-matched, normal healthy females, preferably from the affected families, served as

controls. In each patient, 50 metaphase spreads were scored for SCEs. The mean values of SCEs per metaphase were 5.80, 4.69, and 5.98 in breast cancer patients before surgery, one month after surgery, and after a gap of three years as a follow-up, respectively. A one-way analysis of variance showed that there was a highly significant difference in the frequency of SCE in these patients before surgery, one month after surgical removal of cancerous tissue, and after three years as a follow-up study.

Galloway and Evans (1975) applied the SCE technique to human cells to examine the distribution of SCE between different people and within different chromosomes. The results show: (1) That there were no large differences in the incidence of SCE between blood leukocyte chromosomes from male and female adults and newborn, and that similar frequencies were found in cells from two patients with Ataxia telangiectasia which, nevertheless, showed the typical increases in chromosomal aberrations. (2) The distribution of SCE between chromosomes in the complement was found to be proportional to chromosome length, although the smaller chromosomes were under-represented, but not significantly so. (3) The distribution of SCE within chromosomes was non-random, with a deficiency in the centromeric and excess in the mid-arm regions. There was no evidence for an excess of SCE in chromosome regions rich in AT DNA sequences. (4) The frequency of SCE is to some extent dependent on BrdU concentration, but the influence of concentration is minimal in the range of 1-160 μM. Human cells exposed over two cell cycles at which higher BrdU levels have around 14 SCE per cell - a frequency virtually identical with that observed in cultured cells from the Chinese hamster, wallaby, rat and kangaroo.

Information on a possible association between SCE and cancer is controversial. Studies of SCE frequency in lymphocytes have proven to be useful to evaluate the exposure of living organisms to mutagens or carcinogens. Husum *et al.* (1981) examined SCE frequency in peripheral blood lymphocytes of 131 women patients affected with breast tumour and observed an average 9.39 ± 0.17 SCE/cell in 52 patients with carcinoma of the breast and 9.88 ± 0.18 SCE/cell in 79 patients with non-malignant fibroadenomatosis. This is significantly different to healthy controls.

Phenytoin (PHT) is a widely prescribed anti-epileptic drug. Kaul and Goyle (1999) used SCE frequency assay to investigate its potential to interact with genetic material in a set

of 30 epileptic patients aged 10 to 30 years prior to and following the administration of PHT over a period of nine months. In this study 40 control subjects were used in relation to age, sex, duration of drug therapy, and plasma concentration of PHT. Plasma levels of the PHT were measured by biochemical assay in epileptic patients before and after the PHT therapy. The peripheral blood lymphocytes were cultured and harvested at 72 h. The frequency of SCE was significantly higher in both age groups (10 to 20 and 21 to 30 years) for PHT-treated epileptics compared to PHT-untreated and control subjects. However, there were no considerable variations in SCE findings between the control and PHT-untreated patients. Between the two age groups, a significantly higher SCE frequency was observed in PHT-treated patients in the older age group (21 to 30 years). Mean SCE frequency did not differ between the male and female in the controls, PHT-untreated, or treated epileptics. Correlation between the plasma concentration of PHT and the incidence of SCE among 30 patients was not significant. PHT monotherapy appears to have a genotoxic effect as expressed by the induction of increased SCE rates in treated epileptics, while disease does not play any role in inducing genetic damage as shown by no difference in SCE frequencies between control subjects and PHT-untreated epileptic patients.

Determination of SCE frequency, CA and micronucleus rate of cultured peripheral lymphocytes in 32 patients with lung cancer, 33 miners and 40 non-mining workers in Yunnan Tin Mine was carried out by Hu *et al.* (1987). The results showed that the cancer patients had the highest SCE incidence, CA and micronucleus rate, the non-mining workers had the least and miners on an intermediate level. There was a significant difference of SCE, CAs and micronucleus rate between patients and non-mining workers. They also found that miners had a significantly higher SCE and CA rate but not micronucleus rate as compared with the non-mining workers. They proposed that some carcinogens present in the Yunnan Tin Mine might be responsible for the genetic damages in the miners. Chemical drugs may be considered to contribute to the genetic damage in cancer patients who had a tendency toward an increased SCE, CA and micronucleus rate as compared to the miners, though without reaching statistical significance. It has been suggested that a combination of assays of SCE with CA or MN assay may be a useful index for screening of the high risk population and monitoring the chemical drug prevention of lung cancers in Yunnan Tin Mine.

The spontaneous and MMC-induced SCEs and CAs in cultured lymphocytes from members of "high risk" and "low risk" oesophageal cancer families in Linxian County, China were studied by Wu (1986). The results from this study showed that the frequencies of the spontaneous SCE of "high risk" and "low risk" oesophageal cancer groups were 7.8 – 0.25 and 8.3 – 0.25/per cell. They observed no difference between these two groups. The frequencies of the SCE induced by MMC in "high risk" and "low risk" oesophageal cancer groups were 43.8 – 2.4 and 21.6 – 1.1/per cell. This is a noteworthy difference. Also, the frequencies of the spontaneous and MMC-induced CAs in "high risk" cancer families were higher than those of the "low risk" group.

Mertens *et al.* (1995) investigated the SCE and lymphocyte subsets of children with acute lymphoblastic leukemia (ALL) during chemotherapy. The treatment followed a protocol called ALL-BFM-90. At the time of diagnosis children with ALL showed statistically significant higher SCE frequencies (4.9 – 0.77) than healthy controls (3.6 – 0.93). The *in vivo* effects of cyclophosphamide (CP) resulted in a dramatic increase of the SCE frequency (20.5–3.76). This increased SCE level of lymphocytes might reflect an instability of DNA or a deficiency of DNA repair. One could suggest that lymphocytes of children with ALL might have a higher susceptibility to harmful influences; and this could be a co-factor towards the development of the malignant disease. However, one week after the administration of CP, the SCE rate decreased. This decline of SCE frequency correlated with a severe reduction of the absolute number of T lymphocytes. The observed reduction of SCE frequency may be due to a loss of T lymphocytes, or SCE became repaired during one week.

A study by Baltaci *et al.* (2002) used SCE assays and the COMET assay to study DNA damage in patients with different types of ovarian malignancies and in healthy volunteers. They found that the SCE frequencies of cancer were significantly greater than that of normal controls and that the frequency of exchange in chromosomal groups A, B, and C, which include chromosomes 1-12, was higher than that of the other chromosomal groups in both groups of patients. This is perhaps not surprising considering the larger size of group A, B, and C chromosomes compared to groups D, E, and G. A comparison of the results of the alkaline COMET assay in patients and control subjects showed a significant difference in the number of DNA-damaged cells. And when samples were assayed using the COMET test it was found that the DNA

migration of cells in the women with ovarian malignancies was higher than that of control women. It was therefore concluded that both the SCE and COMET assays could be used successfully to monitor DNA damage in women with ovarian cancer.

The increased risk of neoplasia following cytotoxic therapy for both malignant and non-malignant disease is well known. SCE frequencies in patients receiving such chemotherapy are often elevated, and the persistence of high levels after treatment may provide an indicator for susceptibility to secondary neoplasia. The cytostatic drug razoxane has been used for the treatment of psoriasis, acute myeloid leukemia (AML), and colorectal carcinoma. Prolonged use of this drug, however, has been associated with the subsequent development of AML. Price *et al.* (1992) studied the SCE frequencies of 34 patients with colorectal carcinoma who were receiving or had previously been treated with razoxane. They claimed that no significant increase in SCE levels were observed in the razoxane group compared with either normal controls or untreated patients.

Maurya *et al.* (1987) reported that changes in SCE frequency, the patient s response to anticonvulsant therapy and seizure severity are interrelated. In their study, SCE frequency was scored from PBLs of epileptic patients (untreated and treated) and controls. Significantly higher number of SCEs were observed in samples taken from both treated (recipient of anticonvulsant therapy) and untreated (seizure) epileptic patients than was seen in the normal healthy control group. However, those who received the anticonvulsant therapy had a decreased number of SCEs in comparison to the untreated patients.

Melanomas are highly clonogenic. Genetic variability and polymorphism of tumour cell populations have been reported. However, no direct evidence of mutator activity as a source of genetic polymorphism for melanoma cells has been described. Some intermediates of melanin synthesis are cytotoxic and genotoxic and their mutagenic powers have been described. Miranda *et al.* (1997) observed that the rate of SCE of the line of human melanoma cells varies with the concentration of the melanin precursor L-tyrosine, in the culture medium. An increase of melanin synthesis results in increased SCE rates. The highest values of SCEs are found in melanotic melanoma cells

compared with the amelanotic ones. Observation shows that melanoma cell has higher levels of SCE compared with normal human lymphocytes.

Atalay *et al.* (2000) assessed the impact of malignant mesothelioma on the frequencies of SCE in the pleural effusion cells. Ten patients with mesothelioma and 20 control subjects were included in the study. The SCE frequencies of malignant pleural mesotheliomas were significantly higher than that of the control subjects.

Spontaneous SCE frequencies were measured by Parkes *et al.* (1985) in cells undergoing their second mitosis at 54, 68, 72, 78 or 90 h after PHA stimulation of whole blood cultures from seven normal donors. A consistent pattern of fluctuation of SCE levels was observed between 54 and 78 h. The magnitude of change in SCE frequency between consecutive fixation times was as high as 80% for some donors. These observations support the hypothesis of Snope and Rary (1979) that there are subpopulations of lymphocytes with different spontaneous SCE levels and different proliferation rates. In untreated cancer patients the patterns of change in SCE frequency with time were different from that seen in normal donors. This may be because of quantitative changes in T-lymphocyte sub-populations that have been observed in cancer patients. Changes in SCE frequency of less than about a factor of two observed at a single sampling time may not be indicative of genotoxic events or genetic instability but simply represent changes in the composition of lymphocyte sub-populations and/or in their rates of proliferation *in vitro*.

SCE frequencies were used to compare two types of osteoporosis treatment for genotoxicity by Sahin *et al.* (2002). This study included 57 women, aged between 40 and 64 years. SCE values of patients under estrogen replacement therapy (ERT) or alendronate therapy were compared to controls who never used any drugs for osteoporosis. The difference between the SCE values of women taking ERT and control women was found to be statistically significant. The difference between women taking alendronate and untreated controls was not statistically significant. The author concluded that alendronate does not have genotoxic effects based on SCE frequency, while ERT increases SCE frequencies.

Kayikcioglu *et al.* (2000) evaluated the frequency of SCE during hormone replacement therapy in postmenopausal women. Thirty-four asymptomatic postmenopausal women with a minimum 12 months since their last menstrual period and surgical menopausal women were included in the study. Seventeen patients who were in spontaneous menopause were administered conjugated estrogen and medroxypro-gesterone acetate (group A), and the others who were in surgical menopause were given 17-beta-estradiol only (group B). Peripheral lymphocytes were obtained at the beginning and at the end of the third month of therapy. The mean age of the patients was 50.67 – 4.79 y. There were statistically significant differences in terms of SCE frequencies between pre- and post-treatment levels of both groups. It is likely that estrogens with or without progesterone increase SCE frequency and this issue may be evidence for the increased potential for malignancies.

2.2.6 Human Reproductive Hormones and SCE

Joseph-Lerner *et al.* (1993) tested the hypothesis that sex hormones, particularly steroids, influence the frequency of SCEs in women undergoing ovulation induction for *in vitro* fertilization treatment. These women undergo extreme hormonal changes and therefore serve as a good model for testing the rate of genetic damage due to these changes. The controls were fertile women with regular menstrual cycles who received no hormonal treatment. Peripheral lymphocytes were obtained during different stages of the normal and treated cycles. They found an increased SCE frequency during ovulation time in the controls and during the time of human chorionic gonadotropin administration in the group undergoing ovulation induction. However, in the later group, SCE frequency was significantly higher. SCE frequency was positively correlated with the level of testosterone and follicle stimulating hormone (FSH) in the ovulation induction group and positively correlated with the estradiol level in both groups.

D'Souza *et al.* (1988) observed a higher rate of cellular genetic damage in terms of increased frequencies of SCEs and chromosome aberrations during "ovulatory" and "estrogenic" stages as compared with those of "progestogenic" stages of the menstrual cycle in women and the reported fluctuations in SCEs as a function of time in men. This indicates that *chronobiologic considerations* are essential in the design of studies of

human and animal experiments. It seems that female subjects are more influenced by biological rhythms because of specific hormonal cycles and are sensitive to genetic damage during ovulatory/estrogenic stages of the menstrual cycle. Sharma and Das (1986) have reported an increased frequency of SCEs and CAs in women during the advanced stages of pregnancy. This has been attributed to the high levels of sex steroids present during the last trimester of pregnancy.

Oral contraceptive use has been associated with increased SCEs (Bala Kishna Murthy and Prema, 1979, 1983). However, Husum *et al.* (1982) observed no effect of contraceptives on the frequency of SCE.

2.2.7 Lymphocyte Concentration and SCE

Bender *et al.* (1992b) observed that the lymphocyte concentration in the culture tube leads to variation in SCE frequency. The number of SCEs decreased with the increased number of lymphocytes. Therefore, to conduct SCE experiments it is necessary to standardize the lymphocyte concentration.

2.2.8 Baseline SCE and the Influence of Genetic, Chemical, and Environmental Agents

BrdU itself, like [^{3}H]thymidine (Brewen and Peacock, 1969; Gibson and Prescott, 1972), induces SCEs (Gatti *et al.* 1979; Kato, 1974b; Latt, 1974a; Wolf and Perry, 1974) and may be responsible for most of the baseline SCEs observed in the absence of additional clastogens. However, Stetka and Spahn (1984) demonstrated that replication of BrdU-substituted DNA results in SCE induction, while incorporation of BrdU into nascent DNA does not. The latter phenomenon was reported by Mazrimas and Stetka (1978) and Davidson *et al.* (1980), and was interpreted as an indication that BrdU may induce SCEs by two different mechanisms, one involving incorporation and the other not.

Baseline SCE frequency appears to be directly correlated with genome size, not chromosome number (Kato, 1977). The SCE number within a cell or within a given chromosome is randomly distributed with a few exceptions (Wolf and Perry, 1974;

Chaganti *et al.* 1974; Latt, 1974a; Galloway and Evans, 1975, and Dutrillaux *et al.* 1974). The number of exchanges per chromosome is positively correlated with chromosome length. In man, among small (E, F and G groups) chromosomes there are fewer exchanges than expected (Hsu and Pathak, 1976; Latt, 1974a; Galloway and Evans, 1975; Dutrillaux *et al.* 1974, and Latt *et al.* 1975).

Increased baseline SCE frequencies were observed in some human diseases such as Bloom s Syndrome (Chaganti *et al.* 1974; Shiraishi *et al.* 1976), multiple sclerosis (Sutherland *et al.* 1980; Vijayalaxmi *et al.* 1983; Senecal-Quevillon *et al.* 1986), Down s syndrome (Shubber *et al.* 1991), Schistosoma hematobium (Shubber, 1987), Crohn s disease (Myung-Hee *et al.* 1997) and systemic Lupus erythematosus (Palmer *et al.* 1987). In a recent cytogenetic study with prostate cancer patients (Dhillon and Dhillon, 1998) observed that SCE frequency is significantly higher in prostate cancer patients than in the controls.

Technical factors that influence baseline SCE included the tissue culture medium used, the amount of BrdU in the medium, and the method of differential staining. Cigarette smoking, vaccination, viral infections and therapeutic drugs may also affect baseline SCE, and hence, it is important to check an individual s medical and occupational history in evaluating the SCE levels. Two other factors also have been reported to influence the baseline SCE frequency: the serum used in the culture medium and the culture temperature (Carrano and Natarajan 1988). Das and Sharma (1984) observed that the frequency of SCEs in human lymphocytes increased as a function of culture temperature with a maximum at 40C.

EMFs and SCE

Zwingelberg *et al.* (1993) exposed nine female Wistar rats for seven days or 28 days, 24 h per day to a homogeneous 50 Hz, 30 mT (9.4 T/s) horizontal MF. The calculated internal electric field was 0-170 mV/m assuming an elliptical torso cross-section 5 cm high and 12.5 cm long. Peripheral blood was sampled one day prior to and immediately following exposure. Cyclophosphamide, a potent inducer of SCEs, was used as a positive control. Investigators reported that neither exposure period resulted in a statistically significant increase in SCEs in PBLs in any of the exposed animals when

compared to rates determined prior to exposure. Six additional animals, which were sham-exposed controls, produced similar results.

Antonopoulos *et al.* (1995) incubated human peripheral blood cultures in the presence of an EMF of 50 Hz and 5 mT which lead to a stimulation of the cell cycle of dividing lymphocytes but had no influence on the frequencies of the SCE. Comparative studies with two different exposure systems and with different culture temperatures indicated that the effect on the cell cycle results from the EMF and is not a thermal effect. These data support the assumption that with respect to their suspected carcinogenic effects EMFs have no initiating effects, although they may have promoting effects.

Human lymphocytes culture was exposed by Khalil and Qassem (1991) to a PEMF (50 Hz, 1.05 mT) for 24, 48 and 72 h. They observed a statistically significant suppression of mitotic activity and a higher incidence of CAs. Shorter exposure time (24 and 48 h) had no effect on the cell proliferation index (CPI) or SCE but continuous 72 h exposure exhibited a significant reduction of CPI and an elevation of SCE rate.

Yaguchi *et al.* (1999) evaluated the induction of SCE in cultured mouse m5S cells, which were exposed to 5, 50 and 400 mT. Exposure to 5 mT and 50 mT MF led to a very small increase in the frequency of SCEs, but no significant difference was observed between exposed and unexposed control cells. The cells exposed to 400 mT exhibited a significant elevation of the SCE frequencies suggesting that a 400 mT MF may induce DNA damage.

According to a number of *in vitro* studies, ELF EMFs are unable to induce CAs in white blood cells as measured by the analysis of SCEs (Cohen *et al.* 1986a; Rosenthal and Obe, 1989). SCEs are good indicators of genetic effects induced by chemicals but not by ionizing (Evans, 1977) or non-ionizing radiation (Maes *et al.*1993). A significant increase in DNA synthesis was sometimes observed (Lester and Moore, 1982) as well as an increase in cell division and transcriptional activity (Liboff *et al.* 1984; Goodman *et al.* 1989b).

Rosenthal and Obe (1989) cultured HPBL in the presence of 50 Hz EMFs and observed no alteration of the spontaneous frequencies of SCEs and CAs, but observed an enhancement of the cell cycle progression of HPLs *in vitro.*

Gamma-irradiation and SCE

Shah and Mittal (1987) studied the effects of different doses of gamma (γ)-irradiation on SCE induction in unifiliarly BrdU-substituted DNA in various phases of cell cycle. γ-irradiation-induced SCE frequency was measured by post-irradiation treatment with antimutagen L-cysteine. Perturbation in cellular proliferation kinetics due to γ-irradiation and γ-irradiation plus L-cysteine was also studied. It was observed that γ-irradiation is an efficient inducer of SCE and is most effective in S phase. L-cysteine also causes SCE induction, which is slightly higher than the spontaneous level of SCEs found in HeLa cells. However, post-irradiation addition of L-cysteine reduces SCE frequency in γ-irradiated cultures and this reduction is maximum in G_1 phase irradiated cells. γ-irradiation delayed the mitosis considerably and this delay continued to increase with increasing doses. L-cysteine reduced the delay in cell cycle caused by γ-irradiation.

Influence of Age, Gender, Smoking on SCE

Gender is an important factor for the frequency of the SCE. However, several studies have observed no significant differences between male and female SCE frequencies (Galloway and Evans, 1975; Alhadeff and Cohen, 1976; Crossen *et al.* 1977; Latt and Juergens, 1977; Morgan and Crossen, 1977; Cheng *et al.* 1979; De Arce, 1981; Waksvik *et al.* 1981; Carrano and Moore, 1982; and Livingston *et al.* 1983). Other studies have observed baseline SCE frequencies at approximately 0.5 exchanges per cell higher in females than males (Soper *et al.* 1984; Margolin and Shelby 1985 and Bender *et al.* 1992a). Bender *et al.* (1992a) believed that it might be a consequence of the increased chromosome length (DNA content) represented by the second X-chromosome in females versus the smaller Y-chromosome in males.

Milosevic and Marinkovic (1999) reported the variability in the frequency of SCE in lymphocytes of peripheral blood in relation to age and gender of individuals in a

Yugoslavian population. William *et al.* (1977) studied the incidence of SCE in cultured human lymphocytes from 50 normal individuals using the BrdU-Giemsa technique. The mean SCE frequency per metaphase was 7.9 with a standard deviation of 1.36 and a range of 1-21 and the incidence of exchanges was influenced neither by gender nor by age.

Bukvic *et al.* (2001) studied the possible effect of age, gender and smoking using the micronuclei and SCE frequencies. Peripheral blood obtained from 38 subjects ranging in age from 16 to 63 years and 16 centenarians was used in this study. The mean number of binucleated cells with micronuclei varied as a function of age and gender, particularly in women. Smoking habits had no effect on micronuclei frequency. Gender and smoking habits significantly influenced SCE per cell frequencies, but age had no effect on them.

Rates of SCE in dividing HPBLs were determined and compared between smoking and non-smoking young women between the ages of 16 and 25 by Rowland and Harding (1999). Chromosomes block-stained with Giemsa were also examined for CAs. A striking difference in the frequency of SCE was found between young women who smoked and those who did not. Smokers scored a significantly higher rate and range of SCE than those observed in non-smokers. No statistical difference in the frequency of CAs was found between smokers and non-smokers. The significantly higher frequency of exchange in young smoking women may indicate that initial damage to the DNA in many of these women has probably already occurred, thus causing an increased risk of developing cancer later in life.

Lazutka *et al.* (1992) assessed the effects of alcohol consumption, cigarette smoking and age on SCE frequency in human lymphocytes by means of multiple linear regression. They observed an increase in the rate of SCE, which was associated with alcohol consumption, smoking, and to a small extent age. They concluded that these three confounding factors explain 48% of the inter-personal variation in SCE rates among subjects studied.

The effects of age, gender and smoking on SCE frequency and distribution in human lymphocytes were assessed by means of multiple linear regression (Lazutka *et al.*

1994). Differences in SCE scores were associated with all above variables: SCE increased with age and cigarette smoking intensity, and higher SCE frequencies were observed in females.

Elio (2000) examined a large sample of 4-day old chick embryos from a commercial strain to determine the baseline SCE frequency and the nature of the distribution. A mean frequencies of 1.15 SCE/cell were observed in a total of 6,400 cells from 128 male and female embryos: the SCE frequencies for males and females were similar.

Diet and SCE

The role of diet has not been taken in to consideration when SCE frequencies are studied. However, Bala Kishna Murthy *et al.* (1980) reported that severe protein calorie malnutrition in children is associated with increased lymphocyte SCE frequencies. SCE frequencies were observed to decrease following nutritional rehabilitation.

Occupational Hazards and SCE

Chromosomal aberrations and SCEs in lymphocytes from coke oven workers, an occupational group known to be at increased cancer risk, were studied by Bender *et al.* (1988). It was observed that coke oven workers as a group have a statistically significant increase in the frequency of CAs and SCEs.

SCE frequencies were measured in the peripheral lymphocytes of 104 greenhouse farmers exposed to pesticides and 44 unexposed workers in a study by Shaham *et al.* (2001). The frequency of SCE was significantly higher among the farmers who were exposed compared with the unexposed group.

SCE in blood lymphocytes was determined in 32 male workers occupationally exposed to lead (Pb) and zinc (Zn) and in 20 unexposed controls matched for age and smoking habits by Donmez *et al.* (1998). Exposed workers had higher SCE mean values than control workers. In exposed persons, blood Pb concentrations were also significantly higher than was found in the controls, but the difference between Zn levels in the blood of these groups was not significant. The results indicate that Pb may be genotoxic and

harmful to humans; however, exposure to zinc seem to have no harmful effect on humans.

Lazutka *et al.* (1999) performed cytogenetical analysis of CAs in 175,229 cells from 1,113 individuals, both unexposed and occupationally or environmentally exposed to heavy metals (mercury and lead), organic (styrene, formaldehyde, phenol and benzo[a]pyrene) and inorganic (sulfur and nitrogen oxides, hydrogen and ammonium fluorides) volatile substances and/or ionizing radiation. In addition, 11,250 cells from 225 individuals were scored for the frequency of SCE. Increased frequencies of CAs were observed in all occupationally exposed groups. A principal difference between the exposure to heavy metals and organic substances was found. An increase in the CA frequency was dependent on the duration of exposure to mercury, but not dependent on duration of exposure to styrene, formaldehyde, and phenol. Higher CA incidences were found in lymphocytes of children living in the vicinity of a plant manufacturing phosphate fertilizers. This suggests that children are a sensitive study group for the assessment of environmental exposure.

The genotoxic effects of occupational exposure of 20 nurses who handled cytostatic drugs in medical oncology and haematology units were investigated by Kasuba *et al.* (1999). The effects were evaluated by SCE test. The duration of employment in the units and of exposure to cytostatic compounds ranged from 1 to 31 years. They observed no statistically significant differences in the frequency of SCEs.

Exposure to ionizing radiation was found to cause chromosome breaks and SCEs in Chernobyl clean-up workers. Chromatid breaks, SCEs, dicentric chromosomes, and chromosome translocations were found in workers from Ignalina Nuclear Power Plant. The increased frequency of SCEs in these individuals exposed to ionizing radiation was quite unexpected, as ionizing radiation is not known to cause SCEs. This increase may be attributed to the action of some unrecognized lifestyle or occupational factors, or to be a result of radiation-induced genomic instability.

Tsun-Jen *et al.* (2000) used lymphocyte SCE frequencies as a parameter to investigate the genotoxicity of low-level ethylene dichloride (EDC) and vinyl chloride monomer (VCM) exposure in VCM-manufacturing plant workers. The SCE frequency was

determined for 41 male workers with exposure to VCM and/or EDC and for 20 male workers who were unexposed. Moderate EDC exposure of around one part per million (ppm) corresponded to a significantly greater SCE frequency than was found in the low EDC exposure group. However, VCM exposure of similar level was not associated with increased SCE and concluded that EDC may cause genotoxicity at a relatively low level of exposure.

Shaham *et al.* (2002) studied the SCEs in peripheral lymphocytes of 90 pathology department workers from 14 different hospitals in Israel who were occupationally exposed to formaldehyde (FA) and of 42 unexposed workers from the administrative section of the same hospitals. The mean exposure period to FA was 15.4 years (range 1-39). The results of SCEs are expressed in two variables: (a) the mean number of SCEs per chromosome, and (b) the proportion of high frequency cells (cells with more than eight SCEs). A high correlation was observed between these two variables. The adjusted means of both SCE variables were significantly higher among the exposed group compared with that of the unexposed group.

Chronic exposure to low frequency (LF) noise and whole-body vibration (WBV) induces both physiological and psychological alterations in humans. Silva *et al.* (1999) have shown that long-term occupational exposure to LF noise and WBV produces genotoxic effects in humans expressed as an increase in SCE levels in lymphocytes. SCEs were also analyzed by Silva *et al.* (2002) in spleen lymphocytes of mice exposed to LF noise alone and in combination with WBV for 300 and 600 h. An effect at the cell cycle kinetics level was also investigated. The results revealed significant increases in the mean SCE number per cell and in the proportion of cells with a high frequency of SCEs (HFCs) in lymphocytes of mice submitted to combined noise and WBV over controls. No significant differences were found between single noise-exposed and control mice. A cell cycle delay was observed exclusively in the noise and WBV exposure groups. They concluded that prolonged exposure to LF noise in combination with WBV determines an increase in SCE level in mice while LF noise alone was not effective in SCE induction.

The evaluation of SCE is used to establish the cytogenic damage in subjects exposed to toxic substances. The test is considered to be one of the most sensitive and accurate

indicators of genetic damage and responds to toxic chemicals at low doses. Stea *et al.* (2000) evaluated the incidence of SCE in peripheral lymphocytes of patients with artificial limbs. Subjects with artificial limbs made of titanium-aluminium-vanadium alloys presented a significantly higher SCE number than that of the control population, whereas subjects with artificial limbs made of chrome-cobalt alloy or mixed artificial limbs presented a higher SCE value than the controls, but not significantly so.

Duydu *et al.* (2001) studied inorganic lead exposure in 31 volunteers employed in a storage battery plant. The genotoxicity of lead was measured in terms of SCE. Erythrocyte delta-aminolevulinic acid dehydrogenase (ALAD) activity, urinary delta-aminolevulinic acid (U-ALA), and blood lead levels (PbBs) were also determined to evaluate some possible relations between these lead exposure indicators and the observed SCE frequencies. Blood lead concentration of 36.31 g/decilitre (decilitre = 100 ml) was determined as an average level in the workers. Consequently, decreased ALAD activity in erythrocytes and increased U-ALA excretion was observed in statistically higher PbBs when compared with the control group. A statistically significant correlation was observed between the PbBs and SCE frequencies. Moreover, the correlation between U-ALA excretion and SCE frequencies was relatively higher than the correlation between PbBs and SCE frequencies. They concluded that these results might indicate a possible mechanism of ALA mediation in the genotoxic effects of lead.

Influence of Drugs and Alcohol on SCE

Cocaine is a widely abused drug. Chang-Tze *et al.* (1999) reported that it induces teratogenesis in both humans and animals. Cocaine-induced teratogenesis has been associated with reactive oxygen species (ROS), which are generated by cytochrome P450 during cocaine biotransformation. Since ROS have been reported to induce genotoxicity, it is of interest to determine whether cocaine and/or its metabolites are also genotoxic. In this study, CHO K1 cells were employed as a model system to investigate the genetic toxicity of cocaine in the presence or absence of rat liver S9 fraction. Cocaine-induced cytotoxicity was enhanced when S9 was present, indicating the cytochrome P450 metabolism plays a role in cocaine-mediated cytotoxicity. Cocaine treatments alone induced few chromosome aberrations, while treatments of

cocaine plus S9 caused a significant increase in chromosome aberrations. In contrast, cocaine induced micronuclei formation and hypoxanthine-guanine phosphoribosyltransferase mutation only in the presence of S9. Therefore, cocaine itself is at best a weak clastogen, whereas metabolites of cocaine are truly inducers of clastogenesis and mutagenesis. Cocaine treatments alone also induced a significant increase in SCE frequency, but the addition of S9 did not affect the results. Free radical scavengers, including superoxide dismutase and catalase, efficiently decreased the frequency of cocaine plus S9-induced micronuclei, implying that ROS are indeed important components in cocaine-induced genotoxicity. The observation that non-toxic doses of cocaine can inhibit intercellular metabolic cooperation suggests that cocaine may also be a tumour promoter. The data support the idea that cocaine could possess genotoxicity in addition to its well-known neurotoxicity and teratogenicity.

The induction of SCE by caffeine is very minimal (Kato, 1973; Waksvik *et al.* 1977; Vogel and Bauknecht, 1978; Basler *et al.* 1979; Mourelatos, 1979; and Zamansky *et al.* 1980). However, chronic exposure of human peripheral blood lymphocytes to caffeine has been shown to double the SCE frequency (Guglielmi and Tice, 1980) and retard cell proliferation. In another study caffeine was shown to reduce the inhibitory effect of alkylating agents on replicon progression (Murnane *et al.* 1980). Increased SCEs have also have been reported in alcoholics (Butler and Sanger, 1981).

Hirsch *et al.* (1992) reported that SCE frequencies are significantly affected by coffee drinking habits. A stepwise multiple regression analysis showed that together, smoking and coffee habits entered at the first step accounted for 21% of the observed variance in SCE. Their twin analysis showed that after adjustment of the data set for smoking and other significant predictors, genetic factors accounted for approximately 30% of the variation in SCE rates.

Mycotoxins and SCE

Jia-Sheng and Groopman (1999) reported that mycotoxins are toxic fungal metabolites, which are structurally diverse, common contaminants of the ingredients of animal feed and human food. To date, mycotoxins with carcinogenic potency in experimental animal models include aflatoxins, sterigmatocystin, ochratoxin, fumonisins,

zearalenone, and some *Penicillium* toxins. Most of these carcinogenic mycotoxins are genotoxic agents with the exception of fumonisins, which is currently believed to act by disrupting the signal transduction pathways of the target cells. Aflatoxin B1 (AFB1), a type I known human carcinogen and the most potent genotoxic agent, is mutagenic in many model systems. It has been shown to produce CAs, micronuclei, SCE, unscheduled DNA synthesis, and chromosomal strand breaks, as well as form adducts in rodent and human cells.

SCE Frequency as a pollutant indicator

Etroplus suratensis, a brackish water fish species, has been examined by Paramananda *et al.* (1996) for its applicability as a test organism in screening genotoxic pollutants in aquatic environments. A detailed karyotype study has been carried out and methods developed for demonstrating sister chromatid differentiation (SCD) and SCE. The spontaneous SCE value was found to be 1.79 per metaphase. This study showed that the fish appeared to be a suitable model for *in vivo* cytogenetic assays in pollution monitoring.

Arsenic is one of the few human carcinogens for which there is not yet a reliable animal model for studying cancer. As such, the classification of arsenic as a carcinogen is based upon data derived from human epidemiological studies. Although the mechanisms of action of arsenic as a toxic agent have been known for many years, the inability to cause cancer by arsenic in laboratory animals has confounded the operational characterisation of arsenic as an initiator, promoter, complete carcinogen, or co-carcinogen for humans. Mass (1992) reported that arsenic is a genotoxic agent and it induces CAs, micronuclei and SCE in mammalian cells, as well as neoplastically transforming Syrian Hamster embryo cells. However, it is not a classical point mutagen that causes the exchange in DNA of one nitrogenous base for another.

SCEs were analysed in lymphocytes from 12 control persons and 33 Chernobyl clean-up workers by Lazutka and Dedonyte (1995). The group of Chernobyl clean-up workers consisted of civilians, who were forced to go to Chernobyl to clean up environmental contamination caused by the Chernobyl disaster. On average, they received 0.13 Gy of

external irradiation before returning home. Cytogenetic analyses were performed 6-8 years after the irradiation. Standard cytogenetic techniques were used. Mean SCE frequency was 7.45 SCE/cell in controls and 10.30 SCE/cell in clean-up workers. In addition, increased frequencies of CAs due to exposure at Chernobyl and alcohol consumption were observed. However, there was no correlation between external dose of irradiation and the frequency of CAs. Thus, even 6-8 years after the irradiation, cytogenetic effects in lymphocytes of Chernobyl clean-up workers are still significant.

Ozturk *et al.* (2002) conducted a study to investigate the genotoxic effect of acute overexposure to combustion products originating from coal or wood stoves in patients presented with acute carbon monoxide exposure. The frequency of SCEs and the carboxyhemoglobin concentration was observed in 20 patients without a history of smoking or drug use who had been treated in the Emergency Care Unit of the Istanbul Medical Facility due to acute carbon monoxide exposure. All of these cases were domestic accidents due to malfunctioning coal or wood stoves. The results were compared with a control group of 20 non-smoking, non-drug using healthy individuals matched for age, sex, and absence of other chemical exposure. The mean SCE frequency per metaphase was significantly higher in the study group compared to the control group. No positive correlation was found between the blood carboxyhemoglobin concentration and SCE frequency. They concluded that acute exposure to combustion products of wood or coal is genotoxic to DNA.

Petrochemical Exposure and SCE

SCE frequencies were investigated by Bukvic *et al.* (1998) in the PBLs from 22 men with low average exposure to benzene and 19 control men who had not been exposed. The majority of these men (21 exposed, 19 controls) were also investigated using the micronuclei. The exposed subjects were employed at ten different petrol stations in or near the city (Bari/South Italy). SCE frequencies were significantly related with age and smoking habits, but no relation was observed between SCE and length of employment. Micronuclei frequencies were significantly increased in relation to the length of employment; but no relation was observed when age and smoking habits were taken into consideration.

Summary of SCE Review

In conclusion, it is important to note that the replication fork in S-phase is believed to be the site of SCE formation and SCEs are good indicators of genetic damage. The SCE technique has been used comprehensively and successfully in many studies discussed above to determine the levels of genetic damage caused by several classes of clastogens. This review shows the importance of including the SCE technique in the current study.

2.3 Micronucleus (MN) Assay

In this section, the MN assay that was employed in the current study to measure the degree of malfunctioning of a person's DNA repair system in the G_1 phase of the cell cycle is reviewed. This section includes the definition, origin and genetic basis of micronuclei, and the use of the MN assay as a biomarker. In order to isolate the effect of EMF exposure from the exposure of other environmental agents and lifestyle factors, which might contribute to DNA repair mechanisms, each donor was required to complete a detailed personal questionnaire covering the following: present and past occupational history, exposure history, medical history, smoking history, diet history, and genetic history. Thus, it was considered necessary by the author to include in this review a survey of related studies where MN assay has been performed to monitor the effects of various environmental agents and lifestyle factors on DNA repair.

2.3.1 Introduction

Many endogenous and exogenous agents are known to cause spontaneous cytogenetical or physiological damage in living cells. Chromosomal damage has been linked to severe clinical disorders (Frank and Riccardi, 1977). Therefore, it is important to determine if these agents are capable of inducing such harmful effects in human cells.

The impact of exogenous agents on chromosomal damage has been studied extensively for many years (Raj *et al.* 1983; Yager *et al.* 1988; Surralles & Natarajan, 1997; Fenech *et al.* 1999; Kryscio *et al.* 2001). Cytological examination of metaphase preparations treated with a test agent either *in vivo* or *in vitro* has been the traditional tool used in these studies (Evans, 1976). However, this technique is time consuming and yields

results that are statistically weak due to the relatively low numbers of affected cells that can be scored.

In contrast, techniques such as the MN assay are faster and easy to perform. The existence of micronuclei has been recognized for many years. The linkage between micronuclei and DNA damage was well known to early workers in the radiation field and their occurrence has been frequently mentioned in the early literature (Brenneke and Mather 1937, Heddle *et al.* 1983; Russell and Russell, 1954). Evans *et al.* (1959) made the first attempt to use micronuclei as a monitor of cytogenetic damage by using the frequency of micronuclei to measure the chromosomal damage that had been induced in root-tips by fast neutrons and gamma rays in the presence and absence of oxygen. It was observed that all chromosomal breaks, as well as asymmetrical and incomplete symmetrical exchanges, had produced acentric fragments at mitosis, and that these fragments were frequently excluded from the daughter nuclei. The fragments appeared in the following interphase as micronuclei.

Micronuclei are small extra-nuclear bodies that arise in mitotic cells from acentric chromosomal fragments or whole chromosomes that lag behind in anaphase and are not incorporated into the daughter nucleus (Rieger *et al.* 1968; Evans, 1976 and Fenech, *et al.* 1999). Hence, micronuclei provide a measure of both chromosomal breakage and chromosomal loss. It is worth mentioning here that the mechanistic origin of individual micronuclei can be determined. Micronuclei arising from lagging chromosomes can be identified by the presence of a kinetochore using antikinetochore antibodies or by the presence of centromeric DNA sequences using FISH (Thomson and Perry, 1988; Norppa *et al.* 1993). Micronuclei that do not contain kinetochore/centromeric DNA sequences are interpreted to harbour acentric chromosomal fragments.

2.3.2 Radiation Dose and Micronuclei

Fenech *et al.* (1990) applied the cytokinesis-block MN assay to measure chromosome damage in lymphocytes of 11 cancer patients undergoing fractionated partial-body irradiation. Measurements performed before, during, and after cessation of radiotherapy showed a dose-related increase in micronucleus frequency in each of the patients studied. A general decline in micronuclei frequency was observed during the post-

treatment period down to 57% after 12 months. However, there was considerable variation among individuals.

In another study, the micronuclei of PBLs from radiation-exposed people were monitored by He *et al.* (2000), using the binucleated lymphocyte. Micronuclei frequencies in people with radiation-disease were radiation exposed, and a control group were found to be 1.26%, 0.42% and 0.33%, respectively. The micronuclei frequencies of patients with radiation-disease were significantly higher than other groups. The difference between the radiation-exposed group and the control group was not significant. Meanwhile, CA of the three groups was determined. The results were similar to those previously seen, while the micronuclei frequencies were 2.06%, 0.93% and 0.69%, respectively. CA rate of the radiation-disease group was significantly higher than other groups. The difference between the radiation-exposed group and the control group was not significant. The study indicates that the binucleated lymphocyte is a rapid, sensitive and accurate method, which can be used to monitor a large population exposed to radiation.

Fenech and Morley (1985a) observed no effect of cytochalasin B on micronuclei production and the cytochalasin B method is simple to perform. The *in vivo* micronucleus frequency in normal human individuals was 4.4 – 2.6 micronuclei/500 cytokinesis-blocked binucleated (CB) cells, and for lymphocytes irradiated *in vitro* there was a linear relationship between dose of radiation and number of induced micronuclei.

Kryscio *et al.* (2001) studied the long-term genotoxic effect of occupational radiation exposure of uranium miners. In this study the frequency of micronuclei with and without a centromere were scored using the cytochalasin B technique. It is known that micronuclei can be comprised of acentric fragments and/or whole chromosomes. Micronuclei containing whole chromosomes were identified by means of FISH with a centromere-specific probe. The frequency and percentage of micronuclei with centromeres were analysed in lymphocytes taken from controls and from uranium miners who were exposed to large doses of radiation several decades ago. The miners were subdivided into those with and those without bronchial carcinoma.

It had been shown previously (Bakou *et al.* 2002) that the relative frequency of micronuclei with centromeres decreased with dose, which meant that the number of micronuclei originating from acentric fragments increases. No statistically significant difference in the overall micronuclei frequency was seen between the analysed groups. The fraction of micronuclei with centromeres, however, was highest in lymphocytes of controls (74.6%) followed by healthy miners (62.1%) and those suffering from cancer (55.8%). These results indicated the occurrence of a genomic instability in lymphocytes of miners, especially those with cancer, and it appeared that the low percentage of micronuclei with centromeres might be a marker of genomic instability and cancer predisposition.

It is evident from the discussion above that there is a linear relationship between micronucleus frequency and radiation dose, but the relative frequency of micronuclei with centromeres decreased with higher dose.

2.3.3 Radiosensitivity and Micronuclei

In a study of breast cancer patients, Burrill *et al.* (2000) found that 30% of the patients were sensitive in a lymphocyte assay of radiation-induced chromosome damage (micronucleus induction) compared with 10% of healthy controls. Twenty-two first-degree relatives of 11 sensitive patients had an average micronucleus yield significantly higher than that of 68 controls. In this assay, the authors suggested that radiosensitivity might be an inherited characteristic associated with predisposition to breast cancer.

Erexson *et al.* (1991) compared the radiosensitivity of human, rat and mouse PBLs by analysing frequencies of micronuclei in CB cells. For each species and dose, 4 ml aliquots of whole blood were X-irradiated to obtain doses of 38, 75, 150 or 300 centi-Grey (cGy). Controls were sham-irradiated. After exposure to X-rays, mononuclear leukocytes were isolated using density gradients and cultured in RPMI 1640 medium containing phytohemagglutinin (PHA) to stimulate mitogenesis. At 21 h cytochalasin B was added to produce CB cells, and all cultures were harvested at 52 h post-initiation by cyto-centrifugation. Significant dose-dependent increases in the percentage of

micronucleated CB cells and the number of micronuclei per CB cell were observed in all three species.

2.3.4 Genetic Studies Using the MN Assay

The genetic integrity of human populations is increasingly under threat due to certain industrial activities, which result in exposure to chemical and physical genotoxins. Other factors which can influence genetic damage include life-style factors (diet, smoking, alcohol), various medical therapies, and climatic changes (increased exposure to ultraviolet radiation due to depletion of atmospheric ozone) (Fenech & Neville, 1992). Fortunately, cells of human beings have evolved sophisticated mechanisms to repair any DNA breakages (Braithwaite *et al.* 1998). If this did not happen, one would quickly become ill. If one's DNA repair system is impaired it can be linked to a number of cancers and perhaps a range of illnesses. Indeed some researchers view this as a causal link in tumorigenesis (Wei *et al.* 1996 & Mohrenweiser, 1998).

The increased micronucleus frequencies in human subjects is now under intense evaluation for its association to cancer risk (Chang & Mon-hsiung, 2001; Fenech *et al.* 1999). Many medical laboratories throughout the world conduct tests to monitor the efficiency of a cancer patient's DNA repair system whilst undergoing radiotherapy (Scott, 2000). The technique most commonly applied to measure the efficiency of an individuals DNA repair system is the MN Assay.

Countryman and Heddle (1976) first proposed the use of micronuclei as a measure of chromosomal damage in HPBLs. In recent years this method has been improved with the development of the cytokinesis-blocked binucleated (CB) cell technique, which prevents cytokinesis without inhibiting nuclear division (Fenech and Morley, 1985a and 1985b). This has enabled such cells to be recognized by their binucleate appearance. Cytochalasin B is an inhibitor of actin polymerisation which is required for the formation of the microfilament ring that constricts the cytoplasm between the daughter nuclei during cytokinesis (Carter, 1967). Scott *et al.* improved this method in 1998 and a slightly modified version of it was used in the present study. In this method G_0 lymphocyte cultures were irradiated with a high dose rate (HDR) of X-irradiation, 3.5

Gray (Gy) at 1 Gy min^{-1}. This pulverises the cell's DNA and caused breakages. The irradiated lymphocyte cultures were incubated at 37C under different experimental EMF conditions for a period of six hours to allow for the expression of differential DNA repair (Jones *et al.* 1995), followed by mitogenic stimulation with the mitogen phytohaemagglutinin (PHA). After 24 h of mitogenic stimulation Cytochalasin B was added. In order to achieve the additional advantage of a higher yield of binucleate cells, lymphocytes were harvested at 90 h in lieu of the usual 72 h from the time of mitogenic stimulation.

The MN assay has been used to measure the frequency of micronuclei that have occurred in peripheral blood lymphocytes (PBLs), which acts as a marker for the levels of DNA damage that have been induced in a cell (Fenech and Morley, 1985a; Tucker and Preston, 1996; Miller *at al.* 1997; Kirsch-Volders, 1997; Evans, 1997). The technique has also been used to a lesser extent to study epithelial cells and fibroblasts (Fenech *et al.* 1999).

Using the MN assay it is a relatively simple task to score a large number of cells, compared to the classical metaphase analysis, which gives the MN assay greater statistical power (Fenech *et al.* 1999). Metaphase analysis can be highly tedious with only a small number of cells that can be scored. However, the MN assay allows thousands of cells to be scored in a relatively short time, which will give a statistically stronger result than results gained from metaphase analysis. Most laboratories using the MN assay usually score from 500 up to 2000 CB cells (Bonassi *et al.* 2001). This is a highly recognized assay that provides reliable data when the scoring of micronuclei is limited to those cells that have completed one nuclear division (Fenech and Morley, 1986; Evans, 1997; Fenech, 1997; Fenech and Morley, 1985a).

BRCA1 or BRCA2 Protein

The BRCA1 and BRCA2 gene products are believed to play an important part in the onset and/or development of many sporadic mammary cancers. It has been reported by Welcsh *et al.* (2000) that these two proteins contribute to a centrosome function, which is believed to help maintain the integrity of the chromosome segregation process. This

may mean that a reduced level of the BRCA1 or BRCA2 protein in mammary cells will occasionally lead to non-disjunctional chromosomal loss or gain. Ban *et al.* (2001) reported that spontaneous micronuclei arising from chromosome(s) which fail to be incorporated into the relevant daughter nuclei during mitosis tend to occur more frequently in BRCA1- or BRCA2-defective human cancer cells than in BRCA-positive cancer cells. According to the author, some cases of mammary carcinogenesis might stem from the loss of integrity of chromosome segregation in cells, which have a reduced capacity to express either BRCA1 or BRCA2.

Turner's and Down's Syndrome

In a micronucleus induction study Scarfi *et al.* (1996) studied the PBLs from 15 subjects affected by Turner's syndrome (TS) and between 2 and 24 years of age to evaluate the spontaneous and Mitomycin-C (MMC) induced micronucleus frequency. A group of 15 healthy subjects from the same age group were used as control. As expected, statistically significant differences between spontaneous and MMC-induced micronuclei were found either in TS or in healthy subjects. Unexpectedly, when the two groups of donors were compared, TS subjects showed lower spontaneous and MMC-induced micronucleus frequencies when compared with healthy subjects. Cell proliferation kinetic and cytotoxicity were also measured applying the cytokinesis-block proliferation index (CBPI) and the results showed that the MMC, at the employed concentration, does not induce cell cycle delay both in healthy and in TS donors. When CBPI from TS and healthy donors were compared, a faster proliferation was observed in TS patients in both untreated and MMC-treated cultures.

Similarly, Scarfi *et al.* (1990) studied the PBLs from seven patients with Down's syndrome (DS; trisomy 21) and 14 healthy age-matched controls by the cytokinesis-block method. The spontaneous incidence of micronucleus in lymphocytes from DS subjects was lower than that of control cultures. When lymphocytes were treated with MMC at the beginning of the culture period, an increase in micronucleus formation was found in cells from both DS and control subjects. In DS subjects this increase was much more marked than in control donors. This effect had to be ascribed to cells from older DS subjects (37-55 years old), which showed an MMC-induced micronucleus formation

that was markedly and significantly higher than that observed in cells from younger (9-16 years old) DS subjects. These data indicate that age has to be considered as a major variable when studies on the genetic instability of DS subjects are performed.

2.3.5 Micronucleus Frequency as a Biomarker of Cancer Risk

It is evident from epidemiological studies that long-term risks are associated with human exposure to mutagenic or carcinogenic agents. During the last few decades, many progressive changes have occurred in workplaces, in the environment and in lifestyles. Ultimately, this has resulted in different exposure patterns and exposures to new substances, and therefore new and more sensitive tools should be utilised to investigate cancer risk from these substances. Moreover, there are aspects of metabolism and susceptibility previously unknown or poorly understood, such as metabolic polymorphisms, which can dramatically modify individual responses to toxic and carcinogenic agents. These features have made the identification of etiological factors more difficult, especially for cancers with a long induction period, and the use of traditional epidemiologic outcomes in many cases, such as cancer incidence or mortality, no longer seem sufficient for the evaluation of cancer risk in human populations.

Biomarkers that have been validated for their predictive value may be used for the timely identification of increased cancer risk, which can be used in the prevention or control of disease. The assumption strengthening the use of a biomarker as a surrogate of genetic disease is that the observed relationship between exposure and the marker will translate into a similar relationship between exposure and disease (Schatzkin *et al.* 1990). Confirmation of this assumption is crucial to the validation of the MN assay as a predictor of cancer. However, micronucleus frequency measured by the MN assay may not identify all chromosome damage events; e.g., aberrations such as symmetrical reciprocal translocations are not expressed as micronuclei, but asymmetrical translocations, such as dicentric chromosomes, and their associated acentric fragments may be observed as neoplasmic bridges and micronuclei, respectively.

A causal relationship between micronucleus frequency and cancer risk could be inferred from studies of structural chromosomal aberrations (CA). A huge body of data has been published on the association between CA and neoplastic transformation, which supports the hypothesis that somatic chromosome damage is involved in cancer etiology (Tucker *et al.* 1997). Such damage can occur via chromosome breakage and the relative frequencies of each vary with the inducing chemical.

A direct association between the frequency of micronuclei and cancer development is supported by the following findings: (1) Cheng *et al.* (1996) and Duffaud *et al.* (1997) observed an increased frequency of micronuclei in PBLs in cancer patients. (2) Rudd *et al.* (1988) and Rosin and German (1985) suggested that the subjects affected by congenital diseases such as Bloom syndrome or *Ataxia telangiectasia* have both abnormally high micronucleus frequencies and an increased risk of cancer. (3) Clinical chemo-prevention trials on oral pre-malignancies have used micronuclei in oral mucosa as a surrogate endpoint of cancer (Benner *et al.* 1994; Desai *et al.* 1996). (4) A suggestive correlation exists between carcinogenicity and genotoxicity for some agents able to increase micronucleus frequencies in humans and animals, e.g., ionizing radiation, ethylene oxide, benzene, tobacco smoke (Sorsa *et al.* 1992). (5) Micronucleus frequency is strongly associated with the blood concentration of vitamins and folates, whose deficiencies are associated with increased risks for some cancers (Fenech and Rinaldi, 1995; Fenech *et al.* 1997 and 1998; Blount *et al.* 1997). All the findings mentioned above clearly suggest a causal link between an increase in micronucleus frequency and cancer.

Enhanced sensitivity to the chromosome-damaging effects of ionizing radiation is a feature of many cancer-predisposing conditions. Scott *et al.* (1994) reported that 42% of an unselected series of breast cancer patients and 9% of healthy control subjects with elevated chromosomal radio-sensitivity of lymphocytes irradiated in the G2 phase of the cell cycle. They suggested that in addition to the highly penetrant genes BRCA1 and BRCA2, which confer a very high risk of breast cancer and are carried by about 5% of all breast cancer patients, there are also low-penetrance predisposing genes carried by a much higher proportion of breast cancer patients, a view supported by epidemiological studies (Teare *et al.* 1994; Chen *et al.* 1995). Ideally, testing for the presence of these

putative genes should involve the use of simpler methods than the G2 assay, which requires metaphase analysis of chromosome damage. Scott *et al.* (1998) reported on the use of a simple, rapid MN assay in G0 lymphocytes exposed to high dose rate (HDR) or low dose rate γ-irradiation, with delayed mitogenic stimulation. In this assay, they obtained a good assay reproducibility, particularly with the HDR protocol, which identified 31% (12 out of 39) of breast cancer patients compared with 5% (2 out of 42) of healthy controls as having elevated radiation sensitivity. According to the authors, in the long term, such cytogenetic assays may have the potential for selecting women for intensive screening for breast cancer. This HDR method was also employed during this study to determine whether or not weak EMFs affect the DNA repair system in humans to any degree.

2.3.6 Effects of Age and Gender on Micronucleus Frequency

Several studies (Fenech and Morley, 1986; Bolognesi *et al.* 1997) have investigated the role of age as a confounding factor in cytogenetic bio-monitoring. Research by these groups has found that there is an age-related increase in the frequency of micronuclei.

Fenech and Morley (1986), to overcome the kinetic problems that are found when they have used the human lymphocytes for MN assays, developed the cytokinesis-block micronucleus technique. Using this technique the number of spontaneous micronuclei in lymphocytes from 42 individuals aged between 20 and 85 years was studied and was found to increase at a rate of 4.3% per year. Comparison with the results obtained with the conventional MN assay confirmed that the conventional method markedly underestimates this age effect. The sensitivity of the cytokinesis-block (CB) method was determined by studying the effect of low-dose (less than 50 rad*) X-irradiation. The results indicated that the dose-response was linear and a single *in vitro* exposure to 5 rad of X-rays could be unequivocally detected. They concluded that the CB method was more sensitive and precise than the conventional MN assay and classical metaphase

---

*Rad (rd): A unit of energy absorbed from ionising radiation, equal to 100 ergs per gram or 0.01 joules per kilogram of irradiated material. The Gray (Gy) has replaced it as a standard scientific unit.

analysis, and predicted that the MN assay would be of value for detecting chromosome damage induced *in vivo* by genotoxic agents.

Various authors have reported intra- and inter-individual variations of baseline frequencies of cytogenetic end points in lymphocytes of human populations. Personal characteristics seem to account for a significant proportion of this variability (Fenech, 1999; Bolognesi *et al.* 1997a). For this reason Fenech (personal communication) has established certain criteria for the application of the MN assay. All studies should be designed to allow for the detection of five micronuleated cells per 1,000 binucleated cells between an experimental group and their controls.

Bolognesi *et al.* (1997a) quantitatively evaluated the effect of age on SCE, CA, and micronuclei through the analysis of a population sample that included data from several bio-monitoring studies performed over the last few decades in 12 Italian laboratories. The large size of the data set, more than 2,000 tests for each end point, allowed them to estimate the independent effect of age, gender, smoking habits, occupational exposure, and inter- and intra-laboratory variability. A greater frequency of the mean standardised values with increasing age was observed for all of the end points. A levelling off was evident in the last age classes in the trend of micronuclei frequencies. Frequency ratios (FRs), which express the increase of the cytogenetic damage with respect to the first age classes, 1-19 years, were estimated using Poisson regression analysis after adjustment for potential confounding factors and confirmed the increasing trend by age class for all three end points. The most dramatic increase was observed for micronuclei, with a FR that approaches the values of 2 at the age class 50-59 and remains mainly unchanged thereafter. The trend of FRs for CA is more homogeneous, with a constant rise even in the older classes, whereas the frequency of SCE increases with age to a lesser extent, reaching a plateau in the age class 40-49 and the maximum value of FR in the age class over 70. Their results pointed to an age-related increase of the chromosome damage in lymphocytes and emphasise the need to take into account the potential confounding effect of this variable in the design of bio-monitoring studies based on chromosome damage.

In a biomonitoring study, Bolognesi *et al.* (2002) investigated whether exposure to complex pesticide mixtures in ornamental crop production represents a potential genotoxic risk. Exposed and control subjects were selected in western Liguria (Italy). The area was chosen for its intensive use of pesticides. The main crops produced were roses, mimosas, carnations and chrysanthemums, as ornamental non-edible plants, and tomato, lettuce and basil, as edible ones. The levels of micronuclei were analysed in PBLs of 107 floriculturists (92 men and 15 women) and 61 control subjects (42 men and 19 women). A statistically significant increase in binucleated cells with micronuclei (BNMN) was detected in floriculturists with respect to the control population. The mean number of BNMN varied as a function of gender and age. Smoking habit had no effect on micronuclei frequency. A positive correlation between years of farming and micronuclei frequency in peripheral blood lymphocytes was also observed. The conditions of exposure were also associated with an increase in cytogenetic damage, with a 28% higher micronuclei frequency in greenhouse workers compared with subjects working only outdoors in fields. Workers not using protective measures during high exposure activities showed an increase in micronuclei frequency. Their findings suggest a potential genotoxic risk due to pesticide exposure.

In another study, Bakou *et al.* (2002) used FISH to evaluate spontaneous and aneuploidogen-induced micronucleus frequencies and non-disjunction of chromosomes X and 8 in cultured binucleated lymphocytes of women of two age groups. Demecolcine and vincristine were used as model aneuploidogens to induce micronuclei and chromosome malsegregation. Four of the women were aged between 22-26 (mean 24.3) years and the other four were 47-50 (mean 49.0) years. Pancentromeric FISH was applied to micronuclei to identify chromosomes and double-colour centromeric FISH performed in binucleates of two young and two older women to assess the involvement of chromosomes X and 8 in micronuclei and non-disjunction. This confirmed that age increases the frequencies of micronuclei. Micronuclei that contained entire chromosomes were found predominantly in older females. Age also enhanced micronuclei containing acentric chromosome fragments. The inclusion of chromosomes X and 8 in micronuclei was enhanced by age, and chromosome X was generally over-represented. Non-disjunction of chromosomes X and 8 also increased with age, chromosome X being the more sensitive. Treatment of lymphocytes with vincristine and

demecolcine increased micronucleus frequency and malsegregation of chromosomes X and 8 in both age groups. Comparison of the estimated frequencies of micronucleation and non-disjunction for all human chromosomes showed that non-disjunction is the main type of chromosome malsegregation.

Chromosomal damage as measured by frequency of translocations, acentric fragments, telomere shortening, nondisjunction, chromosome loss, aneuploidy, and micronucleus formation has been shown to increase progressively with age. Fenech *et al.* (1998) used the cytokinesis-block micronucleus technique, which provides an efficient measure of chromosomal breakage and loss, to show that aging can explain at least 25% of the variation in chromosomal damage rate in lymphocytes from both males and females. He also performed cross-sectional and placebo-controlled intervention studies to determine the relationship between the micronucleus frequency in lymphocytes and diet, and blood status for vitamins C, E, B12, and folic acid. Their studies have shown that micronucleus frequency in the 41-60 year age group is significantly lower in vegetarians when compared to non-vegetarians, however the reverse was found to be true in males aged between 20 and 40 years. This was accounted for by a deficient/low B12 status in vegetarian males; there was no difference in the micronucleus frequency of vegetarian and nonvegetarian subjects aged between 61 and 90 years. Results from this study also showed significant negative correlations of micronucleus frequency with folic acid and vitamin B12 but not with vitamin C or vitamin E.

Furthermore, the spontaneous micronucleus yield in lymphocyte cultures (Fenech and Morley, 1985a) from healthy donors aged 0-82 years was estimated at 72 h and 96 h of culture. At both 72 and 96 h there was a positive correlation of micronucleus expression with increasing age, with an approximately four-fold increase in micronuclei in cultures from 80-year-old donors when compared to cultures from newborn donors. Since there is some evidence that the effect of DNA-damaging agents may increase with age, lymphocytes from individuals of various ages were exposed to X-rays and mitomycin C and micronuclei were scored after 72 and 96 h of culture. The total number of micronuclei formed after exposure to these agents were, however, decreased in cells from elderly individuals, most likely due to kinetic differences between the lymphocytes of old and young individuals.

To conclude the above section, the important point to note is that there is a clear indication of age related increase in micronucleus frequency.

2.3.7 MN Assay and the Influence of Chemical and Environmental Agents

EMF Exposure

In an elegant study, Simkó *et al.* (1998) studied the effects of ELF EMFs exposure in humans. In their study, human squamous cell carcinoma cell line (SCL II) and an amniotic fluid cell line (AFC) was used. Cells were exposed to 50 Hz, 0.1-1.0 mT, MFs for durations of 24, 48 and 72 h. Micronucleus frequency and apoptic cells were scored from this study. A statistically significant increase of frequency of micronuclei and the induction of apoptosis in SCL II cells after both 48 h and 72 h of continuous exposure to 50 Hz MFs (0.8 and 1.0 mT) were found. However, exposure of AFC cells to MFs of different intensities and for different exposure times showed no statistically significant differences when compared to controls. Dose-dependent induction of apoptosis and genotoxic effects, resulting in increased micronucleus formation, could be demonstrated in the transformed cell line, whereas the non-transformed cell line did not show statistically significant effects.

Several epidemiological studies (Wertheimer and Leeper, 1979; Savitz *et al.* 1988; and London *et al.* 1991) have suggested that EMFs are associated with increased incidence of cancer. To test the carcinogenic potency of EMFs, the *in vitro* MN assay with Syrian Hamster Embryo (SHE) cells was used as a screening method for genotoxicity in a study by Simkó *et al.* (2001). A 50 Hz, 1mT strength MF was applied either with or without the tumour initiator benzo(a)pyrene (BP) or the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). All three treatments were applied in single, double or triple treatment regimes. MF or TPA (1nM) alone did not affect the number of micronuclei in initiated and non-initiated SHE cells. Changing the schedule of the typical initiation protocol, namely by applying the initiator BP during exposure to MF, results in a 1.8-fold increase in micronuclei formation compared to BP treatment alone. A combined experiment with BP, TPA and MF exposure did not cause further

micronuclei formation. Since initiation during MF exposure caused a significant increase in micronuclei formation, their findings suggest that MFs enhance the initiation process of BP. It was concluded that this MF-enhanced co-carcinogenic effect is caused by an indirect "cell activation" process. The resulting genomic instability is proposed to be due to free radicals and/or to the unscheduled "switching-on" of signal transduction pathways.

Scarfi *et al.* (1999) studied the micronucleus induction and cell proliferation in HPBLs cultured *in vitro* and exposed to a 50 Hz sinusoidal MF for 72 h at different intensities (1.0, 0.75, 0.5, 0.25, and 0.05 mT). A total of 42 healthy human donors, aged between 26 and 54 were used. The cytokinesis-block MN assay results showed no genotoxic effects for the field intensities tested. However, cell proliferation was slightly elevated.

In a separate genotoxic evaluation study, Scarfi *et al.* (1994) exposed HPBL cultures to 50 Hz pulsed MFs in order to evaluate a possible genotoxic effect of such non-ionizing radiation. The genotoxic effect was evaluated in terms of both micronucleus induction and classical CAs; the mitotic index (MI) was also calculated. In this study lymphocytes from five healthy human donors were examined using the tests mentioned above. No genotoxic effects were found, but an increased MI was found in exposed samples compared to the controls.

CAs, micronuclei, and SCEs were analysed by Heimers (2000) in HPBLs of 18 Concord pilots who were exposed to cosmic radiation and 10 controls. It was found that there was an 8-fold significant increase of dicentric chromosomes in the Concord group. The yield of micronuclei was also significantly elevated. However, SCEs in the Concord group did not differ from the controls.

Abramsson-Zetterberg and Grawe (2001) used the flow cytometer-based MN assay to study the effects on the chromosomes of erythroid cells of CBA/Ca mice after an extended exposure to a 50 Hz, 14 μT MF. The study included two different experiments: (a) mice exposed *in utero* during 18 days of their prenatal stage, and (b) adult mice exposed for 18 days. In experiment (a) the mice were exposed 35 days in total and then peripheral blood was drawn from the exposed mice to determine whether

the exposure had a genotoxic effect on the pluripotent erythroid stem cells. About 200,000 polychromatic erythrocytes (PCE) and 200,000 normochromatic erythrocytes (NCE) were analysed from each of 20 exposed mice. The EMF exposure did not significantly change the frequency of micronucleated PCE or NCE in comparison with 20 sham-irradiated mice. There was no difference in the proportion of PCE between exposed and unexposed animals. Similarly, in experiment (b) no differences were seen between EMF-exposed and unexposed adult mice when samples of peripheral blood were taken at the end of exposure and analysed for micronuclei in PCE and NCE. The proportion of PCE was similar in both groups. The results indicate that exposure to EMF does not induce direct or indirect effects on chromosomes in erythroid cells expressed as increased levels of micronucleated erythrocytes of mice. No indications of delayed genetic effects were found.

Lagroye and Poncy (1997) used the cytokinesis-blocked MN assay to investigate a possible amplification of the genotoxic effects of ionizing radiations in cells exposed to combined static or power-frequency EMFs. In this study, rat tracheal epithelial cell lines were used. First, the cells were exposed *in vitro* to ^{60}Co γ-radiation (either 0, 2 or 6 Gy) and then cultured for 24 h in a homogeneous sinusoidal 100 μT EMF (50 Hz) combined with an artificial geomagnetic-like field created by the use of horizontal and vertical pairs of Helmholtz coils. Control cells were cultured in an adjacent incubator where the background EMF was approximately 0.1 μT. Under the *in vitro* experimental conditions, the EMF showed no significant direct effect on micronucleus induction in rat tracheal cell lines. However, an increased frequency of EMF-exposed binucleated cells with micronuclei was observed in cells exposed to 6 Gy of γ-rays compared with controls.

Livingston *et al.* (1991) studied human lymphocytes and Chinese Hamster Ovary (CHO) fibroblasts for cytogenetic and cytotoxic endpoints to determine whether exposure to power frequency (60 Hz) EMFs interfered with normal cell growth and reproduction. An exposure chamber was built in order to apply variable electric current densities of 3, 30, 300, and 3,000 microampere per square centimetre ($\mu A/cm^2$) simultaneously with a fixed MF of 0.22 mT to proliferating cells. The current densities were chosen to bracket those that may be induced in the human body by fields

measured beneath high voltage (765 kV) power transmission lines. The electric current was applied through the media of a cell culture chamber positioned between two stainless steel electrodes but separated from direct contact with the culture media by a salt bridge composed of a 1% agarose gel. The MF was generated using two pairs of Helmholtz coils driven 73 degrees out of phase producing an elliptically polarised MF 36 degrees out of phase with the electric field. The EMFs were measured and mapped inside the cell culture chamber to ensure their uniformity. CHO cells were exposed continuously for 24-96 h (depending on the experiment) and human lymphocytes were exposed continuously for 72 h. The EMFs were monitored throughout the entire treatment period using a multichannel chart recorder to verify continuous application of the desired fields. SCE and micronuclei were monitored to evaluate the potential for genotoxicity. In addition, standard growth curves, clonogenicity, and cell cycle kinetics were analysed to evaluate possible cytotoxic effects. The results showed no effects on both cell types exposed to the EMFs.

The micronucleus can serve as a suitable indicator for the assessment of exposure to potentially genotoxic agents such as radio-frequency electromagnetic radiation (RFR) and the analysis of mitotic activity as an additional parameter for efficient bio-monitoring. In a study by Garaj-Vrhovac (1999), the effects of RFR on the cell kinetics and genome damages in PBLs were determined in lymphocytes of 12 subjects occupationally exposed to RFR. Their results showed an increase in the frequency of micronuclei, as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls.

Haider *et al.* (1994) used the Tradescantia-micronucleus bioassay to determine the genotoxic effects of short-wave EMFs (10-21 MHz) which is used for broadcasting. In their experiment, plant cuttings bearing young flower buds were exposed to this short-wave EMFs for 30 h. A statistically higher micronucleus frequency than controls were observed at all exposure sites except one in the immediate vicinity of the antenna. Hence, they concluded that short-wave radiation from the broadcasting antennae had a clastogenic effect.

Tice *et al.* (2002) studied the potential genotoxicity of radiofrequency signals emitted by cellular telephones. The *in vitro* studies evaluated the induction of DNA and chromosomal damage in human blood leukocytes and lymphocytes, respectively. The signals were voice-modulated 837 MHz signals and produced by an analog signal generator or by a time division multiple access cellular telephone. 837 MHz was generated by a code division multiple access cellular telephone (not voice modulated), and voice modulated 1909.8 MHz was generated by a global system of mobile communication-type personal communication systems cellular telephone.

In this study, DNA damage was assessed in leukocytes using the alkaline (pH>13) single cell gel electrophoresis assay, which is also known as the COMET assay. Chromosomal damage was evaluated in lymphocytes mitogenically stimulated to divide post-exposure using the cytochalasin B-binucleate cell. Cells were exposed at 37 ± 1C, 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, nor did exposure for 3 h induce a significant increase in micronucleated cells among lymphocytes. However, exposure to each of the four RF signal technologies for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase in the frequency of micronucleated lymphocytes. The magnitude of the response (approximately 4-fold) was independent of the technology, the presence or absence of voice modulation, and the frequency (837 vs. 1909.8 MHz). This research demonstrates that under extended exposure conditions, RF signals at an average SAR of at least 5.0 W/kg are capable of inducing chromosomal damage in human lymphocytes.

In another study, Vijayalaxmi *et al.* (2001) studied the chromosomal damage and micronucleus formation in HPBLs exposed *in vitro* to RFR at a cellular telephone frequency (847.74 MHz). In this study, peripheral blood samples collected from four healthy nonsmoking human volunteers were diluted with tissue culture medium and exposed *in vitro* for 24 h to 847.74 MHz RFR (continuous wave), a frequency employed for cellular telephone communications.

A code division multiple access technology was used with a nominal net forward power of 75 W and a nominal power density of 950 W/m^2 (95 mW/cm^2). The mean SAR was

A code division multiple access technology was used with a nominal net forward power of 75 W and a nominal power density of 950 W/m^2 (95 mW/cm^2). The mean SAR was 4.9 or 5.5 W/kg. Blood alliquots that were sham-exposed or exposed *in vitro* to an acute dose of 1.5 Gy of γ-radiation were included in the study as controls. The temperatures of the medium during RFR and sham exposures in the Radial Transmission Line facility were controlled at 37 ± 0.3C. Immediately after the exposures, lymphocytes were cultured at 37 ± 0.3C for 48 or 72 h.

The extent of genetic damage in this study was assessed from the incidence of CAs and micronuclei. The kinetics of cell proliferation was determined from the mitotic indices in 48 h cultures and from the incidence of binucleate cells in 72 h cultures. The data indicated no significant differences between RFR-exposed and sham-exposed lymphocytes with respect to mitotic indices, frequencies of exchange aberrations, excess fragments, binucleate cells, and micronuclei. The response of γ-irradiated lymphocytes was significantly different from that of both RFR-exposed and sham-exposed cells for all of these indices. Thus, the authors concluded that there was no evidence for induction of CAs and micronuclei in HPBLs exposed *in vitro* for 24 h to 847.74 MHz RFR (CDMA) at SARs of 4.9 or 5.5 W/kg.

In a similar study, Vijayalaxmi *et al.* (2001) carried out cytogenetic investigations using HPBL exposed *in vitro* to RFR at a cellular telephone frequency of 835.62 MHz. Freshly collected peripheral blood samples from four healthy human volunteers were diluted with RPMI 1640 tissue culture medium and exposed to 835.62 MHz RFR *in vitro* in T-75 tissue culture flasks for 24 h. This frequency was employed for customer-to-base station transmission of cellular telephone communications. An analog signal was used, and the access technology was frequency division multiple access (continuous wave). A nominal net forward power of 68 W was used, and the nominal power density at the centre of the exposure flask was 860 W/m^2. The mean SAR in the exposure flask was 4.4 or 5.0 W/kg.

Aliquots of diluted blood that were sham-exposed or exposed *in vitro* to an acute dose of 1.50 Gy of γ-radiation were used as negative or positive controls, respectively. Immediately after the exposures, the lymphocytes were stimulated with PHA, a

mitogen, and cultured for 48 or 72 h to determine the extent of genetic damage, as assessed from the frequencies of CAs and micronuclei.

The extent of alteration in the kinetics of cell proliferation was determined from the mitotic indices in 48 h cultures and from the incidence of binucleate cells in 72 h cultures. The data, however, indicated no significant differences between RFR-exposed and sham-exposed lymphocytes with respect to mitotic indices, incidence of exchange aberrations, excess fragments, binucleate cells, and micronuclei. In contrast, the response of the lymphocytes exposed to γ-radiation was significantly different from both RFR- and sham-exposed cells for all of these indices.

In a study by Hook *et al.* (1999) the possible effect of wireless phone signals (analog, TDMA, CDMA and PCS) on micronuclei formation in human lymphocytes was explored. No effects were found after three hours of exposure at SARs of 0, 1, 2.5, 5 or 10 W/kg for any of the signals tested. At 5 W/kg, increases of lesser magnitude were observed from analog and TDMA exposure. Finite Difference Time Domain (FDTD) calculations indicated peak SAR values may have reached as much as twice the average SAR values in parts of the cell pellet and repeat studies were planned to attempt to clarify the findings and determine whether sample heating was a confounding factor. In the following repeat studies the authors observed clear evidence of DNA disruption and micronuclei formation, which means that the cellular DNA had divided unequally.

Vijayalaxmi *et al.* (1997) exposed aliquots of peripheral blood samples collected from two healthy human volunteers to continuous wave 2450 MHz RFR. The exposures were either continuously for a period of 90 min or intermittently for a total period of 90 min (30 min on and 30 min off, repeated three times). Blood aliquots that were sham-exposed or exposed *in vitro* to 150 cGy γ-radiation served as controls.

The continuous wave 2450 MHz RFR was generated with a net forward power of 34.5 W and transmitted from a standard gain rectangular antenna horn in a vertically downward direction. The mean power density at the position of the cells was 5.0 mW/cm^2. The mean SAR calculated by FDTD analysis was 12.46 W/kg. Lymphocytes

There were no significant differences between RFR-exposed and sham-exposed lymphocytes with respect to: (a) mitotic indices; (b) incidence of cells showing chromosome damage; (c) SCE; (d) acentric fragments; (e) binucleate cell, and (f) micronuclei, for either the continuous or intermittent RFR-exposures. In contrast, the response of positive control cells exposed to 150 cGy γ-radiation was significantly different from RFR-exposed and sham-exposed lymphocytes. Thus, there is no evidence for an effect on mitogen-stimulated proliferation kinetics or for excess genotoxicity within 72 h in human blood lymphocytes exposed *in vitro* to 2450 MHz RFR.

Diener and Eberle (1996) investigated the possible effects of RF exposure (400 MHz, 900 MHz, and 1.8 GHz) on gene and chromosome mutations and cell growth. Exposures ranged from 39 to 70 h resulted in no effects on CA rate, SCE, micronucleus frequency or cell proliferation.

Several *in vitro* studies have also been conducted to detect effects of microwave exposure on the genetic material. In one study, Zotti-Martelli *et al.* (2000) investigated the *in vitro* genotoxic effect of microwave exposure in HPBL. Lymphocytes were exposed in G0 to EMFs with different microwave frequencies (2.45 and 7.7GHz) and power density (10, 20 and 30 mW/cm^2) for three times (15, 30 and 60 min). The results showed that microwaves are able to cause cytogenetic damage in human lymphocytes, but only at high power density (30 mW/cm^2) and long exposure times (30 and 60 min).

Fucic *et al.* (1992) chose a new mathematical approach to separate clastogenic from aneugenic activity of three well-known mutagens (vinyl chloride monomer, X-rays and microwaves) on the genome of human somatic cells. The comparison of frequencies of size distribution of micronuclei in the lymphocytes of humans exposed to each of these three mutagens showed that X-rays and microwaves were preferentially clastogens while vinyl chloride monomer showed aneugenic activity as well. Microwaves possess some mutagenic characteristics typical of chemical mutagens.

Garaj-Vrhovac *et al.* (1992) exposed human whole-blood samples to continuous microwave radiation (frequency 7.7 GHz, power density 0.5, 10 and 30 mW/cm^2 for 10, 30 and 60 min). A correlation between specific CAs and the incidence of micronuclei

Garaj-Vrhovac *et al.* (1992) exposed human whole-blood samples to continuous microwave radiation (frequency 7.7 GHz, power density 0.5, 10 and 30 mW/cm^2 for 10, 30 and 60 min). A correlation between specific CAs and the incidence of micronuclei after *in vitro* exposure was observed. In all experimental conditions, the frequency of all types of CAs was significantly higher than in the control samples. In the irradiated samples the presence of dicentric and ring chromosomes was established. The incidence of micronuclei was also higher in the exposed samples. The results of the structural CA test and of the micronucleus test were comparatively analysed. The values obtained showed a positive correlation between micronuclei and specific CAs (acentric fragments and dicentric chromosomes). The results of this study indicated that microwave radiation causes changes in the genome of somatic human cells and that the applied tests are equally sensitive for the detection of the genotoxicity of microwaves.

Cossarizza *et al.* (1989) examined the question of whether EMFs interfere with DNA repair following γ-irradiation. In these experiments lymphocytes were stimulated with PHA for 66 h, subjected to 100 Gy of ^{60}Co γ-radiation, pulsed with [^{3}H]thymidine, and subjected to pulsed EMFs for 6 h. The later 6 h period corresponds to the normal period of radiation damage DNA repair. Their data indicate that post-exposure to EMFs did not interfere with the normal repair processes induced by γ-irradiation.

Micronuclei in lymphocytes have been examined (Livingston *et al.* 1991; Scarfi *et al.* 1991, and 1994) to determine if their electric or pulsed MFs affect chromosomal breakage or induce mis-segregation. The micronuclear test is a sensitive genotoxic marker that "evaluates the frequency of small round bodies derived from chromosome fragments or whole chromosomes whose centromeres have lost their affinity for the mitotic spindle" (Scarfi *et al.*1991). Again, no effects were found in these studies.

Maes *et al.* (1993) and Vijayalaxmi *et al.* (1998) observed an increase in micronucleus in HPBLs and peripheral blood and bone marrow of cancer-prone mice, respectively when they applied much stronger MFs 2.45 GHz microwave RFR. In Maes *et al.* (1993) study, HPBLs were exposed to microwaves at 2.45 GHz. They observed a marked increase in the frequency of CAs (including dicentric chromosomes and acentric fragments) and micronuclei. On the other hand the microwave exposure did not

influence the cell kinetics or the SCE frequency. Formation of dicentric chromosomes is considered to be the "hallmark" of ionizing radiation exposure. However, Vijayalaxmi *et al.* (1998) exposed peripheral blood and bone marrow of cancer-prone mice chronically to 2450 MHz microwave RFR and observed a significant increase in micronuclei.

It is evident from the review of different studies mentioned above that EMFs do influence micronucleus frequency, and this can serve as a suitable indicator for the assessment of exposure to potentially genotoxic agents. Thus, the MN assay was included in the present study.

Exposure to Cytostatic Drugs

Kasuba *et al.* (1999) used the MN assay to study the genotoxic effect of occupational exposure of 20 nurses who handled cytostatic drugs in medical oncology and haematology units. The duration of employment in the units and of exposure to cytostatic drugs ranged from 1 to 31 years. The exposed nurses showed an increased proportion of cells with micronuclei as compared to the control group. Nurses exposed to cytostatic drugs for 20-31 years showed a higher frequency of micronuclei, whereas there was no difference in frequencies between the control group and the group exposed for 1-14 years. The influence of the exposure period proved to be a significant parameter for the MN assay.

To evaluate genotoxicity of cytostatic drugs, 100 employees who were occupationally exposed to cytostatic drugs in hospitals and/or pharmacies, were studied using the MN assay by Hessel *et al.* (2001). A total of 247 peripheral blood samples were collected from different exposure levels. The levels of exposure were "baseline level", i.e., without any cytostatic drugs exposure before recruiting or after at least 3 weeks without cytostatic drugs contact and at three times (cycle 1-3) post-exposure. Blood samples from 60 office employees served as controls.

Statistical analyses were performed under consideration of age, gender and X-ray exposure. The frequency of micronuclei was significantly related to the age of the

subjects. However, there were no significant differences in the frequencies of micronuclei between controls and exposed hospital workers. Similarly, micronucleus rates were not significantly different at the various sampling time points and there was no correlation between duration of employment and micronucleus rates.

In another study, Télez *et al.* (2001) investigated the genotoxic potential of chronic long-term therapy with the antihypertensive drug nimodipine, using SCEs and micronuclei in HPBLs of patients with long-term exposure to this drug. HPBLs of control individuals exposed *in vitro* to nimodipine were also studied to assess the effect of the drug itself. FISH using a centromeric probe was performed to determine the origin of the induced micronuclei. The *in vivo* study was carried out on five patients under antihypertensive treatment with nimodipine. The *in vitro* study was performed on five control individuals by adding the drug to the culture medium at a final concentration similar to the levels found in plasma (controls/medium). The *in vivo* study showed no genotoxic effects of long-term therapy with nimodipine because the frequencies of SCE and micronuclei in exposed patients did not show significant differences as compared with control individuals. A statistically significant increase in the frequency of micronuclei was detected in controls/medium as compared with control individuals without the drug. FISH analysis revealed statistically significant differences with respect to the frequency of centromeric signals in nimodipine-induced micronuclei *in vitro*. With regard to the *in vivo* results, chronic long-term therapy with nimodipine is not associated with increased genotoxicity. The differing results *in vivo* and *in vitro* could be due to extensive metabolism of nimodipine, indicating that the cytogenetic effect observed was due to the drug itself rather than its metabolites or to an adaptive response to nimodipine *in vivo*.

Exposure to Pesticide

The potential cytogenetic damage associated with pesticide use in Greek agricultural workers was evaluated by Pastor *et al.* (2001) using the frequency of micronuclei as bio-markers in lymphocytes of peripheral blood and exfoliated cells of the buccal mucosa. In addition, the effects of pesticide exposure and other variables on the cytokinesis block proliferation index (CBPI) in lymphocytes were also evaluated.

Micronuclei were analysed in 50 agricultural workers exposed to pesticides (30 men and 20 women) and in 66 non-exposed individuals that constituted the control group (41 men and 25 women). The comparison between workers and controls did not reveal any statistical significant difference in the micronuclei frequency for either lymphocytes or buccal cells. Regarding CBPI, the value found in the exposed group was lower than in controls, the difference being statistically significant.

Exposure to Genotoxic Chemicals

Micronucleus frequency from PBLs of two groups of workers from hospital and cyclophosphamide (CP) processing industries who were potentially exposed to CP were analysed using the MN assay by Yager *et al.* (1988). Increased numbers of micronuclei were observed in both exposed groups compared to controls. The finding was independent of the age of the subjects, which was also correlated with micronuclei formation.

Exposure to certain chemical agents in occupational settings has been identified as carcinogenic to the human bladder. Micronucleus analysis in exfoliated urothelial cells is an interesting method for biomonitoring genetic damage in human populations. In one of the few studies to have been performed in an occupational context, Fontana *et al.* (2001) performed the MN assay using exfoliated urothelial cells to examine whether the occupational use of a mineral jelly induced a genotoxic risk for workers employed at a factory producing bearings. The prevalence of micronucleated exfoliated urothelial cells (MNC) was determined in 35 female workers with dermal exposure to the jelly and 41 female controls. They observed a significant occupational effect on the prevalence of MNC. This may be due to the presence of mutagens/carcinogens in the jelly: an aromatic amine, N-phenyl-1-naphthylamine, which is carcinogenic in mice, or sodium nitrite, which is genotoxic in human cell systems. These results suggest that the use of the mineral jelly could present a genotoxic risk for workers and the MN assay on exfoliated cells could be valuable for biological monitoring purposes in occupational contexts as a marker of significant exposure to bladder mutagenic/carcinogenic agents.

Oliveira *et al.* (2001) report on the genotoxicity of the boron neutron capture (BNC) reaction in human metastatic melanoma cells assessed by the MN assay using p-borono-L-phenylalanine (BPA) as the boron delivery agent. Different concentrations of BPA (0.48, 1.2 and 2.4 mM) and different influences of thermal neutrons were studied. Substantial genotoxic potential of alpha and lithium particles generated inside or near the malignant cell by the BNC reaction was observed in a dose-response manner as measured by the frequency of micronucleated binucleated melanoma cells and by the number of micronuclei per binucleated cell. The distribution of the number of micronuclei per micronucleated binucleated cell was also studied. The BNC reaction clearly modifies this distribution by increasing the frequency of micronucleated binucleated cells with two or three micronuclei and consequently decreasing the frequency of micronucleated cells with one micronucleus. A decrease in cell proliferation was also observed which correlated with micronuclei formation.

In a study by Nesti *et al.* (2000), primary liver fibroblasts were studied in a MN assay in combination with FISH using two protocols. In protocol-A (Prot. A), cytochalasin B was added at the end of the treatment time directly to the medium containing the standard compounds, whereas, in protocol-B (Prot. B) the chemical-containing medium was removed and fresh medium with cytochalasin B was added. The study was performed using a fungicidal antibiotic griseofulvin (GF) and the clastogen mitomycin C (MMC) as standard compounds. With both protocols GF induced a significant increase in micronuclei frequency over controls in a dose-related manner at the lower concentrations tested (7.5 and 15 μg/ml). At the highest dose (30 μg/ml) the antibiotic effect was substantially reduced. Micronuclei induction obtained with Prot. A was significantly higher (approximately 3-fold) than with Prot. B. Micronuclei induced by MMC showed a dose- and time-dependent increase in both protocols. In contrast to GF, the greater clastogenic response induced by MMC in human liver fibroblasts was obtained with Prot. B, approximately 3-fold higher than Prot. A and approximately 2-fold with 24 h treatment at 0.17 μg/ml MMC. FISH analysis showed that MMC induced mainly micronuclei containing acentric fragments rather than whole chromosomes. These researchers demonstrated that chemically induced genetic effects are strongly dependent on the cell culture employed, treatment schedule and intra- and post-treatment experimental conditions.

The *in vivo* rodent MN assay is widely used as a cytogenetic assay to detect the clastogenic activity of a chemical *in vivo*. The MN assay is one of three tests recommended by the fourth International Conference on Harmonisation (ICH4) of Genotoxicity Guidelines and as such, many regulatory authorities have accepted it. However, the determination of a positive result in a genotoxicity test, including MN assay, has been an issue of debate among toxicologists and biometricians. Kim *et al.* (2000) compared several statistical procedures that have been suggested for the analysis of MN assay data and indicate which one is the most powerful. The standard protocol of MN assay has at least three dose levels plus the control dose and uses at least four animals per group. For each animal, two thousand polychromatic erythrocytes (PCE) were counted.

In a genotoxicity study, female mice of hybrid strain B6C3F1, 8-10 weeks old were fed on powdered food with or without 2% caffeic acid (Raj *et al.* 1983). Caffeic acid (3,4-dihydroxy cinnamic acid) is widely distributed in plant materials in both free and combined forms and, as such, is a component of the human diet. After one week on these diets, some of each group of mice were injected with 7,12-dimethyl benz[a]anthracene (25 mg/kg) dissolved in dimethyl disulfoxide (DMSO). In the course of separate experiments, bone-marrow samples were taken at various intervals after injection for analysis in the MN assay. From each mouse, 500 polychromatic erythrocytes were scored to determine the frequency of micronuclei. At the time at which the maximum response was observed, which differed between experiments, the frequency of micronuclei induced by 7,12-dimethyl benz[a]anthracene (DMBA) was reduced by 50% by the presence of caffeic acid. The results suggest that caffeic acid provides significant protection against the genotoxicity of DMBA.

Meng and Zhang (1997) studied the frequencies of CA and micronuclei in PBLs of 40 workers at a phosphate fertiliser factory in North China. Hydrogen fluoride (HF) and SiF_4 are the main air pollutants and small amounts of dust containing fluoride, NH_3, and SO_2 were also present in the factory. The mean observed frequencies per 100 metaphases of major CA type (chromosome rings, translocations, and dicentrics) of the workers and the non-exposed controls were 0.91 and 0.24, respectively. The average percentages of lymphocytes with micronuclei of the workers and the controls were 1.55

± 0.71 and 0.62 ± 0.54, respectively. The authors concluded that both the CA and micronuclei frequency of the workers increased with the length of the chemical exposure period.

Anaesthetic Gases

To minimise the possible health risks posed by waste anaesthetic gases the National Institute of Occupational Safety and Health (NIOSH, USA, 1994, publication 94-100) & Health and Safety Executive (HSE, London, 1994, Guidance note EH 40/94) recommends certain exposure limits. Wiesner *et al.* (2001) investigated the genotoxicity of a previously established occupational exposure exceeding these limits (high-level exposure) and of one within these limits (low-level exposure). Genotoxicity was assessed by the formation of micronucleated lymphocytes in 25 anaesthetists and anaesthetic nurses of an Eastern European (High-Level Exposure Group) and a German (Low-Level Exposure Group) university hospital. Each exposed group was compared with a group of non-exposed personnel of the same hospital. Compared with the control group, there was an increased fraction of micronucleated lymphocytes per 1,000 binucleated cells in the High-Level Exposure Group but not in the Low-Level Exposure Group. They conclude that a high-level exposure to inhaled anaesthetics is associated with an increase in chromosome damage, and measures were recommended to decrease exposure levels.

Occupational exposure to anaesthetic gases is associated with various adverse health effects. Genetic material has been shown to be a sensitive target of numerous harmful agents. Rozgaj *et al.* (2001) examined a group of 43 hospital workers of three professions (anesthesiologists, technicians and operating room nurses) and 26 control subjects for CAs, SCEs, and micronucleus frequency. The exposed groups were matched in duration of exposure to anaesthetics, but not in age. An equal ratio between women and men was possible in all groups except nurses. Likewise, the ratio between smokers and non-smokers was also not comparable. An increase in chromosome damage was found in all exposed groups. While the increase in SCE frequency was not significant, CAs and micronucleus frequency increased significantly, showing higher

rates in women. Their results suggest that the micronucleus test is the most sensitive indicator of changes caused by anaesthetic gases.

Air pollution and Micronuclei Frequency

Atmospheric pollution represents a relevant environmental hazard, which has been associated with considerable excess mortality, morbidity, and increased rates of respiratory diseases in humans. To date, more than 3,000 environmental chemical compounds have been identified in the ambient atmosphere, including a variety of mutagenic and/or carcinogenic agents, such as polycyclic aromatic hydrocarbons (PAHs), aromatic amines, and heterocyclic compounds (Bolognesi *et al.* 1997b; Walter & Crinnion, 2000). Positive associations between cytogenetic markers and airborne levels of PAHs have been reported by experimental and human studies. Traffic has been implicated as the major determinant for the concentration of PAHs and, therefore, for the genotoxic activity of urban air.

The Tradescantia/micronuclei test (TRAD/MCN) is a well-validated test for monitoring environmental genotoxicants. These pollutants induce at the early meiotic stage of pollen mother cells chromosome fragments, which become micronuclei at the tetrad stage. The standard test protocol requires several hours of exposure of the inflorescences and a recovery time of about 24 h to reach the early tetrad stage. Since the recovery period represents a critical step of the TRAD/MCN, experiments were performed by Falistocco *et al.* (2000) to establish the appropriate length of time in plants for clone 4430 of the hybrid *T. hirsutiflora* X *T. subacaulis,* which is widely used in environmental monitoring. The aim of the research was to acertain the exact duration of recovery time in order to improve the sensitivity of the TRAD/MCN test. First, studies were performed to select the flowers at the beginning of meiosis, and then anthers were sampled and studied for a period of 48 to 86 h. To study complete meiosis in the plants required approximately 80 h. Second, exposure to genotoxic substances followed by different recovery times was performed in order to demonstrate that the effectiveness of the TRAD/MCN test is closely related to the duration of the recovery time. In order to conduct the tests, the inflorescences were exposed to known mutagens (sodium azide and maleic hydrazide) for 6 h followed by different recovery times (24-

72 h). The results from these experiments showed that the frequency of micronuclei in the pollen mother cells increased with the length of the recovery time.

Similarly, Batalha *et al.* (1999) designed a study to determine the clastogenicity of particulate matter (with an aerodynamic diameter smaller than 10 μm) in the urban polluted air in the city of Sao Paulo. The TRAD/MCN assay was used throughout this study to evaluate the clastogenicity of the extracts of the particulate matter. *Tradescantia pallida* (Rose) Hunt. cv. purpurea, an indigenous cultivar, was used in the TRAD/MCN assay. The efficacy of this plant material for the TRAD/MCN assay was validated with dose-response studies using formaldehyde and beta radiation. Dose-response curves were established with these known mutagens. The extracts of the PM10 particles at concentrations between 5 and 50 ppm induced a dose-related increase in MCN frequencies. The results indicate that *T. pallida* is equally sensitive to mutagens as the standard Tradescantia clone 4430 or 03 and the particulate matter in the urban air are clastogenic to the chromosomes of this plant. Inhalation of these particles by urban dwellers may affect their health by inducing similar genetic damage.

Alcohol

In a genotoxic exposition study, Ramirez and Saldanha (2002) used the MN assay as an indicator of genotoxic exposition, as it is associated with CAs. An increased mutation rate in oral squamous cells, indicated by an increased micronucleus frequency, is also related to the development of oral carcinomas. The frequencies of micronuclei and other metanucleated anomalies in the buccal squamous cells of 30 alcoholics with oral or oropharyngeal carcinomas were evaluated and compared them to a control group of abstinent healthy individuals. Microscopic examination was made of 2,000 cells per individual from each of three distinct areas of the mouth: around the lesion (A), opposite to the lesion (B) and in the upper gingival-labial gutter (C). Area C was used as a control region because of low tumour frequency. A 7-fold increase in micronucleus frequency in region B, 3-fold increases in region A, and a 2-fold increase in C, was observed. The authors concluded that the MN assay could be used for monitoring clinical evaluation, by means of intra- and inter-individual cellular comparisons.

Diet

Fenech & Neville (1992) investigated whether the consumption of cooked meat could induce chromosome damage. In their study, the bone-marrow MN assay was used. They observed no difference in the micronucleus frequency of mice on normal diet (cereal-based, non-purified diet) and mice on normal diet supplemented with microwaved meat. However, supplementing the normal diet with well-done pan-fried meat, or rare charcoal-barbecued meat, or well-done charcoal-barbecued meat produced significant increments in the micronucleus frequency of polychromatic erythrocytes. The increments were of the order of 73%, 90%, and 136%, respectively and they were observed after a 21-day feeding trial. These results suggest that ingestion of well-done pan-fried or barbecued meat may increase genetic damage; however, the accompanying decreased intake of vegetable constituents may have also contributed to the observed changes.

2.3.8 Concentration of Cytochalasin B

Scarfi *et al.* (1993) carried out a study in order to set up a standardised quantitative assay for spontaneous micronuclei in bovine lymphocytes. For this purpose the cytochalasin B micronucleus method, originally proposed by Fenech and Morley (1985a) for human lymphocytes, and was applied to PBLs of 20 healthy cows of the Italian Friesian breed. The results demonstrate that the optimal concentration of cytochalasin B to obtain the highest frequency of binucleated cells was 6 μg/ml. The baseline frequency of spontaneous micronucleus formation in 500 binucleated cells was 12.3 ± 4.1, 3-times higher than that reported in human lymphocytes (Fenech and Morley, 1985a, and Scarfi *et al.* 1991).

As a comparative system, Erexson *et al.* (1987) developed a procedure to test mouse PBLs using the modified technique for assessing micronuclei frequency in human PBLs described by Fenech and Morley (1985a). In this assay, male mice (C57B1/6) were injected with five different doses (0, 2.5, 5.0, 7.5, or 10.0 mg) of diaziquone (AZQ)/kg. After 24 h the mice were bled by cardiac puncture, PBLs were isolated on a Ficoll-density gradient and then cultured in RPMI 1640 medium using 8 μg of PHA/ml. In

some cultures cytochalasin B was added at 21 h during the medium change to block cytokinesis. In other cultures, cytochalasin B was omitted to compare the sensitivity of analysing micronuclei in binucleate versus unblocked mononucleate cells. All doses of AZQ yielded significant increases in micronuclei-containing binucleated PBLs. The use of cytochalasin B in the mouse PBL MN assay increased the sensitivity by approximately 3-fold. The authors concluded that the MN assay in mouse PBLs should be useful in comparative cytogenetic studies of mice and humans.

The review above clearly indicates that environmental agents and lifestyle factors do influence the frequency of micronucleus and thus affect the DNA repair mechanism. In the following section (Section 2.4), the COMET assay which has been employed to assess any possible damage to the DNA as a consequence of ELF EMF exposure is discussed. Section 2.4 includes definitions of comet and COMET assay, mechanism of comet tail formation, use of COMET assay as a tool in the studies of testing genotoxicity, DNA damage, and effects of EMF exposure. It is concluded that the COMET assay should be included in the present study.

2.4 The COMET Assay

2.4.1 Introduction

The COMET assay is one of several methods developed over the last two decades to measure DNA strand breaks produced in individual cells. This assay, which is also known as the single-cell gel assay (SCG) or microgel electrophoresis (MGE), is an effective test for measuring genotoxicity, investigating DNA repair and apoptosis, or monitoring populations for exposure against an environmental agent with clastogenic or genotoxic potentiality.

In 1978, Rydberg and Johanson first estimated DNA damage in individual cells by a novel technique. In their experiment, human lymphocytes were mixed in agarose to make microgels on microscopic slides. They then lysed the cells and unwound DNA using sodium hydroxide. The number of pieces of single stranded DNA that were generated under these alkaline conditions depended upon the number of breaks in the

double stranded DNA. Using acridine orange (AO), an intercalating metachromatic dye (AO fluoresces green when bound to double stranded DNA and red when bound to single stranded DNA at optimum concentrations), they quantified greenness and redness for estimating DNA damage.

In 1984, Östling and Johanson introduced a microgel electrophoresis technique under neutral conditions for detecting DNA damage at the level of a single cell after γ-irradiation. In their technique, cells embedded in agarose were placed on a microscope slide, lysed by detergents and high salt, and the liberated DNA electrophoresed under neutral conditions. Ethidium bromide was used to stain the DNA. The image obtained looked like a "comet" with a distinct head containing intact DNA, and a tail comprising damaged or broken pieces of DNA. Hence the name "COMET" assay was given. The extent of DNA liberated from the head of the comet was a function of the dose of irradiation. However, this procedure is able to detect only DNA double-strand breakages (DSBs). A short time later, the alkaline version of the COMET assay was introduced by Singh *et al.* (1988). In this version of the COMET assay, electrophoresis was performed under high alkaline (pH > 13) conditions for detecting DNA damage in single cells. Unlike the former method, this assay could detect DNA single-strand breakages (SSBs) and DSBs, alkaline-labile sites that are expressed as SSBs, and transient DNA strand breaks arising due to DNA repair processes. Under certain conditions, the assay can also detect DNA-DNA and DNA-protein crosslinking, which appears as a relative decrease in DNA migration compared with concurrent controls (Hartmann *et al.* 2003). Due to its high pH, which denatures the DNA, fragments caused by SSBs are released so they can migrate through a gel when exposed to an electric current. This has made the assay more sensitive than the neutral version and it has become the assay of choice for DNA damage investigators. This assay was used in the current study.

Evidence of DNA damage is gained by observing the cell under an epifluorescent (or similar) microscope and information can be gained using quantitative or qualitative methods. The comet tail can be analysed qualitatively according to its DNA content (indicated by the intensity of the stain) and by comparing this value to control (healthy)

cells. Scoring can be made according to low, medium, or high intensity tail DNA content.

Quantitative methods involve recording values such as tail migration distance, image length, nuclear size, and using these values to calculate the tail moment, which is a good indicator of DNA damage. Several software packages (such as Komet) are available and with the aid of a fluorescent microscope and a digital camera these quantitative values can be quickly analysed.

The COMET assay has widespread applications in genotoxicity testing compared with other assays. This is because: 1) it is highly sensitive for detecting low level of DNA damage; 2) it has widespread applications in genotoxicity testing (Tice, 1995; Anderson *et al.* 1998; Rojas *et al.* 1999); 3) it requires a small number of cells per sample; 4) it is relatively inexpensive and studies can be performed even with a low budget; 5) it is easy to apply; and 6) samples can be prepared and evaluated very quickly by visual scoring or by an image analytical system (Collins *et al.* 1993).

2.4.2 Mechanism of Comet Tail Formation

The application of the alkaline COMET assay in different genotoxicity studies has increased exponentially although the underlying mechanism of comet tail formation has not been entirely resolved. Östling and Johanson (1984) first proposed that strand breaks might be responsible to stretch out DNA loops during electrophoresis, and hence form the comet tail. Relaxation of loops was also proposed to be the primary underlying basis for comet formation under alkaline conditions (Collins *et al.* 1997). On the other hand, Klaude *et al.* (1996) presented an excellent approach to demonstrate that the comet tail under neutral conditions consists of relaxed loops, whereas, comet tails under alkaline conditions consist of DNA fragments.

2.4.3 The COMET Assay in Genotoxicity Testing

DNA strand break assays have been used to test the genotoxicity of various chemical and physical agents in a variety of cells. Peripheral blood is the usual source for screening a large number of subjects exposed to genotoxic agents. DNA single strand breaks and alkali labile sites are easiest to detect and are by far the largest number of lesions in DNA in general. They are relevant to human health as they have been correlated with mutagenicity, teratogenicity, cancer, aging (Newton *et al.* 1989; Elia *et al.* 1991; Chicca *et al.* 1996 and Ames, 1998) and cell death (Sorensen *et al.* 1998).

The majority of X-ray-induced lesions are SSBs or alkali labile sites, which are converted to SSBs under alkaline conditions (Moran and Wallace, 1985) and can be detected by alkaline microgel electrophoresis. Similarly, other carcinogenic chemical or physical agents produce oxidative lesions in DNA and most of these lesions are either DNA SSBs or alkali labile sites (Averbeck *et al.* 1993 and Melvin *et al.* 1998). DNA DSBs are more important but less frequent lesions in DNA. Their repair is more complicated and even a few unrepaired breaks lead to cell death (Tounekti *et al.* 1995; Ahmad *et al.* 1998). DNA DSBs also have been correlated to mutagenicity, carcinogenicity, teratogenicity and aging (Chicca *et al.* 1996; Mayer *et al.* 1989 and Lieber *et al.* 1998).

Many types of cells are suitable for detection of SSBs / alkali labile sites using the alkaline microgel electrophoresis assay. For example, single cells from invertebrates (Salagovic *et al.* 1996) and plants (Koppen and Verschaeve 1996) can be analysed for DNA damage using the COMET assay. The neutral comet method has been used to identify DNA damage in irradiated meats, fish and seeds (Creda *et al.* 1997). The COMET assay is such a sensitive tool to detect DNA damage that SSBs caused by as low as 5 cGy irradiation is possible without the necessity of radiolabeling DNA (Singh *et al.* 1994; Malyapa *et al.* 1998). Sperm cells, however, naturally carry abundant alkali-labile sites (Singh *et al.* 1989). Therefore, it was not possible to see the effects of even very high doses of radiation in well lysed and proteinase-K-treated cells in microgels. In 1997, Singh and Stephens introduced a neutral version of the microgel electrophoresis assay to detect X-ray-induced DNA damage in human lymphocytes (Singh and

Stephens, 1997). Using this neutral version they were able to detect DNA DSBs in sperm cells (Singh and Stephens, 1998).

Cell viability is an important factor in COMET assay procedures and greater than 95% viability should be considered for analysis if only 50 cells per sample are analysed (Singh, 2000). It is not possible to assess cell viability on cells from solid tissues (Singh & Lai, 1998). This is due to the fact that most of the techniques which involve physical separation of cells from tissues, are associated with the disruption of cell membranes at desmosomal (cell to cell junctions) level. In the present study, in each slide, 10 µl of cell suspension having approximately 10,000 cells was mixed with 90 µl of Low Melting Temperature Agarose (LMA 1% per volume, Life Technologies Inc.).

Comparative investigations were performed between the COMET assay and the SCE test, which is known to be very sensitive in detecting chemical mutagens (Hartmann *et al.* 1994; Hartmann and Speit, 1995). For most of the chemicals these researchers observed similar sensitivity for the two assays. The only exceptions were chemicals inducing DNA-crosslinks, which were not sensitively detected with the standard version of the COMET assay. Meanwhile, modifications were introduced which can not only detect DNA-DNA or DNA-protein crosslinks, but even enable the investigator to distinguish between the different types of crosslinks (Pfuhler *et al.* 1996; Merk and Speit, 1999).

In another comparative study, Betti *et al.* (1994) compared the COMET assay and SCE to determine the better method for detecting DNA damage. They used these methods to establish if damage had occurred in the DNA in individuals as a result of smoking. They took samples from one smoker and one non-smoker of the same ages every 3 months for a year and found that the comet tail of lymphocytes taken from the smoker was significantly longer than that of the non-smoker. This meant that the smoker's DNA had been damaged more than that of the non-smoker. When comparing the COMET assay and SCE they were able to detect the effects of smoking in a small sample using the COMET assay where SCE analysis had failed.

2.4.4 EMF Exposure and COMET Assay

Lai and Singh (1997b) exposed live rats to a 60 Hz MF (flux densities 0.1, 0.25 and 0.5 mT) for 2 h. With the aid of the COMET assay method at 4 h post-exposure they observed a dose-dependent increase in DNA strand breaks in brain cells. An increase in single-strand DNA breaks was observed after exposure to MFs of 0.1, 0.25, and 0.5 mT, whereas an increase in double-strand DNA breaks was observed at 0.25 and 0.5 mT. Similarly, exposure to RFR, 2450 MHz, at a whole body specific absorption rate (SAR) of 0.6 and 1.2 W/kg for 2 h caused an increase in both SSBs and DSBs in DNA of brain cells in the rat (Lai and Singh 1995, 1996).

In another EMF study, Ahuja *et al.* (1999) exposed HPBLs to 5 doses (2, 3, 4, 5 and 10 mT, 50 Hz) of MF and they observed significant increase in DNA strand breaks compared to sham-exposed controls in each magnetic flux density except one. In this study comet tail lengths were used as a measure of DNA damage. Using the COMET assay, Svedenstal *et al.* (1999a) also observed an increase in DNA strand breaks in brain cells of mice after 32 days of exposure to MFs at a low intensity of about 8.0 μT. Similarly, an increase in DNA strand breaks in brain cells of mice after 14 days of exposure to a low intensity of 0.5 mT MFs were observed by Svedenstal *et al.* (1999b).

Human diploid fibroblasts were used in a study by Ivancsits *et al.* (2002) in order to evaluate the genotoxic effects of ELF EMFs in the form of DNA SSBs and DSBs. In their study, human diploid fibroblasts were exposed continuously or intermittently (5 min field-on/10 min field-off) to EMFs (1mT, 50 Hz, sinusoidal) for 24 h and then subjected to both the alkaline and the neutral COMET assays. They observed a significant increase in DNA DSBs only for intermittent fields compared to sham-exposed controls. There was no observed difference between the continuous field-exposed and the sham-exposed cells.

2.4.5 Evaluation and Interpretation of Results

The widespread applicability of the COMET assay in genotoxicity testing is reflected by the wealth of data published in the last few years. However, Collins *et al.* (1997) proposed a precaution with the interpretation of increased effects in the COMET assay.

According to them, the COMET assay detects primary DNA lesions, such as DNA strand breaks. These lesions may not be of much relevance since they may be repaired error-free or represent transient repair sites. In order to study comet-effects in more detail, Collins *et al.* (1997) proposed to use lesion-specific enzymes in human monitoring studies. Furthermore, COMET assay data from biomonitoring studies should always be discussed in conjunction with results of other tests run in parallel. Interestingly, in a study by Hartmann *et al.* (1998) with more than 90 individuals they observed increased comet values in leukocytes of waste disposal workers and also detected increased CAs, but observed no correlation between these two parameters.

2.4.6 DNA Damage and Use of COMET Assay

The use of pesticides has been increasing in recent years, resulting in the need for increased production of pesticides. However, some pesticides may represent a hazard to human health, especially by causing cancer. In one study, Grover *et al.* (2003) evaluated the genetic damage in workers employed in pesticide production using the COMET assay. Blood leukocytes of a group of 54 pesticide workers and an equal number of control subjects were used. The two groups had similar mean ages and smoking prevalence. The mean comet tail length was used to measure DNA damage. The exposed workers had significantly greater mean comet tail lengths than those of controls. Smokers had statistically significant larger mean tail lengths than non-smokers did. Further analysis of covariance showed that occupational exposure and smoking had significant effects on mean tail length, whereas age and gender had no effect on DNA damage.

In another study, higher DNA damage was observed by Baltaci *et al.* (1998) in a group of women who suffered from habitual abortion compared to normal controls. A statistically significant difference in the tail lengths was observed using the COMET assay on the blood samples obtained from these groups. Although these women did not suffer from any systematic disorder, other than their abortion history, it seems that chromosomal abnormalities and DNA damage detected by the COMET assay plays an important role in women with a history of recurring abortions. However, it was not determined if emotional stress is a factor in these cases.

One of the most important aspects of cancer is the loss of genomic stability. To assess genomic instability Colleu-Durel *et al.* (2001) studied 19 patients who suffered from sporadic breast cancer. The COMET assay was used to study the lymphocytes of patients before radiotherapy and/or chemotherapy. The study was conducted on cells which were or were not exposed to various levels of irradiation. The results show that the patients have higher baseline values than controls. At an exposure of 2.0 Gy, damage score, the mean tail moment, and the percentage of DNA in the tail increased for both groups but these values are much higher for patients. It is clear from their results that the lymphocyte DNA of cancer patients is more easily damaged than that of controls of the same group, and subsequently, a hypothesis was put forward that the baseline DNA damage reflects a genomic instability in sporadic breast cancer. This instability seems to increase following *in vitro* irradiation.

An interesting study on the use of the COMET assay in studying the relationship between EMF exposure and apoptosis was conducted by Robison *et al.* (2002). They found that EMF exposure protects heat induced apoptosis in human cancer cell lines in a time dependent manner. In their study, HL-60, HL-60R, and Raji cell lines were grown in a 0.15 mT 60 Hz sinusoidal EMF for 4 to 24 h. After induction of apoptosis, cells were analyzed by the neutral comet assay to determine the percentage of apoptotic cells. To discover the duration of this protection, cells were grown in the EMF for 24 h and then removed for 24 to 48 h before heat shock and neutral comet assays were performed. Their results showed that EMF exposure offers significant protection from apoptosis after 12 h of exposure and that protection can last up to 48 h after removal from the EMF. They also demonstrated the effect of the EMF on DNA repair rates. DNA repair data were gathered by exposing the same cell lines to the EMF for 24 h before damaging the exposed cells and non-exposed cells with H_2O_2. Cells were allowed to repair for time periods between 0 and 15 min before analysis using the alkaline comet assay. Their results showed that EMF exposure significantly decreased DNA repair rates in HL-60 and HL-60R cell lines, but not in the Raji cell line.

In a similar study using HL-60 cells, Ding *et al.* (2004) found that although the magnetic field itself (60 Hz, 5mT) could not induce apoptosis, it did exert a promoting effect on H_2O_2-induced cell death.

2.5 Fluorescence *In Situ* Hybridization (FISH)

2.5.1 Introduction

Increased chromosomal translocation is one of the known features of radiation effects in humans, which has the potential to lead to ill health, particularly a variety of cancers or inherited defects which may be passed on to the next generation. In the present study, to investigate the incidence of translocation frequency in PBLs induced by EMF exposure, the fluorescent *in situ* hybridization (FISH) technique was employed. *In situ* hybridization was first introduced in 1969 simultaneously by two groups of scientists (Pardue and Gall, 1969; John *et al.* 1969). The technique has multiple uses in molecular morphology due to its unique capability of visualizing nucleic acid sequences without altering the cell's cytological or chromosomal integrity. FISH uses fluorescent molecules to vividly paint genes or chromosomes. This technique is particularly useful for gene mapping and for identifying chromosomal abnormalities. The usefulness of FISH is also growing rapidly in genomics, cytogenetics, prenatal research, tumour biology, radiation labels, gene mapping, gene amplification, and basic biomedical research.

In principle, the technique is quite straightforward. A stretch of DNA, which matches the area of interest in the genome, is labeled with nucleotides linked to a fluorochrome and this labeled DNA is called the probe. When double stranded DNA in the probe or in the cell is heated, the hydrogen bonds holding the strands together will open up. When the heated probe mixture is put onto heated cellular DNA that has been fixed on a slide, the probe will align with the matching cellular DNA. As the slide cools slowly, new hydrogen bonds will form between the probe strands and the cellular strands. When stimulated with the correct wavelength, the fluorochrome linked to the probe will light up the region of the cellular DNA of interest.

Although a number of different hybridization protocols exist, the variations among them are relatively minor. The basic steps are outlined below: (a) tissue is denatured to create single-stranded DNA; (b) denatured probe is applied to the slide or tissue; (c) slide or tissue is incubated overnight to allow hybridization / reannealing; (d) the hybridization reaction identifies, or labels, target genomic sequences. After washing and possible

signal amplification, their location and size is identified for the cellular DNA of interest and studied by fluorescent microscopy.

2.5.2 Advantage, and Limitations of FISH Analysis

FISH is applicable to several types of tissue preparations: fresh or frozen tissue, cytological preparations, previously Wright-stained smears (or fresh smears stored at -70C), frozen tissue sections, formalin-fixed paraffin-embedded tissue and previously immunostained cells (Krahl, 1999 and Christine *et al.* 2002). The main advantage of FISH lies in its relative rapidity with which it can be performed as compared to conventional metaphase cytogenetic evaluation. FISH also allows analysis of terminally differentiated tumour cells, which never enter mitosis, or tumours with low mitotic rate.

Conventional cytogenetic evaluation of such tumours is difficult or impossible. Another advantage lies in the increased number of cells studied when utilizing FISH, as opposed to metaphase cytogenetics. This ability to study large numbers of cells allows detection of low frequency abnormalities, such as detecting minimal residual disease or early relapse. On the other hand, FISH has limitations too. One of the main limitations of FISH as a molecular tool is signal fading. Furthermore, FISH requires significant technical expertise to obtain consistent results and a comprehensive set up with a good fluorescent microscope, computer, and a digital camera.

In terms of sensitivity and resolution, FISH is better than karyotyping and comparative genomic hybridization (CGH), but worse than polymerase chain reaction (PCR)-based assays for detecting small nucleotide alterations. CGH is a new approach of FISH (Kallioniemi *et al.* 1992) that allows one to screen losses and gains in DNA copy number along the entire genome in a single hybridization (Du Manoir *et al.* 1993). Conventional FISH procedure is limited to alterations of several Mb in size, whereas CGH can be designed to detect single-base mutations. Since FISH probes are typically at least 30 Kb in size, alterations need to be equally large for reliable detection. On the other hand, PCR is more sensitive than FISH for the detection of abnormal fusion transcripts resulting from translocation; it picks up as few as one per million cells.

2.5.3 Translocation Study and the Use of FISH

So far no studies have been reported on the application of FISH in cultured cells exposed to ELF EMFs. Therefore, the survey presented here illustrates the application of the technique in radiation studies using the ionizing section of the electromagnetic spectrum only.

In a radiation study, Lambert *et al.* (2001) used tricolour FISH with whole chromosome-specific probes for chromosomes 2, 4 and 8 for scoring translocations induced by iodine-131 therapy in thyrotoxicosis patients one year after the administration of the radiolabelled compound. The study was carried out on nine patients (one male and eight females). From the genomic translocation frequencies, derived using the Lucas formula, equivalent whole-body doses were calculated, based on the *in vitro* ^{60}Co γ-ray dose-response curve. The average radiation dose obtained for this group of nine patients was 0.79 ± 0.22 Gy.

A total of 101 translocations were observed in 4,864 metaphases in the patient group, 63% being of the two-way type. In the control group used for obtaining dose-response data, nine translocations were observed in 5,278 metaphases, 55% being two-way translocations. Although they observed 12 times higher translocation frequencies in the iodine-therapy patients groups than in the control group involving chromosomes 2, 4 and 8, no correlation was reported between the observed frequency of translocation and administered radioactivity. Furthermore, they observed that chromosome 4 contributed more in the formation of two-way translocation, which was detectable one year after the administration of the radiolabelled compound.

In another study, using FISH, Lucas *et al.* (1999a) studied the relationship between background chromosome translocation frequency and age with translocation frequency measured to a high statistical precision. They also determined the amount of background ionizing radiation which can cause the production of chromosome translocations in a control population. In their study, lymphocytes from 35 healthy control individuals (15 females and 20 males) were used to measure the frequency of

chromosomal translocation. These control subjects were of varying ages, ranging from 0 (cord blood) to 98 years.

They reported that the background translocation frequency in control individuals follows a curvilinear relationship with age. No significant variation was observed between individuals of the same age. Clastogenic processes of normal aging and physiological factors in additional to ionizing radiation play a major role in the production of chromosome translocations in a control population. Background radiation, however, appears to play a minor role in chromosome translocation production in control individuals living near sea level.

Furthermore, a study to detect complete and incomplete chromosomal exchanges (translocations) in human lymphocytes (Deng & Lucas, 1999) combined FISH with pan-telomeric peptide nucleic acid (PNA) and whole chromosome-specific DNA probes. In their study, human lymphocytes were irradiated *in vitro* with 0.9 Gy low dose-rate (0.019 Gy/h) tritium beta-rays.

After hybridization, all three pairs of labelled chromosomes together with 92 telomeres were readily visible. The whole chromosomes 1, 2 and 4 were painted orange, and all telomeres were painted green. Unpainted chromosomes were counterstained with blue. In the observed 680 CAs induced by tritium beta-rays in human lymphocytes after 52 h of culture, no evidence of telomere addition was detected. Incomplete and hidden complete exchanges and terminal deletions were definitively discriminated. According to the authors, this was the pioneer study in the simultaneous detection of telomeres and specific whole chromosomes to analyse accurately the complete and incomplete chromosome exchanges involving painted chromosomes in human lymphocytes.

In a similar study using FISH, Lucas *et al.* (1999) measured the effects of incubation temperature during irradiation, and of donor age, on the *in vitro* induction of chromosomal translocations in human lymphocytes. In their study, six human males were used as lymphocyte donors. Lymphocytes were exposed over 48 h continuously at 37 or 20C to tritium beta-rays or ^{60}Co gamma-rays.

They observed no age-related difference in the alpha coefficients of the fitted induction curves for gamma-ray-exposed lymphocytes obtained from four donors whose ages ranged from 24 to 79 years, or for tritium beta-ray-exposed lymphocytes from two donors aged 36 and 62 years. The conclusion of the study is that the S-ratio (the ratio of induced complete to incomplete translocations) was found to be independent of radiation dose, donor age and exposure temperature.

Natarajan *et al.* (1992) used *in situ* hybridization with chromosome-specific DNA libraries to analyse radiation-induced stable translocations in HPBLs. These data were compared with radiation-induced unstable-type aberrations (dicentrics) in the same samples. The results indicate that far more stable aberrations are induced by radiation in comparison to unstable aberrations.

In another study, Stephan and Pressl (1997) used FISH to determine the yield of symmetric and asymmetric exchange aberrations in metaphase chromosomes after *in vitro* exposure of PBLs to 250 kV X-rays (0-3.0 Gy). For the aberration analyses, chromosomes 2, 4 and 8 and all centromeres were painted. Centric rings amounted to about 8% of the dicentric yield. The proportion of inversions and insertions was about 5% of the total translocations. Regarding the spontaneous levels, the frequency of total induced translocations was higher by a factor of 1.13 than that of dicentrics. The involvement of chromosomes 2, 4 and 8 in translocations is significantly different from the expected ratio concerning physical length. Furthermore, the frequency of translocations was evaluated in three radiation workers who received an accidental radiation exposure 11 years previously. About 75% of the translocations were identified as complete in comparison with 79% in the *in vitro* experiments. In the radiation workers chromosome 2 showed an under-representation in translocations, whereas chromosome 4 was over-represented as in the *in vitro* experiments. The summarized results for the radiation workers showed a mean genomic translocation frequency of 13.4 per 1000 cells. This frequency is not significantly different from the mean frequency of dicentrics which were determined by conventional Fluorescent Plus Giemsa (FPG) staining, after detection of the accidental radiation exposure about 11 years previously (8.6 dic/1000 cells). There were, however, some differences between

individuals affecting this comparison. The distribution patterns of dicentrics showed an over-dispersion, whereas the translocations occurred single in cells.

Hsieh *et al.* (1999) used FISH to measure the alpha coefficient, the initial slope of the translocation dose-response curve, for chromosome translocations in human lymphocytes exposed to chronic ^{60}Co γ-rays at body temperature. In their study, human lymphocytes from two 24-year old male blood donors were exposed to 0, 0.32, 0.62 and 0.92 Gy of chronic ^{60}Co γ-rays under conditions that reduce the metabolic stress to the cells. Chromosome translocation frequencies were measured using FISH with a whole-chromosome probe cocktail specific for chromosomes 1, 2, 4 (orange) and 3, 5, 6 (green). A total of 72,383 metaphases were analysed (33,429 in exposed cells) in two donors. The shape of the dose-response curves for translocations was linear, and alpha coefficient was measured as 0.024 ± 0.002 translocations per cell per Gy for the combined data. The authors concluded that the alpha coefficients measured after chronic exposure were in good agreement with that reported in the literature for acute, low-dose exposure of human lymphocytes to ^{60}Co γ-rays.

In the above review, the utilization of the FISH technique is seen to be comprehensively successful in detecting different types of translocation as a consequence of ionizing radiation exposure, which thus justifies its application in the current study.

Chapter Three

Materials and Methods

3.1 Sister Chromatid Exchange (SCE)

In this study, the frequency of SCEs from two rounds, round-1 (R1) and round-2 (R2), of experiments was studied in peripheral blood lymphocytes from six healthy, non-smoking male blood donors. Donors were selected with similar age, lifestyles, medical history, and dietary history. Ages of the first three donors ranged from 50-56 years and the remaining three donors ranged from 31-44 years. A set of eight experiments was conducted in duplicate with each duplicate experiment using the same strict EMF conditions. These two sets of eight experiments each were called R1 and R2. The eight experiments in R1 were conducted using blood samples from the first three donors and blood samples from the remaining three donors were used in the R2 experiments.

3.1.1 Collection of Blood Samples

Blood samples were obtained (with ethical approval) by venipuncture from the six healthy human male adult donors. Each donor was required to complete a detailed questionnaire (Appendix 2). Donors were also required to complete a consent form (Appendix 3) allowing blood samples (about 10 ml) to be drawn by a qualified haematologist (Mr. Chris Kendrick of the Institute of Molecular BioSciences at Massey University) for analysis. Two heparinised tubes were used to collect blood samples from each donor: one tube was used to perform computerized blood analysis to obtain the white blood count (WBC), and the second tube was used for blood culture. The blood specimens were then gently mixed to prevent clotting.

3.1.2 SCE Protocol

Generation of EMFs

The incubator, which was used to deliver EMFs (Figure 3.1-A), were constructed, calibrated and designed by Mr. B. I. Rapley of the Institute of Technology and Engineering (Massey University, Palmerston North, New Zealand). To generate low power, a laboratory power amplifier, 0.22 V/AMP (Figure 3.1- i) was used, whereas an AC/DC power amplifier was used to generate high power (Figure 3.1-iv). To generate sine and square waves a programmable time base generator was used (Figure 3.1-ii). The oscilloscope (Figure 3.1-iii) was used to check the wave form (sine, square) accuracy. To generate the 'pulsing wave form' a programmable pulse-width modulator (Figure 3.1-v) was used. Culture tubes were housed in a compartment within a pair of Helmholtz coils (Figure 3.1-vi, and 3.1C). A computer (Figure 3.1-vii) was used to generate the 'complex' wave. The incubator temperature (37C) was generated by six 150 W bulbs (Figure 3.1-xi) and was regulated by a thermostat (Figure 3.1- viii). The incubator was placed in an electrically shielded room, and well away from any MF sources.

Field exposure

The culture tubes of controls were placed between a pair of parallel Helmholtz coils (Figure 3.1-B & C) placed at a 20° angle to the horizontal to facilitate cell growth in the incubator. These cells received no exposure (sham) to EMFs. However, the culture tubes of EMF-exposed experiments were placed between the same pair of parallel Helmholtz coils identically to the controls with the exception that during the whole culture time, the culture tubes were exposed to alternating 50 Hz EMFs with the following characteristics:

Wave type	- Sine or Square or Complex
Wave form	- Continuous or Pulsed (4 sec on and 4 sec off)
EMF strengths	- 1μT or 1mT

For the complex experiment, lymphocytes were grown in the presence of a complex MF produced from the back of a computer (Figure 3.1-vi). There was no ambient AC EMFs in the chamber of the incubator where the Helmholtz coils were placed.

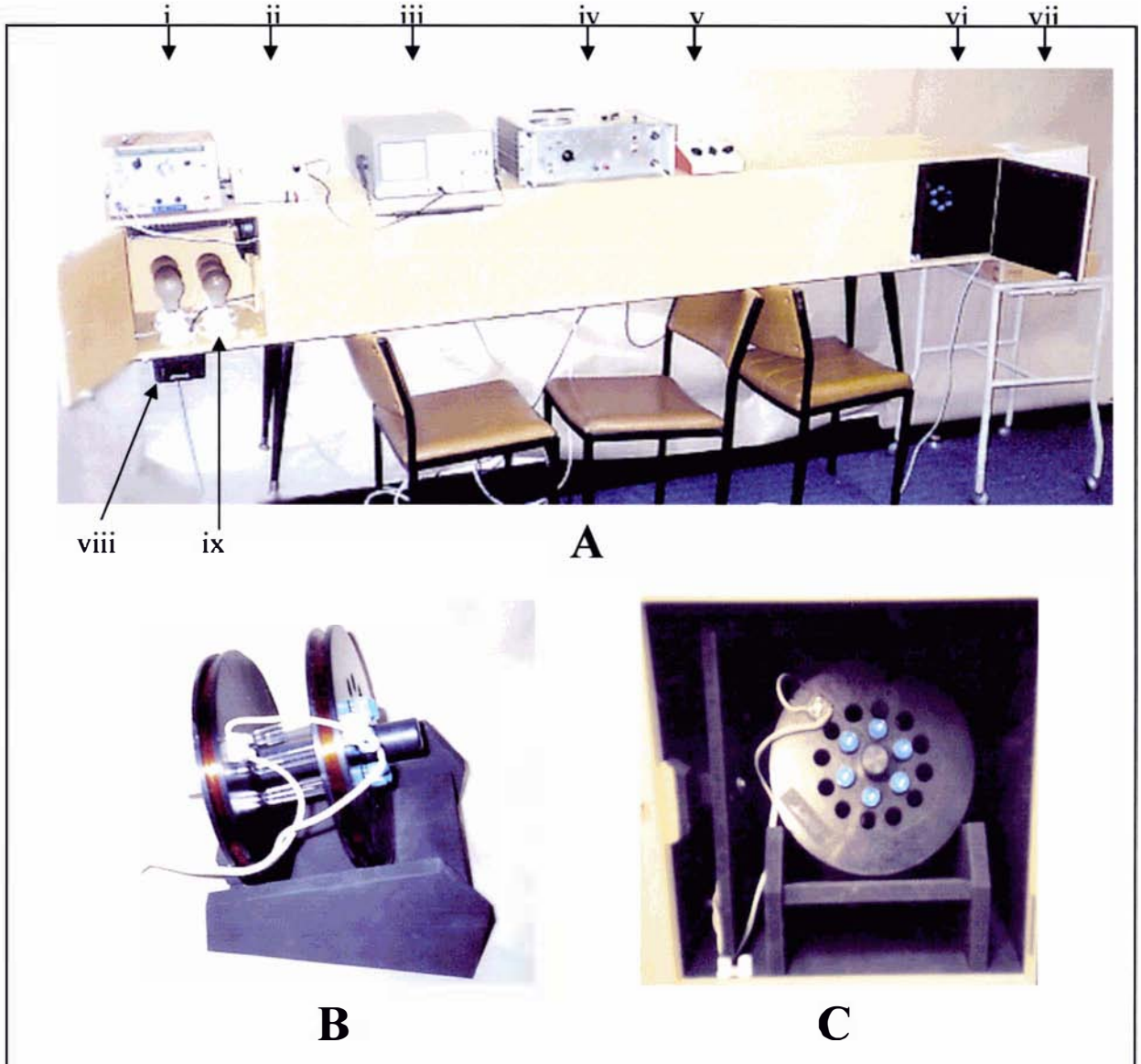


Figure 3.1: A. Incubator apparatus (i) Laboratory power amplifier-low power, 0.22 V/AMP, (ii) Programmable time base generator, (iii) Oscilloscope, (iv) Power amplifier AC/DC-high power, (v) Programmable Pulse-width modulator, (vi) Culture tube compartment with thermostat, and (vii) Computer. **B & C.** Side view and front view of culture tube placement between a pair of Helmholtz coils. (viii) Temperature control of thermostat, and (ix) Bulbs.

Helmholtz coil and EMF

Description of the coil

Wire type	: enameled copper
Wire diameter	: 0.7 mm
Total turns	: 100
Coil diameter	: 180 mm

Coil modeling

The MF produced by the induction coil (described above) was modeled using the EMF simulation programme, MagneSim, developed by Brent Foster and Wyatt Page for Resonance Research. MagneSim models the MFs produced by circular form: single turn; solenoid; Maxwell and Helmholtz pairs. The numeric solution is accurate to within ± 1% of the actual values as determined by measurement (in the 'Z'-axis).

The plane of calculation

The MF produced by a circular form induction coil exists as a three dimensional phenomenon in space surrounding the coil. Circular symmetry exists about the Z (or long) axis, which means that it is only necessary to determine the field in a two dimensional plane. This plane may then be rotated about the Z-axis to model the field in three-dimensional space. The calculation plane is shown in Figure 3.2.

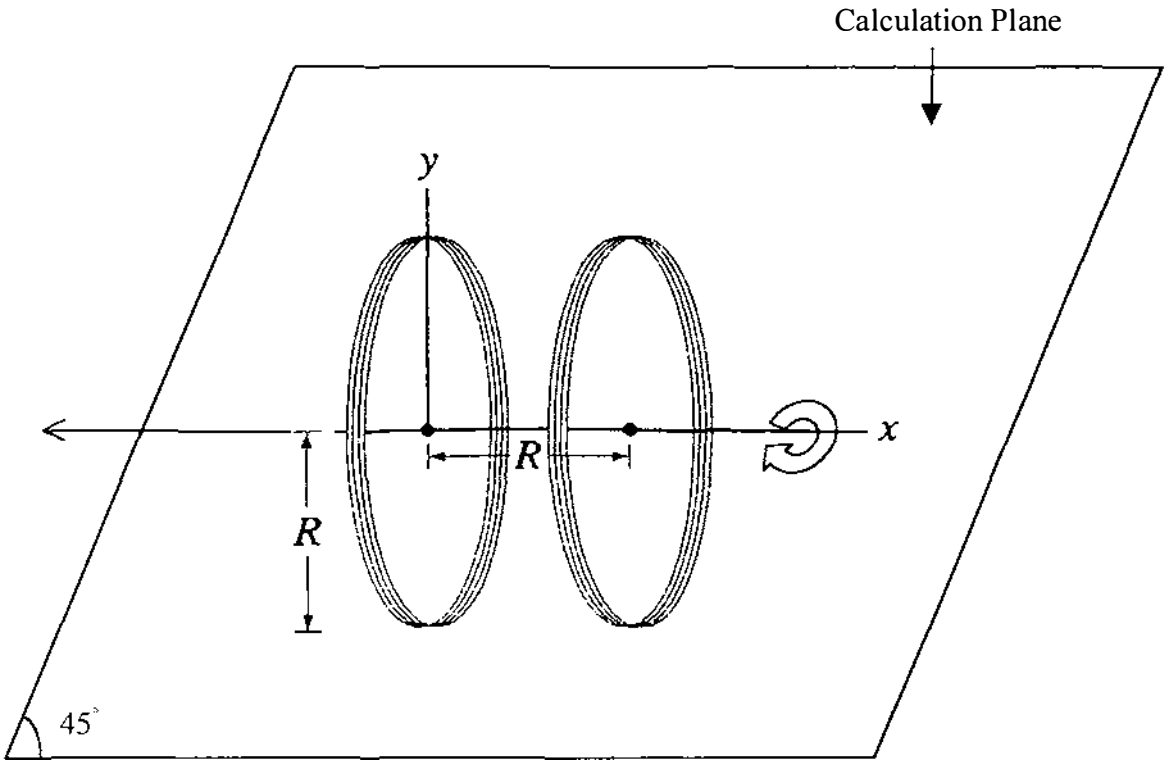


Figure 3.2: Helmholtz coils: Two coils of wire, each with 100 turns and separated by a distance equal to the radius (R), 90 mm.

The calculation matrix

MagneSim models the MF of a coil using a 10x10 matrix of points on a two-dimensional plane about the Z-axis, thus bisecting the coil along the long axis. All points are equally spaced along their axis. However, the x and y axes may be of arbitrary length. The calculation matrix may exist on any part of the calculation plane and is not restricted to having the coil in the centre. Indeed it is possible to choose an area anywhere either within or outside the coil. The only constraint is that the points are automatically equally spaced in the x and y direction. If a calculation point of the MF in the calculation plane falls directly on the geometric x-y position of a turn of wire, MagneSim returns a "?", as the magnitude is, by definition, incalculable.

The EMF vector

To visualize the MF, MagneSim draws a 'lollipop' plot of the MF matrix points on the calculation plane of the coil. The field at each point is represented by a 'lollipop', that is, a point with a stick attached. The 'point' of the lollipop defines the exact geometric position on the calculation plane where the field is calculated, whereas the 'stick' represents the field magnitude and the direction in vector form. The length of the stick represents the magnitude of the field so that the largest value represents two matrix units against which all other field values are auto-scaled. For example, a line drawn from a dot directly parallel to the Z-axis pointing to the right represents a vector at zero degrees Cartesian. The lollipop plot of the coil is shown in Figure 3.3.

Magnitude of the EMF resultant vector

The magnitudes of the MF vectors for the Helmholtz coils used in this research are shown below. The magnitude of resultant vectors inside the two culture tubes (shaded in green colour) from a 1mT and a 1μT exposed experiment are shown in mT (0.9516-1.1326) (Figure 3.4) and in μT (0.9516-1.1326) (Figure 3.5) for the pair of Helmholtz coils.

Direction of the MF resultant vectors

Figure 3.6 shows the directions of the resultant magnetic field vectors in Cartesian coordinates, which are the same for all exposure levels within the Helmholtz coils.

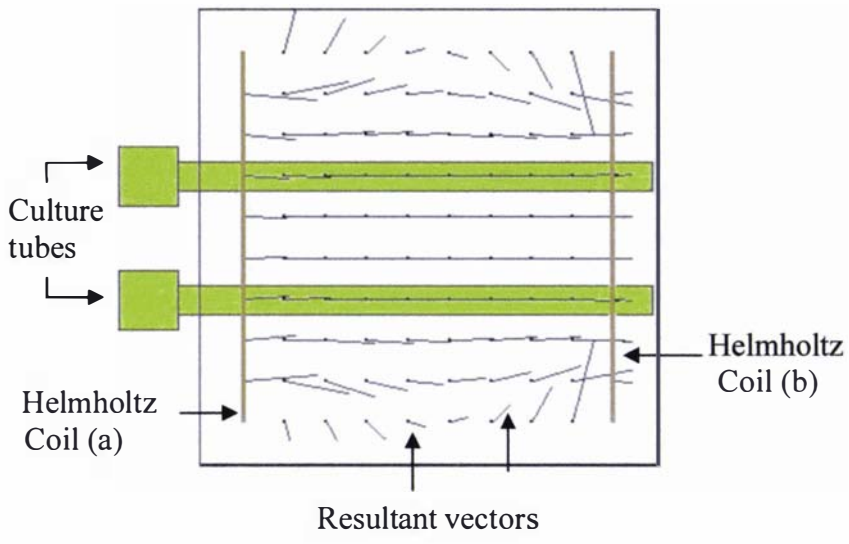


Figure 3.3: Lollipop-plot of the resultant vectors in a longitudinal section through the 'Z-axis' of a Helmholtz coil. The shaded area (green) shows the relative position of the culture tubes with the three-dimensional coil, while the vector arrows display the angle, in the plane of the coil, at the resultant magnetic fields generated. Arrow lengths are auto-scaled to represent the relative field strength in gauss.

?	1.8755	0.8647	0.5308	0.3960	0.3960	0.5308	0.8647	1.8755	?
1.6205	1.4282	1.1130	0.8864	0.7764	0.7764	0.8864	1.1130	1.4282	1.6205
1.1326	1.1219	1.0614	0.9935	0.9516	0.9516	0.9935	1.0614	1.1219	1.1326
0.9960	1.0183	1.0169	1.0047	0.9460	0.9460	1.0047	1.0169	1.0183	0.9960
0.9500	0.9811	0.9954	0.9993	0.9991	0.9991	0.9993	0.9954	0.9811	0.9500
0.9500	0.9811	0.9954	0.9993	0.9991	0.9991	0.9993	0.9954	0.9811	0.9500
0.9960	1.0183	1.1069	1.0047	0.9460	0.9460	1.0047	1.1069	1.0183	0.9960
1.1326	1.1219	1.0614	0.9935	0.9516	0.9516	0.9935	1.0614	1.1219	1.1326
1.6205	1.4282	1.1130	0.8864	0.7764	0.7764	0.8864	1.1130	1.4282	1.6205
?	1.8755	0.8647	0.5308	0.3960	0.3960	0.5308	0.8647	1.8755	?

Figure 3.4: Magnitude of resultant vectors in 'mT' for the pair of Helmholtz coils.

?	1.8755	0.8647	0.5308	0.3960	0.3960	0.5308	0.8647	1.8755	?
1.6205	1.4282	1.1130	0.8864	0.7764	0.7764	0.8864	1.1130	1.4282	1.6205
1.1326	1.1219	1.0614	0.9935	0.9516	0.9516	0.9935	1.0614	1.1219	1.1326
0.9960	1.0183	1.0169	1.0047	0.9460	0.9460	1.0047	1.0169	1.0183	0.9960
0.9500	0.9811	0.9954	0.9993	0.9991	0.9991	0.9993	0.9954	0.9811	0.9500
0.9500	0.9811	0.9954	0.9993	0.9991	0.9991	0.9993	0.9954	0.9811	0.9500
0.9960	1.0183	1.1069	1.0047	0.9460	0.9460	1.0047	1.1069	1.0183	0.9960
1.1326	1.1219	1.0614	0.9935	0.9516	0.9516	0.9935	1.0614	1.1219	1.1326
1.6205	1.4282	1.1130	0.8864	0.7764	0.7764	0.8864	1.1130	1.4282	1.6205
?	1.8755	0.8647	0.5308	0.3960	0.3960	0.5308	0.8647	1.8755	?

Figure 3.5: Magnitude of resultant vectors in 'μT' for the pair of Helmholtz coils.

?	75	61	42	16	344	318	299	285	?
356	11	17	14	5	355	346	343	349	4
355	0	3	3	1	359	357	357	360	5
357	359	360	0	0	360	360	0	1	3
359	359	360	360	0	360	0	0	1	1
1	1	0	0	360	0	360	360	359	359
3	1	0	360	360	0	0	360	359	357
5	360	357	357	359	1	3	3	0	355
4	349	343	346	355	5	14	17	11	356
?	285	299	318	344	16	42	61	75	?

Figure 3.6: Angles in Cartesian co-ordinates of resultant magnetic field vectors relating to the pair of Helmholtz coils.

Warming effect of the Helmholtz coil

One of the most important variables that must be considered when conducting experiments on weak EMFs is temperature. Experiments conducted by the Bioelectromagnetics Research Team at Massey University have previously investigated possible warming effects and possible temperature fluctuations in the design of the Helmholtz coil used in the current series of experiments which were conducted at 37C. The data obtained showed no measurable increase in temperature around the coil at 50 Hz 1mT, the highest strength used in this study. Nevertheless, duplicate experiments have recently been conducted to confirm this finding. Two electronic probes were placed inside the culture chamber: one in the culture tube and one around the coil. The culture chamber was heated by a flow of warm air from a distant source and stabilized at 37C by a thermostat, the temperature at which the PBLs are cultured. Temperature readings were taken over a 24 h period with the coil turned off (control) (Table 1a, Appendix 7) and over another 24 h period with the coil turned on (50 Hz, AC) (Table 1b, Appendix 7). The graphs (Figures1a, 1b, 1c & 1d of Appendix 7) show no detectable differences in temperature fluctuations or warming effects within the chamber with the coil turned off or on. Any warming effect of the coil would indeed be very small (calculations of the heating capacity of the coil show that it contributes less than 1% of the heat to the chamber), and irrespective of this point, an independent heat source is needed to elevate the temperature inside the culture chamber to 37C, which is the temperature required for lymphocyte growth.

Some researchers use a double-wound bi-filar coil in their EMF experiments, the rationale being that such a coil nullifies the magnetic field generated by the current. In our laboratory we designed and tested a simple double-wound, bi-filar coil and could detect a weak magnetic field some distance from the coil. The magnetic field does not disappear entirely. For the experiments conducted in this thesis the decision was made to turn off the current completely for our control experiments, the rationale being that one could then be confident in knowing that all magnetic fields generated by the coil were eradicated entirely.

Lymphocyte cultures

For each experimental series, blood samples from three of the six donors were used. Two culture tubes were set up for each donor. Each tube contained 5 ml of Medium-199 (GibcoBRL, Cat. No. 31100-035), 1 ml of fetal bovine serum, (GibcoBRL, Cat. No. 10093-136), and 0.1 ml of phytohaemagglutinin (PHA) M form (GibcoBRL, Cat. No.10576-015). Using the WBC count, calculations were made to obtain 3.25-million cells/per culture tube by adding approximately 0.3-0.6 ml of blood from the second samples. When conducting the SCE technique it is imperative that the WBC is constant (Bender *et al*, 1992b). 0.05 ml of 10^{-2} M 5-Bromodeoxyuridine (BrdU) (Sigma, B-9285) was added to each culture tube (Falcon, 8 ml polystyrene, round-bottom tube, 13 x 100 mm style). The culture tubes were incubated at 37C for 72 h, which included a treatment with colchicine (0.05%, BDH, Prod. 27805FM) for 1 h.

Harvesting

Harvesting of cells and slide preparation were accomplished using the modified Fluorescence-Plus-Giemsa (FPG) method (Perry and Wolf, 1975). Culture tubes were removed from the incubator after 72 h, mixed gently then centrifuged for 10 min at 1000 rpm. Supernatant was gently removed from the top leaving approximately 1 cm above the pellet. Deposits were mixed thoroughly with a vortex stirrer for 5 sec, to avoid clotting, then resuspended in 5 ml of warm KCl (0.075 M) hypotonic solution at 37C. The tubes were then mixed gently by inversion 6-8 times. The tubes were incubated at 37C in a water bath for 10 min to activate the water transport system (osmosis), which ultimately swells the lymphocytes. The tubes were centrifuged again for 10 min and the supernatant was removed from the top leaving approximately 1 cm above the pellet. Deposits were mixed well again with the vortex stirrer for 3 sec. They were then resuspended in 5 ml of acetic acid (6%) under constant agitation and kept for 5 min at room temperature. Cultures were once again centrifuged and the supernatant removed. Cultures were resuspended in 8 ml of ice cold fixative (Methanol : Acetic Acid = 3 : 1), centrifuged immediately and the supernatant removed. This last step was then repeated. Finally, after removing the supernatant to 5 mm without disturbing the pellet, 2-3 drops of fixative was added to give a cell suspension of light turbidity.

Giemsa block staining

Air-dried, freshly prepared slides were stained with 10% Giemsa (Gurr, BDH, Prod. 350864X) in Sorensen's buffer (pH 6.8) for 7-9 min, then rinsed in Sorensen's buffer for 2-3 min and air-dried. Slides were mounted using DPX. Figure 3.7, shows a Giemsa block stained c-metaphase chromosome spread from a control experiment showing a normal complement of 2n = 46.

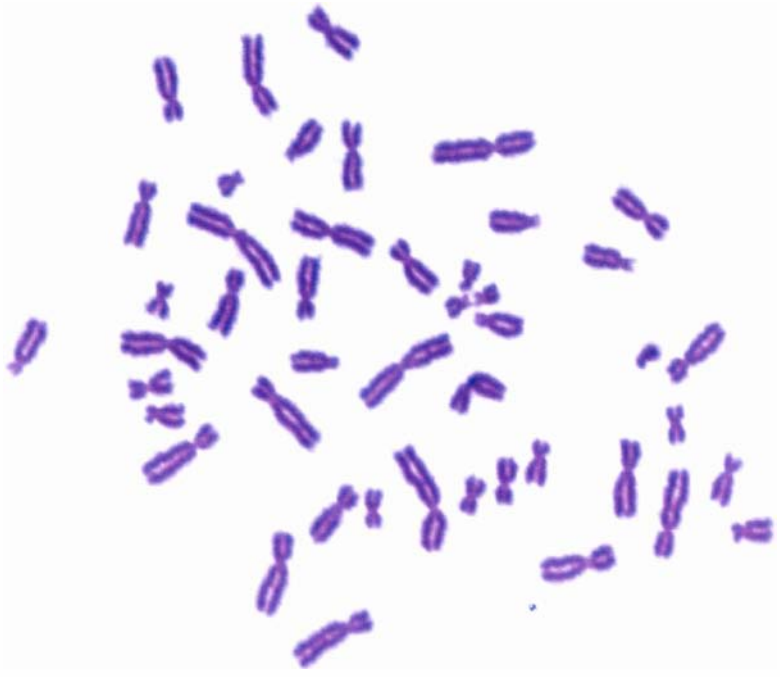


Figure 3.7: A Giemsa-stained c-metaphase chromosome spread of a dividing peripheral blood lymphocyte from a control experiment showing a normal complement of 2n = 46.

Fluorescence-plus-Giemsa (FPG) staining

Fluorescence-plus-Giemsa staining protocol was a modification of methods devised by Wolf and Perry (1975), and Sandberg and AnneMarie (1987). This procedure is based on UV sensitivity of heavily BrdU-labeled DNA. The one week old prepared slides were soaked in Sorensen's buffer solution (pH 6.8) for 5 min, rinsed in distilled water. The air-dried slides were mounted using fluorochrome Hoechst 33258 (bisbenzimide) solution (5 $\mu g\ ml^{-1}$) and covered with coverslips for 30 min (in dark). Hoechst 33258 staining photosensitizes degradation of BrdU-stimulated DNA, creating single-strand nicks. After 30 min coverslips were removed by rinsing with Sorensen's buffer and washed with distilled water (in dark). To avoid chromosome damage, slides were mounted in Macllvaine's buffer (pH 7.0), covered with coverslips, and illuminated to 356 nm UV light for 2.5 h (at a distance of approximately 10 cm). Coverslips were removed by rinsing with Sorensen's buffer and washed with distilled water (in dark). The slides were immediately incubated for 20 min in salt-sodium-citrate buffer (2 x SSC, pH 8.0) at 65C to elute small DNA fragments. Slides were stained in Giemsa (10%) (Gurr; BDH, Prod. 350864X) in Sorensen's buffer (pH 6.8) for 7-10 minutes then rinsed in Sorensen's buffer for 2-3 minutes. Air-dried slides were mounted in DPX.

Scoring criteria

The 10 microscope slides of every donor were randomly coded (a - j) by the researcher to ensure anonymity of samples and examined 'blind' (without knowledge of controls or experimental source). To eliminate bias, an independent person was involved in setting the EMF parameters, which were thus blind to the researcher. The slides were investigated serially. Two hundred consecutive second mitotic metaphase cells per donor, which showed good chromosome morphology, differential staining for SCE and no chromosome overlapping, were selected. The images were captured by a JVC 3-CCD Colour Video Camera using Silicon Graphics and Image Capture software and scored for SCE from the computer screen at 1000X magnification. SCEs were expressed per cell, so it was necessary for a full complement of 46 chromosomes to be present. SCEs were analysed according to guidelines of Swierenga *et al.* (1991) (see Figure 3.8).

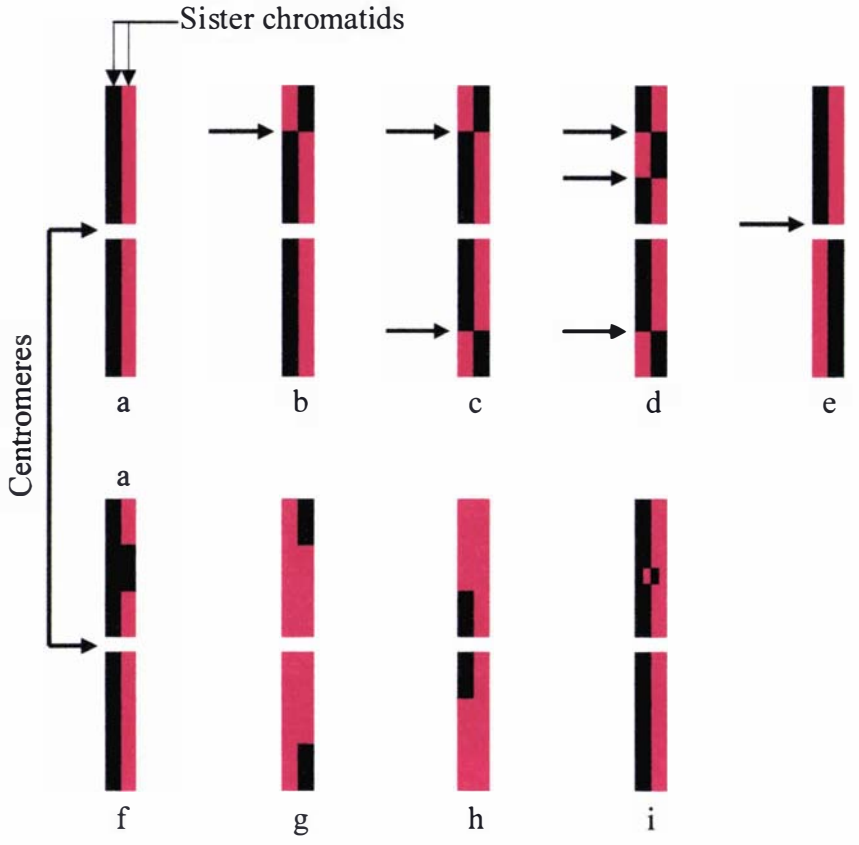


Figure 3.8: Diagrammatic representation of various differential staining patterns observed after incorporation of BrdU into replicating DNA: **(a)** No SCE, **(b)** a single SCE (arrow), **(c)** two SCEs, **(d)** three SCEs, **(e)** SCE at the centromere, **(f)** not counted as SCE, **(g & h)** incorporation of BrdU for more than two complete cycles of DNA synthesis, **(i)** reciprocal pattern of staining less than one chromatid in width and not counted as SCE (Swierenga *et al.*1991).

3.2 Micronuclei Assay (MN assay)

In the present study, the frequency of micronuclei (MN) from one round of experiments was studied in peripheral blood lymphocytes from the same three blood donors used in R1 of the SCE experiments. Only one set of eight experiments was conducted with the same strict EMF conditions as followed in R1 and R2 of the SCE experiments. For the MN assay experiments, the incubator that was used to conduct SCE experiments was used again.

Lymphocyte cultures for all experiments conducted for MN were exposed to 50 Hz sinusoidal (continuous or pulsed) or square (continuous or pulsed) MFs at field strengths of 1μT or 1mT for 90 h. Lymphocyte cultures of three controls were sham exposed. In the twelfth experiment of MN, lymphocyte cultures were exposed to a complex MF produced from the back of a computer, for 90 h. All experiments were performed 'single blind' as followed in the SCE experiments.

3.2.1 Collection of Blood Samples

Fresh blood samples (5 ml) were obtained by venipuncture by the same hematologist, Mr. Chris Kendrick. Only one heparinized tube was used to collect blood samples from each donor. The blood specimen was then gently mixed to prevent clotting.

3.2.2 MN Assay Protocol

Generation of EMFs

The same incubator (Figure 3.1) which was used to deliver EMF intensities for the SCE experiments was also used to conduct MN experiments.

Field exposure

The culture tubes of controls were placed between a pair of parallel Helmholtz coils (Figure 3.1, B and C) placed at a 20° angle to the horizontal in the incubator and received no exposure (sham) to EMFs.

The culture tubes of EMF-exposed experiments were placed between the same pair of parallel Helmholtz coils, identically to the controls, with the exception that during the whole culture time (90 h) the culture tubes were exposed to alternating 50 Hz EMFs with the following characteristics:

Wave type - Sine or Square or Complex
Wave form - Continuous or Pulsed (4 sec on and 4 sec off)
EMF intensities - 1μT or 1mT

Lymphocytes were grown (90 h) in the presence of a complex MF produced from the back of a computer (Figure 3.1-vi). There were no ambient AC EMFs in the chamber of the incubator where the Helmholtz coils were placed.

Preparation of cytochalasin-B stock solution

Cytochalasin-B (Sigma, C-6762) was made up as a stock solution in dimethyl sulphoxide (DMSO) at a concentration of 2 mg/ml and stored at below -20C. The stock solution was thawed and added 24 h after the mitotic stimulation of the lymphocytes at a final concentration of 6μg ml^{-1}.

Lymphocyte cultures

For the MN experiments two culture tubes were set up for each donor from fresh blood samples which were obtained by venipuncture from the donors. 0.5 ml of fresh whole blood was added to culture tubes containing 5 ml of culture medium (4 ml GibcoBRL Medium-199, Cat. No. 31100-035 and 1 ml fetal bovine serum, Cat. No. 10093-136) pre-warmed to 37C. The six culture tubes were placed in a water bath for one h at 37C before being irradiated with X-rays at 3.5 Gy (0.4375 Gy min^{-1}, with a total 8 min exposure time). After irradiation the culture tubes were incubated (in dark) at 37C under different experimental EMF conditions. The controls were sham exposed. All experiments were performed single blind.

Six hours from the point of irradiation the lymphocytes were stimulated to divide using mitogen phytohaemagglutinin (PHA) (M form, GibcoBRL, Cat. No. 10576-015) at a

concentration of 10μg ml^{-1}. Twenty-four h after PHA stimulation, the cytokinesis-blocking agent, cytochalasin-B (Sigma, C-6762) was added to the cell cultures at a concentration of 6μg ml^{-1} to arrest the dividing cells in cytokinesis. First generation post-mitotic cells could subsequently be identified as binucleated cells (Fenech and Morley, 1985a and Scott *et al.* 1998).

Harvesting of lymphocytes, slide preparation

Harvesting of cells and slide preparation was accomplished by the modified method of Fenech and Morley (1985a), and Scott *et al.* (1998). Culture tubes were incubated for 90 h, mixed gently, and centrifuged for 10 min at 1000 rpm. Supernatant was gently removed from the top leaving approximately 1 cm above the pellet. Deposits were mixed thoroughly using a vortex stirrer for 3 sec and then resuspended in 5 ml of a warm hypotonic solution (KCl, 0.075M) at 37C and mixed gently by inverting 6-8 times. Tubes were incubated at 37C in a water bath for 4 min in order to swell the binucleated cells to allow more accurate scoring. Tubes were centrifuged immediately and the supernatant was drawn off to 1 cm above the pellet. Deposits were again mixed using a vortex machine for 3 sec. Lymphocytes were resuspended in 5 ml of fresh ice-cold fixative (Methanol : Acetic Acid = 3 : 1) in three steps, mixed gently by inversion, and centrifuged immediately. The supernatant was then removed and 5 ml of the fixative was added directly, mixed gently, and centrifuged. After centrifugation the supernatant was removed to 5 mm above the bottom of the tube without disturbing the pellet. Two to three drops of fixative were added to give a cell suspension of light turbidity. The cell suspension (3-4 drops) was dropped gently along an acid-alcohol washed (1ml of 1M HCl and 50 ml of 95% ethanol) air-dried slide. Twelve slides were prepared per donor. All harvesting procedures and reagents were at ambient temperature unless otherwise stated.

The slides were stained with 10% Giemsa (Gurr, BDH, Prod. 350864X) in Sorensen's buffer (pH 6.8) for 8 min, then rinsed in Sorensen's buffer for 2-3 min and air-dried. The slides were mounted in DPX.

Coding of slides and scoring of micronuleus

The slides of every donor were randomly coded by the researcher to ensure anonymity of samples and analysed at 400X magnification under a light microscope. In the cytokinesis-blocked method, nuclei are scored only in cells that have been inhibited from undergoing cytokinesis (Figure 3.9). Cytokinesis-blocked (CB) cells are easily recognisable by their binucleate appearance, as they must be dividing cells, which have completed nuclear division but not cytoplasmic division. Approximately 2,000 cells were scored from each of the 3 donors. Figures 3.10, 3.11, and 3.12 illustrate the method used to score micronulei.

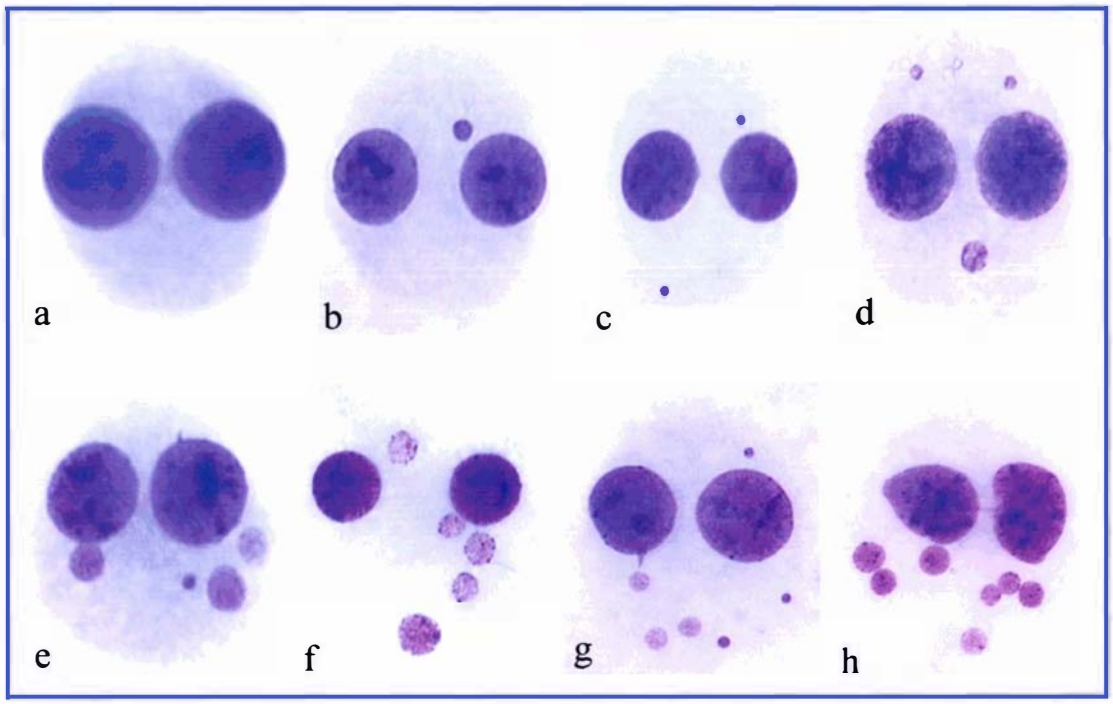


Figure 3.9: Cytokinesis-blocked (CB) binucleated cells (a-h) with varying numbers (0-7) of micronuclei.

Criteria for choosing binucleate cells

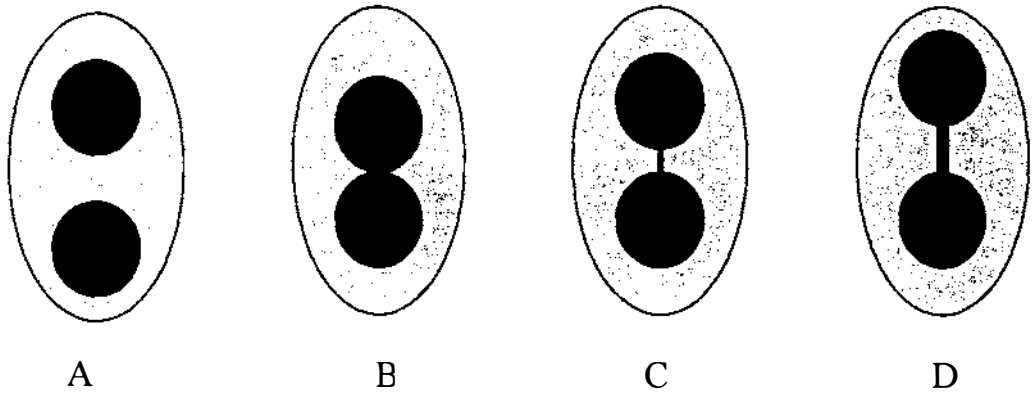


Figure 3.10: Binucleate cells which can be scored for micronuclei, **A.** Ideal binucleate cell; **B.** Binucleate cell with touching nuclei; **C.** Binucleate cell with narrow nucleoplasmic bridge between nuclei; **D.** Binucleate cell with relatively wide nucleplasmic bridge.

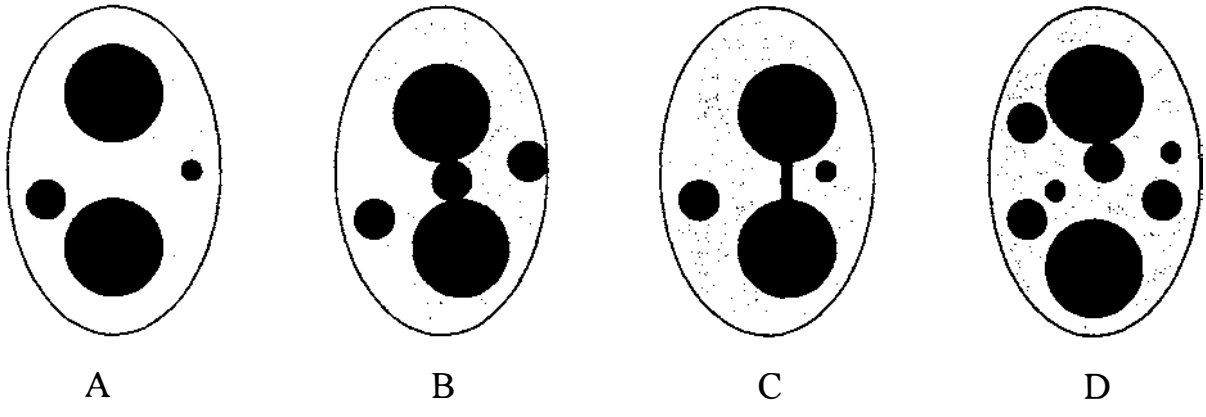


Figure 3.11: Typical appearance and relative size of micronuclei in binucleated cells that meet the scoring criteria: **A.** Cell with 2-micronuclei. The first with one third and second with one ninth the diameter of one of the main nuclei within the cell. **B.** Micronuclei are touching but are not overlapping the main nuclei. **C.** Binucleated cell with nucleoplasmic bridge between the two main micronuclei and, **D.** Binucleated cell with six micronuclei of various size. This type of cell is rarely seen.

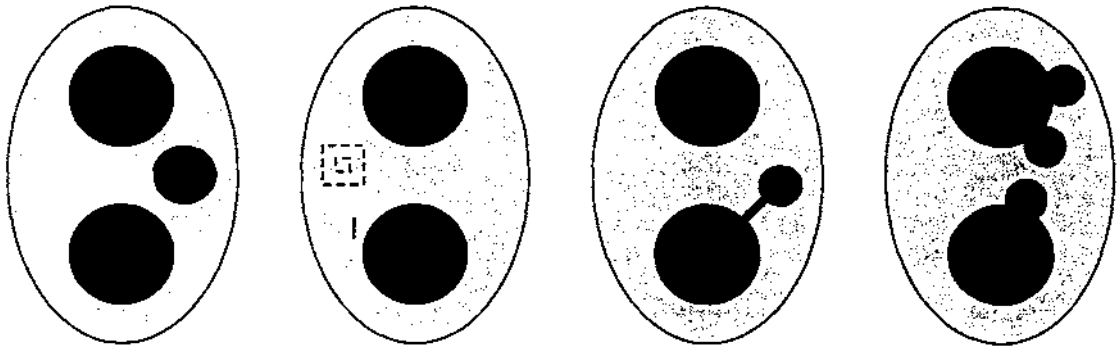


Figure 3.12: Cellular structures which resemble micronuclei but were not scored: **A.** micronuclei with a diameter greater than one third the diameter of the main nuclei, **B.** Dense stippling in a specific region of the cytoplasm, **C.** Extruded nuclear material that appears as a micronucleus with a narrow nucleoplasmic connection to the main nucleus, and **D.** Nuclear blebs which overlap with the main nucleus.

3.3 COMET Assay

In the present study, two COMET assay experiments were carried out in peripheral blood lymphocytes of donor-1 only, which was used in R1 of the SCE study. A total of two experiments were conducted with the same strict EMF conditions that were followed in the SCE and MN experiments.

Lymphocyte cultures were exposed to either a 50 Hz *square continuous* field or a 50 Hz *square pulsed* field (4 sec on and 4 sec off) at a field strength of 1mT for 72 h. The control lymphocyte cultures were sham exposed. Both the COMET assay experiments were conducted single blind.

3.3.1 Materials

The materials used during this COMET assay were stored at room temperature, unless otherwise stated. Sterilization of these materials was not required, unless stated, due to the nature of the assay. If sterilization was an issue, an aseptic technique was used.

Agarose gels

1 % Low melting-point agarose (LMA)

LMAgarose (Life Technologies Inc.)	1 g
1X PBS (Bio-Rad Laboratories)	100 ml

The agarose powder was mixed well with PBS and heated in a microwave with the cap loosened for ~30 sec, or until the solution turned clear. The agarose was prepared as stock and aliquots were melted using a hotplate and transferred into a smaller vial as required for experimental work.

0.5 % Normal melting point agarose (NMA)

NMA Powder (Life Technologies Inc.)	0.5 g
1X PBS (Bio-Rad Laboratories)	100 ml

The agarose powder was mixed well with PBS and heated in a microwave with the cap loosened for 60 sec, or until the solution turned clear. The agarose was prepared as stock and aliquots were melted using a hotplate and transferred into a smaller vial as required for experimental work.

Cell preparation media

Ficoll-Paque media

Ficoll-Paque Plus media was obtained (Amersham Bioscience) and used undiluted. An equal volume of Ficoll was layered beneath a lymphocyte:PBS 1:1 mixture before centrifugation. Ficoll-Paque media was stored at 4C.

1x Phosphate buffered saline (PBS)

10X PBS (No Ca^{2+} or Mg^{2+}) (Bio-Rad Laboratories)	10 ml
Milli-Q Water (MqH_2O)	90 ml

10X concentrated PBS was mixed well with an appropriate volume of mqH_2O and refrigerated.

Trypan blue stain

Trypan blue stain (BDH Chemicals)	0.1 g
DH_2O	10 ml

The stain was mixed well until all powder had dissolved.

DNA stains

4', 6-Diamidino-2-phenylindole (DAPI) stain

Vectashield™ with DAPI was obtained (In Vitro Life Science Technologies Inc.) and used undiluted. DAPI was stored at 4C in the dark to maintain viability.

Molecular biology buffers and solutions

Dimethyl sulfoxide (DMSO)

DMSO was obtained (Life Technologies Inc.) and used undiluted. DMSO was stored at room temperature in the dark.

0.2M EDTA, pH 8

0.5 M EDTA (BDH Laboratories)	6 ml
MqH_2O	9 ml

0.5 M EDTA was diluted with mqH_2O to reach the desired concentration

0.5M EDTA, pH 8

EDTA (BDH Laboratories)	14.61 g
MqH_2O	50 ml
10 M NaOH (BDH Laboratories)	

The solution was mixed well and NaOH was added drop-wise until the desired pH was reached and all powder was dissolved.

70 % Ethanol solution

Absolute ethanol (BDH Laboratories)	350 ml
DH_2O	150 ml

COMET assay materials

All solutions for the COMET assay in this section were made fresh for each sampling day.

Alkaline electrophoresis buffer, ~pH 13

NaOH (BDH Laboratories)	12 g
0.5 M EDTA (BDH Laboratories)	2 ml
MqH_2O	1000 ml

The solution was mixed well until fully dissolved and left to chill at 4C for at least 60 min before use.

Alkaline solution, pH 13

NaOH powder (BDH Laboratories)	0.6 g
0.2 M EDTA (BDH Laboratories)	250 µl
MqH_2O	50 ml

The solution was mixed well until fully dissolved and left to cool at room temperature for at least 60 min before use

Lysis solution

Per 10 slides:	
Lysis solution (Trevigen)	40 ml
DMSO (Life Technologies Inc.)	400 ml

The solution was mixed well by gentle agitation and stored at 4C for at least 30 min before use. The lysis solution contains the following components (Singh *et al*, 1988).

NaCl	146.1 g
EDTA	37.2 g
NaOH	8.0 g
Trizma base	1.2 g

The components were added to approximately 700 ml of dH_2O, and mixed well. NaOH was added to allow powders to dissolve for about 20 min. The pH was adjusted to 10.0 using concentrated NaOH or HCl, before filling to 890 ml with mqH_2O. The solution was stored at room temperature. Before each assay, 35.6 ml of the above solution was measured and 400 µl of Triton X-100 and 4 ml of DMSO was added fresh to a final volume of 40 ml. The solution was then stored at 4C for at least 30 min before use. This protocol is included only for researchers who wish to make their own lysis solution. The lysis solution used in this thesis was obtained commercially.

Cell culture media

Wellcome media (media 199) (GIBCO)	5 ml
AB serum (GIBCOBRL, Cat No. 10093-136)	1 ml
Phytohaemagglutinin (PHA) (GIBCOBRL, Cat No. 10576-015)	0.1 ml

The cell culture media was prepared fresh when required. Aseptic technique was used during the use of this material to prevent bacterial contamination. The total volume of media prepared depended on what was required.

3.3.2 Generation of EMFs and Field Exposure

The same incubator (Figure 3.1) which was used to deliver EMF intensities for the SCE and MN assay experiments was also used to conduct COMET assay experiments. The field exposure conditions and parameter combinations were identical to these used in the SCE study and MN assay.

3.3.3 Collection of Blood Samples

Fresh blood samples (5 ml) were obtained by venipuncture. Only one heparinized tube was used to collect blood samples from the donor. The blood specimen was then gently mixed to prevent clotting.

3.3.4 Lymphocyte Cultures

For the COMET assay experiments, two culture tubes per experiment were established. Each tube contained 5 ml of Medium-199, 1 ml of fetal bovine serum, and 0.1 ml of PHA, M form. Approximately 0.5 ml blood from the fresh sample was added per culture tube. Then the culture tubes were incubated (under sham exposure or EMF exposure) at 37C for 72 h.

3.3.5 Lymphocyte Preparation

After 72 h the lymphocyte cultures were removed from the incubator, the cells were resuspended by gently inverting the tube followed by vortex. In a new 8 ml Falcon^{TM} tube, 3 ml of this lymphocyte culture was carefully layered on to an equal volume of a Ficol–Paque Plus lymphocyte separation media (Amersham Bioscience). This was then centrifuged for 20 min at 800 x g, or until the lymphocytes were completely separated from the erythrocytes and plasma. After centrifugation the sample resembled Figure 3.13. The top layer was a yellowish colour that consisted of plasma, platelets, and a small amount of

erythrocytes and peripheral blood mononulceate cells (PBMCs). The large, clear layer contained the Ficol gradient, and the dark red mass at the bottom of the tube consisted mainly of erythrocytes. A wispy, white "buffy" layer was observed between the plasma layer and the Ficol layer; this layer contained the majority of the lymphocytes. A Pasteur pipette was used to collect the layer in a swirling motion, taking care to collect as little of the upper and lower layers as possible. The lymphocytes were transferred to an 8 ml Falcon tube containing 1 ml of chilled PBS buffer (pH 7), mixed slowly by vortex, and stored on ice. The PBS contained no Ca^{2+} or Mg^{2+} to ensure minimal *in vivo* DNA repair during the assay procedure.

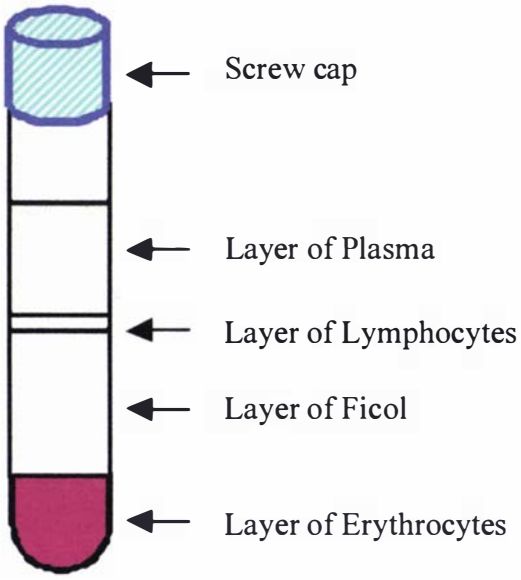


Figure 3.13: Illustrative diagram of what was observed after centrifugation of whole blood during isolation using Ficol, concentration gradient.

3.3.6 Lymphocyte Concentration and Cell Viability

To determine the lymphocyte concentration of the isolated sample, an "Improved Neubauer" haemocytometer was used (Weber Scientific International Ltd). A haemocytometer is a specialised slide with 2 chambers, each with grid patterns that are used to determine the concentration of cells in a medium. Each grid is made up of 9 large squares with sides that are 1 mm in length as illustrated in Figure 3.14.

When the coverslip was placed on the clean haemocytometer there was a slight rainbow appearance over the edges of the coverslip. This indicated that the distance between the

slide and the coverslip is 0.1 mm and therefore able to give an accurate cell count. The large square circled in Figure 3.14 was used for all counts. This square is made up of 25 smaller squares, each with sides that are 0.2 mm in length. The volume contained in the large centre square is 0.1 mm^3 (1 mm x 1 mm x 0.1 mm). There is 1 ml in 1 cm^3, and 1000 mm^3 in 1 cm^3, and 1 x 10^4 (1000 mm^3 x 1 x 10) volumes of the counting square in 1ml. Therefore, by counting the number of cells in the large centre square and multiplying this by 1 x 10^4, it will give the concentration of the cell sample in cells/ml.

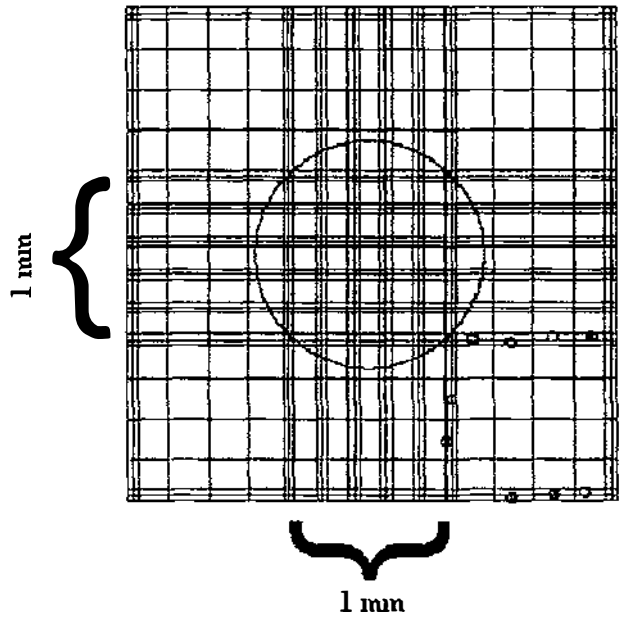


Figure 3.14: Diagram representing the dimensions of a haemocytometer counting chamber.

For this study, the calculation of cell viability was required. Cell concentration and cell viability was determined using trypan blue exclusion and a haemocytometer. One drop of trypan blue stain was added to 1 drop of cell suspension in an Eppendorf tube, mixed well, and allowed to incubate for 1 min. After 1 min approximately 20 µl of the stained cell solution was taken up using a Pasteur pipette and slowly added to the edge of the haemocytometer's coverslip, which is spread across the slide by capillary action. The sample was added until the chamber was filled, taking care not to overfill. The slide was observed under an Olympus® CHA light microscope using 400X magnification to determine the viability of the sample.

Non-viable cells are visible as dark blue structures due to the dye permeating the compromised cell membrane, while viable cells appear to be relatively clear against a darker background. Only cell samples with a cell viability of > 90 % were used for the COMET assay (Visvardis *et al*, 1994). A dilution factor of 2 was taken into consideration when determining lymphocyte concentrations due to addition of trypan blue stain. Lymphocytes were distinguished from erythrocytes by visual morphology only, although identification by other methods can be used, e.g., Turk's stain or using a haemolytic solution.

The total number of cells in the middle counting grid were counted, as well as the number of viable cells. The total cell number was multiplied by 2×10^4 (1×10^4 multiplied by a dilution factor of 2) to give the approximate concentration of cells/ml. For example, if the number of cells in the large centre square was found to be 10, then the concentration of the cell solution is approximately 200,000 or 2×10^5 cells/ml. Cell viability was determined by the number of viable cells divided by the total number of cells counted, all multiplied by 100.

The cell concentration required for the COMET assay is approximately 1×10^5 cells/ml. Samples were diluted with PBS as required, or if the concentration was too low then they were concentrated by centrifugation and resuspended in a lower volume of PBS. Unless otherwise stated, a vortex mixer was used throughout this research for the resuspension of a pellet.

3.3.7 Pre-treatment Method

The current COMET assay slides were prepared from conventional microscope slides using a modified version of the pre-treatment method outlined by Singh *et al* (1988). 26 mm x 76 mm microscope slides (Lomb Scientific Ltd) were first marked at the centre with an electronic engraver to identify the sample number that was to be added to each slide. The slides were then cleaned using a lint-free tissue to remove grease, moisture, and dust, all of which can cause the gel to detach during the assay. An aliquot of electrophoresis-grade, normal melting-temperature agarose (NMA, 0.5 % per volume, Life Technologies Inc.) was melted using a microwave, and 2 aliquots of 75 µl of the molten agarose was pipetted onto each of the slides, 1 at each end. A 22 mm x 22 mm glass microscope coverslip

(Biolab Scientific) was placed on top of each aliquot to act as a type of mould. The slides were stored at 4C for at least 60 min to allow this base layer of agarose to solidify. This layer served as an attachment layer for later agarose gel layers. During seasons of low humidity, such as winter, these gels are prone to drying too fast before a coverslip can be added. To overcome this, slides were placed on top of a slide warmer that had been covered with a damp tissue to create artificial humidity during application of the base gel layer. This change would not affect results, as no cells were present in this layer.

An aliquot of electrophoresis-grade, low melting-temperature Agarose (LMA, 1 % per volume, Life Technologies Inc.) was melted using a boiling water bath. After 10 min the LMA was transferred to a 42C water bath to cool for at least 10 min. Cells were not added until the gel had cooled in order to prevent temperature-associated damage (Speit *et al.* 1999). The coverslip was carefully removed from the base layer by sliding, taking care not to tear or disturb the base agarose layer. Ten µl of the cell solution from the cell suspension, as prepared in Section 3.3.5, was mixed well with 90 µl of the molten LMA by gentle agitation in an Eppendorf tube. Seventy µl of this cell/agarose mixture was added to each of the base gel layers and a fresh coverslip was again placed on top of each of the new layers. The slide was stored at 4C for 20 min to allow the agarose to solidify. The coverslip was again removed and another 70 µl of LMA 42C layer was added. This layer contained no cells and functioned as a protective layer to prevent detachment of the cells from the gel while in solution. A fresh coverslip was then placed on top of this new layer and the slide was stored at 4C for 30 min to allow the agarose to solidify. A diagrammatic representation of the gel layers is seen in Figure 3.15.

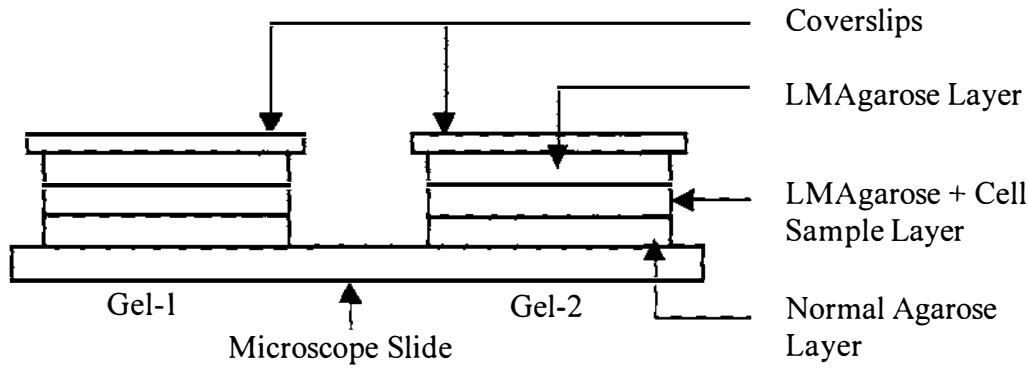


Figure 3.15: Illustrative image showing how the gel layers were formed using the pre-treatment method. Image not to scale.

Two replicate slides were prepared for each sample, each with 2 gels (designated Gel 1 and Gel 2 as illustrated in Figure 3.15) at either end. Each slide was processed 1 step apart from its counterpart to take into account any variability that may occur between sample runs, i.e., while the first slide was immersed in alkaline solution (the second step of the COMET assay) the second slide was immersed in the lysis solution (the first step of the COMET assay). Two gels per slide were prepared as a precaution as the gels would sometimes become detached from the slide while in solution, and hence become unusable. After the gels had solidified the coverslips were carefully removed so that the slides could be subjected to the COMET assay.

3.3.8 The COMET Assay Methodology

The COMET assay was performed using a modified version of the methodology described by Singh *et al.* (1988). All solutions for the COMET assay were prepared at least 1 h before the assay was commenced. Unless otherwise stated, solutions were prepared using mqH_2O. The lysis solution and electrophoresis solution were prepared and stored in a 4C refrigerated room, while the alkaline solution was cooled and stored at room temperature. Samples were kept in the dark during all stages of the assay.

Lysis solution

After the coverslips were removed, the slides were immediately immersed in 40 ml of lysis solution (Trevigen Inc.), which had been chilled at 4C or on ice for at least 60 min before use. The slides were immersed until levels just covered the gels. Immersion was conducted at 4C for 40 min in the dark. The slides were then carefully removed from the solution, drained, and then immediately transferred to the alkaline solution.

Alkaline solution

The slides were immersed in 50 ml of alkaline solution and incubated for 40 min at room temperature, in the dark. At no point during this stage was the container of alkaline solution disturbed or moved, as gels tend to detach from the slides at this point due to the conditions. The slides were then carefully removed and any excess solution was drained off. Slides were immediately transferred to the electrophoresis apparatus.

Alkaline electrophoresis

An electrophoresis apparatus was filled with 1 Litre (L) of chilled, alkaline electrophoresis buffer at least 1 h before use. The volume used will depend upon the apparatus used. Work involving the apparatus was conducted at 4C in the dark to reduce any temperature-related damage due to heating of the solution by the electric current. Heat may affect the results of the assay, or cause the gel to detach from the slide. A maximum of five slides was placed in the centre of the apparatus in each run. The placement of slides in the apparatus is illustrated in Figure 3.16. All slides were placed in the apparatus so that all comet tails were directed from left to right in order to accommodate the COMET assay analysis software that was used to quantify the genetic damage. The settings for electrophoresis were applied at 1 V/cm in length of the electrophoresis apparatus at 300 mA. The apparatus was approximately 30 cm in length and so the voltage applied was 30 V. A current of 300 mA was applied to normalise the volume of electrophoresis buffer used between gel runs. This current is applied regardless of the size of the apparatus. The amount of buffer will affect the electrophoresis current, and so buffer was added or removed until this current was achieved. The voltage was applied for 30 min.

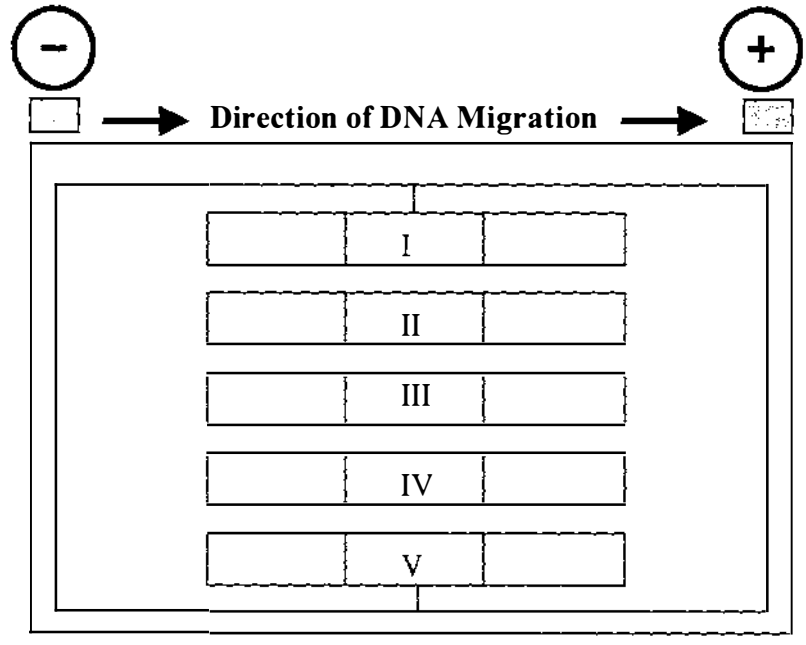


Figure 3.16: Image indicating the placement of microscopic slides in electrophoresis tank.

The slides were then removed from the apparatus and immersed in 70 % ethanol for 5 min. The slides were removed from the ethanol and allowed to dehydrate overnight in a slide

box that contained desiccant (silica, AnalaR). The drying step is important as it brings the cells into the same field of view to allow observation by fluorescent microscopy.

3.3.9 Slide Analysis (Quantitative)

Fluorescent microscopy was utilised to visualise the slides after applying the COMET assay. Various fluorescent DNA stains were trialed to detect comets in this study, such as SYBR Green™, and propidium iodide. Although these stains give clear images, they were very photosensitive and faded quickly, making them unreliable for digital imaging. However, DAPI is a semi-permanent stain and during trials for a suitable stain was found to be very effective in regards to both clarity of image and stability.

Fifteen μl of DAPI was added to the centre of each gel and a coverslip was place on top to allow the stain to spread. The slides were then refrigerated overnight at 4 ^{0}C and observed the following day. All microscope work and digital imaging was conducted using an Olympus® BX51 fluorescent microscope, under 400X magnification. The wavelengths for excitation and emission of DAPI occur at 358 nm and 461 nm, respectively. Therefore, the stain was detected using a U excitation (wide band) filter (abbreviated WU). Digital imaging was conducted using an Optronics MagnaFire S99802 digital camera with MagnaFire™ frame-grabbing software on a 2 GHz Pentium 4 ™ computer with 256 MB of RAM and a 128 MB video card. Cell images were captured using an exposure time of 200 ms throughout the sampling period. Figure 3.17 shows the hardware used during the current study.

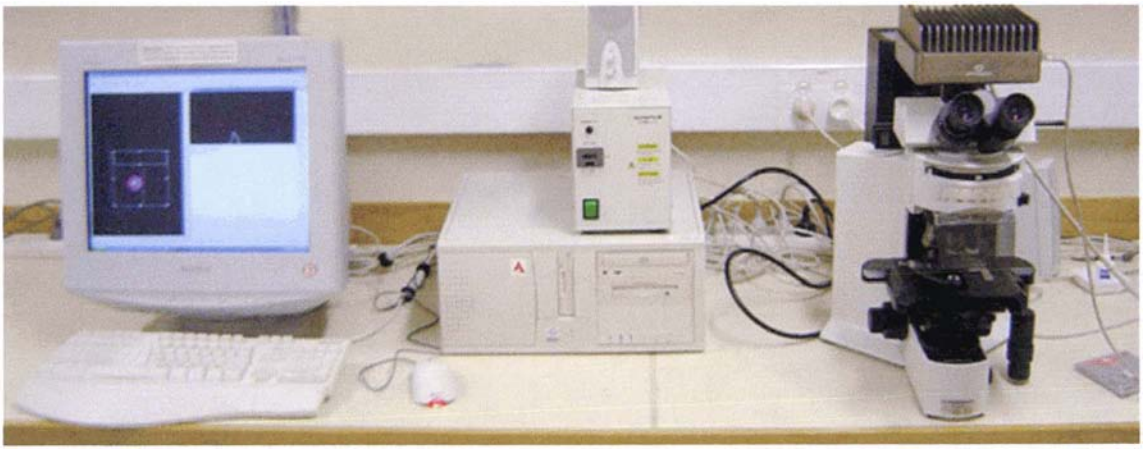


Figure 3.17: Image capturing hardware used in the current study.

In the current study 103, 99 and 70 cells were photographed for the control and two experiments (*square continuous 1mT*, and *square pulsed 1mT*), respectively.

Cell images were captured so that they would accommodate the COMET assay analysis software (The CASP, © Krzysztof Konca). The main requirement is that COMET tails were to be directed from left to right as illustrated in Figure 3.18.

Each cell that was to be captured was required to be free of debris and other cellular material, and be completely separated from other comets (both head and tails) around the perimeter of the cell of interest. A set of 50 random microscope coordinates (X and Y positions) for each of the 2 gels per slide were generated using Microsoft Excel™ (Appendix 4) by determining the perimeter of the 2 gels per slide and the corresponding area of the microscope stage. These coordinates were used in the current study. Once the microscope had been directed to the coordinate, separate images were taken of all comets that could be seen in the field of view that matched the prerequisites outlined in this paragraph.

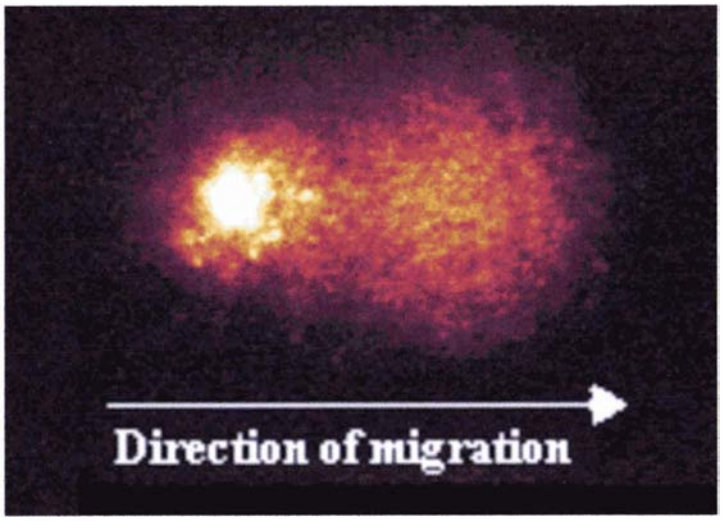


Figure 3.18: Orientation of the cell (directed left to right) for analysis by the CASP software.

Images of comet cells were analysed using The CASP version 1.01 (The COMET Assay Software Project, Krzysztof Konca ©) software package with a 902 MHz Intel Celeron ™ computer, with 256 MB of RAM and a 64 MB video card. The CASP software captures 13 variables from each comet cell, which can be used to determine the extent of the DNA damage.

An explanation of these 13 variables is outlined below:

Head Area - Area of the comet head in pixels (sum of pixels in the head)

Tail Area - Area of the comet tail in pixels (sum of pixels in the tail)

Head DNA - Amount of DNA in the comet head (derived from the sum of intensities of pixels in the head)

Tail DNA - Amount of DNA in the comet tail (derived from the sum of intensities of pixels in the tail)

Head DNA% - The Percentage of the cell's total DNA that is located in the comet head

Tail DNA% - The Percentage of the cell's total DNA that is located in the comet tail

Head Radius - Radius of the comet head (in pixels)

Tail Length - Length of the comet tail measured from right border of head area to end of tail (in pixels)

Comet Length - Length of the entire comet from the left border of head area to the end of tail (in pixels)

Head MeanX - Centre of gravity of DNA in the head (x-coordinate)

Tail MeanX - Centre of gravity of DNA in the tail (x-coordinate)

Tail Moment - TailDNA% x TailLength ([percent of DNA in the tail] x [tail length])

Olive Tail Moment (OTM) - TailDNA% x (TailMeanX-HeadMeanX) ([percent of DNA in the tail] x [distance between the centre of gravity of DNA in the tail and the of centre of gravity of DNA in the head in x-direction])

A pictorial explanation of some of these variables is illustrated in Figure 3.19.

Of the 13 variables, only 3 have been found to be the most indicative of genetic damage. The comet's Tail Length (TL) was used for many years due to its ease of measurement via an eyepiece micrometer. Tail moment (TM) and Olive Tail Moment (OTM) (Olive *et al.* 1990) have become more prominent in recent literature. These 3 variables were used as a measure of genetic damage in the current study.

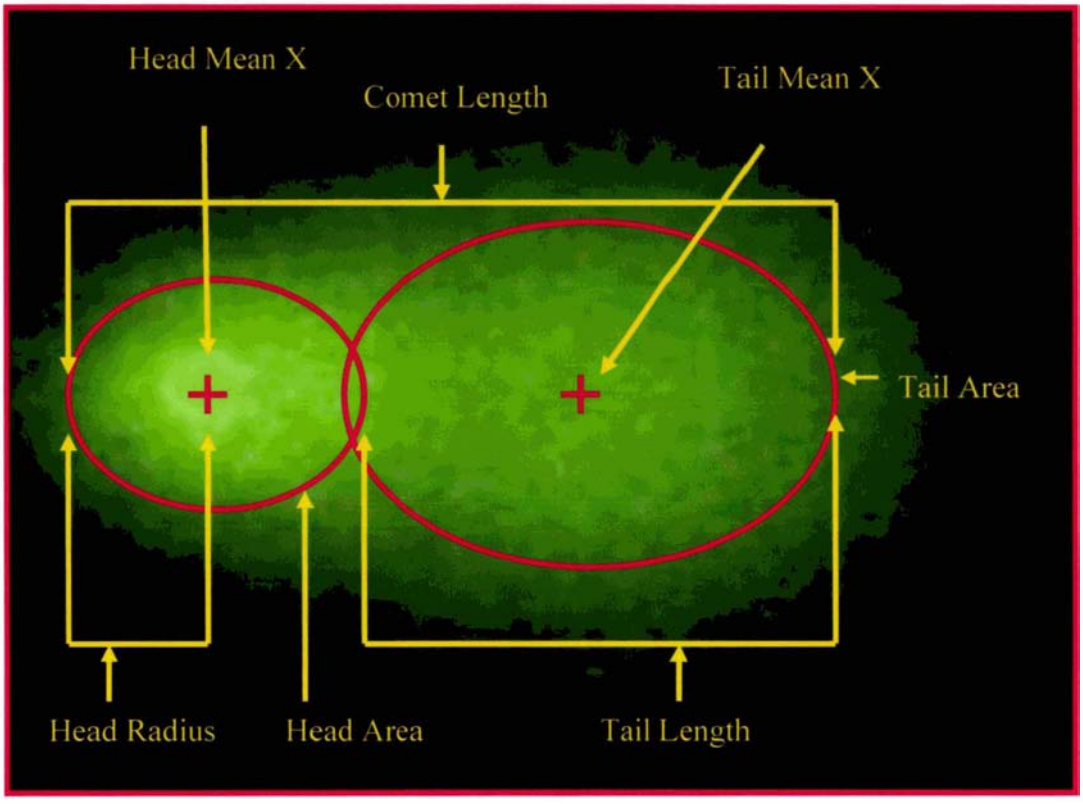


Figure 3.19: Image outlining 7 of the 13 different variables that are measured by the CASP™ software.

3.4 Fluorescent *in situ* Hybridization (FISH)

In the present study, FISH experiments were carried out in peripheral blood lymphocytes of donor-1 only, who was studied in R1 of the SCE study. One experiment was conducted with the same strict EMF conditions as followed in the SCE, MN assay and COMET assay experiments.

Lymphocyte cultures of the experiment conducted for FISH were exposed to a 50 Hz square continuous MF at the field strength of 1 mT for 72 h. The lymphocyte cultures of the control were sham exposed.

3.4.1 Collection of Blood Samples

Fresh blood samples (5 ml) were obtained by venipuncture. Only one heparinized tube was used to collect blood samples from the donor. The blood specimen was then gently mixed to prevent clotting.

3.4.2 FISH Protocol

Generation of EMFs and Field Exposure

The same incubator (Figure 3.1) which was used to deliver EMF intensities for the SCE, MN and COMET assay experiments was also used to conduct FISH experiments. All field parameters and exposure conditions were identical as followed in the SCE, MN assay and COMET assay experiments.

Lymphocyte cultures

Two culture tubes for both control and experiment were established from a fresh blood sample. Each tube contained 5 ml of Medium-199 (GibcoBRL, Cat. No. 31100-035), 1 ml of fetal bovine serum, (GibcoBRL, Cat. No. 10093-136), and 0.1 ml of phytohaemagglutinin (PHA) M form (GibcoBRL, Cat. No.10576-015). Using the WBC count, calculations were made to obtain 3.25-million cells per culture tube by adding approximately 0.3-0.6 ml of blood from the sample. The culture tubes were incubated (under sham exposure or EMF exposure) at 37C for 72 h, which included a 1 h treatment with colchicine (0.05%, BDH, Prod. 27805FM).

Lymphocyte harvesting

Harvesting of lymphocytes was accomplished by the same method as used for the SCE experiments with a little modification. After final centrifugation the supernatant was removed to 5 mm above the bottom of the tubes without disturbing the pellet. Then the pellets of the two tubes were transferred to one Eppendorf (1.5 ml) tube, filled with fresh fixative and stored in the freezer (at –20C) until slides were required for chromosome painting.

Slide preparation and fluorescence in situ hybridization

Day-one:

Lymphocytes dropping on pre-cleaned slide

Prior to dropping of lymphocytes on slides, Eppendorf tubes containing dividing lymphocytes were thawed at ambient temperature for approximately 10-15 mins. Each tube was subjected to brief vortex and then spun for 5-10 sec using a microcentrifuge (Model No. C-1200, 220V/50 Hz, National Labnet Co). After centrifugation, the supernatant was removed to approximately 5 mm above the bottom of the tubes without disturbing the pellet. A single drop of the lymphocyte was then placed on a pre-cleaned slide and the area of the droplet was marked with an electronic engraver on the specimen slide. The slide was then kept at ambient temperature in the dark for 24 h.

Day-two:

Pre-hybridisation slide washing and drying

Nine clean Coplin jars (J) with lids were placed in the bio-hood. Each of the jars (from J-1 to j-5) were filled with 70 ml of freshly prepared solution of 70%, 85%, 95%, 100%, 100% alcohol, respectively. J-6 was filled with 70 ml of Roticlear solution, and the final three Jars (J-7 to J-9) were filled with 100% alcohol. The slides were immersed in each solution (from J-1 to J-5) then for 10, 10, 10, 10 and 30 min. The slides were then immersed in Roticlear solution (J-6) for 30 min followed by 10 min in each of final three jars (J-7 to J-9). Slides were then transferred to a hot plate at 37C for 30 min.

Preparation of probe mixture and *in situ* hybridisation

For the FISH analysis, chromosomes 2, 3 and 5 were painted green by *in situ* hybridisation with whole chromosome probes (Vysis) labelled with fluoresce in isothiocyanate (FITC). The centromeres were labelled red with Cy3-pancentromeric probe (Cambio). The rest of the chromosomes were stained with the blue fluorescent dye, DAPI. The co-denaturation

process was used in this study; this simplifies FISH by combining denaturation of probe mixture and specimen into a single step.

In a sterile Eppendorf tube, a cocktail of the probes and hybridization buffer was prepared to a total of 10 µl in the following ratios:

Pancentromeric probe (PCP)	1 µl
Chromosome 2 probe	1 µl
Chromosome 3 probe	1 µl
Chromosome 5 probe	1 µl
Hybridization buffer	6 µl
Total	10 µl

The probe cocktail was then mixed thoroughly by centrifuging the Eppendorf tubes for 3-5 sec with a micro-centrifuge, followed by vortex for 3-5 sec, and then centrifugation again. Ten µl of the probe mixture was placed on the targeted area of the sample slide, covered with a 22 mm x 22 mm coverslip (Biolab Scientific), and then sealed with rubber cement (vulcanizing rubber solution). The slides were then kept in the dark at an ambient temperature for 15-30 mins to dry the rubber cement. The slides were then placed onto a micro-heating system (Vysis HYBrite^{TM} hybridization system) at 75C for 5 min to allow denaturation of the probes and DNA. The slides were then placed on moist tissue in a closed plastic container overnight at 37C in a thermostat control room to allow hybridisation.

Day-three:

Rapid post hybridisation wash, drying and counterstain

Post hybridisation wash for probes was carried out by immersing the slides in 0.4 x SSC / 3% NP 40 (1 x SSC = 150 mM NaCl, 15 mM sodium citrate) for 2 min at 71C and 1 min in 2 x SSC / 0.1% NP 40 at ambient temperature. After draining off excess liquid and drying for 30 min, the slides were stained with 15 µl of DAPI. Slides were covered with glass

coverslips (22 x 22 mm), sealed with nail polish, and kept in the dark for 10-15 min. The slides were stored at 4C until analysis (between 1 and 24 h).

Cytogenetic evaluation

For the analysis of translocation (shift of any green across to a blue chromosome) 545 cells from control and 506 cells from EMF-exposed experiments were scored. The formulas, proposed by Lucas *et al.* (1992) and Straume and Lucas (1993), could be used for comparison of data obtained with different chromosome cocktails. These formulas are based on the assumption that the probability of a chromosome being involved in a particular exchange aberration is proportional to its DNA content.

Chapter Four

Results

4.1 Sister Chromatid Exchange (SCE)

In the present study, the frequency of SCEs from two rounds, round-1 (R1) and round-2 (R2), of experiments were studied in peripheral blood lymphocytes (PBLs) from six healthy, non-smoking male blood donors. Donors were selected with similar lifestyles, medical history, and dietary history. Ages of the first three donors were 50-56 years and the remaining three donors were 31-44 years. The background information of each donor was obtained through a detailed questionnaire but a careful analysis of these data showed no record of any confounding factor(s), which could influence the results. None of the participants was a smoker or substantial consumer of alcohol or caffeine, which are the main factors that could influence the results.

A set of eight experiments was conducted in duplicate, with each duplicate experiment using the same strict EMF conditions. The eight experiments in R1 were conducted using blood samples from the first three donors, and blood samples from the remaining three donors were used in the R2 experiments. A specially designed and built EMF field generator (incubator) was used to generate different EMF waves (sine and square), different EMF flux densities, one weak (1μT), and one strong (1mT), and continuous or pulsed fields (4 sec on and 4 sec off).

Lymphocyte cultures in eight experiments conducted in each round were exposed to 50 Hz sinusoidal (continuous or pulsed) or square (continuous or pulsed) MFs at field strengths of 1μT or 1mT for 72 h. Lymphocyte cultures of three controls were sham exposed in each round. In the twelfth experiment of each round, lymphocyte cultures were exposed to a complex MF, produced from the back of a computer, for 72 h.

To eliminate bias, an independent person was involved in setting the EMF parameters, which were unknown to the researcher. A total of 5145 and 6141 c-metaphase cells with clear exchanges were analysed in R1 and R2.

4.1.1 Analysis of Round-1 (SCE)

Initially, the data of R1 were examined by collapsing across all experimental conditions so that the mean number of SCEs in all experimental conditions could be compared with the control condition. Table 4.1 summarises the data for the number of cells analysed (N), the mean SCE and standard deviations of the control group and that of the experimental group. The data were analysed by *t*-test to see whether there was any difference between the mean SCE for the control group and that of the combined experimental groups.

A statistically significant difference was observed between the mean SCE of the control group and that of the entire set of experimental groups, $t\ (5143) = 4.09$, $P < .001$.

Table 4.1: Overall mean SCE and standard deviations of the control group and that of the entire set of experimental groups for R1.

Expt.	N	Mean	Std. Deviation
Control	1554	8.92	3.19
Experimental	3591	9.34	3.49

Does the result shown in Table 4.1 apply to all three donors? Figure 4.1 shows the overall results but for each donor separately. D-1 (donor-1) and D-2 showed a significant difference $t\ (1608) = 4.09$, $P < .001$, and $t\ (1947) = 4.33$, $P < .001$, respectively, while D-3 showed only a small increase in SCE per/cell for the exposed conditions. This difference was not significant ($t < 1$).

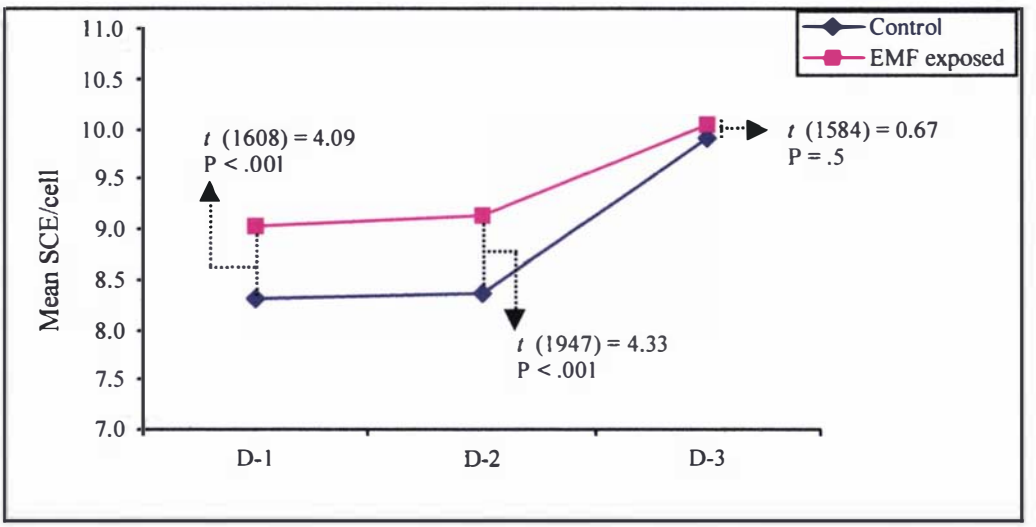


Figure 4.1: Graph of mean SCE of control groups and EMF exposed groups of three donors in R1.

Figure 4.2 shows an example of a Giemsa stained complement from a sham-exposed control experiment showing 11 SCEs (arrowheads). Figure 4.3 is a similar complement but from an EMF-exposed experiment which shows 20 SCE (arrows). However, in some spreads both the chromatids in some chromosomes show light or pale colour (Fig. 4.4). Figure 4.4 is an example of a cell that has undergone three rounds of DNA replication in the presence of BrdU.

Table 4.2 summarises the data in R1 on the number of cells observed for SCE (N), mean SCE and standard deviations for all eight experiments, controls, and complex. The mean SCE values of the three controls and complex experiment for R1 are 8.92 and 9.34, respectively. The mean SCE values range from 8.08 for *sine continuous 1μT* to 10.03 for *square continuous 1mT*. It is also notable that the mean SCE value increases with an increase of field strength in all parameter combinations except for a downward trend in *sine pulsed 1μT and 1mT* (9.55 and 8.82, respectively). The lowest standard deviation (2.73) was observed in the experiment *sine continuous 1μT,* and the highest (4.59) for the experiment *square pulsed 1mT,* but a comparatively uniform standard deviation was observed for all other experiments (2.98-3.67).

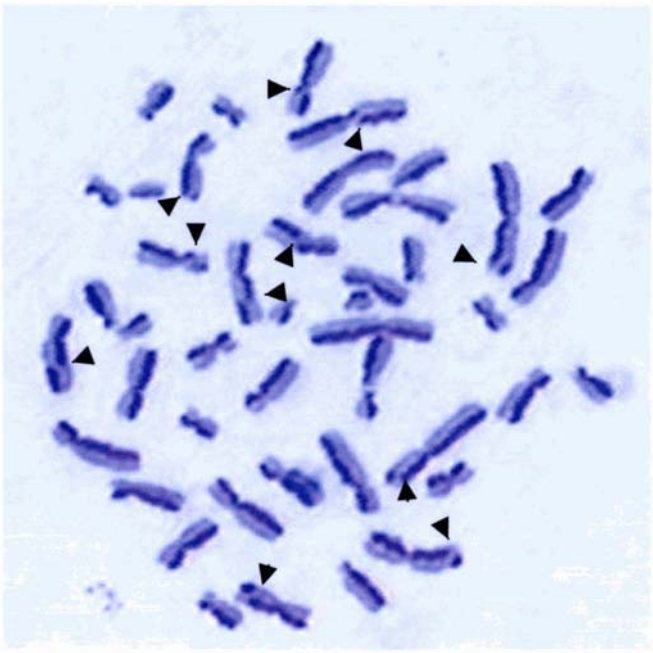


Figure 4.2: A c-metaphase chromosome spread of a dividing peripheral blood lymphocyte from a sham exposed control that has undergone two rounds of DNA replication in the presence of BrdU, showing 11 SCEs (arrowheads).

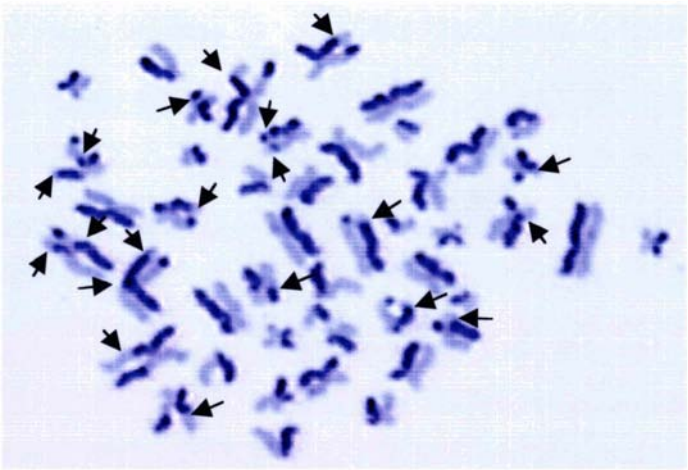


Figure 4.3: A c-metaphase chromosome spread of a dividing peripheral blood lymphocyte from an EMF exposed experiment that has undergone two rounds of DNA replication in the presence of BrdU, showing 20 SCEs (arrows).

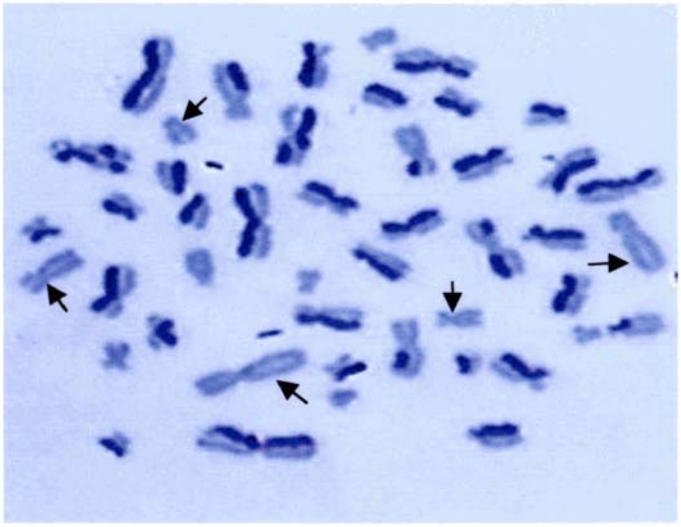


Figure 4.4: A c-metaphase chromosome spread of a dividing peripheral blood lymphocyte that has undergone three rounds of DNA replication in the presence of BrdU, showing both the chromatids light or pale (arrows).

Table 4.2: Mean SCE and standard deviations of all experiments in R1 based on all three donors.

Wave	Form	Strength (T)	Mean (SCE)	Std. Deviation	N
Sine	Continuous	1μ	8.08	2.73	87
Sine	Continuous	1m	9.62	3.22	600
Sine	Pulsed	1μ	9.55	3.07	443
Sine	Pulsed	1m	8.82	3.12	600
Square	Continuous	1μ	9.09	2.98	600
Square	Continuous	1m	10.03	3.67	600
Square	Pulsed	1μ	8.70	3.28	79
Square	Pulsed	1m	9.28	4.59	495
Complex			9.34	3.01	87
Control (x3)			8.92	3.19	1554
Total					5145

Note: T = Tesla.

These mean differences were subjected to a 4-way ANOVA (analysis of variance) test (Table 4.3). To reiterate for clarity, Wave (sinusoidal, square), Form (continuous, pulsed), Strength (1μT, 1mT) and Donor (D-1, D-2, D-3) were the factors. There was a significant main effect for Strength, F (1, 24) = 16.64, P = .01 and a near significant main effect for Wave, F (1, 24) = 7.04, P = .09. However, the main effect for Strength was qualified by a near significant Form x Strength interaction, F (1, 24) = 10.29, P = .08. This interaction is shown graphically in Figure 4.7. Follow up t-tests showed that for the continuous wave form the SCE/cell count was significantly greater for the 1mT field strength compared to the1 μT field strength, t (1885) = 5.51, P < .001, whereas the 1 μT field produced a higher SCE count than the 1mT field for the pulsed wave, t (1615) = 2.01, P = .04 (see Figure 4.7).

Table 4.3: Overall ANOVA for R1.

Source	df	F	P
Intercept	1	692.83	0.001
Wave	1	7.04	0.09**
Form	1	1.15	0.38
Strength	1	16.64	0.01*
Donor	2	-	- ***
Wave x Form	1	2.55	0.24
Wave x Strength	1	0.30	0.88
Form x Strength	1	10.29	0.08**
Wave x Form x Strength	1	1.33	0.36
Wave x Donor	2	0.13	0.89
Form x Donor	2	2.89	0.98
Wave x Form x Donor	2	0.38	0.72
Strength x Donor	2	0.03	0.98
Wave x Strength x Donor	2	1.89	0.35
Form x Strength x Donor	2	0.69	0.59
Wave x Form x Strength x Donor	2	3.29	0.04*

Note: (* = significant, ** = Near significant, and *** = The SPSS could not calculate because it cannot compute the error degrees of freedom using Satterthwaite's method.

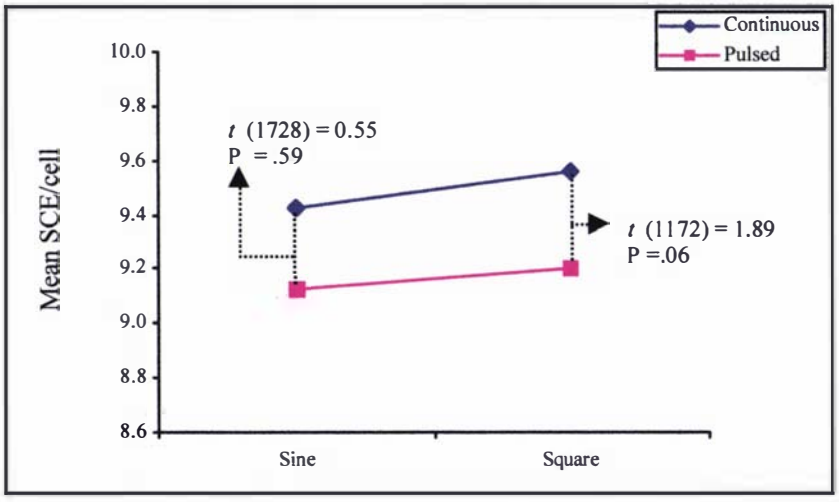


Figure 4.5: Graph of mean SCE of different Wave and Form in R1

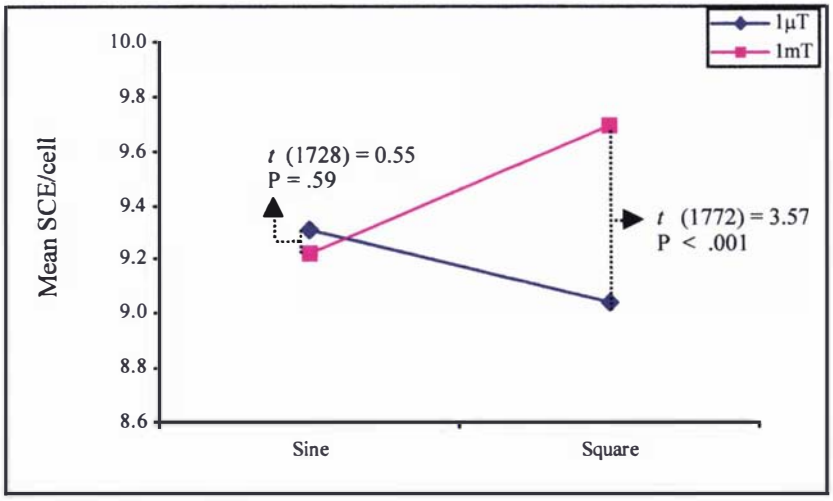


Figure 4.6: Graph of mean SCE of different Wave and Strength in R1

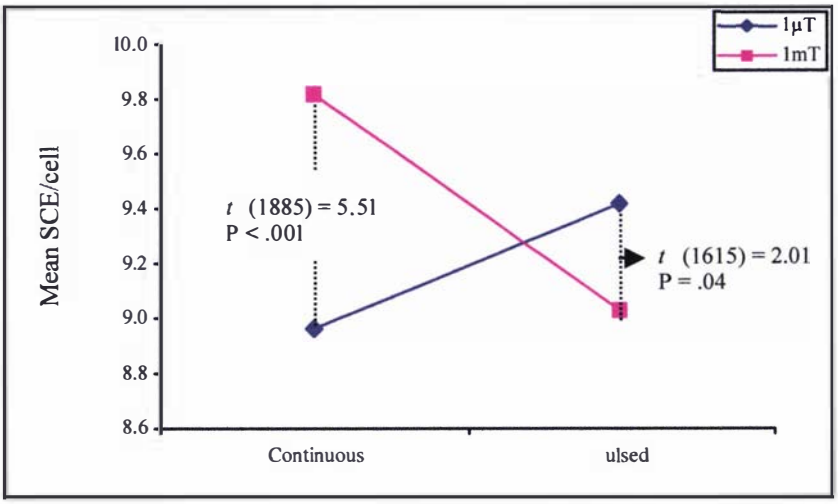


Figure 4.7: Graph of mean SCE of different Form and Strength in R1

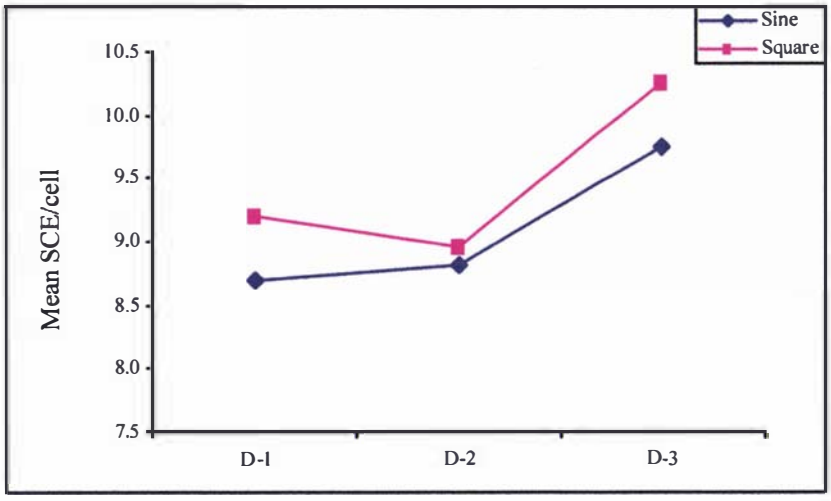


Figure 4.8: Graph of mean SCE of different Wave and three Donors.

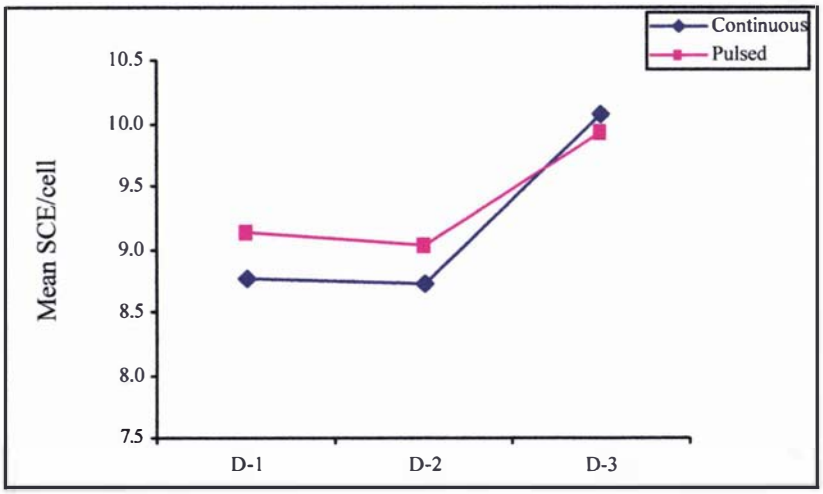


Figure 4.9: Graph of mean SCE of different Form and three Donors.

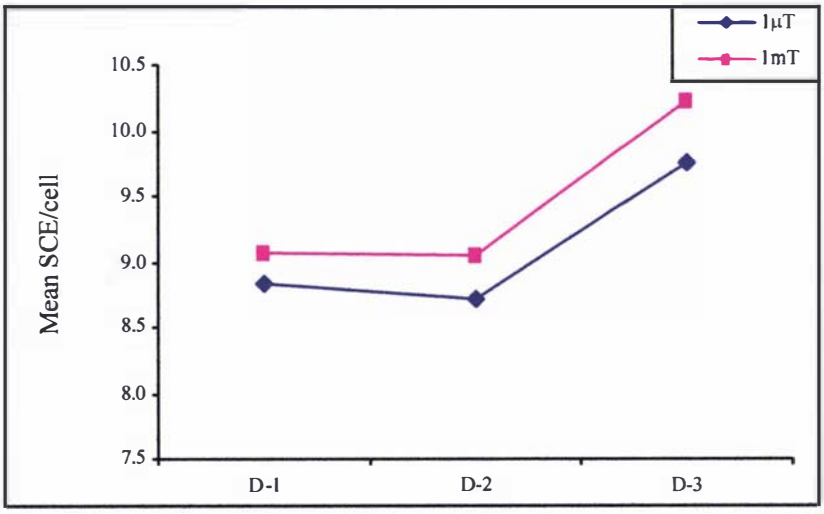


Figure 4.10: Graph of mean SCE of different Strength and three Donors.

There was also a 4-way interaction across Wave, Form, Strength, and Donor, F (2, 24) = 3.29, P = .04, suggesting that the EMF effect is somewhat dependant on all four factors.

The pair-wise interactions involved can be seen in Figures 4.5 - 4.10. By far the biggest component of this interaction was the contribution made by the Form x Strength interaction (Figure 4.7) and the Wave x Strength interaction (Figure 4.6). Concerning the donor's contribution, D-3 always had the highest number of SCE/cell compared with the other two donors.

4.1.2 Analysis of Round-2 (SCE)

As in R1, the data of R2 were also examined by collapsing across all experimental conditions so that the mean number of SCEs in all experimental conditions could be compared with the control condition. Table 4.4 summarises the data for the SCE means and standard deviations of the control group and that of the experimental group. The data were again analysed by t-test to see whether there was any major difference between the mean SCE for the control group and that of the combined experimental groups.

Table 4.4: Overall mean SCE and standard deviations of the control group and that of the entire set of experimental groups for R2.

Expt.	N	Mean	Std. Deviation
Control	1535	9.66	3.72
Experimental	4606	10.17	3.79

A statistically significant difference was observed between the mean SCE of the control group and that of the entire set of experimental groups, t (6139) = 4.57, P < .001.

Similar to R1, Figure 4.11 shows that the difference is mainly due to donors D-4 and D-6, D-5 making a minor contribution. D-6 and D-4 showed a significant difference, t (1686) = 4.45, P < .001, and t (2280) = 2.81, P < .01, respectively, while D-5 showed

small increase in SCE / cell for the exposed conditions. This difference was not significant ($t = 1.12$).

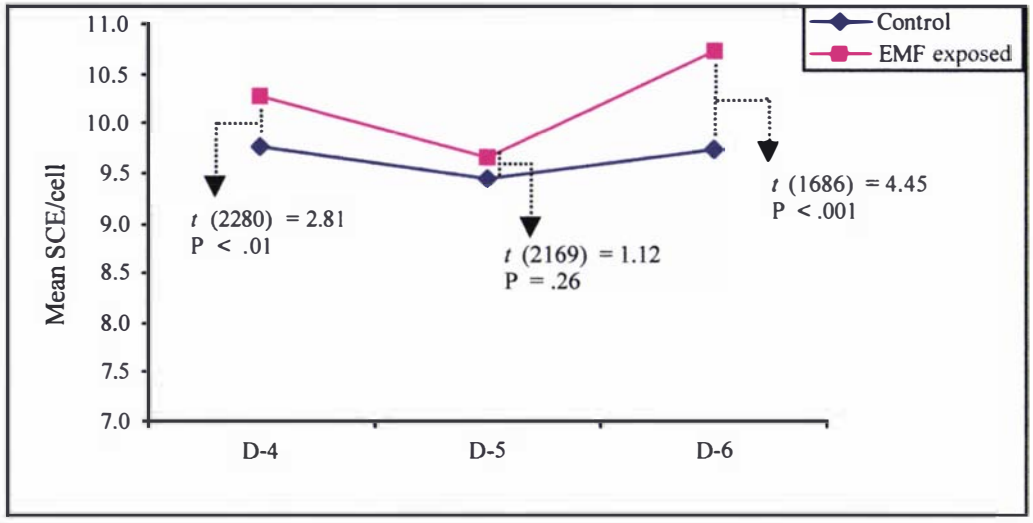


Figure 4.11: Graph of mean SCE of control groups and EMF-exposed groups of three donors in R2.

In summary, looking at Figures 4.1 and 4.11 it can be seen that four of six donors showed statistically significant changes between the control and exposed conditions. However, even though the remaining two donors did not yield significance, the data showed a trend towards an effect from EMF exposure.

Table 4.5 summarises the data in R2 on the number of cells observed for SCE (N), mean SCEs and standard deviations for all 12 experiments. It is worth mentioning here that the overall mean SCE value in R2 is higher than that in R1. The mean SCE value of the three control experiments and complex experiment for R2 are 9.66 and 9.46, respectively. The mean SCE values range from 9.66 for *square pulsed 1mT* to 10.61 for *sine pulsed 1μT*. It is also notable, in contrast to R1, that the mean SCE value decreases with an increase of field strength in all parameter combinations except for an upward trend between *sine continuous 1μT* and *1mT* (9.98 and 10.21, respectively). The standard deviations differ little across all the experiments (3.52-4.09), with the standard deviation value of the experiment *sine pulsed 1μT* being the highest.

Table 4.5: Mean SCEs and standard deviations of all experiments in R2, based on all three donors.

Wave	Form	Strength (T)	Mean (SCE)	Std. Deviation	N
Sine	Continuous	1μ	9.98	3.58	508
Sine	Continuous	1m	10.21	3.85	521
Sine	Pulsed	1μ	10.61	4.09	516
Sine	Pulsed	1m	9.98	3.62	473
Square	Continuous	1μ	10.39	3.86	600
Square	Continuous	1m	9.89	3.83	600
Square	Pulsed	1μ	9.83	3.66	600
Square	Pulsed	1m	9.66	3.74	600
Complex			9.46	3.52	188
Control (x3)			9.66	3.72	1535
Total					6141

These mean differences were subjected to a 4-way ANOVA test (Table 4.6) as in R1. No significant difference for any of the main effects, Wave, Form, Strength or Donor was observed. However, there was a significant Wave x Form interaction, F (1, 24)= 90.62, P < .01. This interaction is shown visually in Figure 4.12. Follow up t-tests showed that there was a significant difference in SCE count between Continuous and Pulsed for the Square wave, t (2398) = 4.45, P < .001, but no difference for the Sine wave, t (2016) = 1.28, P = .19.

Comparing R1 with R2, it will be recalled that there was a near significant interaction between Form and Strength (P = .08) in R1, Table 4.3. Table 4.6 shows that while this interaction did not reach significance in R2 (P = 0.16) there was a trend towards an interaction. This can be seen in Figure 4.13, which can be compared with Figure 4.7. The main feature to note is that in both cases the trend is exactly the same. For a Continuous wave form, there was a greater SCE/cell for the 1mT field compared to the 1μT field, whereas the reverse was true for the Pulsed wave form.

The largest difference between R1 and R2 involved the two factors, Wave and Form. There was a significant Wave x Form interaction in R2, whereas in R1 this interaction showed only a small trend, if anything.

Table 4.6: Overall ANOVA for R2.

Source	df	F	P
Intercept	1	1143.82	0.001
Wave	1	3.63	0.17
Form	1	2.77	0.24
Strength	1	0.01	0.95
Donor	2	8.58	0.23
Wave x Form	1	90.62	0.01*
Wave x Strength	1	0.69	0.49
Form x Strength	1	4.66	0.16
Wave x Form x Strength	1	0.35	0.61
Wave x Donor	2	3.42	0.99
Form x Donor	2	-	- **
Wave x Form x Donor	2	0.06	0.94
Strength x Donor	2	2.22	0.67
Wave x Strength x Donor	2	0.96	0.51
Form x Strength x Donor	2	0.61	0.62
Wave x Form x Strength x Donor	2	2.07	0.13

Note: * = significant, ** = SPSS could not calculate because it cannot compute the error degrees of freedom using Satterthwaite's method.

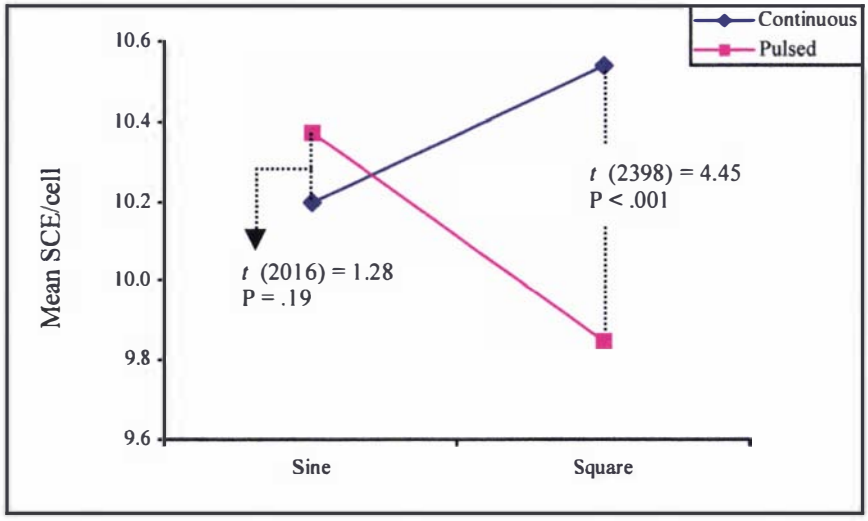


Figure 4.12: Graph of mean SCE of different Wave and Form in R2.

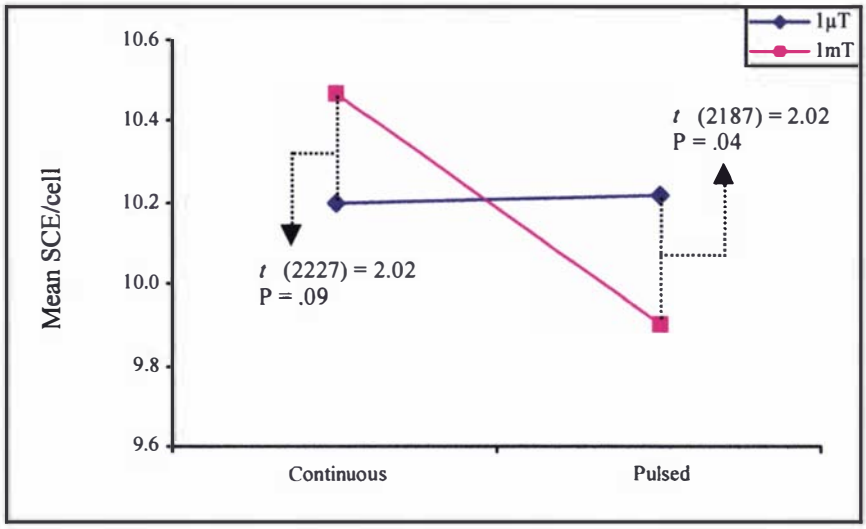


Figure 4.13: Graph of mean SCE of different Form and Strength in R2.

4.1.3 Complex MF

Table 4.7 summarises the number of cells analysed (N), the mean SCEs and standard deviations of the mean of the control and complex experiment in R1. No statistically significant difference, t (1639) = 1.20, P = 0.23, was found between the control and complex experiment in the mean frequency of SCE of R1. This experiment was duplicated in R2. As Table 4.8 shows, R2 produces the same result for this complex field, t (1721) = 0.70, P = 0.48.

Table 4.7: Overall SCE means of control and complex experiment in R1.

Expt.	N	Mean	Std. Deviation
Complex	87	9.34	3.01
Control (x3)	1554	8.92	3.19

Table 4.8: Overall SCE mean of control and complex experiments in R2.

Expt.	N	Mean	Std. Deviation
Complex	188	9.46	3.52
Control (x3)	1535	9.66	3.72

It is particularly notable, however, that the number of dividing cells cultured in a complex field in both R1 and R2 was considerably reduced (complex N = 87 and control N = 518 in R1; Complex N = 188 and control N = 511 in R2). Unfortunately, no reliable data to compare mitotic indexes are available. Nevertheless, the smaller number of dividing cells in slides prepared from lymphocytes cultured in a complex field is interesting, a matter returned to in the Discussion.

4.2 Micronucleus Assay (MN Assay)

In the MN assay, the frequency of micronuclei from one set of eight experiments was studied using PBLs from the same three blood donors used in R1 of the SCE experiments. The same strict EMF conditions used in R1 and R2 of the SCE experiments were followed. For the MN experiments, the same incubator that was used to conduct the SCE experiments was used again (See section 3.2).

Initially, the data of MN experiments were examined by collapsing across all experimental conditions so that the *mean number of micronucleated cytokinesis-blocked (CB) cells per 100 CB cells* (M_a) in all experimental conditions could be compared with those of the control condition. Table 4.9 summarises the data for the number of CB cells analysed (N), M_a, and standard deviations of the control group and that of the experimental groups. The data were analysed by *t*-test to see whether there was any major difference between the means for the control group and that of the combined experimental groups.

A statistically significant difference was observed between the mean, M_a, of the control group and that of the entire set of experimental groups, t (34) = 2.37, P = .03.

Table 4.9: M_as and standard deviations of control group and that of the entire set of experimental groups.

Expt.	N	M_a	Std. Deviation
Control	18466	19.39	5.28
Experimental	50216	25.06	8.43

Figure 4.14 shows the overall results for each donor separately. Interestingly, none of the differences between control and experimental conditions for any donor reached significance, t (10) = 1.36, P = .20, t (10) = 0.28, P = 0.78 and t (10) = 1.74, P = .11, for D1, D2 and D3, respectively. Even though there were no significant differences here, it can be noted (Figure 4.14) that the differences between control and experimental conditions for all three donors were in the direction of an EMF effect. However, as for the SCE analysis (R1), one donor (D-2) had little effect on the outcome. Analysing more cells, or using more subjects, would have increased the level of statistical power. The clear trends seen in Figure 4.14 may then have reached significance.

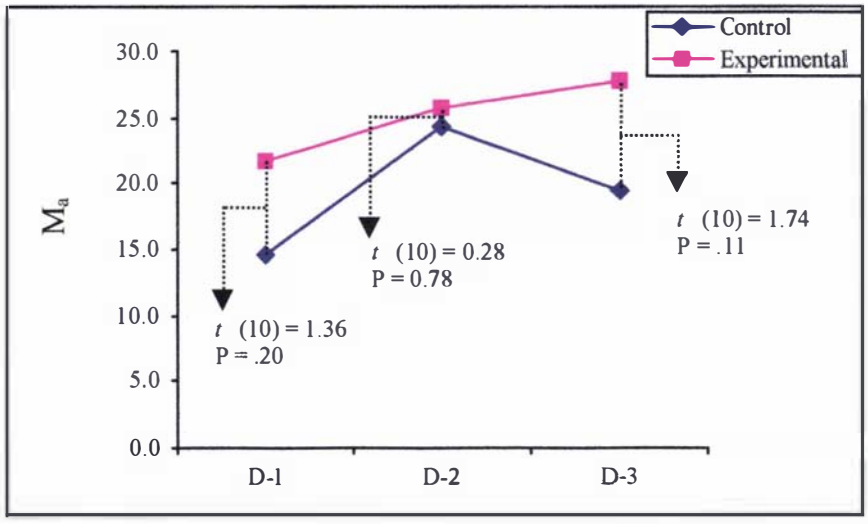


Figure 4.14: Graph of M_a of control groups and EMF-exposed groups for three donors.

Table 4.10 summarises the MN data for complex, three controls, and eight experiments. The average M_a value of the three controls and complex experiment for MN are 19.39 and 18.84, respectively. The M_a value differs considerably among all the experiments (16.70-35.18), with the M_a value of the experiment *Square pulsed 1mT* being the highest. It is also notable that the M_a value increases with an increase of field strength in two parameter combinations *Sine continuous1 μT and 1mT* (16.70 to 24.02), and *Square pulsed 1 μT and 1mT* (33.57 to 35.18), while the other two parameter combinations showed downward trends, *sine pulsed 1 μT and 1mT* (27.15 to 17.69), *and Square continuous1 μT and 1mT* (24.98 to 22.57). The standard deviation (SD) of M_a for the experiment *sine pulsed 1mT* was lowest (3.08), and highest (12.29) for the experiment *Sine continuous 1mT*, but a fairly uniform deviation was observed among all other experiments (4.05-7.40). The reason for the high SD in *Sine continuous 1mT* (12.29) was due to an unusually high number of CB cells with micronuclei found in D-3.

Table 4.10: M_a's and standard deviations of all experiments based on all three donors. N= Total number of CB cells observed.

Wave	Form	Strength (T)	M_a	Std. Deviation	N
Sine	Continuous	1μ	16.70	5.90	6129
Sine	Continuous	1m	24.02	12.29	6095
Sine	Pulsed	1μ	27.15	7.40	6106
Sine	Pulsed	1m	17.69	3.08	6061
Square	Continuous	1μ	24.98	4.93	6322
Square	Continuous	1m	22.57	5.34	6345
Square	Pulsed	1μ	33.57	4.52	6086
Square	Pulsed	1m	35.18	4.05	7072
Complex			18.84	4.27	6100
Control (x3)			19.39	6.67	18,466
Total					74,782

Table 4.11: Overall ANOVA for M_a.

Source	df	F	P
Intercept	1	202.74	0.005
Wave	1	3.74	0.19
Form	1	3.28	0.21
Strength	1	0.17	0.71
Donor	2	0.38	0.71
Wave x Form	1	3.20	0.22
Wave x Strength	1	0.06	0.82
Form x Strength	1	5.00	0.15*
Wave x Form x Strength	1	1.99	0.29
Wave x Donor	2	-	- ***
Form x Donor	2	-	- ***
Wave x Form x Donor	2	0.28	0.78
Strength x Donor	2	-	-***
Wave x Strength x Donor	2	0.06	0.94
Form x Strength x Donor	2	-	-***
Wave x Form x Strength x Donor	2	12.59	0.001**

* = Near significant, ** = Highly significant, and *** = SPSS could not calculate because it cannot compute the error degrees of freedom using Satterthwaite's method.

The only statistically significant outcome was a 4-way interaction involving Wave, Form, Strength and Donors $F(2, 24) = 12.59$, $P < .001$.

The pair wise interactions can be seen in Figures 4.15 through 4.20. Figure 4.17 shows a significant difference in M_a, $t(10) = 3.25$, $P < .01$, in Pulsed wave form between the Square wave and Sine wave. Figures 4.18 to 4.20 show data for each donor separately, and it can be seen that D-3 is not contributing at all to M_a. Figures 4.18 and 4.19 show substantial differences between Sine and Square wave and Continuous and Pulsed wave forms. However, Strength had no effect on any donor.

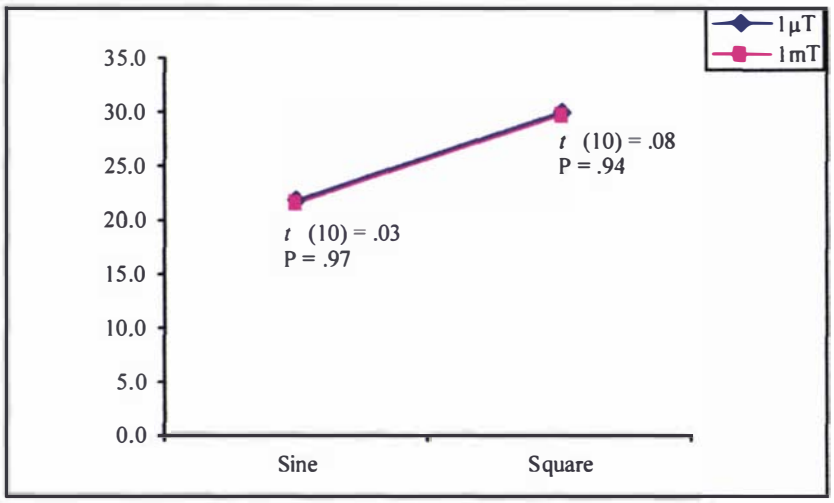


Figure 4.15: Graph of M_a of different Wave and Strength.

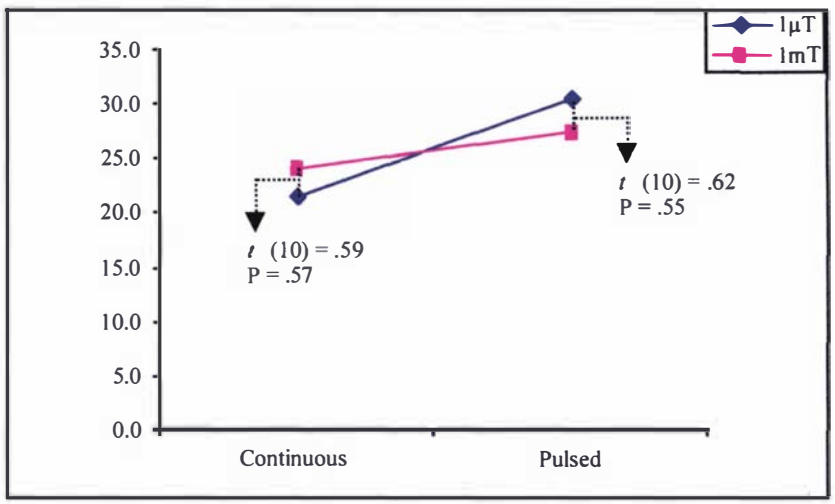


Figure 4.16: Graph of M_a of different Form and Strength.

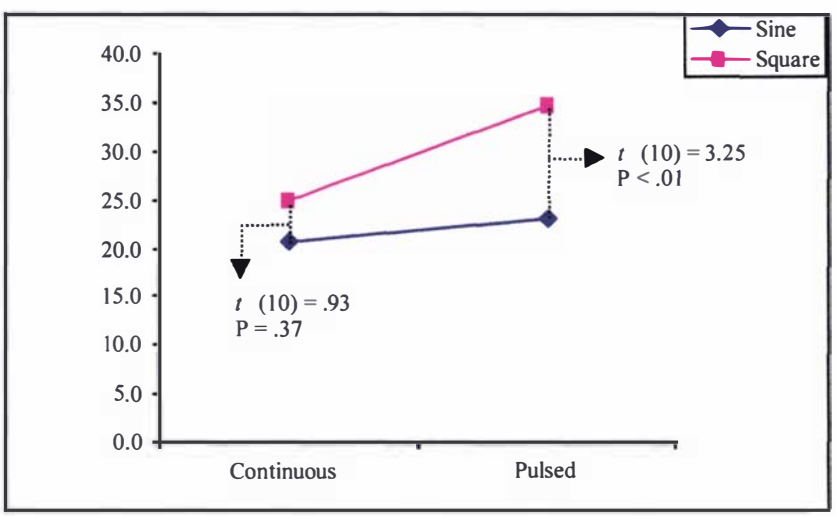


Figure 4.17: Graph of M_a of different Form and Wave.

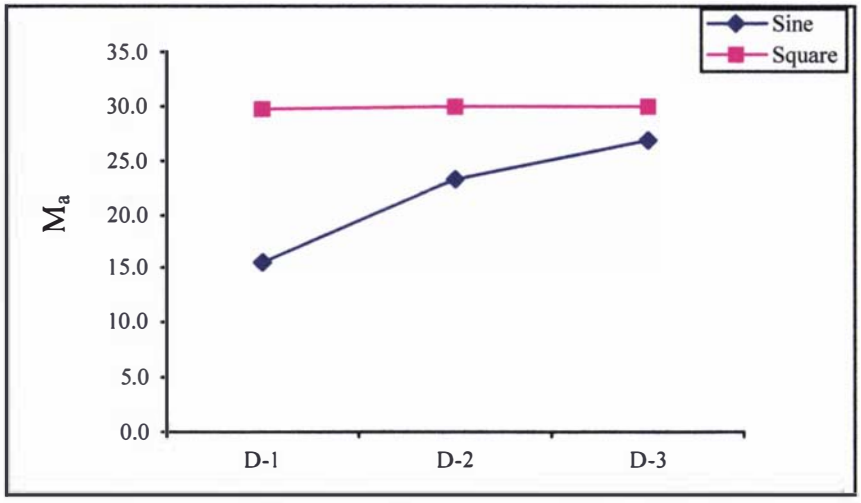


Figure 4.18: Graph of M_a of different Wave and three Donors.

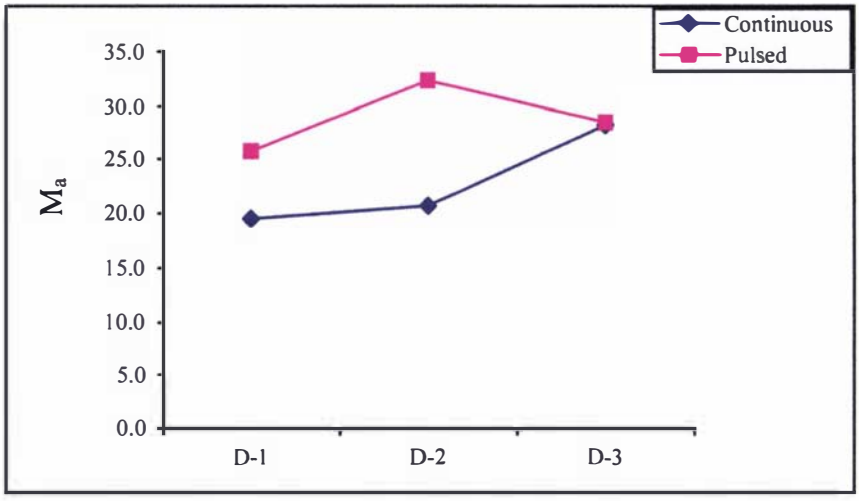


Figure 4.19: Graph of M_a of different Form and three Donors.

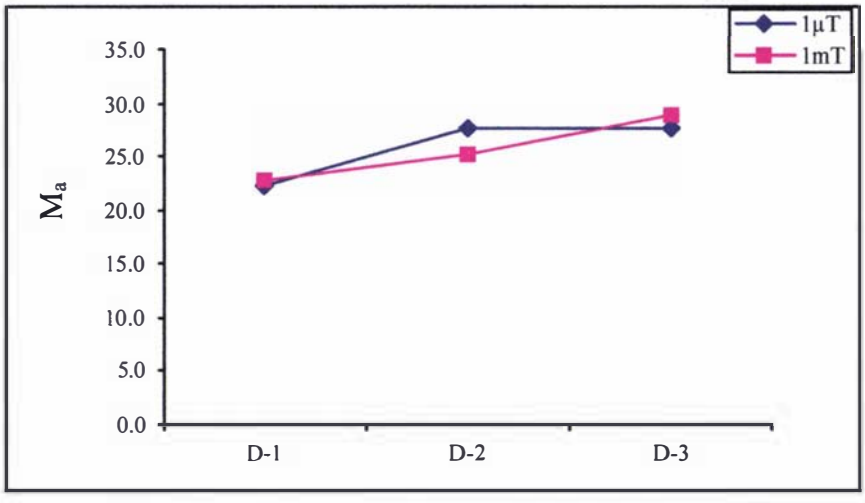


Figure 4.20: Graph of M_a of different Strength and three Donors.

The data of MN experiments were also examined by collapsing across all experimental conditions so that the *mean number of micronuclei per 100 CB cells* (M_b) in all experimental condition could be compared with those of the control condition. Table 4.12 summarises the data for the number of CB cells analysed (N), M_b, and the standard deviations of the control group and the experimental groups. The data were analysed by *t*-test to see whether there was any major difference between the means for the control group and that of the combined experimental groups.

Table 4.12: M_b's and standard deviations of control groups and that of the entire set of experimental groups.

Expt.	N	M_b	Std. Deviation
Control	18466	23.80	6.97
Experimental	50216	32.02	13.06

A statistically significant difference was observed between M_b of the control group and that of the entire set of experimental groups, $t\,(34) = 2.40$, $P < .01$.

Does the result shown in Table 4.12 apply to all three donors? Figure 4.21 shows the results for each donor separately.

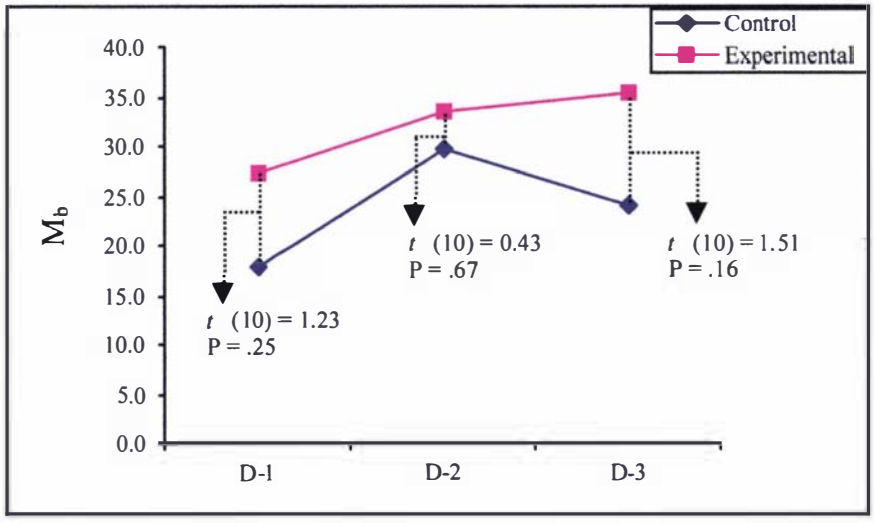


Figure 4.21: Graph of M_b of control groups and EMF-exposed groups for three donors.

Again, none of the differences between control and experimental conditions for the donors reached significance, t (10) = 1.23, P = .25, t (10) = 0.43, P = .67 and t (10) = 1.51, P = .16, for D-1, D-2 and D-3, respectively.

Table 4.13 summarises the data for complex, three controls and nine experiments. Average M_b values of the three controls and complex experiment for MN is 23.80 and 21.66, respectively. The M_b value differs considerably across all the experiments (19.70- 48.98), with the M_b value of the experiment, *Square pulsed 1mT* being the highest. It is also notable that the M_b value decreases with an increase of field strength in two parameter combinations, *Sine pulsed 1μT and 1mT* (34.96-20.44), and *Square continuous 1μT and 1mT* (31.39-27.66). The other two-parameter combinations showed upward trends, *sine continuous 1μT and 1mT* (19.70-29.27), and *Square pulsed 1μT and 1mT* (45.38-48.98). The standard deviations of M_b for the two experiments were the highest (15.10 and 11.76, respectively) for *Sine continuous 1mT*, *Sine pulsed 1μT,* and lowest for *Sine pulsed 1mT* (3.99), but a uniform deviation was observed among all other experiments (5.15-9.27). The reason for the high standard deviation in *Sine continuous 1mT* (15.10) was due to an unusually high number of CB cells with varying number of micronuclei observed in D-3.

Table 4.13: M_bs and standard deviations of all experiments based on all three donors. N= Total number of CB cells observed.

Wave	Form	Strength (T)	M_b	Std. Deviation	N
Sine	Continuous	1μ	19.70	7.87	6129
Sine	Continuous	1m	29.27	15.10	6095
Sine	Pulsed	1μ	34.96	11.76	6106
Sine	Pulsed	1m	20.44	3.99	6061
Square	Continuous	1μ	31.39	7.21	6322
Square	Continuous	1m	27.66	7.97	6345
Square	Pulsed	1μ	45.38	8.20	6086
Square	Pulsed	1m	48.98	7.94	7072
Complex			21.66	5.15	6100
Control (x3)			23.80	9.27	18,466
Total					74,782

Table 4.14 shows that the only statistically significant outcome was a 4-way interaction involving Wave, Form, Strength and Donors F (2, 24) = 12.47, P < .001, the same result that was obtained using M_a. Figure 4.24 shows that one main source of this interaction was the Form x Wave interaction. It can be seen that a significant difference, *t* (10) = 3.33, P < .01, in Pulsed wave form between the Square wave and Sine wave (Figure 4.24) occurred. Figures 4.25 to 4.27 show data for each donor separately, and it can be seen that D-3 makes little contribution, as for M_a. Thus, the factor, Donor, plays an important part in the observed 4-way interaction.

Table 4.14: Overall ANOVA for M_b.

Source	df	F	P
Intercept	1	176.77	0.006
Wave	1	5.64	0.14*
Form	1	3.82	0.19
Strength	1	0.36	0.61
Donor	2	0.32	0.74
Wave x Form	1	6.18	0.13*
Wave x Strength	1	1.33	0.37
Form x Strength	1	2.02	0.29
Wave x Form x Strength	1	2.13	0.28
Wave x Donor	2	-	-***
Form x Donor	2	-	-***
Wave x Form x Donor	2	0.17	0.85
Strength x Donor	2	-	-***
Wave x Strength x Donor	2	0.04	0.96
Form x Strength x Donor	2	-	-***
Wave x Form x Strength x Donor	2	12.47	0.001**

* = Near significant, ** = Highly significant, and *** = SPSS could not calculate because it cannot compute the error degrees of freedom using Satterthwaite's method.

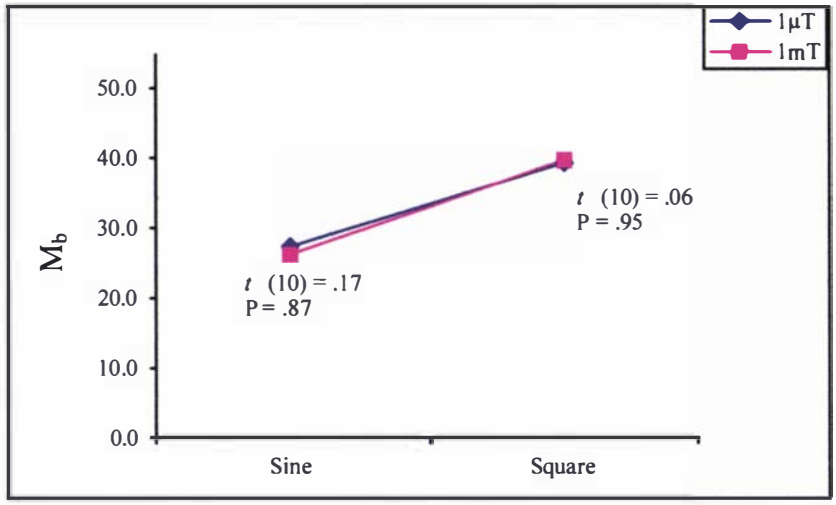


Figure 4.22: Graph of M_b of different Wave and Strength.

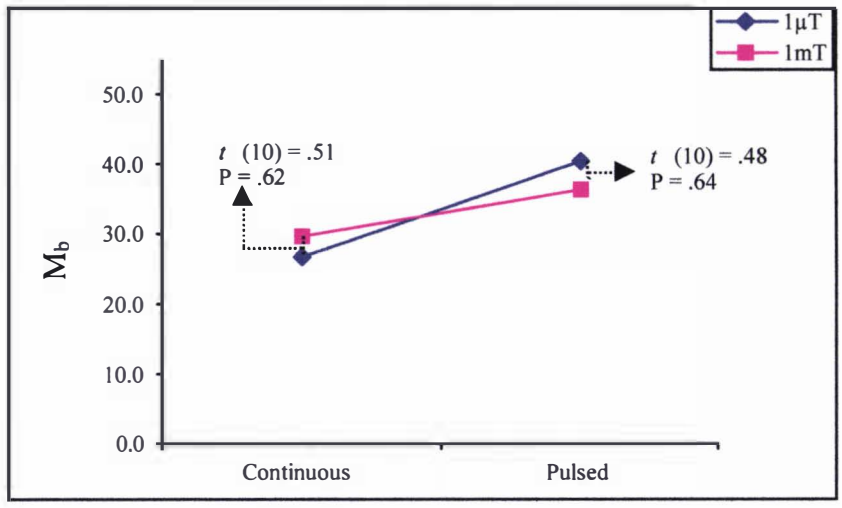


Figure 4.23: Graph of M_b of different Form and Strength.

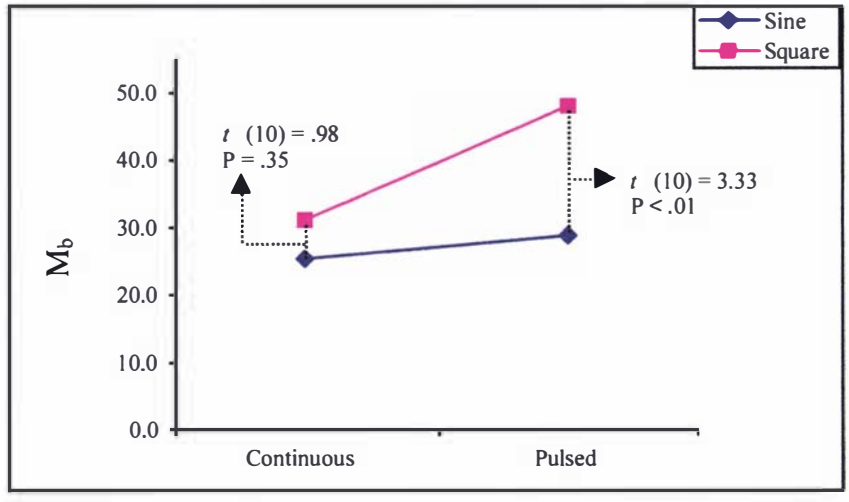


Figure 4.24: Graph of M_b of different Form and Wave.

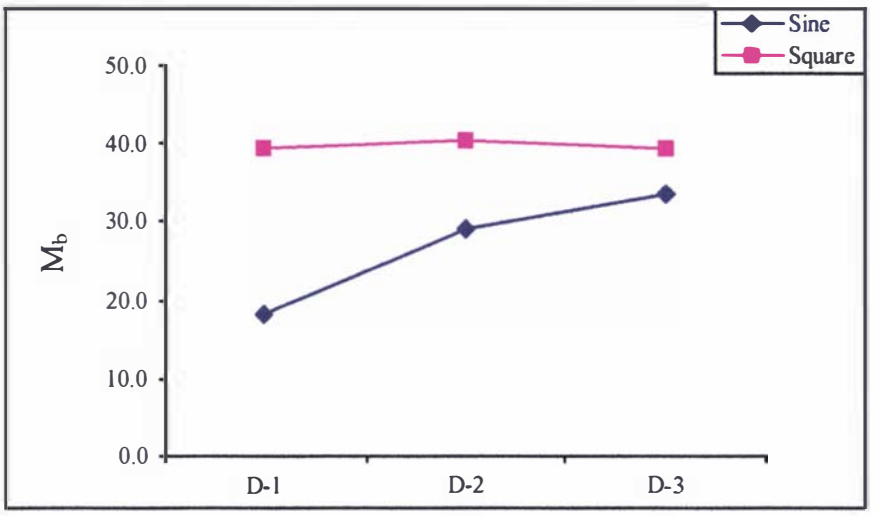


Figure 4.25: Graph of M_b of different Wave and three Donors.

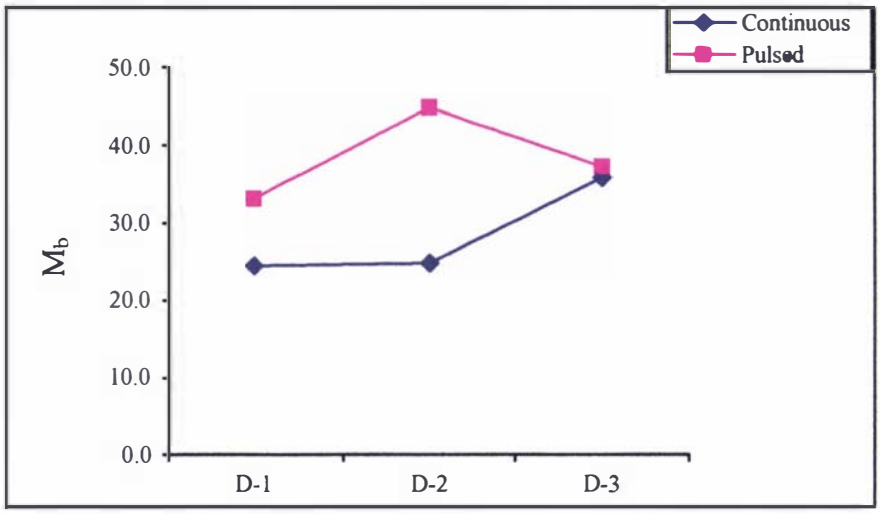


Figure 4.26: Graph of M_b of different Form and three Donors.

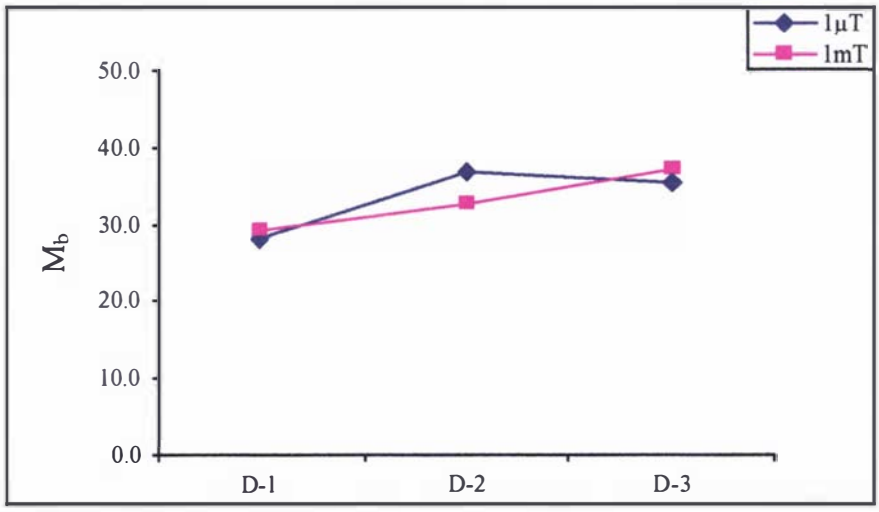


Figure 4.27: Graph of M_b of different Strength and three Donors.

4.2.1 Complex MF

Table 4.15 summarises the data for the number of cells analysed (N), M_a, and standard deviation of the mean of the control and complex experiment. No statistically significant difference was observed between the mean, M_a, of control and complex experiment, t (10) = 0.16, P = 0.88. A simlar result was found for M_b (Table 4.16), t (10) = 0.48, P = 0.64.

Table 4.15: Overall M_a of control and complex experiment of MN assay.

Expt.	N	M_a	Std. Deviation
Complex	6,100	18.84	4.53
Control (x3)	18,466	19.39	5.28

Table 4.16: Overall M_b of control and complex experiment of MN.

Expt.	N	M_b	Std. Deviation
Complex	6,100	21.66	5.59
Control (x3)	18,466	23.80	6.97

4.3 COMET Assay

The COMET assay experiments in the current study were performed in peripheral blood lymphocytes of one donor (D-1) only, who took part in R1 of the SCE study. Two lymphocyte culture tubes were established for each of the two experiments and control. Lymphocyte cultures of the two experiments conducted for the COMET assay were exposed to 50 Hz *square continuous* or *pulsed* (4 sec on and 4 sec off) MFs at the field strength 1mT for 72 h. The lymphocyte cultures of the control were sham exposed. Both the COMET assay experiments and control were conducted single blind. A total of 272 comets were analysed for the control and those two experiments. Two cell images are shown in Figure 4.28, which are typical of their respective conditions: (a) shows an

undamaged cell that was typical of the control conditions, whereas (b) shows a damaged cell that was typical of the EMF exposed conditions.

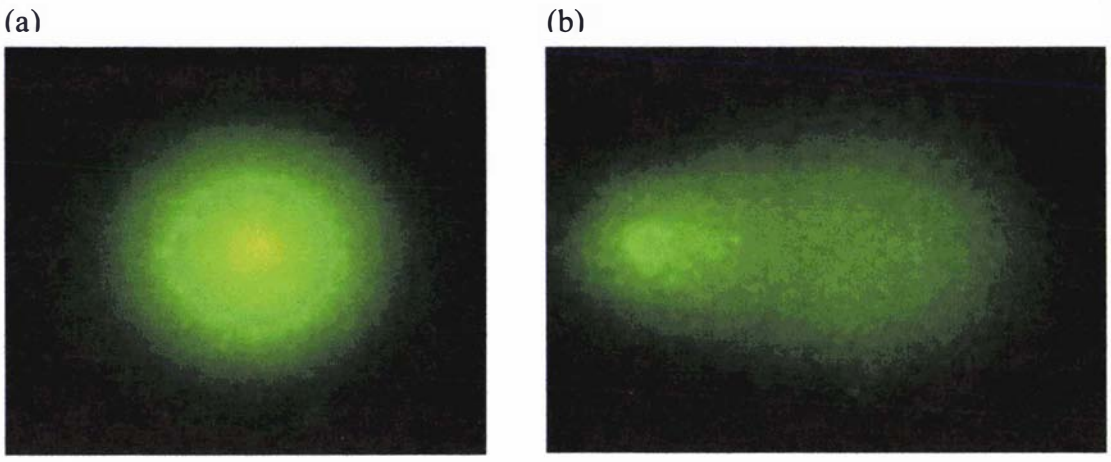


Figure 4.28: Photographs showing single-strand DNA migration pattern of individual peripheral blood lymphocytes from D-1. (a) Control Sample - This cell is typical of the control sample. It is undamaged and will most likely be found in a negative control; (b) Experimental - This cell is typical of the experimental samples. This cell has DNA that is extremely damaged. It is most likely apoptotic.

Table 4.17 summarises the data of the COMET assay experiments on the number of comets observed (N), mean Tail length, Tail moment and Olive tail moment for control, *Square continuous 1mT* and *Square pulsed 1mT*. The mean Tail length values range from 33.99 for *Square pulsed 1mT* to 44.02 for *Square continuous 1mT*. The Tail moment values range from 4.14 for control to 14.96 for *Square continuous 1mT,* and similarly, Olive tail moment values range from 3.76 for control to 10.12 for *Square continuous 1mT*. The standard deviation for Tail length values range from 24.39 for control to 38.87 for *Square continuous 1mT*. The standard deviation for Tail moment values range from 10.33 to 28.07 for *Square continuous 1mT,* and similarly, the Olive tail moment values standard deviation values range from 6.51 for *Square pulsed 1mT* to 15.83 for *Square continuous 1mT*. It is important to note here that *Square continuous 1mT* has the highest mean values for all three parameters (Tail length, Tail moment and Olive tail moment).

Table 4.17: Summary results of three parameters (Tail length, Tail moment and Olive tail moment) of the COMET assay experiments.

Parameters	Experiments	Mean	Std. Deviation	N
Tail length	Control	34.45	24.39	103
	Square continuous 1 mT	44.02	38.87	99
	Square pulsed 1 mT	33.99	30.23	70
Tail moment	Control	4.14	10.33	103
	Square continuous 1 mT	14.96	28.07	99
	Square pulsed 1 mT	9.03	14.92	70
Olive tail moment	Control	3.76	6.87	103
	Square continuous 1 mT	10.12	15.83	99
	Square pulsed 1 mT	5.67	6.51	70

The mean differences between each pair of conditions were analysed using *t*-tests (see Table 4.18). Statistically significant differences were observed for the mean Tail length, mean Tail moment, mean Olive tail moment between control and *Square continuous 1mT,* $P < .04$, $P < .001$, and $P < .001$, respectively. It should be noted that all these outcomes involve the continuous waveform. However, there was also statistically significant difference for the mean Tail moment between control and *square pulsed 1mT,* $P < .02$. Furthermore, there was a significant difference between the two waveforms, $P < .01$. These differences are also shown graphically in Figure 4.29.

Table 4.18: *t*-tests for all three parameters (Tail length, Tail moment and Olive tail moment) between the three COMET assay experiments.

Parameters	Experiments	*t*	df	Sig. (2-tailed)	Mean difference
Tail length	Control vs Sq. cont. 1mT	2.09	200	.04	9.57
	Control vs Sq. pulsed 1mT	0.11	171	.92	0.46
	Sq. cont. 1mT vs Sq. pulsed 1mT	1.81	167	.07	10.03
Tail moment	Control vs Sq. cont. 1mT	3.61	200	.001	10.82
	Control vs Sq. pulsed 1mT	2.38	171	.02	4.89
	Sq. cont. 1mT vs Sq. pulsed 1mT	1.78	167	.08	5.94
Olive tail moment	Control vs Sq. cont. 1mT	3.68	200	.001	6.36
	Control vs Sq. pulsed 1mT	1.84	171	.07	1.91
	Sq. cont. 1mT vs Sq. pulsed 1mT	2.51	167	.01	4.45

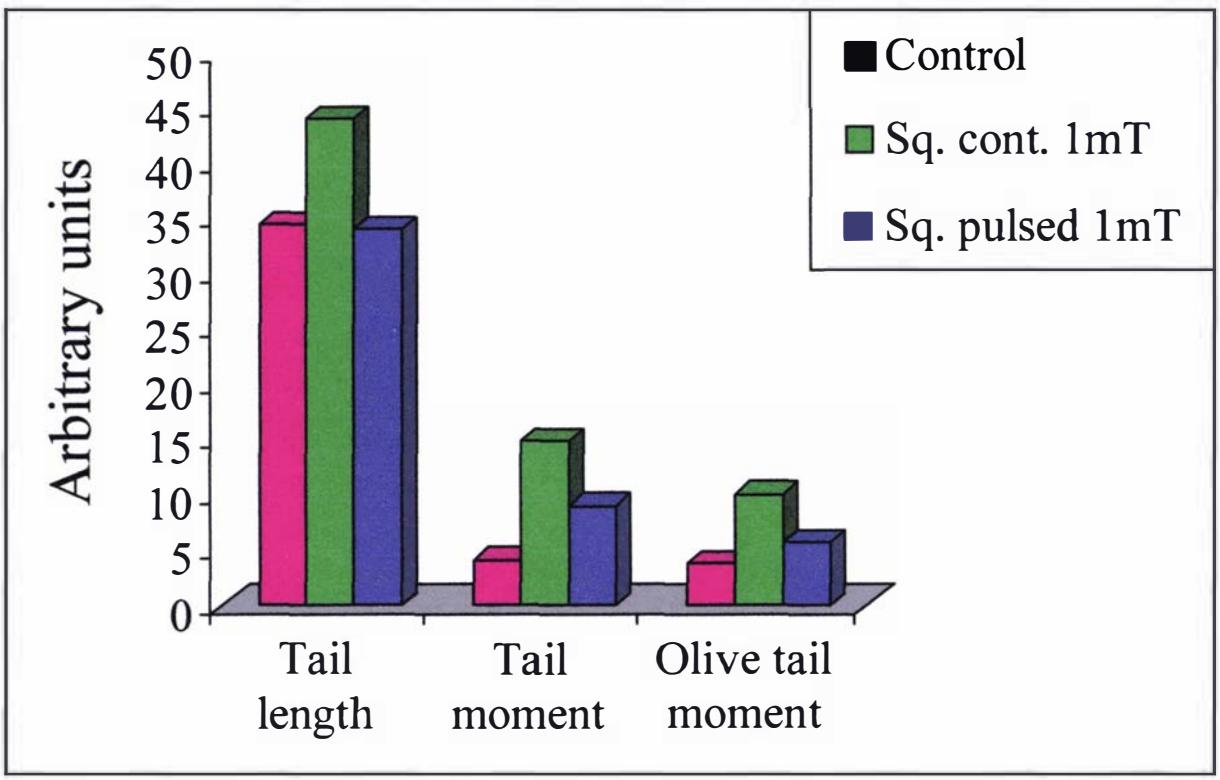


Figure 4.29: Mean values for Tail length, Tail moment, Olive tail moment for control, *Square continuous 1mT* and *Square pulsed 1mT*.

4.4 Fluorescent *in situ* Hybridization (FISH)

The FISH experiments were performed on peripheral blood lymphocytes for only one donor (D-1) in the current study. Only one FISH experiment was conducted because of time and resource constraints.

Two lymphocyte cultures for both the experiment and the control were established. Lymphocyte cultures of the FISH experiment were exposed to a 50 Hz *square continuous 1mT* MF for 72 h. Lymphocyte cultures of the control experiment were sham exposed. A total of 1051 metaphase cells were analysed in the experiment and the control conditions (Table 4.19). No translocations or dicentric chromosomes were observed. Figure 4.30 shows a cell image typical of the control conditions.

Table 4.19: Summary results of control and one FISH experiment.

Experiments	No. of cells observed	No. of translocations observed
Control	545	0
Square continuous 1mT	506	0

4.5 Chromosomal Anomalies

In the current study, the main aim was to investigate the biological effects of EMFs on the HPBL. Although it affects the frequency of SCE, frequency of micronucleus, and causes single DNA strand breakage (SSB), it is the chromosomal anomalies that are of particular interest. Of these, endoreduplication is the strangest phenomenon observed in PBL cultures of all donors studied. Figures 1 (a & b) and 2 (a & b) of Appendix 5, shows the result of endoreduplication in cultured lymphocytes at metaphase. The chromosomes have replicated but were unable to separate correctly at anaphase, thus resulting in this abnormal configuration.

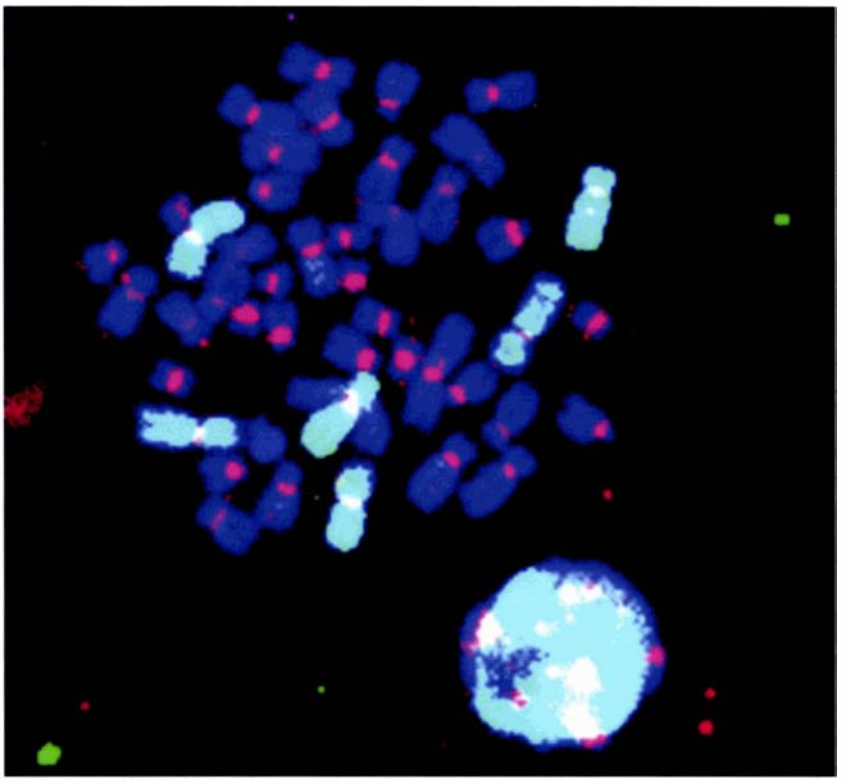


Figure 4.30: Photographs showing a human peripheral blood lymphocyte cell with six whole chromosome (2, 3 and 5) labelled with FITC (green), centromeres labelled red with Pan Centromeric Probe (PCP, Cambio) and rest of the chromosome labelled with DAPI in the analysis of metaphase cells.

Among other anomalies; multiple SCEs (3 & 4) are shown in Figure 3 (a & b), and chromosome complexes formed by 2-pairs of chromosomes are shown in Figure 4 (a & b) of Appendix 5, respectively.

Chapter Five

Discussion

In this section the author explores the significance of the findings in this study as they relate to the current body of knowledge about EMF effects on cells, in particular on the genetic machinery. Because this area is highly contentious, the researcher was cognisant of the fact that the study had to be conducted under strict conditions, i.e., sufficient power to detect any effects if they existed, ensure that the fields to which the lymphocytes were exposed in all experiments were clearly defined and, most importantly, demonstrated to be so, and that the study be conducted blind. In addition, the researcher wanted to determine whether the results obtained could be repeated and in different subjects. For this reason, the study was conducted twice (Round 1 and Round 2) for the initial main focus on SCE. Limited time and limited resources did not permit the whole study to be repeated for the MN assay, COMET assay and FISH. Thousands of cells were examined and scored which involved a considerable effort, but this meant that any conclusions drawn were supported by reasonably secure data.

5.1 SCE Analysis

The SCE experiments for both rounds (R1 and R2) were conducted to evaluate whether or not weak EMFs affect the frequency of SCE, which is used as an indicator of clastogenicity, or genotoxicity, in blood lymphocytes of humans. Six donors took part in this study (identified as D-1 to D-6). The first three donors (D-1 to D-3) were included in R1, whilst D4-D6 were included in the replicate set of experiments (R2). For all experiments, BrdU-treated G0 lymphocyte cultures that were subjected to both control and experimental conditions were placed in a specially designed incubator at 37C. The incubator was placed in an electrically quiet room where stray magnetic and electric fields as measured with a standard Gaussmeter, were negligible. Lymphocyte cultures of experimental conditions were exposed each to a certain MF continuously for 3 days while the lymphocyte cultures of the control experiments were sham-exposed (no EMF field). In an additional experiment, lymphocytes from all donors were grown in the presence of a complex MF produced from the back of a computer. Lymphocytes were harvested at the end of a 72 h incubation period, which included 1 h of treatment with

colchicine. Approximately 200 second mitotic metaphase cells for SCE were scored per donor, per experiment.

The SCE results show that ELF EMFs do have an effect on human chromosomes. These results, however, are sometimes conflicting and difficult to interpret. While a significant difference between the experimental and sham control conditions was observed, there were mixed results throughout the different parameter combinations as well as inconsistencies in and across both rounds of experiments.

The overall means of SCE/cell of the controls and that of the entire set of experimentals for R1 and R2 are shown in Tables 4.1 and 4.4. The overall mean of SCE/cell for the controls in R1 and R2 were 8.92 and 9.66, respectively, whereas the overall mean of SCE/cell of the entire set of experimentals in R1 and R2 were 9.34 and 10.17, respectively. A *t*-test showed that there was a significant increase ($P < .001$) in the number of SCEs/cell in the grouped experimental conditions compared to the controls in both R1 and R2. The important point to note here is the repetition of an overall increase in the number of SCE/cell as a consequence of EMF exposure in R2 that was previously observed in R1.

SCE results were analysed further to determine whether all six donors contributed equally to the results of R1 and R2. The results showed significant increases in the formation of SCE/cell under grouped experimental conditions compared to the sham exposed controls for four donors out of six, while the other two donors showed only a small increase in the effects for the experimental conditions. However, even though these remaining two donors did not yield significance results, the data showed a trend towards an effect from EMF exposure (Figures 4.1 and 4.11).

A key element in this body of work was the adoption of a reductionist approach to try and establish whether any particular factor or combination of factors could affect the genetic machinery. Unfortunately the results of the SCE study are complex and interpreting the results is not easy. Nevertheless, a close examination shows some interesting patterns. First, in R1 the mean SCE values increased with increasing EMF field strength in all parameter combinations except for a downward trend in *sine pulsed* $1\mu T$ to $1mT$ (Table 4.2), while the SCE values for R2 are largely the reverse.

Paradoxically, the SCE values decreased in R2 with an increase of EMF field strength in all parameter combinations except for an upward trend between *sine continuous* $1\mu T$ and $1mT$ (Table 4.5). Thus, the mean SCE values increased with increasing EMF field strength ($1\mu T$ to $1mT$) in both R1 and R2 but only for *sine continuous*. The trend of increasing SCE frequency for *sine continuous* with increasing field strength was exactly the same in both rounds. But for *sine pulsed* the mean SCE value was downward for both R1 and R2 with increasing field strength. For both *square continuous* and *square pulsed* the SCE value was upward in R1 and downward in R2 with increasing field strength. It would appear from these data that there are no straightforward conclusions that can be drawn concerning a linear increase in EMF field strength and genetic effect, although a closer examination may indicate that some subtle effects may be occurring.

Interactions

A detailed analysis of parameter interactions showed the most interesting results. Figure 4.5 shows that a *square continuous* field produces a near significant higher number of SCEs per cell in R1 ($P = 0.06$). Similarly, we can see from Figure 4.12 in R2 that the same *square continuous* field indeed produces a significantly higher number of SCEs per cell. Going a step further, Figure 4.6 shows that a *square* wave produces a significantly higher number of SCEs per cell with increasing field strength, and Figure 4.7 shows that a *continuous* form produces a significantly higher number of SCEs per cell with increasing strength. Thus, it appears that a *square continuous* field with increasing strength may be of importance here in producing a higher number of SCEs per cell.

The author notes that the SCE frequency of 10.61 scored for a *sine pulsed* field at 1μT in R2 was the highest recorded, which theoretically could mean that this field has the greatest clastogenic effect of all parameters, but caution is warranted here; the highest SCE frequency in R1 was 10.03 for a *square continuous* field, and the SCE frequency of 10.39 for a *square continuous* field in R2 (albeit a different strength) was the second highest in this latter round. The possible importance of a square field generating a clastogenic effect became even more apparent with subsequent experimentation using the Micronucleus assay and COMET assay.

Complex field

The mean SCE value of the three control experiments and *complex* experiment for R1 and R2 are shown in Tables 4.7 and 4.8, respectively. No statistically significant difference was observed in either R1 or R2 between the mean SCE/cell of the control groups and that of the complex field. The author notes, however, that despite attempting to culture lymphocytes on three separate occasions in a complex field, difficulty was always encountered in obtaining a sufficient number of dividing metaphase cells. Only 87 cells could be scored in R1, and only 188 cells in R2, in all 3 subjects. A similar reduction has also been noted by Nagy and Fischl (2004) in the sporulation of fungi. The low mitotic index observed in the present study severely limits the statistical power of the observations and consequently restricts the conclusions that can be drawn. Nevertheless, a repeatedly low mitotic index must be a cause for concern, despite the lack of significant differences in SCE frequencies between the complex-field-exposed cultures and the controls. This raises questions concerning health issues and could be the focus of a more intensive study in the future.

EMF exposures and SCE

The overall increase in number of SCE/cell as a consequence of EMF exposure that was observed in the current study is in good agreement with the results reported previously by Khalil and Qassem (1991). They similarly showed that *in vitro* exposure of human cells to EMFs resulted in a significant increase in frequency of SCE. In their study, PHA-stimulated lymphocytes were exposed to a pulsed field (50 Hz, 1.05 mT peak; pulse duration, 10 msec) for 24 h, 48 h, and 72 h. No significant alterations in the baseline frequency of SCE were observed in cultures exposed to EMFs for 24 h or 48 h. In contrast, however, a statistically significant 2-fold increase in the frequency of SCE was observed in cultures continuously exposed for 72 h. This is the only report so far available which shows this positive result using SCE analysis on extremely weak MFs. It is interesting to note, however, that Yaguchi *et al.* (1999) also observed an increase in SCE in mouse m5S chromosomes when they applied much stronger MFs (5-400 mT). Furthermore, several other studies of human (Garcia-Sagredo and Monteagudo, 1991; Nordenson *et al.* 1994) as well as animal (Lai and Singh 1997a) cells exposed to ELF EMFs have also shown clastogenic effects via different methods.

In Garcia-Sagredo and Monteagudo's (1991) study, HPBLs were exposed to pulsed EMFs at 4 mT and a statistically significant increase in frequency of CAs was observed. More recently, Nordenson *et al.* (1994) exposed human amniotic cells to a sinusoidal 50 Hz, 30 μT pulsed MF (15 sec on / 15 sec off) for 72 h, and similarly observed a significant increase in the frequency of CAs. Among exposed cells, the aberration frequency was 4% compared to 2% in sham-exposed cells. The same authors observed a similar increase in CA in another series of eight experiments in a 2 sec on / 2 sec off pulsed field (Nordenson *et al.* 1994). The rationale used to explain EMF effects observed with pulsed fields but not continuous fields is sometimes attributed to a phenomenon called "habituation", or in this case a lack of habituation. According to this theory, cells can become habituated to a constant continuous field and thus do not show a response. With pulsed fields, the opposite prevails: the view expressed is that cells do not become habituated and therefore do show a response. No mechanism as to how habituation could be achieved has been documented that the current author is aware of, and whilst agreeing that the idea is attractive, no evidence was found in the present study to support the theory.

Conflicting results

In the current study conflicting results were observed in some parameters between the two successive rounds of identical experimentation. Sometimes a significant effect was observed in R1 but not in R2 and vice versa. Such contradictions are not unusual in this field of study as is illustrated by the following examples. DNA strand breaks which are a common form of DNA damage were observed in brain cells of rats in a study conducted by Lai and Singh (1997a). In their study, rats were exposed for 2 h to a 60 Hz MF (flux densities 0.1, 0.25 and 0.5 mT) and they observed a dose-dependent increase in DNA strand breaks in the brain cells at 4 h post-exposure. An increase in single-strand DNA breaks was also observed in all three flux densities, but double-strand DNA breaks were observed only at the higher parameters of exposure (0.25 and 0.5 mT). On the other hand, other authors have reported that ELF EMFs affect neither the frequency of SCE nor CA in HPBLs. Rosenthal and Obe (1989) exposed mitogen-stimulated adult peripheral lymphocytes to a sinusoidal 50 Hz field from 0.1 mT to 7.5 mT and reported no effect of EMF on the frequency of both chromosome breaks and SCEs. A similar study using a pulsed MF at 1.0, 2.0, and 4.0 mT, applied as a quasirectangular pulse of

26 μsec, in 5 msec trains, repeated at 14 Hz, also showed no effect on SCE (Garcia-Sagredo *et al.* 1990).

Similar findings of null EMF effects were also reported by Antonopoulos *et al.* (1995) and Cohen *et al.* (1986a, b). HPBLs were exposed to 5 mT, 50 Hz MF, in the former study, but in the latter, the same were exposed to 60 Hz, circulatory polarized fields at an electric current density of 30 $\mu A/cm^2$ and/or MFs at 0.1 and 0.2 mT. In both studies no effect of magnetic, electric, or a combined EMF was observed on the frequency of SCE, mitotic rate or chromosome breakage.

In a continuation of null EMF effects, an animal genotoxic study performed by Livingston *et al.* (1991) on Chinese hamster ovary fibroblasts and PHA-stimulated adult and newborn lymphocytes were exposed to a fixed MF of 0.22 mT, with 60 Hz electric fields (current densities of 3, 30, 300, and 3000 $\mu A/cm^2$). No significant effects on SCE were observed in any cell type or field examined. In a similar study, exposures of Chinese hamster cells to pulsed EMFs from 0.18-2.5 mT did not show any increase in the baseline frequency of SCE (Takahashi *et al.* 1987). The data from several other studies have indicated an absence of significant differences in the incidence of CA, SCE as well as micronucleus frequency between RFR-exposed and sham-exposed cells (Vijayalaxmi *et al.* 1997, 2001a, b; d'Ambrosio *et al.* 2002; Bisht *et al.* 2002).

EMF effects non-reproducible

As noted previously, although an overall effect was observed in the present SCE study, different parameter combinations in R1 and R2 did not yield exactly duplicate results. This is perplexing. Other workers have encountered similar difficulties in reproducing their own positive findings. Maes *et al.* (1996, 1997) reported a significant increase in CA and a weak effect on SCEs in RFR-exposed human blood lymphocytes but this was not confirmed in their own subsequent investigation (Maes *et al.* 2001). Similarly, subsequent studies by other investigators have sometimes not confirmed previous observations. For example, Lai and Singh (1995, 1996, and 1997a) observed an *in vivo* significant increase in DNA single and double strand breaks in brain cells of rats, when they were exposed to 2.45 GHz, but this was not confirmed in several subsequent

studies using rodent and human cells (Malayapa *et al.* 1997a, b, 1998; Maes *et al.* 1997; Vijayalaxmi *et al.* 2000; Li *et al.* 2001; Tice *et al.* 2002).

Donor

Two groups of three healthy donors were used in the present SCE study. The intention of performing the entire experiments twice with a different set of donors in each round was to determine whether observed effects could be replicated in different people. A key point to note is that the results did show significant differences between control and combined experimental conditions in both rounds. The frequency of SCE was significantly higher in the exposed group in both rounds ($P < .001$). From these data the conclusion can be drawn that certain EMFs can exert a biological effect, but the effects vary. This could be due to a chance event or may be attributed to variability of response in the different donors. It is recognized that the sample size of the two groups is too small to draw any firm conclusion, but then, it was not the aim of the overall study to compare individual effects. The aim of the current study was to observe cellular effects.

Subtle response of cells to EMFs

The controversy relating to variability of response to EMFs could be due to the subtle response of cells to EMFs and this could explain the conflicting results sometimes obtained when experimental replications are attempted. Some of the problems affecting reproducibility have been addressed by Delgado *et al.* (1982). Their study is one of the best examples of a concerted attempt to reproduce an EMF bio-effect involving six independent laboratories trying to replicate the developmental alterations on chicks. In the Delgado experiments, chicken eggs that were exposed to pulsed MFs (10, 100, and 1000 Hz, 0.12 to 1.2 μT) exhibited significant developmental malformations. In the replicate studies all investigators used identical equipment constructed in one laboratory and followed similar but not identical protocols. Fertilized eggs were evaluated for fertility, development, morphology, and stage of maturity. Ten eggs were sham-exposed and ten eggs were exposed to a unipolar pulsed MF (500 msec pulse, 100 pulses / sec, 1μT peak). Because the fertilized eggs were obtained from a local market supplier, the genetic characteristics of the eggs were not controlled. In five of six laboratories more embryos exhibited structural anomalies than did the sham-exposed controls. Statistically

significant differences, however, were observed only in two laboratories; a third laboratory had marginally significant differences (Berman *et al.* 1990). When the data were pooled from all laboratories, approximately 25% of exposed embryos showed abnormalities compared to 19% for sham-exposed controls.

The disconcerting lesson from the study described above is that subtle factors yet to be identified may play a dominant role in bio-effect studies of EMFs. In Delgado *et al.'s* study an effort was made by all of the research groups to reproduce the original experiments as closely as possible and yet the results were still ambiguous. As mentioned above, one possible explanation for the overall lack of agreement might be the genetic variability in the eggs. Support for this suggestion was obtained by Litovitz *et al.* (1993), who, using conditions and end points similar to those in Berman's study, report evidence that changes in the breeding flock may be a strong determinant as to whether or not an EMF-related defect is observed. In some experiments involving multiple replications with eggs from one breeding flock, a strong effect was obtained each time the experiment was performed. In other experiments, using eggs from the same supplier but a different breeding flock, no defects were ever obtained. These data strongly suggest that a yet to be defined factor in a supposedly homogeneously bred stock may be involved in susceptibility to EMFs.

SCE is a sensitive marker for genotoxicity

Although the relationship between SCE, DNA damage and carcinogenesis is not well understood, the SCE technique is still recognised as a sensitive marker for genotoxicity. It is generally believed that induction of SCEs represents the interchange of DNA replication products at apparently homologous loci involving DNA breakage and reunion (Latt *et al.* 1981). In the present study, acute exposure to a 50 Hz MF resulted in a significant overall increase in the frequencies of SCEs in both rounds. This result suggests that DNA breakage might be enhanced by ELF EMFs, as shown by Lai and Singh (1997a). As noted previously, they found by using the COMET assay that acute exposure to a 60 Hz ELF EMF could significantly increase the amounts of single- and double-stranded DNA breakages in the brain cells of rats. Therefore, the presence of both an increase in the frequency of SCEs and the increase in the levels of DNA

damage detected by the COMET assay may indicate that both tests are mechanistically related to effects of exposure to ELF EMFs.

SCE mechanism

If EMFs can exert an effect on the genetic machinery, a central question then arises: what is the mechanism by which this effect occurs? There were only a few important clues to an SCE mechanism before the rapid influx of BrdU-incorporation SCE-studies. From his autoradiographic studies, Taylor (1958) reported that a double-strand exchange was involved in SCEs. Wolff *et al.* (1974) subsequently showed that lesions induced in DNA had to pass through an S phase before they could result in SCEs, and Kato (1974a) likewise noted that SCE induction maximized during the period of DNA synthesis. Considering the above findings the replication bypass model for SCE was originally proposed in 1975 to explain DNA crosslink-induced SCEs, since it appeared that: i) crosslinking agents were the most potent inducers of SCEs (Perry and Evans, 1975; Latt, 1974a); ii) crosslinks would inhibit or prevent the normal separation of DNA strands as required for bidirectional replication (Geiduschek, 1961); and iii) that crosslink repair required an undamaged parallel DNA duplex in order to complete a recombinational exchange (Cole, 1973 and 1974).

On this basis, Shafer (1977) proposed a model in which SCEs could result from the completion of DNA replication at the site of an unrepaired DNA crosslink. The most important characteristic of this proposed model was that SCEs could result from a series of sequential events that might occur as bidirectional replication encounters a crosslink. It is interesting to mention here that the induction of DNA crosslinks was observed in rat brain cells when exposed to a 60 Hz MF (Singh and Lai, 1998). The effects of these MFs were also compared with those of a known DNA crosslink-inducing agent, mitomycin C. The pattern of effects was similar between these two agents. Therefore, the presence of a significant increase in the frequency of SCEs in the present study could be the ultimate product of ELF EMF exposure via DNA-crosslinks. Of all the models proposed linking SCEs with ELF EMF effects (as covered in the Literature Review), this one appears to be the soundest.

Free radicals and melatonin

Free radicals are known to be an important factor in cell proliferation and hence to be cancer promoters (Burdon and Rice-Evans, 1989; Rose and Bode, 1993). The production of free radicals (.OH) is a consequence of the utilization of oxygen by all organisms. About 1-2% of inspired oxygen ends up as toxic free radicals, which can damage macromolecules such as DNA, proteins and lipids. This damage is referred to as oxidative stress. On the other hand, the pineal hormone, melatonin, eliminates free radicals through its most efficient natural cell protection and anti-oxidative stress ability in our bodies (Reiter *et al.* 1994, and 2001; Hardeland *et al.* 1993; Vijayalaxmi *et al.* 1995; Omura *et al.* 1993).

Melatonin's DNA damage inhibitory activity by eliminating free radicals can be seen in the *in vivo* study using safrole that was conducted by Tan *et al.* (1993). Safrole is a known chemical carcinogen, and damages DNA by inducing the production of large numbers of oxygen free radicals. However, melatonin inhibits DNA damage induced by safrole *in vivo*. Tan *et al.* (1993) injected rats with safrole alone and observed extensive DNA damage after 24 h. However, when melatonin was injected along with safrole, the DNA damage was reduced by 99%. Lai and Singh (1997b, c) have also observed similar *in vivo* effects of melatonin. In their studies, rats were treated with melatonin before exposure to EMFs (ELF EMF and RFR) and damaging effects of EMFs on DNA previously observed were now blocked. Vijayalaxmi *et al.* (1995) similarly observed that melatonin protects human blood lymphocytes from radiation-induced chromosome damage. In their study, cells of human peripheral blood were treated *in vitro* with increasing concentrations of melatonin (0.5 or 1.0 or 2.0 mM) for 20 min at 37 ± 1C and then exposed to 150 cGy gamma-radiation from a ^{137}Cs source. The lymphocytes which were pre-treated with melatonin exhibited a significant concentration-dependent decrease in the frequency of radiation-induced chromosome damage as compared with the irradiated cells which did not receive the pre-treatment.

On the other hand, it has been reported from the results of many previous studies that exposure of human cells (Pflunger and Minder, 1996; Arnetz *et al.* 1996; Wilson *et al.* 1990; Graham *et al.* 1994; Davis, 1997; Wood *et al.* 1998; Burch *et al.* 1997, 1998, 1999, 2000; and Griefahn *et al.* 2002) as well as animal cells (Rosen *et al.* 1998; Olcese

et al. 1985; Kato *et al.* 1993; Yellon, 1991, 1994) to ELF EMFs can reduce the amount of melatonin. Wood *et al.* (1998) also reported that MF generated by a square wave significantly reduced melatonin level compared to MF generated by sinusoidal waveforms. This suggests that ELF EMF exposure enhances free radical activity in cells through the suppression of melatonin production, which in turn leads to DNA damage and ultimate production of a higher number of SCEs per cell.

MF exposure limits

In the present research, fields of 1μT and 1mT were deliberately selected for study in order to cover the range of exposure normally encountered by humans. The ELF magnetic flux density in the environment varies over a wide range. For example, household and office levels can vary from 0.01-1μT. Intermittently, levels can reach more than 10 μT. Levels near a power transmission line can be 10-30 μT, whereas the magnetic flux density can vary between 0.1 and 1mT near some electrical appliances (e.g., electric blankets, hair dryers). Much higher levels are expected in occupational exposures (Bernhardt, 1985; Gauger, 1984; Tenforde and Kaune, 1987). Recommended maximum levels also vary. For example, the interim guidelines of the International Nonionizing Radiation Committee of the International Radiation Protection Association (INIRC/IRPA, 1990) for occupational situations are 0.5 mT for workday exposure and 5 mT for short-term (2 h) exposure, whereas for the general public they are 0.1 mT for 24 h/day exposure and 1mT for exposure of a few h per day. The National Radiological Protection Board (NRPB) of England recommends a limit of 2 mT for both occupational and general public exposure to ELF EMFs (NRPB, 1989). In the current study, exposure to ELF EMFs with a magnetic flux density ranging from 1μT to 1mT produced significantly higher SCEs/cell than the sham-exposed controls. These flux densities are much higher than the majority of people encounter in daily life. However, they are still within the limits contained in current MF exposure guidelines of the INIRC/IRPA and NRPB and can be encountered in both public and occupational situations.

kT- problem

One of the criticisms sometimes directed towards suggestions of ELF EMFs being able to exert a biological effect is that the fields are far too weak. The *kT* of the fields is much lower than the *kT* inside the cells; this is an indisputable fact. However, over the last 20 years, biologists have continued to conduct research in this area, and the evidence is compelling that weak EMFs can exert a biological effect. The number of studies which confirm this are too many to ignore. As Hill (1965) stated long ago, it is not necessary to identify a biological mechanism for classifying a causal effect of ELF EMFs. Nevertheless, this point has been the main bone of contention by skeptics who dismiss claims of a biological effect on the grounds that a mechanism is not evident. The author of the current treatise acknowledges this point and recognizes that it is helpful to strengthen the argument (of an effect) beyond doubt if a plausible biological mechanism of EMF interaction can be identified and supported by sound scientific research.

EMF mechanism

Mechanisms of interaction between EMFs and biological systems have been studied for decades and a number of innovative models have been proposed (see earlier review Section 2.1.2). Although there is no generally accepted mechanism of EMF interaction to explain how EMFs can affect biological systems, there is a general agreement that the primary site of interaction is the cell membrane. Signal transduction is the basis for cell communication. It is a general process in which a ligand molecule binds to its receptor site on the cell surface triggering a cascade of molecular interactions in the cell membrane and inside the cell leading to enzyme activation, gene induction, protein synthesis which eventually leads to growth, differentiation and cell proliferation. Liburdy (1995) hypothesised that EMFs interact with the membrane, triggering an early event in the signal transduction cascade, such as calcium ion influx. The calcium ion influx process has been confirmed experimentally (Liburdy, 1992a, b; and Zimmerman *et al.* 1990) in the absence of a release of calcium from intracellular stores. These results implicated the cell membrane as being involved in the EMF interaction.

Adair (1991) has attempted to address the question of ELF EMFs being too weak to influence cell behaviour that they are too weak to be recognized and are thus lost in the general thermal and environmental noise. Adair advanced the opinion that ELF signals need amplification if they are to influence the action of messengers, antibody and cancer promoter molecules at their membrane receptor sites. There have been several hypotheses as to how these frequencies can be amplified in biological systems. Kruglikov and Dertinger (1994) proposed that the signals are amplified via the phenomenon of stochastic resonance, a view that is gaining currency (Astumian *et al.* 1995), and that this amplification occurs at the cell membrane in ion channels. The ion channels can form a network, which is capable of amplifying the signal by several orders of magnitude.

The concept of stochastic resonance (SR) first put forward in the seminal paper by Benzi *et al.* (1981) has ultimately been proposed as a possible mechanism through which weak EMF fields could be detected in biological systems (Kruglikov and Dertinger, 1994). Stochastic resonance is the phenomenon whereby the addition of an optimal level of noise to a weak information-carrying input to certain non-linear systems can enhance the information content at their outputs.

In some systems, for example, such as radio receivers, turning up the volume in order to hear a faint signal amidst much noise usually only results in turning up the noise as well. However, in other systems increasing the amount of ambient noise actually enhances the signal-to-noise ratio through a complicated non-linear cooperation between the system and detector. This phenomenon has been shown to exist in various physical systems, but has only recently received support in biology (Bezrukov & Vodyanoy, 1997). The noise fluctuations might be stochastic but the detection of a desired signal can be maximised by tuning the noise. Of all the theories advanced, this appears to be the most plausible mechanism to explain how weak forces can be enhanced to a level where an increase in SCE can be observed.

In the following section the findings of the MN assay are discussed and an attempt is made to establish any common EMF effects between the SCE technique and MN assay.

5.2 MN Assay

MN assay experiments were conducted in the present study to ascertain whether or not weak EMFs affect the DNA repair system in humans to any degree. In this study, both control and experimental G0 lymphocyte cultures, established from fresh blood, were irradiated with 3.5 Gy X-rays and placed immediately in an incubator at 37C. Following irradiation, a rest period of 6 h was allowed to repair the damaged DNA at G0. After this 6 h period, the lymphocytes were stimulated with the mitogen PHA. Twenty-four hours after this stimulation, the cytokinesis-blocking agent Cytochalasin-B was then added in order to capture binucleate cells with any micronuclei arising from MF exposure. Experimental lymphocyte cultures were exposed to different MFs for the entire culture period (90 h after addition of PHA), whereas the control lymphocyte cultures were sham-exposed.

The results of the micronucleus study showed ELF EMF effects similar to that seen in the SCE study. There was a significant difference between the pooled experimental groups and the control, but mixed results throughout the different parameter combinations for both the *mean number of micronucleated CB cells/100 CB cells* (M_a) and the *mean number of micronuclei/100 CB cells* (M_b).

M_a and M_b results for all experimental conditions and the control are summarised in Tables 4.9 and 4.12. The overall means of the controls and experimentals for M_a were 19.39 and 25.06, respectively, whereas the overall means of the controls and experimentals for M_b were 23.80 and 32.02, respectively. As with the SCE study, a *t*-test showed that there was a significant increase in M_a and M_b in the grouped experimental conditions compared to the controls. The most important point to reiterate is the observation of an overall increase in mean value of both M_a and M_b in the experimentals compared to the controls as a consequence of EMF exposure. These results strongly support the view that ELF EMFs affect the efficiency of DNA repair in cultured PBLs.

Comparing the effects of the different types of fields is not straightforward, but nevertheless interesting in its complexity. First, let us examine those parameters with the greatest effects. A *Square pulsed* field at strengths of both 1μT and 1mT produced

the highest frequency of M_a (33.57 for 1μT and to 35.18 for 1mT) and M_b (45.38 for 1μT and 48.98 for 1mT). These figures are convincingly higher than the average of the 3 controls (19.33 for M_a and 23.74 for M_b). This could be interpreted as a *Square pulsed* field affecting DNA repair in HPBLs greater than any other parameter combination. Interestingly, it was noted previously that the highest SCE frequency in R1 was 10.03 for a *square continuous* field, and the SCE frequency of 10.39 for a *square continuous* field in R2 (albeit a different strength) was the second highest in this latter round. It is tempting here to draw the conclusion that a square wave has a more general damaging effect on DNA, bearing in mind that one must proceed with caution in interpreting these data, not least in acknowledging that the SCE test and the MN assay are measuring two quite different phenomena. Nevertheless, one could argue that the commonality here between the SCE and MN assays is a square wave and that a *square pulsed* field in particular has the greatest effect on the DNA repair system. Having made this point, however, it should also be noted (which also reinforces the perplexity one encounters in this field of scientific endeavour) that a *sine pulsed* field at 1μT produced a relatively high M_b frequency (34.96) and interestingly, a *sine pulsed* field at 1μT produced the highest SCE frequency (10.61) of any parameter.

Interactions

A comparison between the results observed in the SCE study with that of the MN assay shows other interesting parallel features. Table 4.11 shows a near significant interaction between Form and Strength, the same as that seen in Table 4.3 in R1 of the SCE experiments. Also, as noted previously, a *Square pulsed* field showed an increase in M_a and M_b values with an increase in field strength from 1μT to 1mT, the same trend as that observed in R1 of the SCE study. (SCE means in R2 for *square pulsed 1μT* and *square pulsed 1mT* were 9.83 and 9.66, respectively, which is a negligible difference.)

What can one infer from the above parallel observations? Although the SCE test and the MN assay measure quite different cellular phenomena, the similar trends in effects could indicate that a common mechanism is operating at the molecular level. Furthermore, the importance of these parallel observations become even more interesting when we compare the results of the COMET assay in a later section.

Energy of square waves

The rationale for a square wave being the strongest in its biological effect may be due to its higher power output than a sine wave. The higher power-generating capacity of square waves was reported by both Loss (2001) and Islam (2003). The root mean square (RMS) average, which describes the energy within the sine and square waves, was calculated using instantaneous voltages measured over a complete cycle (Islam, 2003 and Loss, 2001). They observed that a sine wave has a RMS value 0.707 times that of its peak value but the same RMS average value for a square wave is equal to its peak value which is 1.0 (see appendix 6).

In sine waves, the voltage increases smoothly from the negative maximum to the positive maximum and back again. Square waves, on the other hand, do not increase in voltage smoothly, but shift suddenly from negative to positive, stay there for half a cycle, then jump to full negative and stay there for half a cycle, repetitively. Three important points can be drawn from the above discussion: 1) the RMS voltage output of a square wave is higher than a sine wave, which might contribute to a higher magnetic flux density; 2) a square wave changes its voltage abruptly which might cause energy splatter; 3) while a sine wave has a single frequency in it (50 Hz in New Zealand), a square wave contains many higher frequencies in it as well, called *harmonics,* which can cause problems; and moreover 4) pulsing also causes extra energy splatter. It is a plausible conjecture that these four factors of square waves (50 Hz) might influence enzymatic processes involved in DNA repair, leading to an accumulation of DNA strand breaks and thereby inducing a higher number of micronuclei. On the other hand, these might also influence the enzymatic processes to induce more cross-links, which might lead to a higher number of SCE per cell.

Donor

In the current MN assay study only one round of eight experiments was performed (because of time constraints) using the same first group of three donors (D-1 to D-3) which was used in R1 of the SCE study. The rationale for conducting the MN assay was to establish whether or not weak EMFs affect DNA repair mechanisms in a similar fashion to the SCE study, which yielded inconsistent results among the different

parameter combinations, both within and between rounds. Indeed, the MN assay experiments produced similar inconsistent results among different parameter combination as seen in the SCE study, even though the overall frequency of micronuclei was significantly higher in the exposed group. Further analysis on the donor contribution towards these results showed that none of the differences between control and experimental conditions for any one donor reached significance. Even though there were no significant differences between control and experimental conditions for each of the three donors, all observations were in the direction of an EMF effect. Only when the results were pooled was an effect observed. From these data the conclusion was drawn that certain EMFs can exert a biological effect on DNA repair mechanisms, but the effects vary. This could be due to a chance event or may be attributed to variability of response in the different donors.

Complex field

Attempts were also taken to see whether there was any difference between the M_a and M_b of the control and that of a complex field (Tables 4.13 and 4.14). No statistically significant difference was observed between the means of each of M_a and M_b of the controls compared to the complex field. From these observations one can tentatively deduce that a complex field, at least the field produced from the rear of the particular computer used in this experiment, causes no damage to DNA repair mechanisms in dividing PBLs. However, as previously noted with the SCE experiments, difficulty was always encountered in obtaining a sufficient number of dividing cells.

Positive and null EMF effects

In the current study, the results show that acute exposure to a 50 Hz MF causes an overall increase in micronucleus frequency as a consequence of EMF exposure which is in agreement with results reported previously by Simko *et al.* (1998). They similarly showed that *in vitro* exposure of human squamous carcinoma cell line (SCL II) to a sinusoidal 50 Hz MF (0.8 and 1mT) resulted in a significant increase in the frequency of micronuclei. In their study, they examined the effects of ELF-EMFs in a human squamous cell carcinoma cell line (SCL II) and in a human amniotic fluid cell line (AFC) for different durations (24, 48, and 72 h) and different field intensities (0.1-1mT)

on micronucleus formation and induction of apoptosis. A statistically significant increase in micronucleus frequency and induction of apoptosis in SCL II cells after 48-h and 72-h continuous exposure to 50 Hz MF (0.8 and 1mT) was found. On the other hand, at lower (0.1 and 0.5 mT) field intensities, no effects could be detected. Exposure of AFC cells to EMF of different intensities and for different exposure times showed no statistically significant differences when compared with the controls. This study by Simko is the only report so far available at 50 Hz which shows a significant increase in micronucleus frequency using the MN assay as a marker for effects of extremely weak MFs, although Scarfi *et al.* (1997a) observed an increase in micronucleus frequency when cultured human lymphocytes were exposed to MFs at 100 Hz.

It is interesting to note, however, that Maes *et al.* (1993) and Vijayalaxmi *et al.* (1998) also observed an increase in micronucleus frequency in HPBLs and peripheral blood and bone marrow of cancer-prone mice, respectively when they applied much stronger MFs (2450 MHz microwave RFR). In Maes *et al.* (1993) study, HPBLs were exposed to microwaves at 2450 MHz. They observed a marked increase in the frequency of CAs (including dicentric chromosomes and acentric fragments) and micronuclei. On the other hand, the microwave exposure did not influence either the cell kinetics or the SCE frequency. Similarly, Vijayalaxmi *et al.* (1998) exposed peripheral blood and bone marrow of cancer-prone mice chronically to 2450 MHz microwave RFR and observed a significant increase in micronuclei.

Intriguingly, Tofani *et al.* (1995) claimed that a DC field can induce the formation of micronuclei. In their *in vitro* study, when human peripheral lymphocytes were exposed to 32 or 50 Hz (75 or 100 μT) with no DC component, no micronuclei were induced. However, when the lymphocytes were exposed to the 32 Hz field with a 42 μT DC component, micronuclei were induced.

The results of most other studies on micronucleus formation due to EMF exposure show a null effect in micronucleus frequency. For example, Scarfi *et al.* (1991, 1993, 1994) were consistently unable to detect an increase in micronucleus frequency in normal cultured human lymphocytes exposed to a 50 Hz pulsed MF or a sinusoidal electrical field. Similarly, Paile *et al.* (1995) exposed human peripheral lymphocytes to 50 Hz, 30 μT, 300 μT, and 1mT MF for 65 h. They observed no statistically significant effect on

the frequency of micronuclei. Interestingly, however, cells from patients with Turner's syndrome showed an 80-100% increase in micronuclei after similar exposure (Scarfi *et al.* 1997).

Hypothesis involving DNA repair

ELF MFs do not have enough energy to break chemical bonds directly in DNA molecules. A possible explanation of the present observations is that 50 Hz MFs, in particular a square pulsed field, might affect the enzymatic processes involved in DNA repair, leading to an accumulation of DNA strand breaks. This hypothesis is supported by a recent report by Phillips *et al.* (1995) that acute exposure to a 60 Hz MF significantly affected the activity of poly-ADP-ribose polymerization, an enzymatic activity involved in DNA repair. A similar effect on poly-ADP-ribose polymerization has also been observed by Sarker *et al.* (1994) in brain cells of rats after chronic exposure to a 50 Hz MF.

Furthermore, the results gained in the current study suggest that either the cell's DNA repair mechanism is affected in such a way that it was unable to repair any DNA damage that was induced by the ELF EMF, or the damage was so severe that although the cell was able to repair some of the strand breakages, not all damage induced could be repaired. Although the true cause behind the increased numbers of micronuclei observed in the current study cannot be elucidated, the results of the present study support the view that weak EMFs can affect DNA repair.

In the following section (5.3 COMET assay) the author discusses the findings of the COMET assay and endeavours to establish any common EMF effects between the SCE technique, MN assay and COMET assay. Furthermore, the author explores whether the EMF effects observed with the SCE technique, MN assay and COMET assay are ultimately controlled by the same mechanism.

5.3 COMET Assay

The application of the COMET assay in both *in vitro* and *in vivo* studies has been shown to be a reliable measure of genetic damage induced by genotoxic agents. Damage induced by physical, chemical or environmental agents in different cell systems can be accurately and sensitively assessed by this assay (Ostling and Johanson, 1984; Singh *et al.* 1988, 1990, 1991, 1994, Singh, 2000; Vijayalaxmi *et al.* 1992, 1993 and Betti *et al.* 1994). Moreover, the COMET assay seems highly reproducible in the same subject and is an effective tool for the evaluation of DNA damage induced by chemotherapy (Ostling *et al.* 1987; Tice *et al.* 1992) or by syndromes involving defects in excision repair, such as *Xeroderma pigmentosum* (Green *et al.* 1992). This assay is also highly sensitive in revealing DNA-damaging lifestyle factors such as smoking (Anderson *et al.* 1994). Damage by UV radiation has been examined extensively using the alkaline COMET assay (Green *et al.* 1992; Gedik *et al.* 1992; Arlett *et al.* 1993). There is also some indication that the alkaline COMET assay can detect DNA repair deficiency after irradiation of lymphocytes from patients with systemic lupus erythematosus and rheumatoid arthritis (McCurdy *et al.* 1997). For these reasons, a total of two COMET assay experiments (*square continuous 1mT* and *square pulsed 1mT*) were conducted to evaluate whether or not weak EMFs damage the DNA to any degree in humans. Scarcity of time and resources are the reasons why only two COMET assay experiments were conducted. However, the results of the SCE study and MN assay showed that a square continuous and square pulsed 1mT MF produced significant effects. This was the rationale for choosing these fields to conduct the same experiments with the COMET assay.

In the current study, both control and experimental lymphocytes were cultured in an incubator at 37C. Lymphocyte cultures of the experimentals were exposed to each of the two MFs while the lymphocyte cultures of the controls were unexposed (sham) for 72 h. Cell suspensions were prepared from these cultures and the lymphocytes were resuspended in low melting agarose then layered onto slides precoated with a 0.5% normal melting-temperature agarose. The cells were lysed with a concentrated salt solution in order to remove cellular proteins and liberate the damaged DNA. The liberated DNA was subjected to unwinding under alkaline conditions to allow DNA supercoils to relax and express DNA single strand breaks and alkali labile sites.

Electrophoresis was then performed also under highly alkaline conditions (pH > 13), which allowed the broken ends to migrate under the effect of an electric field, towards the anode. After neutralization, the nuclei were stained with a fluorescent DNA stain, DAPI. The slides were then kept in a humidified slide chamber until they were scored. A total of 70-103 comets were captured. Only three parameters: Tail Length (in pixels), Tail moment and Olive Tail Moment (arbitrary units) were analysed and used to assess DNA damage.

Similar EMF effects

In the current study, the results showed that exposure for 72 h to a 50 Hz, *square continuous 1mT* or a *square pulsed 1mT* MF caused a significant increase in single-strand DNA breaks in HPBLs. This is in good agreement with the results reported previously by Lai and Singh (1995, 1996 and 1997a) and Ivancsits *et al.* (2002). While Lai and Singh observed effects of 60 Hz MF exposure on DNA in brain cells of the rat, Ivancsits *et al.* (2002) observed effects of intermittent ELF EMFs, 50 Hz, sinusoidal, on human diploid fibroblasts. In Lai and Singh's study, when rats were exposed for 2 h to an EMF at intensities of 0.1, 0.25, or 0.5 mT, they observed single strand breaks, whereas an increase in double strand breaks was observed at 0.25 and 0.5 mT, but not 0.1 mT. The effect was proportional to the intensity of the MF (Lai and Singh, 1997a). In Ivancsits *et al.* (2002) study, human diploid fibroblasts were exposed to continuous or pulsed (5 min-on / 10 min-off) for 24 h to a 1mT EMF intensity. They observed a significant increase in DNA strand breaks due to pulsed exposure, mainly DSBs as compared to non-exposed controls. Similarly, exposure to RFR, 2450 MHz, at a whole body specific absorption rate (SAR) of 0.6 and 1.2 W/kg for 2 h caused an increase in both single and double strand breaks in DNA of brain cells in the rat (Lai and Singh 1995, 1996).

In addition to the above example, Ahuja *et al.* (1999) and Svedenstal *et al.* (1999a, b) also reported an increase in DNA strand breaks in cells after MF exposure. In Ahuja *et al.*'s (1999) study, HPBLs were exposed to five different field strengths (2, 3, 4, 5 and 10 mT, all at 50 Hz) and they observed a significant increase in DNA strand breaks in each magnetic flux density except one. Interestingly, Svedenstal *et al.* (1999a) observed an increase in DNA strand breaks in brain cells of mice after 32 days of exposure to MF

at a low intensity of about 8 μT. Similarly, Svedenstal *et al.* (1999b) also observed an increase in DNA strand breaks in brain cells of mice after 14 days of exposure to a low intensity of 0.5 mT MFs.

A square wave in particular appears to exert multiple effects across all three tests (SCE, MN and COMET) which ostensibly measure different molecular phenomena. It is interesting to recall that in an earlier discussion the current author noted the widely-accepted damaging effect of free radicals on macromolecules such as DNA, proteins and lipids (see p220 *Free radicals and melatonin*). The findings of Wood *et al.* (1998) were also noted (p221), who reported that MFs generated by a square wave significantly reduced melatonin levels, which are thought to inhibit the damaging effect of free radicals. The author also noted (p219) the possible link between MF exposure, DNA cross-links and SCE induction. One can only conjecture about the possibility of a common molecular mechanism operating here, but the current author offers the speculative view in the following section that free radicals, formed as a consequence of MF exposure, may have a wide range of effects on the genetic machinery. This may conceivably involve the formation of DNA cross-links, accumulation of DNA strand breaks, interference with DNA repair, resulting ultimately in genetic damage.

EMF effects: a possible explanation

It is an accepted fact that the energy from ELF EMFs is incapable of breaking chemical bonds directly in DNA molecules. A possible explanation of the present observations in this thesis is that a 50 Hz MF might initiate an iron-mediated process, known as the Fenton reaction, which increases hydroxyl free radical formation in cells, leading to DNA strand breaks (Christophersen *et al.* 1991), and perhaps even widespread effects on the genetic apparatus as seen with the SCE, MN and COMET assays. To diverge briefly, the Fenton reaction is an iron-mediated process. Cells with high iron intake such as proliferating cells, brain cells, peripheral blood cells, and cells infected by DNA virus may be more susceptible to the effects of EMF because a high metabolic rate generates high amounts of hydrogen peroxide via the mitochondrial electron transport pathway (oxidative phosphorylation). For proliferating cells, the most vulnerable time may be during the /S phases of the cell cycle when transferrin receptors are expressed and iron influx is high. This coincides with when DNA repair occurs with the MN assay and

when SCEs are initiated. Hydroxyl radicals are generated from hydrogen peroxide via the Fenton reaction, which is the iron-salt-dependent decomposition of di-hydrogen peroxide, generating the highly reactive hydroxyl radical. The equation that illustrates the Fenton reaction is, $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$ (Wedrychowski *et al.* 1986). Returning to the central argument, the above hypothesis is supported by recent reports (Lai and Singh, 1997b, c and Lai, 2001), which state that treatment of rats before exposure with melatonin (a free radical scavenger) blocked the effects of EMF (ELF-EMF and RFR) on DNA. This suggests that EMFs enhance free radical activity in cells, which in turn leads to DNA damage. They also observed that EMF exposure caused DNA-protein and DNA-DNA crosslinks (Singh and Lai, 1998) and increased rates of apoptosis and necrosis in brain cells of rats. Furthermore, they observed that pre-treating rats with an iron-chelator could block the effects of EMF exposure on DNA. This means, in the presence of an iron chelator, the Fenton reaction can no longer activate and produce free radicals and consequently yield no DNA damage. Further detailed investigation is undoubtedly required to substantiate the conjecture that the effects of free radicals represent the common theme in the results observed in the present study, which is discussed in the Future Research section on page.

5.4 FISH

Translocations are often referred to as stable CAs as they may remain reasonably constant for years (Buckton *et al.* 1967, 1978; Buckton, 1983; Lucas, 1997; Lloyd *et al.* 1998). Increased CA frequency is a well-known feature of ionising radiation in humans. The scoring of chromosomal translocations in HPBLs has long been established as a method for personal dosimetry in radiological protection (IAEA, 1986). Exciting progress, however, has been made in this area over the last decade. The recent development of FISH methods has brought the objective scoring of translocations to a new level (Pinkel *et al.* 1988; Lucas *et al.* 1989), ushering in a new era in the detection of stable aberrations. In the current study, of course, the effects of *non*-ionising radiation exposure over 3 days were investigated. Nevertheless, in view of the above developments, FISH was employed to investigate the incidence of translocation frequency in PBLs, should they be induced by ELF EMF exposure. The aim was scientifically sound even if the results were somewhat predictable.

An extensive literature search showed no reports of translocations occurring in humans or any other model system as a result of ELF EMF exposure. It seems surprising to this author that no such studies have been performed. Perhaps the absence of publications is a consequence of investigators not finding any evidence of major CAs arising from exposure to weak EMFs. This would not be totally unexpected as the forces involved to break a chromosome and thereby give rise to a translocation are much greater than the forces required to disrupt molecular events at the replication fork, for instance, and thus affect the frequency of SCE. Certainly the results obtained in the current study support the view that ELF EMFs are too weak to affect whole chromosome structure. Although only one field (*square continuous 1mT*) was studied, a count of 506 cells showed no evidence of translocations occurring in PBLs subjected to this exposure.

5.5 Future Research

In the field of EMF research, perhaps the most outstanding question that remains to be answered with certainty is how weak EMFs exert their effects at the molecular level. In other words, "What is the mechanism by which weak EMFs exert their biological effect?" One of the exciting ideas advanced by the author of the current thesis is that free radicals, formed as a consequence of MF exposure, may have a wide range of effects on the genetic machinery. The possible role of free radicals may conceivably involve the formation of DNA cross-links, accumulation of DNA strand breaks, interference with DNA repair, resulting ultimately in genetic damage. Simko *et al.* (2004) have also recently proposed a mechanism where EMFs indirectly affect the DNA by way of free radical production, the speculation being on the basis of experiments they conducted in rats. Free radicals are widely known to cause genetic damage (Tan *et al.* 1993). Simko's group, however, have not tested this mechanism. That EMFs may induce the production of an increased number of free radicals, could herald a breakthrough in providing a long sought-after model by which very weak EMFs affect genetic material as observed in this thesis. Future research should be conducted to test this hypothesis, especially as it relates to assays such as sister chromatid exchange, micronucleus assay and COMET assay, all of which are known to detect genotoxic/ clastogenic effects.

According to my proposed model, exposure to certain 50 Hz MFs increases the number of free radicals, which are known genotoxic elements. My hypothesis is that when dividing PBLs are cultured in a suspected clastogenic EMF field, this results in a higher frequency of sister chromatid exchange, a higher number of micronuclei and an increase in DNA degradation as a consequence of exposure to free radicals. If this is true, then exposure of PBLs to a free radical scavenger (e.g. melatonin) when growing in the presence of a selected EMF field, should reduce the amount of genetic damage.

To test this hypothesis, I propose that three assays be applied: sister chromatid exchange, the radiation-induced MN assay and the COMET assay. The aim of these investigations is to investigate whether the application of melatonin (a known free radical scavenger) to an PBL culture can reduce the effect of free radicals allegedly produced by 50 Hz MF exposure.

First, however, we should confirm by way of a surrogate experiment that free radicals can cause an increase in genotoxic damage as observed by the above three assays. Safrole is a well-known chemical carcinogen which is known to damage DNA by inducing the production of large numbers of free radicals (Tan *et al.* 1993). In my proposed study, a PBL culture should be grown in the presence of safrole which would thus expose dividing blood lymphocytes to a large number of free radicals. I predict that this would result in a higher frequency of sister chromatid exchange, a higher number of micronuclei and increased DNA degradation as compared to an unexposed control culture.

As mentioned, melatonin is a free radical scavenger and is thereby a known protector of genetic damage. If melatonin is added to the PBL culture along with safrole, the result should show a decrease in genetic damage frequencies compared to the safrole-administered culture. If this predicted result from these experiments is observed, then this would support the view that free radical damage of DNA may be mitigated by melatonin.

The next step in this investigation would be to culture PBLs in the presence of melatonin and certain EMFs which have been shown to give rise to genetic damage. The assumption here is that the observed relationship between exposure to safrole and

melatonin will translate into a similar relationship between exposure to EMF and melatonin.

In the first instance a square pulsed 50 Hz 1mT field should be tested. If my assumption is correct, then exposure of lymphocytes to a combined melatonin/EMF flux density should result in a decrease in frequency of sister chromatid exchange, a decrease in frequency of micronuclei and less DNA degradation as compared to the results already obtained from exposing the lymphocytes to the EMF alone. Even then, however, one would have to be cautious in drawing conclusions from this investigation, as cause and effect would still not have been established. Nevertheless, experimentation with different concentrations of safrole and melatonin could be a fruitful avenue to pursue in depth.

Chapter Six

Summary and Limitations

6.1 Summary

Current evidence suggests that 50 Hz weak EMFs can influence cell processes. A comparison between the results observed in the SCE study with that of the MN assay show interesting parallel features. In the SCE study, an overall significant increase in the number of SCEs/cell in the grouped experimental conditions compared to the controls in both rounds was observed. Similarly, the MN assay also showed an overall significant increase of *mean number of micronucleated CB cells/100 CB cells* (M_a) and *mean number of micronuclei/100 CB cells* (M_b) in the grouped experimental conditions compared to the controls. Although results from both the studies (SCE study and MN assay) showed an overall EMF effect, the relation between effect and applied field intensities is neither simple nor linear.

From a detailed analysis of SCE data, it appears that a *square continuous* field with increasing field strength produces a higher number of SCEs per cell, although the highest SCE frequency (10.61) was scored for a *sine pulsed* field at 1μT in R2.

In the MN assay, a *square pulsed* field showed an increase in M_a and M_b values with an increase in field strength, the same trend as that observed in R1 of the SCE study. Moreover, the highest SCE frequency in R1 was 10.03 for a *square continuous* field, and the SCE frequency of 10.39 for a *square continuous* field in R2 (albeit a different strength) was the second highest in this latter round. But in the MN assay a *square pulsed* field with increasing EMF strength showed the greatest effect on the DNA repair system.

It is tempting here to draw the conclusion that a square wave has a more general damaging effect on DNA, bearing in mind that one must proceed with caution in interpreting these data, not least in acknowledging that the SCE test and the MN assay are measuring two quite different phenomena. Nevertheless, one could argue that the

commonality in effect here between the SCE and MN assays is a square wave and that a *square pulsed* field in particular has the greatest effect on the DNA repair system.

Furthermore, the importance of these parallel observations becomes even more interesting when we compare them with the results of the COMET assay. The COMET assay also showed that both a *1mT square* field (*continuous or pulsed*) resulted in significant fragmentation of the DNA. On the other hand, a FISH analysis failed to show any translocations.

The results obtained in this study lead one to the conclusion that a square wave may exert multiple effects across all three tests (SCE, MN and COMET) which presumably measure different molecular phenomena. From the literature it appears that free radicals have the potential to damage DNA, protein and lipids. Square waves significantly reduced melatonin levels, which are thought to inhibit the damaging effect of free radicals. With reference to these facts one can speculate upon the involvement of a common molecular mechanism. Formation of free radicals as a consequence of MF exposure seems to be a plausible common mechanism, which may have a wide range of effects on the genetic machinery. This may conceivably involve the formation of DNA cross-links, accumulation of DNA strand breaks, interference with DNA repair, resulting ultimately in genetic damage.

With regard to the null hypothesis, 3 of the 4 tests applied (SCE, MN and COMET), during this research do not support the null hypothesis originally stated, which is thus rejected. From the data gathered in this study, the author concludes that ELF EMFs do have a clastogenic effect on human chromosomes.

One of the most exciting developments in this thesis that should be followed up in subsequent research is a thorough investigation into the possible production of free radicals in the presence of certain EMFs and whether this is the cause of the genetic effects observed in the current study.

6.2 Limitations of Present Study

A number of limitations became apparent in the present study, which could be overcome in further research.

1. In the current SCE study two different groups of three healthy blood donors were used when replicating the EMF experiments. The rationale for conducting the entire experiments twice with a different set of donors in each round was to determine whether observed effects could be replicated, as well as in different people. The limitation in doing this is the risk of detecting possible variability of response between donors, which could impact on interpreting apparently conflicting results.

   It is very important to note that the results did show significant differences between control and exposed conditions in both rounds. From these results the conclusion was drawn that certain EMFs can exert a biological effect, but the effects vary. This could be due to a chance event or may be attributed to variability of response in the different donors. It is obvious that the sample size of the two groups is too small to draw any firm conclusion, but then, it was not the aim of the overall study to compare individual effects. The aim of the current study was to observe cellular effects.

   In further EMF studies, in order to observe individual effects a concerted attempt using a larger number of donors and involving more than two independent laboratories could be useful. Certainly this would be a task beyond that of one person if all the current tests were conducted. In the replicate studies all investigators should use identical equipment constructed in one laboratory and they should follow identical protocols.

2. The author notes that the overall SCE reading is higher in R2 than R1, which might be due to individual susceptibility to the marker used, BrdU. This was also noted by Morgan and Crossen (1977). Replacement of BrdU with a different marker could be helpful in solving possible differences in individual susceptibilities towards the marker. But at present there is no other marker available to replace BrdU in this type of experiment.

3. No statistically significant difference was observed in either R1 or R2 between the mean SCE/cell of the control groups and that of the complex field. The author notes, however, that despite attempting to culture lymphocytes on three separate occasions in a complex field, difficulty was always encountered in obtaining a sufficient number of dividing metaphase cells. Only 87 cells could be scored in R1, and only 188 cells in R2, in all 3 donors. This low mitotic index severely limits the statistical power of the observations and consequently restricts the conclusions that can be drawn. Nevertheless, a repeatedly low mitotic index must be a cause for concern, despite the lack of significant differences in SCE frequencies between the complex-field-exposed cultures and the controls. This raises questions concerning health issues and could be the focus of a more intensive study in the future.

4. In the MN assay, steps were also taken to determine whether there was any difference between the M_a and M_b of the control and that of a complex field. No statistically significant difference was observed between the mean of both M_a and M_b of the control and complex experiment. From these observations one can tentatively deduce that a complex field, at least the field produced from the rear of the particular computer used in this experiment, causes no damage to DNA repair mechanisms in dividing peripheral blood lymphocytes. Appropriately designed multi-laboratory international validation studies are necessary to gather information on additional potential sources of variability and on the intra- or inter laboratory reproducibility of MN assay results.

5. In the current MN assay study, only one round of eight experiments was performed. The current author scored micronuclei from a large number of binucleate cells per experiment, which was time consuming without having access to an automated system. Insufficient time limited the current author in the extent of his study, although according to Fenech *et al.* (1999), other end-points such as apoptosis, necrosis, and cytostasis should also be considered when interpreting controversial data.

6. There seem to be problems associated with using cytochalasin B to produce binucleate cells. Shimizu *et al.* (1998) observed the formation of micronuclei as a

result of gene amplification in which the cell eliminates excess amplified DNA directly from the nucleus by nuclear budding during S phase. This is known as mitotic slippage. Elhajouji *et al.* (1997) also observed that the application of specific mitotic spindle inhibitors might cause mitotic slippage leading to polyploid nuclei and micronuclei in mononucleated cells. The concern here is that the frequency of micronuclei observed in the present EMF study may be artificially elevated by cytochalasin B, even given the use of controls. Therefore, it would be useful to study further the possible effects of cytochalasin B.

7. In view of the current findings and the contradictory published reports, it is important that more investigations be conducted using various types of cells from both humans and animals under different experimental conditions to resolve the controversy of ELF EMF bio-effects.

References.

Abe S, & Sasaki M. 1977. Chromosome aberration and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. *J. Natl.Cancer Inst.* 58:1635-1641.

Abe S, & Sasaki M. 1982. SCE as an index of *Mutagenesis* and/or *Carcinogenesis. In* "Sister Chromatid Exchange", A. A. Sandberg, (Ed.) Alan R. Liss. Inc., New York, pp. 461-514.

Able KP, & Able MA. 1995. Interactions in the flexible orientation system of a migratory bird. *Nature* 375:230-232.

Abramovsky I, Vorsanger G, & Hirschhorn K. 1978. Sister chromatid exchange induced by X-ray of human lymphocytes and the effect of L-cysteine. *Mutation Res.* 50:93-100.

Abramsson-Zetterberg L, & Grawe J. 2001. Extended exposure of adult and fetal mice to 50 Hz magnetic field does not increase the incidence of micronuclei in erythrocytes. *Bioelectromagnetics* 22(5):351-357.

Adair R. 1991. Constraints on biological effects of weak extremely-low frequency electromagnetic fields. *Phys. Rev.* A43:1039-1048.

Adair R. 1992a. Criticism of Lednev's mechanism for the influence of weak magnetic fields on biological systems. *Bioelectromagnetics* 13:231-235.

Adey WR. 1990. Nonlinear electrodynamics in cell membrane transductive coupling. *In* "Membrane Transport and Information Storage" (Aloria RC, Curtain CC, & Gordon LM. eds.), pp. 1-27. Alan R. Liss, New York.

Ahmad SI, Kirk SH, & Eisenstark A. 1998. Thymine metabolism and thymineless death in prokaryotes and eukaryotes. *Ann. Rev. Microbiol.* 52:591-625.

Ahuja YR, Vijayashree B, Saran R, Jayashri EL, Manoranjani JK, & Bhargava SC. 1999. *In vitro* effects of low-level, low-frequency electromagnetic fields on DNA damage in human leucocytes by comet assay. *Indian J. Biochem. Biophys.* 36:318-322.

Alhadeff B, & Cohen MM. 1976. Frequency and distribution of sister chromatid exchanges in human peripheral lymphocytes. *Isr. J. med. Sci.*12:1440-1447.

Alkon DL, & Rasmussen H. 1988. A spatial-temporal model of cell activation. *Science* 239:998-1005.

Ames BN. 1998. Micronutrients prevent cancer and delay aging. *Toxicology Letters.* 102-103:5-18.

Anderson LE. 1991. ELF: Exposure levels, bioeffects, and epidemiology. *Health Phys.* 61:41-46.

Anderson D, Yu TW, Phillips BJ, & Schmezer P. 1994. The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the COMET assay. *Mutation Res.* 307:261-271.

Anderson D, Yu TW, McGregor DB. 1998. COMET assay responses as indicators of carcinogen exposure. *Mutagenesis* 13:539-555.

Andrew C, & Bassett L. 1995. *Bioelectromagnetics* in the service of medicine. Electromagnetic Fields, Biological Interactions and Mechanisms, Edited by Martin Blank, American Chemical Society, Washington, D.C. pp 497.

Antonopoulos A, Yang B, Stamm A, Heller WD, & Obe G. 1995. Cytological effects of 50-Hz electromagnetic fields on human lymphocytes *in vitro*. *Mutation Res.* 346:151-157.

Arlett CF, Lowe JE, Harcourt SA, Waugh AP, Cole J, Roza L, Diffey BL, Mori T, Nikaido O, & Green MH. 1993. Hypersensitivity of human lymphocytes to UV-B and solar irradiation. *Cancer Res.* 53:609-614.

Arnetz BB, & Berg M. 1996. Melatonin and Andrenocorticotropic Hormone levels in video display unit workers during work and leisure. *J. Occup. Med.* 38(11):1108-1110.

Astumian RD, Weaver JC, & Adair RK. 1995. Rectification and signal averaging of weak electrical fields by biological cells. *Proc. Acad. Sci. USA* 92:3740-3743.

Atalay F, Baltaci V, Alpas I, Savas I, Atikcan S, & Balci S. 2000. Sister chromatid exchange rate from pleural fluid cells in patients with malignant mesothelioma. *Mutation Res.* Genetic Toxicology & Environmental *Mutagenesis* 465(1-2):159-163.

Averbeck D, Polasa K, Buisson JP, Bensasson R, Rougee M, Cadet J, Ravanat JL, Perin F, Vigny P, & Demerseman P. 1993. Guanine is the target for direct ionization damage in DNA, as detected using excision enzymes. *Mutation Res.* 287:165-179.

Azanza MS, & Del Moral A. 1994. Cell membrane biochemistry and neurobiological approach to Biomagnetism. *Prog. Neurobiol.* 44:517-601.

Bakou K, Stephanou G, Andrianopoulos1C, & Demopoulos NA. 2002. Spontaneous and spindle poison-induced micronuclei and chromosome non-disjunction in cytokinesis-blocked lymphocytes from two age groups of women. *Mutagenesis* 117(3):233-239.

Bala Kishna Murthy P, & Prema. 1979. Sister chromatid exchanges in oral contraceptive users. *Mutation Res.* 68:149-152.

Bala Kishna Murthy P, & Prema. 1983. Further studies on sister chromatid exchange frequency in users of hormonal contraceptives. *Mutation Res.* 119:351-354.

Bala Kishna Murthy P, Bhaskaram P, & Srikantia SG. 1980. Sister chromatid exchanges in protein energy malnutrition. *Hum. Genet.* 55:405-406.

Baldi MI, Benedetti P, Mattoccia E, & Tocchini-Valentini GP. 1980. *In vitro* catenation and decatenation of DNA and a novel eucaryotic ATP-dependent topoisomerase. *Cell* 20(2):461-467.

Baltaci V, Aygun N, Akyol D, Karakaya AE, & Sardas S. 1998. Chromosomal aberrations and alkaline comet Assay in families with habitual abortion. *Mutation Res.* 417:47-55.

Baltaci V, Kayikciolu F, Alpas I, Zeynelolu H, & Haberal A. 2002. Sister chromatid exchange rate and alkaline comet assay scores in patients with ovarian cancer. *Gynecologic Oncology* 84(1):62-66.

Ban S, Shinohara T, Hirai Y, Moritaku Y, Cologne JB, & MacPhee DG. 2001. Chromosomal instability in BRCA1- or BRCA2-defective human cancer cells detected by spontaneous micronucleus assay. *Mutation Res.* 474(1-2):15-23.

Basler A, Bachmann U, Roszinsky-Kocher G, & Rohrborn G. 1979. Effects of caffeine on sister chromatid exchanges (SCE) *in vivo. Mutation Res.* 59:209-214.

Batalha JRF, Guimaraes ET, Lobo DJA, Lichtenfels AJFC, Deurv H, Carvalho A, Alves ES, Domingos M, Rodrigues GS, & Saldiva PHN. 1999. Exploring the clastogenic effects of air pollutants in Sao Paulo (Brazil) using the Tradescantia micronuclei assay. *Mutation Res.* 426(2):229-232.

Bauchinger M, Braselmann H, Savage JRK, Natarajan AT, Terzoudi GI, Pantelias GE, Darroudi F, Figgitt M, Griffin CS, Knehr S, Okladnikova ND, Santos S, & Snigiryova G. 2001. Collaborative exercise on the use of FISH chromosome painting for retrospective biodosimetry of Mayak nuclear-industrial personnel. *Int. J. Radiat. Biol.* 77(3):259-267.

Beason RC, & Brennan WJ. 1986. Natural and indiced magnetization in the bobolink (Dolichonyx oryzivorus). *J. Exp. Biol.* 125:49-56.

Bender MA, Leonard RC, Jr. White O, Costantino JP, & Redmond CK. 1988. *Chromosomal* aberrations and sister-chromatid exchanges in lymphocytes from coke oven workers. *Mutation Res.* 206(1):11-16.

Bender MA, Preston RJ, Leonard RC, Pyatt BE, & Gooch PC. 1992a. On the distribution of spontaneous SCE in human peripheral blood lymphocytes. *Mutation Res.* 281:227-232.

Bender MA, Preston RJ, Leonard RC, Pyatt BE, & Gooch PC. 1992b. Influence of white blood cell count on SCE frequency in peripheral lymphocytes. *Mutation Res.* 283(2):87-89.

Ben-Hur E, & Elkind MM. 1972. Damage and repair of DNA in 5-bromodeoxyuridine-labeled Chinese hamster cells exposed to fluorescent light. *Biophys. J.* 12:636-647.

Beniashvili DSH, Bilanishvili VG, & Menabde MZ. 1991. The effect of low-frequency electromagnetic fields on the development of experimental mammary tumors. *Voprosy Onkologii.* 37(9-10):937-941.

Benjamin J. 1994. Genes V. Oxford University press, pp. 1272.

Benzi R, Sutera A, & Vulpiani A. 1981. The mechanism of stochastic resonance. *J. Phys.* A14:453-458.

Benner SE, Lippman SM, Wargovich MJ, Lee JJ, Velasco M, Martin JW, Toth BB, & Hong WK. 1994. Micronuclei a biomarker for chemoprevention trials: results of a randomized study in oral pre-malignancy. *Int. J. Cancer* 59:457-459.

Berman E, Chacon L, House D, Koch BA, Leal J, Lovstrup S, Mantiply E, Martin AH, Martucci GI, Mild KH, Monahan JC, Sandstrom M, Shamsaifar MK, Tell R, Bernhardt JH, & Kossel F. 1985. Recommendations for the safe use of NMR equipment. *Clinical Physics & Physiological Measurement* 6(1):65-74.

Betti C, Davini T, Giannessi L, Loprieno N, & Barale R. 1994. Microgel electrophoresis assay (comet test) and SCE analysis in human lymphocytes from 100 normal subjects. *Mutation Res.* 307:323-333.

Bezrukov SM, & Vodyanoy I. 1997. Stochastic resonance in non-dynamical systems without response thresholds. *Nature* 385:319-321.

Binhi VN. 1997. Interference of ion quantum states within a protein explains weak magnetic field's effect on biosystems. *Electro-Magnetobiology* 16(3):203–214, 1997.

Binhi VN. 1997a. The mechanism of magnetosensitive binding of ions by some proteins. *Biophysics* 42(2):317–322.

Bisht KS, Moros EG, Straube WL, Baty JD, & Roti Roti JL. 2002. The effect of 835.62 MHz FDMA or 847.74 MHz CDMA modulated radiofrequency radiation on the induction of micronuclei in C3H 10T(1/2) cells. *Radiat. Res.* 157(5):506-515.

Blackman CF. 1996. Independent replication of the 12 mG magnetic field effect on melatonin and MCF-7 cells in vitro. BEMS Meeting Victoria, British Columbia, Canada. Abstract A-1-2.

Blackman CF, Benane SG, House DE, & Joines WT. 1985a. Effects of ELF (1-120 Hz) and modulated (50 Hz) RF fields on the efflux of calcium ions from brain tissue. *Bioelectromagnetics* (N.Y.) 6:1-11.

Blackman CF, Benane SG, Rabinowitz JR, House DE, & Joines WT. 1985b. A role for the magnetic field in the radiation-induced efflux of calcium ions from brain tissue *in vitro*. *Bioelectromagnetics* (N.Y.) 6:327-338.

Blackman CF, Benane SG, Elliot DJ, House DE, & Pollock MM. 1988. Influence of electromagnetic fields on the efflux of calcium ions from brain tissue in vitro: a three-model? Analysis consistent with the frequency response up to 510 Hz. *Bioelectromagnetics* 9:215-227.

Blackman CF, Blanchard JP, Benane SG, & House DE. 1994. Emperical test of an ion parametric resonance model for magnetic field interactions with PC-12 cells. *Bioelectromagnetics* 15:239-260.

Blakemore RP. 1975. Magnetotactic bacteria. *Science* 190:377–379.

Blakemore RP. 1982. Magnetotactic Bacteria. *Ann. Rev. Microbiol.* 36:217-238.

Blanchard JP, & Blackman CF. 1994. Clarification and application of an ion parametric resonance model for magnetic field interactions with biological systems. *Bioelectromagnetics* 15:217-238.

Blank M. 1995. Electromagnetic Fields: Biological Interactions and Mechanisms. American Chemical Society, Washington, DC. Advances in chemistry series 250: pp. 1-497.

Bloom SE. 1978. *In*: Hollander, and F. J. de Serres (Eds.), Chemical Mutagens: Principles and Methods for Their Direction, Vol. 5. Plenum, New York, pp. 203-232.

Bloom SE, & Hsu TC. 1975. Differential fluorescence of sister chromatids in chicken embryos exposed to 5-bromodeoxyuridine. *Chromosoma* 51:261-267.

Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, & Ames B. 1997. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc. Natl. Acad. Sci. USA* 94:3290-3295.

Blumenthal AB, Kriegstein HJ, & Hogness DS. 1974. The units of DNA replicationin *Drosophila melanogaster* chromosomes. *Cold Spring Harbor Symp. Quant. Biol.* 38:205-223.

Bolognesi C, Abbondandolo A, Barale R, Casalone R, Dalpra L, De Ferrari M, Degrassi F, Forni A, Lamberti L, Lando C, Migliore L, Padovani P, Pasquini R, Puntoni R, Sbrana I, Stella M, & Bonassi S. 1997a. Age-related increase of baseline frequencies of sister chromatid exchanges, chromosome aberrations, and micronuclei in human lymphocytes. *Cancer Epidemiology, Biomarkers & Prevention* 6(4):249-256.

Bolognesi C, Merlo F, Rabboni R, Valerio F, & Abbondandolo A. 1997b. Cytogenetic biomonitoring in traffic police workers: micronucleus test in peripheral blood lymphocytes. *Environmental & Molecular Mutagenesis* 30(4):396-402.

Bolognesi C, Perrone E, & Landini E. 2002. Micronucleus monitoring of a floriculturist population from western Liguria, Italy. *Mutagenesis* 17(5):391-397.

Bonassi S, Fenech M, Lando C, Lin YP, Ceppi M, Chang WP, Holland N, Kirsch-Volders M, Zeiger E, Ban S, Barale R, Bigatti MP, Bolognesi C, Jia C, Di Giorgio M, Ferguson LR, Fucic A, Lima OG, Hrelia P, Krishnaja AP, Lee TK, Migliore L, Mikhalevich L, Mirkova E, Mosesso P, Muller WU, Odagiri Y, Scarffi MR, Szabova E, Vorobtsova I, Vral A, & Zijno A. 2001. Human MicroNucleus project: international database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. *Environmental & Molecular Mutagenesis* 37(1):31-45.

Braithwaite E, Wu X, & Wang Z. 1998. Repair of DNA lesions induced by polycyclic aromatic hydrocarbons in human cell free exracts: involvement of two excision repair mechanisms *in vitro*. *Carcinogenesis* 19:1239-1246.

Brandon C, Khan A, Lai H, & Lin C. 1996. The application of electromagnetic energy to the treatment of neurological and psychiatric diseases. Biological Effects of Magnetic Fields and Electromagnetic Fields, Edited by S. Ueno. Plenum Press, New York. p243.

Brewen JG, & Peacock WJ. 1969. The effect of tritiated thymidine on sister-chromatid exchange in a ring chromosome. *Mutation Res.* 7(3):433-440.

Bruckner-Lea C, Durney CH, Janata J, Rappaport C, & Kaminski M. 1992. Calcium binding to metallochromic dyes and calmodulin in the presence of combined AC-DC magnetic fields. *Bioelectromagnetics* 13:147-162.

Buckton KE, Court Brown WM, & Smith PG. 1967. Further studies on chromosome aberration production after whole-body irradiation in man. *Nature* 214:470-473.

Buckton KE, Hamilton GE, Paton L, & Langlands AO. 1978. Chromosome aberrations in irradiated ankylosing spondylitis patients. *In* H.J. Evans and D.C. Lloyd (Eds.), Mutagen-induced Chromosome Damage in Man (Edinburgh: Edinburgh University Press), pp. 142-150.

Buckton KE. 1983. Chromosome aberrations in patients treated with X-irradiation for ankylosing spondylitis. *In* T. Ishihara and M. S. Sasaki (Eds.), Radiation-induced Chromosome Aberration in Man (New York: Allan R. Liss), pp.491-511.

Bukvic N, Bavaro P, Elia G, Cassano F, Fanelli M, & Guanti G. 1998. Sister chromatid exchange (SCE) and micronucleus (MN) frequencies in lymphocytes of gasoline station attendants. *Mutation Res.* 415(1-2):25-33.

Bukvic N, Gentile M, Susca F, Fanelli M, Serio G, Buonadonna L, Capurso A, & Guanti G. 2001. Sex chromosome loss, micronuclei, sister chromatid exchange and aging: a study including 16 centenarians. *Mutation Res.* 498(1-2):159-67.

Burch JB, Reif JS, Pittrat, CA, Keefe TJ, & Yost MG. 1997. "Cellular telephone use and excretion of a urinary melatonin metabolite". *In* Annual Review of Research in Biological Effects of electric and magnetic fields from the generation, delivery and use of electricity, San Diego, CA, Nov. 9-13, p-52.

Burch JB, Reif JS, Yost MG, Keefe TJ, & Pittrat CA. 1998. Nocturnal excretion of urinary melatonin metabolite among utility workers. *Scand J. Work Environ. Health* 24(3):183-189.

Burch JB, Reif JS, Yost MG, Keefe TJ, & Pittrat CA. 1999. Reduced excretion of a melatonin metabolite among workers exposed to 60 Hz magnetic fields *Am. J. Epidemiol.* 150(1):27-36.

Burch JB, Reif JS, Noonan CW, & Yost MG. 2000. Melatonin metabolite levels in workers exposed to 60-Hz magnetic fields: work in substations and with 3-phase conductors. *J. Occup. Environ. Med.* 42(2):136-142.

Burdon RH, & Rice-Evans C. 1989. Free radicals and the regulation of mamalian cell proliferation. *Free Radic. Res. Commun.* 6:345-358.

Burrill W, Barber JB, Roberts SA, Bulman B & Scott D. 2000. Heritability of *Chromosomal* radiosensitivity in breast cancer patients: a pilot study with the lymphocyte micronucleus assay. *Int. J. Radiat. Biol.* 76(12):1617-1619.

Butler MG, & Sanger WG. 1981. Increased frequency of sister-chromatid exchanges in alcoholics. *Mutation Res.* 85:71-76.

Byus CV, Lundak RL, Fletcher RM, & Adey WR. 1984. Alteration in protein kinase activity following exposure of cultured human lymphocytes to modulated microwave fields. *Bioelectromagnetics* 5:341-351.

Cain CD, Adey WR, & Luben RA. 1987. Evidence that pulsed electromagnetic fields inhibit coupling of adenylate cyclase by parathyroid hormone in bone cells. *J. Bone Mineral Res.* 2:437-441.

Canfield JN, Belford RI, Debrunner PG, & Schukten KJ. 1994. A perturbation theory treatment of oscillating magnetic fields in the radical pair mechanism. *Chem.Phys.*182:1-18.

Carrano AV, & Moore II DH. 1982. The rational and methodology for quantifying sister chromatid exchange in humans, *In* J. A. Headle (Ed.), Mutagenicity: New Horizons in Genetic Toxicology, Academic Press, New York, pp. 267-304.

Carrano AV, & Natarajan AT. 1988. Considerations for population monitoring using cytogenetic techniques. *Mutation Res.* 204:379-406.

Carrano AV, & Thompson LH. 1982. Sister chromatid exchange and gene mutation. *Cytogenet. Cell Genet.* 33:57-61.

Carrano AV, Thompson LH, Lindl PA, & Minkler JL. 1978. Sister chromatid exchange as an indicator of mutagenesis. *Nature* 271:551-553.

Carrano AV, Thompson LH, Stetka DG, Minkler JL, Mazrimas JA, & Fong S. 1979. DNA crosslinking, sister-chromatid exchange and specific-locus mutations. *Mutation Res.* 63(1):175-188.

Carter SB. 1967. Effects of cytochalasins on mammalian cells. *Nature* 213:261-264.

Catena C, Conti D, Del Nero A, & Righi E. 1993. Cytogenetic dosimetry: dose-effect curves and a methodological comparison. *Medicina del Lavoro.* 84(2):133-146.

Chaganti RSK, Schonberg S, & German J. 1974. A manifold increase in sister chromatid exchanges in Bloom's Syndrome lymphocytes. *Proc. Natl. Acad. Sci. USA* 71:4508-4512.

Chang-Tze YR, Te-Chang L, Wang TC & Jih-Heng L. 1999. Genetic toxicity of cocaine. *Carcinogenesis* (Oxford) 20(7):1193-1199.

Chen G, Upham BL, Sun W, Chang CC,Rothwell EJ, Chen KM, Yamasaki H, & Trosko JE. 2000. Effect of electromagnetic field exposure on chemically induced differentiation of Friend erythroleukemia cells. *Environ. Health Perspect* 108:967-972.

Chen GL, Yang L, Rowe TC, Halligan BD, Tewey KM & Liu LF. 1984. Nonintercalative antitumor drugs interfere with the breakage-reunion reaction of mammalian DNA topoisomerase II. *J. Biol. Chem.* 259:13560-13566.

Chen PL, Sellers TA, Rich SS, Potter JD, & Folsom AR. 1995. Segregation analysis of breast cancer in a population-based samle of postmenopausal probands. *Genet. Epidemiol.* 12:401-415.

Cheng TJ, Christiani DC, Xu X, Wain JC, Wiencke JK, & Kelsey KT. 1996. Increased micronucleus frequency in lymphocytes from smokers with lung cancer. *Mutation Res.* 349:43-50.

Cheng W, Mulvihill JJ, Greene MH, Pickle LW, Tasi S, & Whang-Peng J. 1979. Sister chromatid exchanges and chromosomes in chronic myelogenous leukemia and cancer families. *Int. j. Cancers* 23:8-13.

Cherry N. 2000. Criticism of the health assessment in the icnirp guidelines for radiofrequency and microwave radiation (100 khz - 300 ghz). *EMF (guru); Cellular Phone:* http://www.emfguru.org/CellPhone/cherry2/ICNIRP-2.htm

Chicca MC, Nesti C, Muzzoli M, Pasetti P, & Pinamonti S. 1996. Correlation between age and DNA damage detected by FADU in human peripheral blood lymphocytes. *Mutation Res.* 316(5-6):201-208.

Christine E, Fuller MD, & Perry A. 2002. Fluorescence in situ hybridization (FISH) in diagnostic and investigative neuropathology. *Brain Pathol.* 12:67-86.

Christophersen AG, Jun H, Jørgensen K, & Skibsted LH. 1991. Photobleaching of astaxanthin and canthaxanthin: quantum-yields dependence of solvent, temperature, and wavelength of irradiation in relation to packageing and storage of carotenoid pigmented salmonoids. *Z. Lebensm Unters Forsch* 192:433-439.

Cleaver JE. 1981. Correlations between sister chromatid exchange frequencies and replication sizes, a model for the mechanism of SCE production. *Exp. Cell Res.* 136:27-30.

Cohen MM, Kunska A, Astemborski JA, & McCulloch D. 1986a. The effect of low-level 60-Hz electromagnetic fields on human lymphoid cells. II. Sister-chromatid exchanges in peripheral lymphocytes and lymphoblastoid cell lines. *Mutation Res.* 172:177-184.

Cohen MM, Kunska A, Astemborski JA, McCulloch D, & Paskewitz DA. 1986b. The effect of low-level 60-Hz electromagnetic fields on human lymphoid cells. I. Mitotic rate and chromosome breakage in human peripheral lymphocytes. *Bioelectromagnetics* (N.Y.) 7:415-423.

Cole RS. 1973. Repair of DNA containing interstrand crosslinks in *Escherichia coli*: sequential excision and recombination. *Proc. Natl. Acad. Sci. USA* 71:3162-3166.

Cole RS. 1974. Psoralen crosslink in DNA: Formation, consequences and repair. *In* Schenck GO (Ed.): "Progress in Photobiology". Frankfurt: Deutsche Gesellschaft fur Lichtforschung, pp 19-21.

Colleu-Durel S, Guitton N, Nourgalieva K, Leveque J, Danic B, & Chenal CTI. 2001. Genomic Instability and breast cancer. *Oncology Reports* 8(5):1001-1005.

Collins AR, Duthie SJ, & Dobson VL. 1993. Direct enzymatic detection of endogenous base damage in human lymphocyte DNA. *Carcinogen* 14:1733-1735.

Collins AR, Dobson VL, Dusinska M, Kennedy G, & Stetina R.1997. The comet assay: what can it really tell us? *Mutation Res.* 375:183-193.

Cook PR, & Brazell IA. 1976. Detection and repair of single-strand breaks in nuclear DNA. *Nature* 263:679-682.

Cortes-Gutierrez EI, Cerda-Flores MR, & Leal-Garza CH. 2000. Sister chromatid exchanges in peripheral lymphocytes from women with carcinoma of the uterine cervix. *Cancer Genetics & Cytogenetics* 122(2):121-123.

Cossarizza A, Monti D, Sola P, Moschini G, Cadossi R, Bersani F, & Franceschi C. 1989. DNA repair after gamma irradiation in lymphocytes exposed to low-frequency pulsed electromagnetic fields. *Radiat. Res.* 118(1):161-168.

Coulton LA, & Berker AT. 1993. Magnetic fields and intracellular calcium: Effects on lymphocytes exposed to conditions for 'cyclotron resonance'. *Physics in Medicine and Biology* 36(3):347-360.

Countryman RI, & Heddle JA. 1976. The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. *Mutation Res.* 41:321-332.

Creda H, Delincee H, Haine H, & Rupp H. 1997. The DNA 'COMET assay' as a rapid screening technique to control irradiated food. *Mutation Res.* 375:167-181.

Cridland NA, Haylock RG, & Saunders RD. 1999a. 50 Hz magnetic field exposure alters onset of S-phase in normal human fibroblasts. *Bioelectromagnetics* 20(7):446-452.

Cridland NA, Sabour NR, & Saunders RD. 1999b. Effects of 50 Hz magnetic field exposure on the rate of RNA synthesis by normal human fibroblasts. *Int. J. Radiat. Biol.* 75(5):647-654.

Crossen PE, Drets ME, Arrighi FE & Johnston DA. 1977. Analysis of the frequency and distribution of sister chromatid exchanges in cultured human lymphocytes. *Hum. Genet.* 35 :245-352.

Czyz J, Nikolova T, Schuderer J, Kuster N, & Wobus AM. 2004. Non-thermal effects of power-line magnetic fields (50 Hz) on gene expression levels of pluripotent embryonic stem cells-the role of tumour suppressor p53. *Mutation Res.* 557(1):63-74.

Dacha M, Accorsi A, Pierotti C, Vetrano F, Mantovani R, Guidi G, Conti R, & Nicolini P. 1993. Studies on the possible biological effects of 50 Hz electric and/or magnetic fields: evaluation of some glycolytic enzymes, glycolytic flux, energy and oxido-reductive potentials in human erythrocytes exposed *in vitro* to power frequency fields. *Bioelectromagnetics* 14:383-391.

d'Ambrosio G, Massa R, Scarfì MR, & Zeni O. 2002. Cytogenetic damage in human lymphocytes following GMSK phase modulated microwave exposure. *Bioelectromagnetics* 23(1):7-13,

Das BC, & Sharma T. 1984. Effects of temperature on the frequency of sister chromatid exchanges (SCEs) in peripheral blood lymphocytes of man and muntjac. *Environ. Mutagen.* 6:25-31.

Davidson RL, Kaufman ER, Dougherty CP, Quellette AM, DiFolco CM, & Latt SA. 1980. Induction of sister chromatid exchanges by BrdU is largely independent of the BrdU content of DNA. *Nature* 284:74-76.

Davis JG, Bennet WR, Brady JV, Brent RL, Gordis L, Gordon WE, Greenhouse SW, Reiter RJ, Stein GS, Susskind C, & Trichopoulos D. 1992. Health effects of low frequency electric and magnetic fields, Oak Ridge Associated Universities, Oak Ridge.

Davis S. 1997. Weak residential Magnetic Fields affect Melatonin in Humans. Microwave News, Nov/Dec 1997.

Davis S, Kaune WT, Mirick DK, Chen C, & Stevens RG. 2001. Residential magnetic fields, light-at-night, and nocturnal urinary 6-sulfatoxymelatonin concentration in women. *Am. J. Epidemiol.* 154(7):591-600.

De Arce MA. 1981. The effect of donor sex and age on the number of sister chromatid exchanges in human lymphocytes growing *in vitro*. *Hum. Genet.* 57:83-85.

de Weerd-Kastelein EA, Keijzer W, Rainaldi G, & Bootsma D. 1977. Induction of sister chromatid exchanges in *Xeroderma pigmentosum* cells after exposure to ultraviolet light. *Mutation Res.* 45:253-261.

Delgado MR, Leal J, Monteagudo JL, & Garcia MG. 1982. Embryological changes induced weak, extremely low frequency electromagnetic fields. *J.Anat.*134:533-551.

Demers PA, Thomas DB, Rosenblatt KA, Jimenez LM, McTiernan A, Stalsberg H, Stemhagen A, Thompson WD, McCrea-Crnen MF, Satariano W, Austin DF, Isacson P, Greenberg RS, Key C, Kolonel LN, & West DW. 1991. Occupational exposure to electromagnetic fields and breast cancer in men. *Am. J. Epidemiol.* 13:340-347.

Deng W, & Lucas JN. 1999. Combined FISH with pan-telomeric PNA and whole chromosome-specific DNA probes to detect complete and incomplete *Chromosoma*l exchanges in human lymphocytes. *Int. J. Radiat. Biol.* 75(9):1107-1112.

Desai SS, Ghaisas SD, Jakhi SD, & Bhide SV. 1996. Cytogenetic damage in exfoliated oral mucosal cells and circulating lymphocytes of patients suffereng from precancerous oral lesions. *Cancer Lett.* 109:9-14.

Dhillon VS, & Dhillon IK. 1998. Chromosome aberrations and sister chromatid exchange studies in patients with prostate cancer: possible evidence of chromosome instability. *Cancer Genet. Cytogenet.* 100(2):143-147.

Diener S, Eberle P. 1996. Cell proliferation, sister-chromatid exchange, chromasolam aberrations, micronuclei and mutation rate. Electronic Compatability of Biological Systems 1997. Newsletter Edition Wissenschaft Nr.4, 1996.

Dillehay LE, Jacobson-Kram D, & Williams JR. 1989. DNA topoisomerases and models of sister-chromatid exchange. *Mutation Res.* 215:15-23.

Ding GR, Takehisa N, & Junji M. 2002. Exposure to power frequency magnetic fields and X-rays induces GAP-43 gene expression in human glioma MO54 cells. *Bioelectromagnetics* 23(8):586-591.

Ding GR, Nakahara T, Hirose H, Koyama S, Yakashima Y, & Miyakoshi J. 2004. Extremely low frequency magnetic fields and the promotion of H2O2-induced cell death in HL-60 cells. *Int. J. Radiat. Biol.* 80:317-324.

Donmez H, Dursun N, Ozkul Y, & Demirtas H. 1998. Increased sister chromatid exchanges in workers exposed to occupational lead and zinc. *Biologic. Trace. Elem. Res.* 61(1):105-109.

Douglas JK, Wilkins L, Pantazelou E, & Moss E. 1993. Noise enhancement of information transfer in crayfish mechanoreceptor by stochastic resonance. *Nature* 365:337-340.

D'Souza D, Thomas IM, & Das BC. 1988. Variation in spontaneous *Chromosoma*l damage as a function of biologic rhythms in women. *Hum. Genet.* 79(1):83-85.

Du Manoir S, Schröck E, Bentz M, Speicher MR, Joos S, Ried T, Lichter P, & Cremer T. 1993. Quantitative analysis of comperative genomic hybridization. *Cytometry* 19:27-41.

Dubrov AP. 1978. The geomagnetic field and life: Geomagnetobiology. Plenum, New York.

Duffaud F, Orsiere T, Villani P, Pelissier AL, Volot F, Favre R, & Botta A. 1997. Comparison between micronucleated lymphocyte rates observed in healthy subjects and cancer patients. *Mutagenesis* 12:227-231.

Durney CH, Kaminski M, Anderson AA, Bruckner-lea C, Janata J, & Rappaport C. 1992. Investigation of AC-DC manetic field effects in planar phospholipid bilayers. *Bioelectromagnetics* 13:19-33.

Dutrillaux B, Fosse AM, Prievr M, & LeJeune. 1974. Analyses des echanges de chromatids dans les cellules somatiques humaines. *Chromosoma* (Berl.) 48:327-340.

Duydu Y, Suzen HS, Aydin A, Cander O, Uysal H, Isimer A & Vural N. 2001. Correlation between lead exposure indicators and sister chromatid exchange (SCE) frequencies in lymphocytes from inorganic lead exposed workers. *Archives of Environmental Contamination & Toxicology* 41(2):241-246.

Eeva T, & Millard S. 1992. Sister chromatid exchanges and mitotic crossing over. Human Chromosomes, Structure, behavior, and Effects. 3rd. Ed. Springer-Verlag New York Inc. pp. 376.

Elhajouji A, Tibaldi F, & Kirsch-Volders M. 1997. Indication for tresholds of chromosome non-disjunction versus chromosome lagging induced by spindle inhibitors *in vitro* in human lymphocytes. *Mutagenesis* 12:133-140.

Elia MC, DeLuca JG, & Bradley MO. 1991. Significance and measurement of DNA double strand breaks in mammalian cells. *Pharmacology & Therapeutics* 51(3):291-327.

Elio A. 2000. Spontaneous sister chromatid exchanges in chicken embryos. *Hereditas* 133:263-266.

EPA (Environmental Protection Agency, U.S.A.). 1992. EMF in your environment.

Erexson GL, Kligerman AD, & Allen JW. 1987. Diaziquone-induced micronuclei in cytochalasin B-blocked mouse peripheral blood lymphocytes. *Mutation Res.* 178 (1):117-122.

Erexson GL, Kligerman AD, Bryant MF, Sontag MR, & Halperin EC. 1991. Induction of micronuclei by x-radiation in human mouse and rat peripheral blood lymphocytes. *Mutation Res.* 253 (2):193-198.

Evans HJ. 1976. *In* A. Hollaender (Ed.), Chemical Mutagens: Principles and Methods for their Detection. Vol. 4, plenum, New York, pp. 1-29.

Evans HJ. 1977. Molecular mechanisms in the induction of chromosome aberrations. *In* Scott D, Bridges BA, Sobels FH, eds, Progress in Genetic Toxicology, Elsevier, Amesterdam:57-74.

Evans HJ. 1997. Historical perspectives on the development of the *in vitro* micronucleus test: a personal view. *Mutation Res.* 392:5-10.

Evans HJ, Neary GJ, & Williamson FS. 1959. The relative biological efficiency of single doses of fast neutrons and gamma-rays of *Vicia faba* roots and the effect of oxygen. Part II. Chromosome damage: the production of micronuclei. *Intl. J. Radiat. Biol.* 3:216-229.

Falistocco E, Torricelli R, Feretti D, Zerbini I, Zani C, & Monarca S. 2000. Enhancement of micronuclei frequency in the Tradescantia/micronuclei test using a long recovery time. *Hereditas* 133(2):171-174.

Fenech M. 1997. The advantages and disadvantages of the cytokinesis-block micronucleus method. *Mutation Res.* 392(1-2):11-18.

Fenech M. 1998. Chromosomal Damage Rate, Aging, and Diet. *Annals of the New York Academy of Sciences* 854:23-36.

Fenech M. 1999. Micronucleus frequency in human lymphocytes is related to plasma vitamin B12 and homocysteine. *Mutation Res.* 428(1-2):299-304.

Fenech M, & Morley AA. 1985a. Measurements of micronuclei in lymphocytes. *Mutation Res.* 147:29-36.

Fenech M, & Morley AA. 1985b. The effect of donor age on spontaneous and induced micronuclei. *Mutation Res.* 148:99-105.

Fenech M, & Morley AA. 1986. Cytokinesis-block micronucleus method in human lymphocytes: effect of *in vivo* aging and low dose X-irradiation. *Mutation Res.* 161(2):193-198.

Fenech M, & Neville S. 1992. Micronucleus induction in bone-marrow cells following consumption of cooked beef in mice. Preliminary investigations. *Mutation Res.* 281(1):3-10.

Fenech M, & Rinaldi J. 1995. A comparison of lymphocyte micronuclei and plasma micronutrients in vegetarians and non-vegetarians. *Carcinogenesis* 16:223-230.

Fenech M, Aitken C, & Rinaldi JR. 1998. Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. *Carcinogenesis* 19:1163-1171.

Fenech M, Crott J, Turner S, & Brown S. 1999. Necrosis, apoptosis, cytostasis and DNA damage in human lymphocytes measured simulteneously within the cytokinesis-block micronucleus assay: decription of the method and results for hydrogen peroxide. *Mutagenesis* 14(6):605-612.

Fenech M, Denham J, Francis W, & Morley AA. 1990. Micronuclei in cytokinesis-blocked lymphocytes of cancer patients following fractionated partial-body radiotherapy. *Intl. J. Radiat. Biol.* 57(2):373-383.

Fenech M, Dreosti IE, & Rinaldi JR. 1997. Folate, vitamin B12, homocysteine status and chromosome damage rate in lymphocytes of older men. *Carcinogenesis* 18:1329-1336.

Fenech M, Holland N, Chang WP, Zeiger E, & Bonassi S. 1999. The HUMN project: an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. *Mutation Res.* 428:271-283.

Feychting M, & Ahlbom A. 1993. Magnetic fields and cancer in children residing near Swedish high-voltage power lines. *Am. J. Epidemiol*. 138:467-481.

Fiorani M, Cantoni O, Sestili P, Conti RC, Nicolini P, Vetrano F, & Dacha M. 1992. Electric and/or magnetic field effects on DNA structure and function in cultured human cells. *Mutation Res.* 282:25-29.

Fisher SJ, Dulling J, & Smith SD. 1986. Effect of a pulsed electromagnetic field on plasma membrane protein glycosylation. *Bioelectromagnetics* 5:253-267.

Fontana L, Lasfargues G, Ughetto S, Rogier S, Masdieu E, Lafaure M, Aublet-Cuvelier B & Catilina P. 2001. The micronucleus assay in human exfoliated urothelial cells: application in a genotoxicity study of workers exposed to a mineral jelly containing sodium nitrite and N-phenyl-1-naphthylamine. *Mutagenesis* 16(5):449-452.

Fontanesi G, Traina GC, Giancecchi F, Tartaglia I, Rotini R, Virogili B, Cadossi R, Ceccherelli C, & Marino AA. 1986. Slow healing fractures: can they be prevented? Results of electrical stimulation in fibular osteotomies in rats and diaphyseal fractures of the tibia in humans. *Italian J. Orthopaedics and Traumatology* 12:371-386.

Fornace AJ Jr, Nagasawa H, & Little JB. 1980. Relationship of DNA repair to chromosome aberrations sister chromatid exchanges and survival during liquid-holding recovery in X-irradiated mammalian cells. *Mutation Res.* 70:323-336.

Heppner FH, & Haffner JD. 1974. 'Communication in bird flocks: an electromagnetic model', *In* biologic and clinical effects of low-frequency magnetic and electric fields, Ed. Frank J, & Riccardi VM. 1977. "The 11q -- syndrome". An eighth case of terminal deletion of the long arm of chromosome eleven. *Hum. Genet.* 35:241-246.

Frankel RB, Bazylinski DA, Johnson MS, & Taylor BL. 1997. Magneto-aerotaxis in marine coccoid bacteria. *Biophys. J.* 73:994–1000.

Frisch VK. 1967. The dance language and orientation of bees. Harvard University Press, Cambridge, MA.

Frohlich H, & Kremer F. 1983. (Eds.) Coherent Excitations in Biological Systems. Springer-Verlag, New York.

Fucic A, Garaj-Vrhovac V, Skara M, & Dimitrovic B. 1992. X-rays, microwaves and vinyl chloride monomer: their clastogenic and aneugenic activity, using the micronucleus assay on human lymphocytes. *Mutation Res.* 282(4):265-271.

Galloway SM, & Evans HJ. 1975. Sister chromatid exchange in human chromosomes from normal individuals and patients with ataxia telangiectasia. *Cytogenetics & Cell Genetics* 15(1):17-29.

Galt S, Sandblom J, Mamnerius Y, Hojevik P, Saalman E, & Rappaport C. 1993. Experimental search for combined AC and DC magnetic field effects on ion channels. *Bioelectromagnetics* 14:315-327.

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

Garcia-Sagredo JM, & Monteagudo JL. 1991. Effect of low-level pulsed electromagnetic fields on human chromosomes *in vitro:* analysis of Chromosomal aberrations. *Hereditas* 115:9-11.

Garcia-Sagredo JM, Parado LA, & Monteagudo JL. 1990. Effect on SCE in human chromosomes *in vitro* of low-level pulsed magnetic fields. Environ. *Mol. Mutagen.* 16:185-188.

Gatti M, Pimpinelli S, & Oliviery G. 1974. The frequency and distribution of isolabeling in Chinese Hamster chromosomes after exposure to X-rays. *Mutation Res.* 23:229-238.

Gatti M, Pimpinelli S, & Oliviery G. 1979. Lack of spontaneous sister chromatid exchanges in somatic cells of Drosophila melanogaster. *Genetics* 91:255-274.

Gauger JR. 1984. Household appliance magnetic field survey; IIT Res. Inst. Rep.No. Eo6549-3; Naval electronic systems command; Washington, DC.

Gedik CM, Ewen SW, & Collins AR. 1992. Single cell gel electrophoresis applied to the analysis of UV-C damage and its repair in human cells. *Int. J. Radiat. Biol.* 62:313-320.

Geiduschek EP. 1961. "Reversible" DNA. *Proc. Natl. Acad. Sci. USA* 47:950-955.

German J, Archibald R, & Bloom D. 1965. Chromosomal breakage in a rare and probably genetically determined syndrome of man. *Science* 148:506-507.

German J, Crippa LP, & Bloom D. 1974. Bloom's Syndrome III: Analysis of the chromosome aberration characteristic of this disorder. German, J., R. Archibald, D. Bloom (1965). *Chromosoma* (Berl.) 48:361-366.

Ghadire M, & Mahnaz A. 2002. Developmental changes in Drosophila melanogaster following exposure to alternating electromagnetic fields. *Bioelectromagnetics* 23(6):416-420.

Giancoli DC. 2000. Physics for scientists and engineers. Prentice Hall, Upper Saddle River, New Jersey, pp 976.

Gibson DA, & Prescott DM. 1974. Frequency and sites of sister chromatid exchanges in rat kangaroo chromosomes. *Expt. Cell Res.* 86(1):209-214.

Goodman R, & Henderson A. 1991. Trnscription and translation in cells exposed to extremely low frequency electromagnetic fields. *Bioelectrochem. Bioenerg.* 25:335-355.

Goodman R, & Henderson A. 1992. Exposure of human cells to electromagnetic fields: Effect of time and field strength on transcript levels. *Electro-Magnetobiol.* 11:11-28.

Goodman R, Bassett CAL, & Henderson A. 1983. Pulsing electromagnetic fields induce cellular transcription. *Science* 220:1283-1285.

Goodman R, Wei LX, Xu JC, & Henderson A. 1989b. Exposure of human cells to low frequency electromagnetic fields results in quantitative changes in transcripts. *Biochem. Biophys. Acta.* 1009:216-220.

Goodman R, Weisbrot D, Uluc A, & Henderson A. 1992. Transcription in *Drosophila melanogaster* Salivary gland cells is altered following exposure to low-frequency electromagnetic fields: analysis of chromosome 3R. *Bioelectromagnetics* 13:111-118.

Goodman EM, Greenebaum B, & Marron MT. 1995. Effects of Electromagnetic Fields on Molecules and Cells. *Int. Rev. Cytol.* 158: 279-338

Gould JL. 1980. The case for magnetic sensitivity in birds and bees (such as it is). *American Scientist* 68: 256-267.

Graham C, Cook MR, Cohen HD, & Gerkovich MM. 1994. A dose response study of human exposure to 60 Hz electric and magnetic fields. *Bioelectromagnetics* 15:447-463.

Graham C, Sastre A, Cook MR, Gerkovich, & Mary M. 2001. Examination of the melatonin hypothesis in women exposed at night to EMF or bright light. *Environ. Health Perss* 109(5):501-507.

Grundler W, Kaiser F, Keilmann F, & Walleczek J. 1992. Mechanisms of electromagnetic interaction with cellular systems. *Naturwissenschaften* 79:551–559.

Grandolfo M, Santini MT, Vecchia P, Bonincontro A, Camett C, & Indovina PL. 1991. Non-linear dependence of the electric properties of chick embryo myoblast membranes exposed to sinusoidal 50 Hz magnetic field. *Int. J. Radiat. Biol.* 60:877-890.

Green MHL, Lowe JE, Harcourt SA, Akinluyi P, Rowe T, Cole J, Anstey AV, & Arlett CF. 1992. UV-C sensitivity on unstimulated human lymphocytes from normal and *Xederma pigmentosum* donors in comet assay: a potential diagnostic technique. *Mutation Res.* 273:137-144.

Greene JJ, Skowronski WJ, Mullins JM, & Nardone RM. 1991. Delineation of electric and magnetic field effects of extremely low frequency electromagnetic radiation on transcription. *Biochem. Biophys. Res. Commun.* 174:742-749.

Griefahn B, Kunemund C, Blaszkewicz M, Lerchl A, & Degen GH. 2002. Effects of electromagnetic radiation (bright light, extremely low-frequency magnetic fields, infrared radiation) on the circadian rhythm of melatonin synthesis, rectal temperature, and heartrate. *Ind. Health.* 40(4):320-327.

Griffin DR. 1944. The sensory basis of bird navigation. *Rev. Biol.* 19:15-31.

Griffin DR. 1952. Bird navigation. *Biol. Rev. Cambridge Philos. Soc.* 27:359-390.

Grissom CB. 1995. Magnetic field effects in biology: A survey of possible mechanisms with emphasis on radial-pair recombination. *Chem. Rev.* 95:3-24.

Grover KP, Danadevil M, Mahboob R, Rozatil B, & Banu S. 2003. Evaluation of genetic damage in workers employed in pesticide production utilizing the Comet assay. *Mutagenesis* 2:201-205.

Guglielmi GE, & Tice RR. 1980. Caffeine induces sister chromatid exchanges and inhibits cellular proliferation in human peripheral lymphocyte cultures. *Am. J. Hum. Genet.* 155A(abstr.).

Haider T, Knasmueller S, Kundi M, & Haider M. 1994. Clastogenic effects of radio-frequency radiations on chromosomes of Tradescantia. *Mutation Res.* 324(1-2):65-68.

Hand R, & German J. 1975. A retarded rate of DNA chain growth in Bloom's syndrome. *Proc. Natl. Acad. Sci. USA* 72(2):758-62.

Hardeland R, Reiter RJ, Poeggeler B, & Tan DX. 1993. The significance of the metabolism of the neurohormone melatonin: antioxidative protection and formation of bioactive substances. *NeuroScience & Biobehavioral Rev.* 17(3):347-357.

Harland JD, & Liburdy RP. 1996. ELF inhibition of Melatonin and Tamoxifen action on MCF-7 cell proliferation: Field parameters. BEMS Meeting Victoria, British Columbia, Canada. Abstract A-1-1.

Hartmann A, & Speit G. 1995. Genotoxic effects of chemicals in the single cell gel (SCG) test with human blood cells in relation to the induction of sister chromatid exchanges (SCE). *Mutation Res.* 346:49-56.

Hartmann A, Fender H, & Speit G. 1998. A comparative biomonitoring study of workers of a waste disposal site using cytogenetic tests and the comet (single cell gel) assay. *Environ. Mol. Mutagen.* 32:17-24.

Hartmann A, Plappert U, Raddatz K, Grünert-Fuchs M, & Speit G. 1994. Does physical activity induce DNA damage? *Mutagenesis* 9:269-272.

Hartmann A, Agurell E, Beevers C, Brendler-Schwaab S, Burlinson B, Clay P, Collins A, Smith A, Speit G, Thybaud V, & Tice RR. 2003. Recommendations for conducting the *in vivo* alkaline Comet assay. *Mutagenesis* 18:45-51.

He JL, Jin HY, Jin LF, & Gao SY. 2000. Monitoring of human exposure to radiation with the binucleated lymphocyte micronucleus assay. *Biomed. Environ. Sci.* 13(1):32-36.

Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW, & Salamone MF. 1983. The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environ. Prot. Agency Gene-Tox Prog. *Mutation Res.* 123:61-118.

Heimers A. 2000. Chromosome aberration analysis in Concorde pilots. *Mutation Res.* 467(2):169-176.

Hessel H, Radon K, Pethran A, Maisch B, Grobmair S, Sautter I, & Fruhmann G. 2001. The genotoxic risk of hospital, pharmacy and medical personnel occupationally exposed to cytostatic drugs-evaluation by the micronucleus assay. *Mutation Res.* 497(1-2):101-109.

Hill BH Jr. 1965. A medieval German wound man. Wellcome MS 49. *Journal of the History of Medicine & Allied Sciences* 20(4):334-357.

Hiroaki Y, Takehiro T, Hiroki M, Hidemi O, Seiichi K, Hiroyuki Y, Chiharu S, Setsu H, Toshio M, Taku Y, Fumio M, Hideki H, & Yoshihisa O. 2002. No effect of extremely low-frequency magnetic field observed on cell growth or initial response of cell proliferation in human cancer cell lines. *Bioelectromagnetics* 23(5):355-368.

Hirsch BA. Sentz KK. & McGue M. 1992. Genetic and environmental influences on baseline SCE. *Environ. Mol. Mutagenesis* 20(1):2-11.

Holliday R. 1964. A mechanism for gene conversion in fungi. *Genet. Res. Camb.* 5:1158-1160.

Hoo JJ, & Parslow MI. 1979. Relation between the SCE points and the DNA replication bands. *Chromosoma* 73(1):67-74.

Hook GJ, Vasquez M, Blackwell DM, Clancy JJ, Donner EM, Tice RR, & McRee D. 1999. Genotoxicity of RF fields generated from analog, TDMA, CDMA and PCS technology evaluated using a single-cell gell electrophoresis and the Cytochalasin B micronucleus (CB-MN) assay. Presented at BEMS in Long Beach, California.

Hsiang YH, Hertzberg R, Hecht S, & Liu LF. 1985. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J. Biol. Chem.* 260:14873-14878.

Hsieh T, & Brutlag D. 1980. ATP-dependent DNA topoisonmerase from D. melanogaster reversibly catenates duplex DNA rings. *Cell* 21(1):115-125.

Hsieh WA, Deng W, Chang WP, Galvan N, Owens CL, Morrison DP, Gale KL, & Lucas JN. 1999. Alpha coefficient of dose-response for chromosome translocations measured by FISH in human lymphocytes exposed to chronic ^{60}Co γ-rays at body temperature. *Int. J. Radiat. Biol.* 75(4):435-439.

Hsu Chin-Yuan, & Chia-Wei Li. 1994. Magnetoreception in honey bees. *Science* 265: 95-97.

Hsu TC, & Pathak S. 1976. Differential rates of sister chromatid exchanges between euchromatin and heterochromatin. *Chromosoma* 58(3):269-273.

Hu GG, Luo XM, Liu J, Liu X, Gu M, Mao BL, Chen ZY, & Wang LJ. 1987. Sister chromatid exchanges (SCE), Chromosomal aberrations and micronuclei of cultured peripheral lymphocytes of patients with lung cancer, miners and non-mining workers of the tin mine in Yunnan (Chinese). Chung-Hua Chung Liu Tsa Chih (*Chinese Journal of Oncology*) 9(1):29-32.

Husum B, Wulf HC, & Niebuhr E. 1981. Sister-chromatid exchanges in lymphocytes in women with cancer of the breast. *Mutation Res.* 85:357-362.

Husum B, Wulf HC, & Niebuhr E. 1982. Normal sister chromatid exchanges in oral contraceptives users. *Mutation Res.* 103:161-164.

Hutchinson F. 1973. The lesions produced by ultraviolet light in DNA containing 5-bromouracil. *Q. Rev. Biophys.* 6:201-246.

IAEA (International Atomic Energy Agency, Viena). 1986. Biological Dosimetry: Chromosomal Aberration Analysis for Dose Assessment. *Technical Report Series No.* 260.

Ikushima T, & Wolff S. 1974. Sister chromatid exchanges induced by light flashes to 5-bromodeoxyuridine- and 5-iodeoxyuridine-substituted Chinese hamster chromosomes. *Expt. Cell Res.* 87:15-19.

Ishii Y, & Bender MA. 1980. Effects of inhibitors of DNA synthesis on spontaneous and ultraviolet light induced sister chromatid exchanges in Chinese hamster cells. *Mutation Res.*79:19-32.

Islam M. 2003. Ac signals, the signal generator, and the oscilloscope. http://www.utdallas.edu/~mxi033000/ee1102/experiment_03.htm

Ivancsits S, Diem E, Pilger A, Rudiger HW, & Jahn O. 2002. Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts. *Mutation Res.* 519(1-2):1-13.

Jan JE, Espezel H, & Appleton RE. 1994. The Treatment of Sleep Disorders with Melatonin. *Developmental Medicine and Child Neurology* 36:91-107.

Jia-Sheng W, & Groopman JD. 1999. DNA damage by mycotoxins. *Mutation Res.* 424(1-2):167-181.

John H, Birnstiel M, & Jones K. 1969. RNA-DNA hybrids at the cytological level. *Nature* 223:582-587.

Johnson, CJ. 2004. A cytogenetic study of New Zealand Nuclear Test Veterans: The COMET assay. Master of Science Thesis (unpublished), Massey University, Palmerston North.

Jones LA, Scott D, Cowan R, & Roberts SA. 1995. Abnormal radiosensitivity of lymphocytes from breast cancer patients with excessive normal tissue damage after radiotherapy: chromosome aberrations after low dose-rate irradiation. *Int. J. Radiat. Biol.* 67:519-528.

Joseph-Lerner N, Fejgin M, Ben-Nun I, Legum C, & Amiel A. 1993. The correlation between the frequency of sister-chromatid exchange and human reproductive hormones. *Mutation Res.* 300(3-4):247-252.

Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, & Pinkel D. 1992. Compartive genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 258:818-821.

Kanda N. 1982. Spontaneous sister chromatid exchanges *in vivo*. *In* Sandberg A. A. (Ed.) Sister chromatid exchange. Liss, New York, pp 279-296.

Kasuba V, Rozgaj R, & Garaj VV. 1999. Analysis of sister chromatid exchange and micronuclei in peripheral blood lymphocytes of nurses handling cytostatic drugs. *J. Applied Toxicology* 19(6):401-404.

Kato H. 1973. Induction of sister chromatid exchange by UV light and its inhibition by caffeine. *Exp. Cell Res.* 82:382-390.

Kato H. 1974a. Possible role of DNA synthesis in function of sister chromatid exchanges. *Nature* (London) 252:739-741.

Kato H. 1974b. Spontaneous sister chromatid exchanges detected by a BrdU-labelling method. *Nature* (London) 251:70-72.

Kato H. 1974c. Is isolabeling a false image? *Exp. Cell Res.* 89:416-420.

Kato H. 1977. Mechanisms for sister chromatid exchanges and their relation to the production of Chromosomal aberrations. *Chromosoma* 59:179-191.

Kato H. 1980. Evidence that the replication point is the site of sister chromatid exchange. *Cancer Genet. Cytogenet.* 2:69-73.

Kato MK, Honma T, Shigemitsu T, & Shiga Y. 1993. Effects of exposure to circularly polarized 50-Hz magnetic field on plasma and pineal melatonin levels in rats. *Bioelectromagnetics* 14:97-106.

Kato MK, Honma T, Shigemitsu T, & Shiga Y. 1994. Horizontal or vertical 50-Hz, 1 T magnetic fields have no effect on pineal gland or plasma melatonin concentrations of albino rats. *Neurosci. Lett.* 168:205-208.

Kaul A, & Goyle S. 1999. Genotoxicity of the anticonvulsant drug phenytoin (PHT): A follow-up study of PHT-untreated epileptic patients. I. Sister chromatid exchange (SCE) analysis. *Teratogenesis, Carcinogenesis, & Mutagenesis* 19(1):61-72.

Kayikcioglu F, Gunes M, Baltaci V, Kocak M, Alpas I, & Haberal A. 2000. Sister-chromatid exchange frequencies in postmenopausal hormone replacement patients. *Mutation Res.* 452(1):37-39.

Khalil AM, & Qassem W. 1991. Cytogenetic effects of pulsing electromagnetic fields on human lymphocytes *in vitro*: chromosome aberrations, sister-chromatid exchanges and cell kinetics. *Mutation Res.* 247:141-146.

Kihlman BA. 1975. Sister chromatid exchanges in *Vicia faba*. II. Effects of thiotepa, caffeine and 8-ethoxycaffeine on the frequency of SCEs. *Chromosoma* (Berl.) 51:11-18.

Kikkawa U, Kishimoto A, & Nishizuka Y. 1989. The protein kinase C family: heterogeneity and its implications. *Annu. Rev. Biochem.* 58:31-44.

Kim BS, Cho M, & Kim HJ. 2000. Statistical analysis of *in vivo* rodent micronucleus assay. *Mutation Res.* 469(2):233-41.

Kirschvink JL, & Kobayashi-Kirschvink A. 1991. Is geomagnetic sensitivity real? Replication of the Walker-Bitterman conditioning experiment in honeybees. *Am. Zoologist* 31(1):169-185.

Kirschvink JL, Dizon AE, & Westphal JA. 1986. Evidence from strandings for geomagnetic sensitivity in cetaceans. *J. Exp. Biol.* 120:1-24.

Kirschvink JL, Kobayshi-Kirschvink A, & Kirschvink SJ. 1992. Magnetite biomineralization in the human brain. *Proc. Nat. Acad. Sci. USA* 89:7683-7687.

Kirschvink JL, Kuwajima T, Ueno S, Kirschvink SJ, Diaz-Ricci JC, Morales A, Barwig S, & Quinn K. 1992a. Discrimination of low-frequency magnetic fields by honeybees: Biophysics and experimental tests. *In* Sensory Transduction (Ed. D. P.Corey and S. D. Roper), pp. 225-240. Soceity of General Physiologists, 45th annual symposium. Rockefeller University Press: New York.

Kirschvink JL, Padmanabha S, Boyce CK, & Oglesby J. 1997. Measurement of the threshold sensitivity of honeybees to weak, extremely low-frquency magnetic fields. *J. Expt. Biol.* 200:1363-1368.

Kirschvink JL, Walker MM, Chang SB, Dizon AE, & Peterson KA. 1985. Chains of single domain magnetite particles in chinook salmon, *Oncorhynchus tshawytscha. J. Comp. Physiol.* 157:375-381.

Kirschvink JL, Walker MM, & Diebel CE. 2001. Magnetite-based magnetoreception. *Current Opinion in Neurobiology* 11(4):462-467.

Kirsch-Volders M. (Ed). 1997. The CB *in vitro* micronucleus assay in human lymphocytes. *Mutation Res.* 392 (Special Issue 1 & 2).

Kisliuk M, & Islay J. 1977. Influence of an additional magnetic field on hornet nest architecture. *Experimentia* 37(7):885-887.

Klaude M, Ericson S, Nygren J, & Ahnström G. 1996. The COMET assay: mechanisms and technical considerations. *Mutation Res.* 363:89-96.

Kligerman AD. 1979. Induction of sister chromatid exchanges in the central mudminnow following *in vivo* exposure to mutagenic agents. *Mutation Res.* 64:205-217.

Kligerman AD. & Bloom SE. 1976. Sister chromatid differentiation and exchanges in adult mudminnows (Umbra limi) after *in vivo* exposure to 5-bromodeoxyuridine. *Chromosoma* 56(2):101-109.

Kligerman AD, Bloom SE, & Howell WM. 1975. Umbra limi: a model for the study of chromosome aberrations in fishes. *Mutation Res.* 31:225-233.

Kobayashi AK, Kirschvink JL, & Nesson MH. 1995. Ferromagnetism and EMFs. *Nature* 374 (6518):123.

Kodama Y, Pawel D, Nakamura N, Preston D, Honda T, Itoh M, Nakano M, Ohtaki K, Funamoto S, & Awa AA. 2001. Stable chromosome aberrations in atomic bomb survivors: Results from 25 years of investigation. *Radiat. Res.* 156(4):337-346.

Koppen G, & Verschaeve L. 1996. The alkaline comet test on plant cells: a new genotoxicity test for DNA strand breaks in *Vicia faba* root cells. *Mutation Res.* 360:193-200.

Krahl L. 1999. Detection of chromosome abnormalities: let's go FISH-ing. PSA consult. Vol.II (8).

Kram D. 1982. *In utero* analysis of sister chromatid exchanges *In* A. A. Sandberg (Ed.). Sister Chromatid Exchange. Liss, New York, pp. 425-460.

Kreuzer KN, & Cozzarelli NR. 1980. Formation and resolution of DNA catenanes by DNA gyrase. *Cell* 20(1):245-254.

Kruglikov IL, & Dertinger H. 1994. Stochastic resonance as a possible mechanism of amplification of weak electric signals in living cells. *Bioelectromagnetics* 15:539-547.

Kryscio A, Ulrich Muller WU, Wojcik A, Kotschy N, Grobelny S, & Streffer C. 2001. A cytogenetic analysis of the long-term effect of uranium mining on peripheral lymphocytes using the micronucleus-centromere assay. *Int. J. Radiat. Biol.* 77(11):1087-1093.

Kumlin T, Alhonen L, Janne J, Lang S, Kosma VM, & Juutilainen J. 1998. Epidermal ornithine decarboxylase and polyamines in mice exposed to 50 Hz magnetic fields and UV radiation. *Bioelectromagnetics* 19(6):388-391.

Lagroye I, & Poncy JL. 1997. The effect of 50 Hz electromagnetic fields on the formation of micronuclei in rodent cell lines exposed to gamma radiation. *Int. J. Radiat. Biol.* 72(2):249-254.

Lai H. 2001. Genetic effects of non-ionizing electromagnetic fields. A paper presented at the "International Workshop on Biological Effects of Ionizing Radiation, Electromagnetic Fields and Chemical Toxic Agents" in Sinaia, Romania, October 2-6.

Lai H, & Singh NP. 1995. Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. *Bioelectromagnetics.* 16(3):207-210.

Lai H, & Singh NP. 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, & Singh NP. 1997a. Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells. *Bioelectromagnetics* 18:156-165.

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

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

Lambert V, Thierens H, Monsieurs M, Roncancio C, & Laurent C. 2001. Translocation frequencies measured in patients one year after radioactive iodine therapy for thyrotoxicosis. *Int. J. Radiat. Biol.* 77(6):679-685.

Latt SA. 1973. Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. *Proc. Natl. Acad. Sci. USA* 70:3395-3399.

Latt SA. 1974a. Localization of sister chromatid exchanges in human chromosomes. *Science* 185:74.

Latt SA. 1981a. Sister chromatid exchange in human chromosomes. *Science* 185:74-76.

Latt SA. 1981b. Sister chromatid exchange formation. *Ann. Rev. Genet.* 15:11-55.

Latt SA, Juergens LA. 1977. Determinants of sister chromatid exchange frequencies in human chromosomes, *In* E. B. Hook and I. H. Porter (Eds.), Population Cytogenetics: Studies in Humans, Academic Press, New York, pp. 217-236.

Latt SA, & Loveday KS. 1978. Characterization of sister chromatid exchange induction by 8-methoxypsoralen plus near-UV light. *Cytogenet. Cell Genet.* 21:184-200.

Latt SA, George YS, & Gray JW. 1977. Flow cytometric analysis of bromodeoxyuridine-substituted cells stained with 33258 Hoechst. *Journal of Histochemistry & Cytochemistry* 25(7):927-934.

Latt SA, Stetten G, Juergens LA, Buchanan GR, & Gerald PS. 1975. Induction by alkylating agents of sister chromatid exchanges and chromatid breaks in Fanconi's anemia. *Proc. Natl Acad. Sci. USA* 72(10):4066-4070.

Latt SA, Allen JW, Bloom SE, Carrano AV, Falke E, Kram D, & Schneider E. 1981. Sister chromatid exchanges: a report of the GENETOX program. *Mutation Res.* 87:17-62.

Latt SA, Schrek RR, D'Andrea A, Kaiser TN, Schlesinger F, Lester S, & Sakai K. 1984. Detection significance and mechanism of sister-chromatid exchange formation: past experiments, current concepts, future chalenges, *In* Sister chromatid exchanges, 25 years of experimental research. Plenum, New York, pp. 11-40.

Lawley PD. 1974. Some chemical aspects of dose-response relationships in alkylation mutagenesis. *Mutation Res.* 23:283-295.

Lawrence AF, & Adey WR. 1982. Nonlinear wave mechanisms in interactions between excitable tissues and electromagnetic fields. *Neurol. Res.* 4:115-153.

Lazutka JR, & Dedonyte V. 1995. Increased frequency of sister chromatid exchanges in lymphocytes of Chernobyl clean-up workers. *Int. J. Radiat. Biol.* 67(6):671-676.

Lazutka JR, Dedonyte V, & Lekevicius RK. 1992. Sister chromatid exchanges in lymphocytes of normal and alcoholic subjects. *Experientia* 48(5):508-512.

Lazutka J R, Dedonyte V, & Krapavickaite D. 1994. Sister-chromatid exchanges and their distribution in human lymphocytes in relation to age, sex and smoking. *Mutation Res.* 306(2):173-80.

Lazutka JR, Lekevicius R, Dedonyte V, Maciuleviciute-Gervers L, Mierauskiene J, S. Rudaitiene J, & Slapsyte G. 1999. Chromosome aberrations and sister chromatid exchanges in Lithunian population: Effects of occupational and environmental exposures. *Mutation Res.* 445(2):225-39.

Lednev VV. 1991. Possible mechanism for the influence of weak magnetic fields on biological systems. *Bioelectromagnetics* 12(2):71-75.

Leask MJM. 1977. A physiological mechanism for magnetic field detection by migratory birds and homing pigeons. *Nature* 267:144.

Lerchl KO, Nonaka, & Reiter RJ. 1990. Pineal gland "magnetosensitivity" is a consequence of induced electric eddy currents. *J. Pineal Res.* 10:109–116.

Lester JR, & Moore DF. 1982. Cancer mortality and air force bases. *J. Bioelectricity* 1:77-82.

Levallois P, Dumont M, Touitou Y, Gingras S, Masse B, Gauvin D, Kroger E, Bourdages M, & Douville P. 2001. Effects of electric and magnetic fields from high-power lines on female urinary excretion of 6-sulfatoxymelatonin. *Am. J. Epidemiol.* 154:601-609.

Li L, Bisht KS, & Zhang P. 2001. Measurement of DNA damage in mammalian cells exposed to radiofrequency fields at high SAR *in vitro*. *Radiat. Res.* 156:328-332.

Liboff AR. 1985. Geomagnetic cyclotron resonance in living cells. *J. Biol. Phys.* 13:99.

Liboff AR, & Parkinson WC. 1991. Search for ion cyclotron resonance in an Na+ - transport system. *Bioelectromagnetics* 12:77-83.

Liboff AR, Williums T, Strong DM & Wister R. 1984. Time-varying magnetic fields: effects on DNA synthesis. *Science* 223:818-820.

Liboff AR, Rozek RJ, Sherman ML, Mcleod BR, & Smith SD. 1987. 45-Ca^{++} cyclotron resonance in human lymphocytes. *J. Bioelectricity* 6:13-22.

Liburdy RP. 1992a. Calcium signalling in lymphocytes and ELF fields: Evidence for an electric field metric and a site of interaction involving the calcium ion channel. *FEBS Lett.* 301:53-59.

Liburdy RP. 1992b. Calcium signalling in lymphocytes and ELF fields: evidence for an electric field metric and a site of interaction involving the calcium ion channel. *FEBS Lett.* 301(1):53-59.

Liburdy RP. 1995. Cellular studies and interaction mechanisms of extremely low frequency fields. *Radio Science* 30:179-203.

Liburdy RP, & Afzal SMJ. 1996. Inhibition of melatonin's action of MCF-7 cell proliferation by magnetic fields associated with Video Display Terminals: A preliminary study. BEMS Meeting Victoria, British Columbia, Canada. Abstract A-1-3.

Liburdy RP, Sloma TR, Sokolic R, & Yaswen P. 1993. ELF magnetic fields, breast cancer and melatonin: 60 Hz fields block melatonin's oncostatic action on ER+ breast cancer cell proliferation. *J. Pineal Res.* 14(2):89-97.

Lieber MR. 1998. Pathological and physiological double-strand breaks: roles in cancer, aging, and the immune system. *Am. J. Pathol.* 153(5):1323-1332.

Lin MS, & Davidson RL. 1974. Centric fusion, satellite DNA, and DNA polarity in mouse chromosomes. *Science* 185:1179-1181.

Lindstrom E, Lindstrom P, Berglund A, Mild KH, & Lundgren E. 1993. Intracellular calcium oscillations induced in a T-cell line by weak 50 Hz magnetic field. *J. Cell Physiol.* 156:395-398.

Lin-Xiang W, Goodman R, & Henderson A. 1990. Changes in levels of c-myc and histone H2B following exposure of cells to low frequency sinusoidal electromagnetic fields: Evidence for a window effect. *Bioelectromagnetics* 11:269-272.

Litovitz TA, Montrose CJ, Goodman R, & Elson EC. 1990. Amplitude windows and transiently augmented transcription from exposure to electromagnetic fields. *Bioelectromagnetics* 11:297-312.

Litovitz TA, Farrell JM, Krause D, Doinov P, & Montrose CJ. 1993. Superimposing electromagnetic noise blocks the alteration of ornithine decarboxylase activity in developing chick embryos caused by a 60 Hz sinusoidal field . Bioelectromagn. 15th Annu. Meet. Los Angeles, p. 95.

Littlefield LG, Colyer SP, Joiner EE, & DuFrain RJ. 1979. Sister chromatid exchange in human lymphocytes exposed to joining radiation during G_0. *Radiat. Res.* 78:514-521.

Livingston GK, Cannon LA, Bishop DT, Johnson P, & Fineman RM. 1983. Sister chromatid exchange : variation of age, sex, smoking and breast cancer status. *Cancer Genet. Cytogenet.* 8:289-299.

Livingston GK, Witt KL, Gandhi OP, Chatterjee I, & Roti JL. 1991. Reproductive integrity of mammalian cells exposed to power frequency electromagnetic fields. *Environ. Mol. Mutagen.* 17(1):49-58.

Lloyd DC, Moquet JE, Oram S, Edwards AA, & Lucas JN. 1998. Accidental intake of tritiated water: a cytogenetic follow-up case on translocation stability and dose reconstruction. *Int. J. Radiat. Biol.* 73:543-547.

London SJ, Thomas DC, Bowman JD, Sobel E, Cheng TC, & Peters JM. 1991. Exposure to residential electric and magnetic fields and risk of child leukemia. *Am. J. Epidemiol.* 134:923-937.

Loomis DP, Savitz DA, & Ananth CV. 1994. Breast cancer mortality among female electrical workers in the United States. *J. Natl. Cancer Inst.* 86: 921-925.

Löscher W. Mevissen M. Lehmacher W. & Stamm A. 1993. Tumor promotion in a breast cancer model by exposure to a weak alternating magnetic field. *Cancer Lett.* 71(1-3):75-81.

Loss R. 2001. Physical measurements 101: introduction to AC signals. Lecture notes, University of Technology, Perth, Western Australia.

Luben RA. 1991. Effects of low-energy electromagnetic fields (pulsed and DC) on membrane signal transduction processes in biological systems. *Health Phys.* 61:15-28.

Luben RA, Cain CD, Chen MCY, Rosen DM, & Adey WR. 1982. Inhibition of parathyroid hormone actions on bone cells in culture by induced low energy electromagnetic fields. *Proc. Natl. Acad. Sci. USA* 79:4180-4184.

Lucas JN. 1997. Dose reconstruction for individuals exposed to ionizing radiation using chromosome painting. *Radiat. Res.* 148:33-38.

Lucas JN, Tenjin T, Straume T, Pinkel D, Moore II, Litt M, & Gray JW. 1989. Rapid determination of human chromosome translocation frequency using a pair chromosome-specific DNA probes. *Int. J. Radiat. Biol.* 56:35-44.

Lucas JN, Awa A, Straume T, Poggensee M, Kodoma Y, Nakano M, Ohtaki K, Weier HU, Pinkel D, Gray J, & Littlefield G. 1992. Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation. *Int. J. Radiat. Biol.* 62: 53-63.

Lucas JN, Deng W, Hsieh WA, Galvan N, Gale KL, & Morrison DP. 1999. Exposure temperature, but not donor age, is a confounding factor for *in vitro* translocation production by chronic irradiation. *Int. J. Radiat. Biol* 75(6):673-680.

Lucas JN, Deng W, Moore D, Hill F, Wade M, Lewis A, Sailes F, Kramer C, Hsieh A, & Galvan N. 1999a. Background ionizing radiation plays a minor role in the production of chromosome translocations in a control population. *Int. J. Radiat. Biol.* 75(7):819-827.

Maes A, Collier M, Verschaeve L. 2001. Cytogenetic effects of 900 MHz (GSM) microwaves on human lymphocytes. *Bioelectromagnetics* 22(2):91-96.

Maes A, Collier M, Slaets D, & Verschaeve L. 1996. 954 MHz microwaves enhance the mutagenic properties of mitomycin C. *Environ. Mol. Mutagen.* 28(1):26-30.

Maes A, Verschaeve L, Arroyo A, De Wagter C, & Vercruyssen L. 1993. *In vitro* cytogenetic effects of 2450 MHz waves on human peripheral blood lymphocytes. *Bioelectromagnetics* 14:495-501.

Maes A, Collier M, Van Gorp U, Vandoninck S, Verschaeve LC. 1997. Cytogenetic effects of 935.2-MHz (GSM) microwaves alone and in combination with mitomycin. *Mutation Res.* 393(1-2):151-156.

Maes A, Collier M, Vandoninck S, Scarpa P, & Verschaeve L. 2000. Cytogenetic effects of 50 Hz magnetic fields of different magnetic flux densities. *Bioelectromagnetics* 21(8):589-596.

Maes A, Collier M, Slaets D, & Verschaeve L. 1995. Cytogenetic effects of microwaves from mobile communication frequencies (954 MHz). *Electro-Magnetobiol.* 14:91-98.

Malayapa RS, Bi C, Ahern EW, & Roti Roti JL. 1998. Detection of DNA damage by the alkaline COMET assay after exposure to low-dose gamma radiation. *Radiat. Res.* 149:396-400.

Malayapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, & Roti-Roti J. 1997a. Measurement of DNA damage following exposure to EMR in the cellular communications frequency band (835.62 and 847.74 MHz). *Radiat. Res.* 148: 618-627

Malayapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, & Roti Roti JL. 1997b. Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation. *Radiat. Res.* 148(6):608-617.

Male JC. 1992. Biological effects of magnetic fields: a possible mechanism? *Biologist* 39(3): 87-89.

Mann S, Sparks NHC, Walker MM, & Kirschvink JL. 1988. Ultrastructure, morphology and organization of biogenic magnetite from sockeye salmon, Oncorhynchus nerka: Implications for magnetoreception. *J. Exp. Biol.* 140:35-49

Manni V, Lisi A, Rieti S, Serafino A, Ledda M, Giuliani L, Sacco D, D'Emilia E, & Grimaldi S. 2004. Low electromagnetic field (50 Hz) induces differentiation on primary human oral keratinocytes (HOK). *Bioelectromagnetics* 25(2):118-126.

Margolin BH, & Shelby MD. 1985. Sister chromatid exchanges: A reexamination of the evidence for sex and race differences in humans. *Environ. Mutagen* 7 Suppl. 4:63-72.

Maria HL, Heather SR, Helen RZ, James WA, & John CH. 1989. Evidence for Chromosomal replicons as units of sister chromatid exchanges. *Chromosoma* (Berl) 98:69-76.

Martin H, & Lindauer M. 1977. Der Einfluss der Ermagnetfeld und Schwereorientierung der Honigbiene. *J. Comp. Physiol.* 122:145-187.

Martin H, Korall H, & Forster B. 1989. Magnetic field effects on activity and ageing in honeybees. *Bioelectromagnetics* 9:393-396.

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* 24:82-90.

Mass MJ. 1992. Human *Carcinogenesis* by arsenic. *Environmental Geochemistry & Health* 14 (2):49-54.

Matanoski GM, Breysse PN, & Elliott EA. 1991. Electromagnetic field exposure and male breast cancer. *Lancet* 337:737.

Mattei MD, Caruso A, Traina GC, Pezzetti F, Baroni T, & Sollazzo V. 1999. Correlation between pulsed electromagnetic fields exposure time and cell proliferation increase in human osteosarcoma cell lines and human normal osteoblast cells in vitro. *Bioelectromagnetics* 20:177-182.

Maurya AK, Rout U, & Roy D. 1987. Seizure induced changes in sister chromatid exchanges frequency in epioleptic patients. *Cellular & Molecular Biology* (Oxford) 33(2):237-246.

Mayer PJ. Lange CS, Bradley MO, & Nichols WW. 1989. Age-dependant decline in rejoining of X-ray-induced DNA double strand breaks in normal human lymphocytes. *Mutation Res.* 219:95-100.

Mazrimas JA, & Stetka DG. 1978. Direct evidence for the role of the incorporated BrdU in the induction of sister chromatid exchanges. *Exp. Cell Res.* 117:23-30.

McCann J, Dietrich F, Rafferty C, & Martin AO. 1993. A critical review of the genotoxic potential of electric and magnetic fields. *Mutation Res.* 297:61-95.

McCurdy D, Tai L, Frias S, & Wang Z. 1997. Delayed repair of DNA damage by ionizing radiation in cells from patients with juvenile systemic lupus erythematosus and rheumatoid arthritis. *Radiat. Res.* 147:48-54.

Mertens R, Rubbert F. & Buessing A. 1995. Childhood acute lymphoblastic leukemia (ALL): Sister chromatid exchange (SCE) frequency and lymphocyte subpopulations during therapy. *Leukemia* (Basingstoke) 9(3):501-505.

Melvin T, Cunniffe SM, O'Neill P, Parker AW, & Roldan-Arjona T. 1998. Guanine is the target for direct ionisation damage in DNA, as detected using excision enzymes. *Nucleic Acids Research* 26(21):4935-4942.

Meng Z, & Zhang B. 1997. Chromosomal aberrations and micronuclei in lymphocytes of workers at a phosphate fertilizer factory. *Mutation Res.* 393(3):283-288.

Merk O. & Speit G. 1999. Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. *Environ. Mol. Mutagen.* 33:167-172.

Mevissen M, Kietzmann M, & Loscher W. 1995. *In vivo* exposure of rats to a weak alternating magnetic field increases ornithine decarboxylase activity in the mammary gland by a similar extent as the carcinogen DMBA. *Cancer Lett.* 90(2):207-214.

Mevissen M, Stamm A, Buntenkotter S, Zwingelberg R, Wahnschaffe U, & Loscher W. 1993. Effects of magnetic fields on mammary tumor development induced by 7,12-dimethylbenz(a)anthracene in rats. *Bioelectromagnetics* 14(2):131-143.

Milham S. 1982. Mortality from Leukemia in Workers Exposed to Electric and Magnetic Fields. *New England J. Medicine* 307:249-250.

Miller B, Albertini S, Locher F, Thybaud V, & Lorge E. 1997. Comparative evaluation of the *in vitro* micronucleus test and the in vitro chromosome aberration test: industrial experience. *Mutation Res.* 392:45-59.

Milosevic DO, & Marinkovic D. 1999. Frequencies of sister-chromatid exchange (SCE) and proliferation indexes (PRI) in lymphocytes of individuals of different age and sex in a Yugoslavian population. *Bulletin Academie Serbe des Sciences et des Arts Classe des Sciences Mathematiques et Naturelles Sciences Naturelles* 38:37-45.

Miranda M, Ligas C, Amicarelli F, D'Alessandro E, Brisdelli F, Zarivi O, & Poma A. 1997. Sister chromatid exchange (SCE) rates in human melanoma cells as an index of mutagenesis. *Mutagenesis* 12(4):233-236.

Monti MG, Pernecco L, Moruzzi MS, Battini R, Zaniol P, & Barbiroli B. 1991. Effect of ELF pulse electromagnetic fields on protein kinase C activation process in HL-60 leukemia cells. *Journal of Bioelectricity* 10: 119-130.

Moran E, & Wallace SS. 1985. Photobiological activities of 1,6-dioxapyrene in pro- and eukaryotic cells. *Mutation Res.* 146:229-241.

Morgan WF, & Crossen PE. 1977. The incidence of sister chromatid exchanges in cultured human lymphocytes. *Mutation Res.* 42:305-312.

Mourelatos DC. 1979. Enhancement by caffeine of sister chromatid exchange frequency induced by antineoplastic agents in human lymphocytes. *Experientia* 35:822-824.

Murnane JP, Byfield JE, Ward JF, & Calabro-jones P. 1980. Effects of methylated xanthines on mamalian cells treated with bifunctional alkylating agents. *Nature* 285:326-329.

Murphy JC, Kaden DA, Warren J, & Sivak A. 1993. Power frequency electric and magnetic fields: a review of genetic toxicology. *Mutation Res.* 296:221-240.

Myung-Hee K, Dieter G, & Ibrahim E. 1997. Increased sister chromatid exchanges in peripheral lymphocytes of patients with Crohn's disease. *Mutation Res.* 381:141-148.

Nagy P & Fischl G. 2004. Effect of static magnetic field on growth and sporulation of some plant pathogenic fungi. *Bioelectromagnetics* 25:316-318.

Nair I, Morgan MG, & Florig HK. 1989. Biological effects of power frequency electric and magnetic fields. Rep. No. OTA-BP-E-53, Office of Technology Assessment, U.S. Government Printing office, Washington, DC.

Nakano M, Kodama Y, Ohtaki K, Itoh M, Delongchamp R, Awa AA, & Nakamura N. 2001. Detection of stable chromosome aberrations by FISH in A-bomb survivors: comparison with previous solid Giemsa staining data on the same 230 individuals. *Int. J. Radiat. Biol.* 77(9):971-977.

Natarajan AT, Vyas RC, Darroudi F, & Vermeulen S. 1992. Frequencies of x-ray-induced chromosome translocations in human peripheral lymphocytes as detected by *in-situ* hybridization using chromosome-specific DNA libraries. *Int. J. Radiat. Biol.* 61(2):199-204.

Nesti C, Trippi F, Scarpato R, Migliore L & Turchi G. 2000. Cytokinesis-block micronucleus assay in primary human liver fibroblasts exposed to griseofulvin and mitomycin C. *Mutagenesis* 15(2):143-147.

Newton RK, Ducore JM, & Sohal RS. 1989. Effect of age on endogenous DNA single-strand breakage, strand break induction and repair in the adult housefly, Musca domestica. *Mutation Res.* 219(2):113-120.

Nichols WW, Bradt CI, Toji LH, Godley M, & Sigawa M. 1978. Induction of SCE by transformation with simian virus 40 (SV-40). *Cancer Res.* 38:906-910.

NIOSH Facts: EMFs in the workplace. 1995. A fact sheet from the National Institute for Occupational Safety and Health, U.S.A. Free from the NIOSH hotline (0800) 356-4674.

Nordenson I, Mild KH, Andersson G, & Sandstrom M. 1994. Chromosomal aberrations in human amniotic cells after intermittent exposure to (50 Hz) magnetic fields. *Bioelectromagnetics* 15:293-301.

Nordenson I, Mild KH, Ostman U, & Ljungberg H. 1988. Chromosome effects in lymphocytes of 400 kV-substation workers. *Radiat. Environ Biophys* 27(1):39-47.

Nordenson I, Hansson KM, Nordstrom S, Sweins A, & Birke E. 1984. Clastogenic effects in human lymphocytes of power frequency electric fields: *in vivo* and *in vitro* studies. *Radiat. Environ. Biophys.* 23:191-201.

Norppa H, Renzi L, & Lindholm C. 1993. Detection of whole chromosomes in micronuclei of cytokinesis-blocked human lymphocytes by antikinetochore staining and *in situ* hybridization. *Mutagenesis* 8:519-525.

NRC, USA. 1996. Possible health effects of exposure to residential electric and magnetic fields. Nat. Res. Council, Washington: Nat. Acad. Press.

NRPB (National Radiological Protection Board), UK. 1992. Electromagnetic fields and the risk of cancer: Report of an advisory group on non-ionizing radiation. (Vol. 3, No. 1). Chilton, England.

Olcese J, Reuss S, & Vollrath L. 1985. Evidence for the involvement of the visual system in mediating magnetic field effects on pineal melatonin synthesis in the rat. *Brain Res.* 333(2):382-384.

Olive PL, Banath JP, & Durand RE. 1990. Detection of etoposide resistance by measuring DNA damage in individual chinese hamster cells. *J. Natl. Cancer Inst.* 82:779-783.

Oliveira NG, Castro M, Rodrigues AS, Goncalves IC, Cassapo R, Fernandes AP, Chaveca T, Toscano-Rico JM, & Rueff J. 2001. Evaluation of the genotoxic effects of the boron neutron capture reaction in human melanoma cells using the cytokinesis block micronucleus assay. *Mutagenesis* 16(5):369-375.

Omura Y, Losco BM, & Takeshige C. 1993. Non-invasive evaluation of the effects of opening & closing of eyes, and of exposure to a minute light beam, as well as to electrical or magnetic field on the melatonin, serotonin, & other neuro-transmitters of human pineal gland representation areas & the heart. *Acupunct. Electrother. Res.* 18(2):125-151.

Östling O, & Johanson KJ. 1984. Microelectrophoretic study of radiation induced DNA damages in individual mammalian cells, Biochem. *Biophys. Res. Commun.* 123:291-298.

Östling O, & Johanson KJ. Blomquist E, & Hagelqvist E. 1987. DNA damage in clinical radiation therapy studied by microelectrophoresis in single tumor cells. *Acta Oncologica* 26:45-48.

Ozturk S, Vatansever S, Cefle K, Palanduz S, Guler K, Erten N, Erk O, Karan MA, & Tascioglu C. 2002. Acute wood or coal exposure with carbon monoxide intoxication induces sister chromatid exchange. *Journal of Toxicology-Clinical Toxicology* 40(2):115-20.

Paile W, Jokela K, Koivistoinen A, & Salomaa S. 1995. Effects of 50 Hz sinusoidal magnetic fields and spark discharges on human lymphocytes *in vitro. Bioelectrochem. Bioenergetics* 36:15-22.

Painter RB. 1980. Replication model for sister chromatid exchange. *Mutation Res.* 70:337-341.

Palmer RG, Dore CJ, Henderson L, & Denman AM. 1987. Sister-chromatid exchange frequencies in fibroblasts and lymphocytes of patients with systemic lupus erythematosus. *Mutation Res.* 177:125-132.

Paramananda D, George J, & Gupta ID. 1996. Demonstration of sister chromatid differentiation and sister chromatid exchanges in Etroplus suratensis (Bloch). *Indian Journal of Experimental Biology* 34(12):1229-1232.

Pardue ML, & Gall JG. 1969. Molecular hybridization of radioactive DNA to the DNA of cytological preparations. *Proc. Natl. Acad. Sci. USA* 64:600-604.

Parkes DJ, Scott D, & Stewart A. 1985. Changes in spontaneous SCE frequencies as a function of sampling time in lymphocytes from normal donors and cancer patients. *Mutation Res.* 147(3):113-22.

Parkinson WC, & Hanks CT. 1989. Search for cyclotron resonance in cells *in vitro. Bioelectromagnetics* 10:129-145.

Pastor S, Gutierrez S, Creus A, Xamena N, Piperakis S, & Marcos R. 2001. Cytogenetic analysis of Greek farmers using the micronucleus assay in peripheral lymphocytes and buccal cells. *Mutagenesis* 16(6):539-545.

Patterson MC, Smith BP, Lohman PHM, Anderson AK, & Fishman L. 1976. Defective excision repair of gamma-ray-damaged DNA in human (Ataxia telangiectasia) fibroblasts. *Nature* 260:444-447.

Perera FP, Motzer RJ, Tang D, Reed E, Parker R, Warburton D, Oneill P, Albertini R, & Bigbee WL. 1992. Multiple biological markers in germ cell tumor patients treated with platinum based chemotherapy. *Cancer Res.* 52 (13):3558-3565.

Perry P, & Evans HJ. 1975. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature* (London) 258:121-124.

Perry P, & Wolff S. 1974. New giemsa method for the differential staining of sister chromatids. *Nature* (London). 251:156-158.

Pesch G, & Pesch C. 1979. Proc. Environ. Mutagen Sco, New Orleans, L. A. March 8-12, p. 102.

Pfluger DH, & Minder CE. 1996. Effects of exposure to 16.7 Hz magnetic fields on urinary 6-hydroxymelatonin sulfate excretion of Swiss railway workers. *J. Pineal Res.* 21:91-100.

Pfuhler S, & Wolf HU. 1996. Detection of DNA-crosslinking agents with the alkaline COMET assay. *Environ. Mol. Mutagen.* 27:196-201.

Phillips JB. 1977. Use of the Earth's magnetic field by orientating cave salamanders (Eurycea lucifuga). *J. Comp. Physiol.* 121:273-288.

Phillips JB. 1986. Magnetic compass orientation in the eastern red-spotted newt (*Notophthalmus viridescens*). *J. Comp. Physiol.* 158:103-109.

Phillips JB, & Sayeed O. 1993. Wavelength-dependent effects of light on magnetic compass orientation in Drosophila melanogaster. *J. Comp. Physiol.* 172(3):303-308.

Phillips JL, Campell-Beachler M, Ishida-Jones T, Haggren W, & Adey WR. 1995. Exposure of PC12 cells to a 1 G sinusoidal magnetic field at 60 Hz: Effect on the activity of the DNA repair enzyme poly(ADP-ribose) polymerase, Paper presented at the Annual Review of Research on Biological Effects of Electric and Magnetic Fields from the Generation, Delivery and Use of Electricity, Palm Springs, CA, November 12-16.

Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas JN, & Gray J. 1988. Fluorescence *in situ* hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocation of chromosome 4. *Proc. Natl. Acad. Sci. USA* 85:9138-9142.

Pommier Y, Zwelling LA, Kao-Shan CS, Whang-Peng J, & Bradley MO. 1985. Correlations between intercalator-induced DNA strand breaks and sister chromatid exchanges, mutations, and cytotoxicity in Chinese hamster cells. *Cancer Res.* 45:3143-2149.

Popescu NC, & Dipaolo JA. 1982. The relevance of SCE to the induction of neoplastic cell transformation, *In* A. A. Sandberg (ed.), Sister Chromatid Exchange. Liss, New York, pp. 425-460.

Popescu NC, Turnbull D, & Dipaolo JA. 1977. Sister chromatid exchange and chromosome aberration analysis with the use of several carcinogens and non-carcinogens. *J. Natl. Cancer Inst.* 59:289-293.

Povirk LF, & Painter RB. 1976. The effect of 313 nanometer light on initiation of replicons in mammalian cell DNA containing bromodioxyuridine. *Biochim. Biophys. Acta* 432:267-272.

Power line-Health link acknowledged (November 25, 1994). The Evening Standard, Manawatu, PN, p.3.

Price CM, Hagger D, Evans M, Kanfer EJ, Shephard J, Topham C, Cassell PG, & Haworth C. 1992. Sister chromatid exchange (SCE) frequency in lymphocytes of patients with colorectal carcinoma treated with razoxane. *Cancer Detection & Prevention.* 16(4):221-223.

Quinn TP, & Brannon EL. 1982. The use of celestial and magnetic cues by orienting Sockeye salmon smolts. *J. Comp. Physiol.* 147:547-552.

Raj AS, Heddle JA, Newmark HL, & Katz M. 1983. Caffeic acid as an inhibitor of DMBA-induced *Chromosoma*l breakage in mice assessed by bone-marrow micronucleus test. *Mutation Res.* 124(3-4):247-253.

Ramirez A, & Saldanha PH. 2002. Micronucleus investigation of alcoholic patients with oral carcinomas. *Genet. Mol. Res.* 1 (3):246-260.

Rapley BI, Rowland RE, Page WH, & Podd JV. 1998. Influence of extremely low frequency magnetic fields on chromosomes and the mitotic cycle in Vicia faba L., the broad bean. *Bioelectromagnetics* 19:152-161.

Reiter RJ. 1994. Melatonin suppression by static and extremely low frequency electromagnetic fields: relationship to the reported increased incidence of cancer. *Rev. Environ. Health* 10(3-4):171-186.

Reiter RJ. 2000. Summary of international symposium entitled 'low frequency EMF, visible light, melatonin and cancer'. Cologne International Symposium, May 4-5.

Reiter RJ, Poeggeler B, & Tan DX. 1993. Antioxidant capacity of melatonin: a novel action not requiring a receptor. *Neuroendocrinol Letter* 15:103-116.

Reiter RJ, Tan DX, Poeggeler B, Menendez-Pelaez A, Chen LD, & Saarela S. 1994. Melatonin as a free radical scavenger: implications for aging and age-related diseases. *Ann. New York Acad. Sci.* 719:1-12.

Reiter RJ, Tan DX, Manchester LC, & Qi W. 2001. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence. *Cell Biochem. & Biophys.* 34(2):237-256.

Reynolds RJ, Natarajan AT, & Lohman PHM. 1979. Micrococcus luteus UV-endonuclease sensitive sites and sister chromatid exchanges in Chinese hamster ovary cells. *Mutation Res.* 64:353-356.

Rieger R, Michaelis A, & Green M. 1968. A Glossary of Genetics and Cytogenetics. 3rd. ED., Allen and Unwin, London. 507 pp.

Robison JG, Pendleton AR, Monson KO, Murray BK, & O'Neill KL. 2002. Decreased DNA repair rates and protection from heat induced apoptosis mediated by electromagnetic field exposure. Bioelectromagnetics 23:106-112.

Rogers WR, Reiter RJ, & Orr JL. 1997. Effects of exposure to 60-Hz EMF on melatonin in nonhuman primates might depend on specific aspects of field exposure. *In* The Melatonin Hypothesis. (Stevens, R. G. Wilson, B.W., Anderson, L. E., eds). Battelle Press, Colombus Ohio. pp 429-478.

Rojas E, Lopez MC, & Valverde M. 1999. Single cell gel electrophoresis assay: methodology and applications. *J. Chromatography B.* 722:225-254.

Rose RC, & Bode AM. 1993. Biology of free radical scavengers: an evaluation of ascorbate. *FASEB J.* 7:1135-1142.

Rosen LA, Barber I, & Lyle DB. 1998. A 0.5 G, 60 Hz magnetic field suppresses melatonin production in pinealocytes. *Bioelectromagnetics* 19:123-127.

Rosenthal M, & Obe G. 1989. Effects of 50 Hz electromagnetic fields on proliferation and Chromosomal alterations in human peripheral lymphocytes untreated or pretreated with chemical mutagens. *Mutation Res.* 210:329-335.

Rosin MP, & German J. 1985. Evidence for chromosome instability *in vivo* in Bloom syndrome: increased numbers of micronuclei in exfoliated cells. *Hum. Genet.* 71:187-191.

Rowland RE, & Harding KM. 1999. Increased sister chromatid exchange in the peripheral blood lymphocytes of young women who smoke cigarettes. *Hereditas* 131(2):143-146.

Rozgaj R, Kaubal V, & Jazbec AA. 2001. Preliminary study of cytogenetic damage in personnel exposed to anesthetic gases. *Mutagenesis* 2:139-143.

Rudd NL, Hoar DI, Greentree CL, Dimnik LS, & Hennig UG. 1988. Micronucleus assay in human fibroblasts: a measure of spontaneous Chromosomal instability and mutagen hypersensitivity. *Environ. Mol. Mutagen.* 12:3-13.

Russell LB, & Russell WE. 1954. Cold Spring Harbor Symp. *Quant. Biol.* 19:50-59.

Rydberg B, & Johanson KJ. 1978. Estimation of DNA strand breaks in single mammalian cells, *In* P.C. Hanawalt, E.C. Friedberg and C.F. Fox (Eds.), DNA repair mechanisms, Academic Press, New York, pp. 465-468.

Sagan LA. 1992. Epidemiological and laboratory studies of power frequency electric and magnetic fields. *J. Am. Med. Assoc.* (JAMA) 268:625-629.

Sahin FI, Sahin I, Ergun MA, & Saracoglu MA. 2000. Effects of estrogen and alendronate on sister chromatid exchange (SCE) frequencies in postmenopausal osteoporosis patients. *Int. J. Gynaecol. & Obstet.* 71(1):49-52.

Salagovic J, Gilles J, Verschaeve L, & Kalini I. 1996. The COMET assay for the detection of genotoxic damage in the earthworm: a promising tool for assessing the biological hazards of pollution sites. *Folia Biologica (Praha)* 42:17-21.

Sandberg AA. 1982. Sister chromatid exchange in human states. *In* Sandberg A. A. (Ed.) Sister chromatid exchange. Liss, New York, pp 619-651.

Sandberg AA, & AnneMarie WB. 1987. Sister chromatid exchange. Chromosomes and chromatin, Volume II: 123-146.

Sandberg AA, Becher R, & Gibas Z. 1984. Value and significance of SCE in human leukemia and cancer. *Basic Life Sciences* 29 Pt. B:825-838.

Sarkar S, & Ali SJ. 1994. Effect of low power microwave on the mouse genome: a direct DNA analysis. *Mutation Res.* 320:141-147.

Savitz DA, & Ahlbom A. 1994. Epidemiologic evidence on cancer in relation to residential and occupational exposures. *In* Carpenter DO, & Ayrapclyan S. (Eds), Biological effects of electric and magnetic fields. Acad. Press. San Diego. pp 233-261.

Savitz DA, Wachtel H, Barnes F, John EM, & Tvrdik JG. 1988. Case-control study of childhood cancer and exposure to 60 Hz magnetic fields. *Am. J. epidemiol.* 128:21-38.

Scaiano JC, Cozens FL, & McClean J. 1994a. Model for the rationalization of magnetic field effects *in vivo.* Application of the radical-pair mehanism to biological systems. *Photochem. Photobiol.* 59:585-598.

Scaiano JC, Mohtat N, Cozens FL, McClean J, & Thansandte A. 1994b. Application of the radical-pair mehanism to free radicals in organized systems: Can the effects of 60 Hz be predicted from studies under static fields? *Bioelectromagnetics* 15:549-554.

Scarfì MR, Cossarizza A, Monti D, Bersani F, Zannotti M, Lioi MB, & Franceschi C. 1990. Age-related increase of mitomycin C-induced micronuclei in lymphocytes from Down's syndrome subjects. *Mutation Res.* 237(5-6):247-252.

Scarfì MR, Bersani F, Cossarizza A, Monti D, Castelani G, Cadossi R, Franceschetti G, & Franceschi C. 1991. Spontaneous and mitomycin-C-induced micronuclei in human lymphocytes exposed to extremely low frequency pulsed magnetic fields. *Biochem. Biophys. Res. Commun.* 176:194-200.

Scarfì MR, Lioi MB, Di Berardino D, Zeni O, Coviello AM, & Matassino D. 1993. Measurement of micronuclei by cytokinesis-block method in bovine lymphocytes. *Mutation Res.* 289(2):291-295.

Scarfì MR, Bersani F, Cossarizza A, Monti D, Zeni O, Lioi MB, Franceschetti G, & Franceschi C. 1993. 50 Hz AC sinusoidal electric fields do not exert genotoxic effects (micronucleus formation) in human lymphocytes. *Radiat. Res.* 135:64-68.

Scarfi MR, Lioi MB, Zeni O, Franceschetti G, Franceschi C, & Bersani F. 1994. Lack of Chromosomal aberration and micronucleus induction in human lymphocytes exposed to pulsed magnetic fields. *Mutation Res.* 306(2):129-133.

Scarfi MR, Prisco F, Bersani F, Lioi MB, Zeni O, Di Pietro R, Franceschi C, Motta M, Iafusco D, & Stoppoloni G. 1996. Spontaneous and mitomycin-C-induced micronuclei in lymphocytes from subjects affected by Turner's syndrome. *Mutation Res.* 357(1-2):183-190.

Scarfi MR, Prisco F, Lioi MB, Zeni O, Della MN, Di Pietro R, Franceschi C, Iafusco D, Motta M, & Bersani F. 1997. Cytogenetic effects induced by extremely low frequency pulsed magnetic fields in lymphocytes from Turner's syndrome subjects. *Bioelectrochem. & Bioenerg.* 43(2):221-226.

Scarfi MR, Prisco F, Lioi MB, Zeni O, Della Noce M, Di Pietro R, Franceschi C, Iafusco D, Motta M, & Bersani F. 1997a. Cytogenetic effects induced by extremely low frequency pulsed magnetic fields in lymphocytes from Turner's syndrome subjects. *Bioelectrochem. & Bioenerg.* 43(2):221-226.

Scarfi MR, Lioi MB, Zeni O. Della Noce M, Franceschi C, & Bersani F. 1999. Micronucleus frequency and cell proliferation in human lymphocytes exposed to 50 Hz sinusoidal magnetic fields. *Health Physics* 76(3):244-250.

Schatzkin A, Freedman LS, Shiffman MH, & Dawsey SM. 1990. Validation of intermediate end points in cancer research. *J. Natl. Cancer Inst.* 82:1746-1752.

Schimmelpfeng J, & Dertinger H. 1997. Action of a 50 Hz magnetic field on proliferation of cells in culture. *Bioelectromagnetics* 18:177–183.

Schubert I, & Rieger R. 1981. Sister chromatid exchanges and heterochromatin. *Hum. Genet.* 57:119-130.

Schubert I, Kuenzel G, Bretschneider H, Rieger R, & Nicoloff H. 1980. Sister chromatid exchanges in barley. *Theoretical & Applied Genetics* 56(1-2):1-4.

Schulten K. 1982. Magnetic field effects in chemistry and biology. Adv. *Solid State Phys.* 22:61-83.

Schulten K, & Windemeuth A. 1986. *In* biophysical effects of steady magnetic fields; Maret G, Boccara N, Kiepenheuer J. Eds.: Springler-Verlag: New York, 1986, pp 99-106.

Schvartzman JB, & Gutierrez C. 1980. The relationship between the cell time available for repair and the effectiveness of a damaging treatment in provoking the formation of sister chromatid exchanges. *Mutation Res.*72:483-490.

Schvartzman JB, Cortes F, Gonzalez-Fernandez A, Gutierrez C, & Lopez-Saez JF. 1979. On the nature of sister chromatid exchanges in 5-bromodeoxyuridine substituted chromosomes. *Genetics* 92:1251-1253.

Scott D. 2000. Chromosomal radiosensitivity, cancer predisposition and response to radiotherapy. *Strahlentherapie und Onkologie* 176(5):229-234.

Scott D, Hu Q, & Roberts SA. 1996. Dose-rate sparing for micronucleus induction in lymphocytes of controls and ataxia-telangiectasia heterozygotes exposed to 60Co gamma-irradiation *in vitro*. *Int. J. Radiat. Biol.* 70(5):521-527.

Scott D, Spreadborough A, Levine E, & Roberts SA. 1994. Genetic predisposition in breast cancer. *The Lancet* 344:1444.

Scott D, Barber JBP, Levine EL, Burrill W, & Roberts SA. 1998. Radiation-induced micronucleus induction in lymphocytes identifies a high frequency of radiosensitive cases among breast cancer patients: a test for predisposition. *British J. Cancer* 77(4): 614-620.

Selmaoui B, & Touitou Y. 1995. Sinusoidal 50-Hz magnetic fields depress rat pineal NAT activity and serum melatonin. Role of duration and intensity of exposure. *Life Sci.* 57: 1351-1358.

Semm P, Schneider T, & Vollrath L. 1980. Effects of an earth-strength magnetic field on electrical activity of pineal cells. *Nature* 288:607-608.

Senecal-Quevillon M, Pierre D, & Claude-Lise R. 1986. Analysis of sister-chromatid exchanges (SCEs) in familial and sporadic multiple sclerosis. *Mutation Res.* 161:65-74.

Setlow RB, & Setlow JK. 1972. Effects of radiation on polynucleotides. *Ann. Rev. Biophys. Bioeng.* 1:293-346.

Shafer DA. 1977. Replication bypass model of sister chromatid exchanges and implications for Bloom's syndrome and Fanconi's anemia. *Hum. Genet.* 39:177-190.

Shafer DA. 1982. Alternate replication bypass mechanisms for sister chromatid exchange formation. *Prog. Topics Cytogenet.* 2:67-98.

Shafer DA, Hollingsworth F, Tadayon F, & Falek A. 1976. Giemsa replication (GR) banding of metaphase chromosomes. *Mam. Chrom. Newsltr.* 17:62.

Shaffer M. 1993. Melatonin could help elderly sleep better. *Medical Tribune* 15.

Shah VC, & Mittal SC. 1987. Studies on sister chromatid exchange (SCE) induction by Gamma-irradiation and protective effect of L-cysteine in HELA cells. *Strahlentherapie und Onkologie* 163(1):54-60.

Shaham J, Kaufman Z, Gurvich R, & Levi Z. 2001. Frequency of sister-chromatid exchange among greenhouse farmers exposed to pesticides. *Mutation Res.* 491(1-2):71-80.

Shaham J, Gurvich R, & Kaufman Z. 2002. Sister chromatid exchange in pathology staff occupationally exposed to formaldehyde. *Mutation Res.* 514(1-2):115-123.

Sharma T, & Das BC. 1986. Higher incidence of spontaneous sister-chromatid exchanges (SCEs) and X-ray induced chromosome aberrations in peripheral blood lymphocytes during pregnancy. *Mutation Res.* 174:27-33.

Shimizu N, Itoh N, Utiyama H, & Wahl GM. 1998. Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during S phase. *J. Cell Biol.* 140:1307-1320.

Shin-ichi H, Sotoshi Y, Osamu K, Yoshirou G, Masatoshi K, Tomokazu M, Hideto Y, Shigeru S, Kazuo B, Ryuji H, & Hiroshi Y. 2001. Effects of high ELF magnetic fields on enzyme-catalyzed DNA and RNA synthesis *in vitro* and on a cell-free DNA mismatch repair. *Bioelectromagnetics* 22(4):260-266.

Shiraishi Y, Freeman AI, & Sandberg AA. 1976. Increased sister chromatid exchange in bone marrow and blood from Bloom's Syndrome. *Cytogenet. Cell Genet.* 17:163-173.

Shubber EK. 1987. Sister-chromatid exchanges in lymphocytes from patients with Schistosoma hematobium. *Mutation Res.* 180:91-99.

Shubber EK, Hamami HA, Allak BMA, & Khaleel AH. 1991. Sister-chromatid exchanges in lymphocytes from infants with Down's syndrome. *Mutation Res.* 248:61-72.

Shuvalova LA, Ostrovskaya MV, Sosunov VA, & Lednev VV. 1991. Influence of a weak magnetic field under conditions of paramagnetic resonance on the rate of calmodulin-dependent phosphorylation of myosin in solution. *Proc. Natl. Acad. Sci. USSR* 317:227.

Silva MJ, Carothers A, Castelo-Branco NA, Dias A, & Boavida MG. 1999. Sister chromatid exchange analysis in workers exposed to noise and vibration. *Aviation Space & Environmental Medicine* 70(3 Pt 2):A40-45.

Silva MJ, Dias A, Barreta A, Nogueira PJ, Castelo-Branco NA, & Boavida MG. 2002. Low frequency noise and whole-body vibration cause increased levels of sister chromatid exchange in splenocytes of exposed mice. *Teratogenesis, Carcinogenesis, & Mutagenesis* 22(3):195-203.

Simko M, Kriehuber R, Weiss DG, & Luben RA. 1998. Effects of 50 Hz EMF exposure on micronucleus formation and apoptosis in transformed and nontransformed human cell lines. *Bioelectromagnetics* 19(2):85-91.

Simko M, Richard D, Kriehuber R, & Weiss DG. 2001. Micronucleus induction in Syrian hamster embryo cells following exposure to 50 Hz magnetic fields, benzo(a)pyrene, and TPA *in vitro*. *Mutation Res.* 495(1-2):43-50.

Simko M, & Mattsson MO. 2004. Extremely low frequency electromagnetic fields as effectors of cellular responses in vitro: Possible immune cell activation. *J Cell Biochem.* 93(1):83-92.

Simon NJ. 1992. Biological effects of static magnetic fields: a review. International Cryogenic Materials Commission, Inc., Boulder, Colorado. p 284.

Singh NP. 2000. Microgels for estimation of DNA strand breaks, DNA protein crosslinks and apoptosis. *Mutation Res.* 455:111-127.

Singh N, & Lai H. 1998. 60 Hz magnetic field exposure induces DNA crosslinks in rat brain cells. *Mutation Res.* 400(1-2):313-320.

Singh NP, & Stephens RE. 1997. Microgel electrophoresis: sensitivity, mechanisms, and DNA electrostretching. *Mutation Res.* 383(2):167-175.

Singh NP, & Stephens RE. 1998. X-ray induced DNA double-strand breaks in human sperm. *Mutagenesis* 13(1):75-79.

Singh NP, Stephens RE, & Schneider EL. 1994. Modifications of alkaline microgel eletrophoresis for sensitive detection of DNA damage. *Int. J. Radiat. Biol.* 66:23-28.

Singh NP, McCoy MT, Tice RR, & Schnider EL. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.* 175:184-191.

Singh NP, Tice RR, Stephens RE & Schnider EL. 1991. A microgel electrophoresis technique for the direct quantitation of DNA damage and repair in individual fibroblasts cultured on microscope slides, *Mutation Res.* 252:289-296.

Singh NP, Danner DB, Tice RR, Brant L, & Schneider EL. 1990. DNA damage and repair with age in individual human lymphocytes, *Mutation Res.* 237:123-130.

Singh NP, Danner DB, Tice RR, McCoy MT, Collins GD, & Schneider EL. 1989. Abundant alkali labile sites in DNA of human and mouse sperm. *Exp. Cell Res.* 184:461-470.

Smith SD, Mcleod BR, Liboff AR, & Cooksey K. 1987. Calcium cyclotron and diatom mobility. *Bioelectromagnetics* 8:215-227.

Snope AJ, & Rary JM. 1979. Cell-cycle duration and sister chromatid exchange frequency in cultured human lymphocytes. *Mutation Res.* 63(2):345-349.

Solomon E, & Bobrow M. 1975. Sister chromatid exchanges. A sensitive assay of agents damaging human chromosomes. *Mutation Res.* 30:273-278.

Soper KA, Stolley PD, Galloway SM, Smith JG, Nichols WW, & Wolman SR. 1984. Sister chromatid exchange (SCE) report on control subjects in a study of occupationally exposed workers. *Mutation Res.* 129:77-88.

Sorensen M, Sehested M, Christensen IJ, Larsen JK, & Jensen PB. 1998. Low-level resistance to camptothecin in a human small-cell lung cancer cell line without reduction in DNA topoisomerase I or drug-induced cleavable complex formation. *British J. Cancer* 77(12):2152-2161.

Sorsa M, Wilbourn J, & Vainio H. 1992. Human cytogenetic damage as a predictor of cancer risk *In* Vainio H, Magee PN, McGregor DB, & McMichael AJ. (Eds.), Mechanisms of *Carcinogenesis* in Risk Identification. IARC Scientific Publications No. 116, pp.543-554.

Sou S, Takabayashi T, Ozawa N, Sho K, Sasamoto K, Yajima A, & Suzuki M. 1986. Spontaneous and MMC induced SCE in lymphocytes from patients with cervical cancer. *Tohoku J. Exp. Med.* 149(4):417-423.

Speit G, Trenz K, Schutz P, Rothfuss A, & Merk O. 1999. The influence of temperature during alkaline treatment and electrophoresis on results obtained with the COMET assay. *Toxicology Letters* 110: 73-78.

Speit G, & Hartmann A. 1999. The COMET assay (single-cell gel test): a sensitive genotoxicity test for the detection of DNA damage and repair protocols 113:203-212.

Stange BC, Rowland RE, Rapley BI, & Podd JV. 2002. ELF magnetic fields increase amino acid uptake into Vicia faba L. roots and alter ion movement across the plasma membrane. *Bioelectromagnetics* 23(5):347-354.

Stea S, Visentin M, Granchi D, Savarino L, Dallari D, Gualtieri G, Rollo G, Toni A, Pizzoferrato A, & Montanaro L. 2000. Sister chromatid exchange in patients with joint prostheses. *J. Arthroplasty* 15(6):772-777.

Stephan G, & Pressl S. 1997. Chromosome aberrations in human lymphocytes analysed by fluorescence *in situ* hybridization after *in vitro* irradiation, and in radiation workers, 11 years after an accidental radiation exposure. *Int. J. Radiat. Biol.* 71(3):293-299.

Stevens RG. 1987. Electric power use and breast cancer: An hypothesis. *Am. J. Epidemiology* 125: 556-561.

Stevens RG, Davis S, Thomas DB, Anderson LE, & Wilson BW. 1992. Electric Power, Pineal Function and the Risk of Breast Cancer: A Hypothesis. *FASEB J.* 6:853-860.

Stetka DG. 1979. Further analysis of the replication bypass model for sister chromatid exchange. *Human genet.* 49:63-69.

Stetka DG, & Jr. Spahn MC. 1984. Sister chromatid exchanges are induced by replication of 5-bromodeoxyuridine-substituted DNA templates, but not by incorporation of 5-bromodeoxyuridine into nascent DNA. *Mutation Res.* 140(1):33-42.

Straume T, & Lucas JN. 1993. A comparison of the yields of translocations and dicentrics measured using fluorescence *in situ* hybridization. *Int. J. Radiat. Biol.* 64: 185-187.

Surralles J, & Natarajan AT. 1997. Human lymphocytes micronucleus assay in Europe: an international survey. *Mutation Res.* 392:165-174.

Sutherland GR, Baker E, Seshadri RS & Black A. 1980. Increased sister chromatid exchange in multiple sclerosis. *New Engl. J. Med.* 303(19):1126.

Svedenstal BM, Johanson KJ, Mattsson MO, & Paulsson LE. 1999a. DNA damage, cell kinetics and ODC activities studied in CBA mice exposed to electromagnetic fields generated by transmission lines. *In Vivo* 13(6):507-513.

Svedenstal BM, Johanson KJ, & Mild KH. 1999b. DNA damage induced in brain cells of CBA mice exposed to magnetic fields. *In Vivo* 13(6):551-552.

Sweirenga SHH, Heddle JA, Signal EA, Gilman JPW, Brillinger RC, Douglas GR, & Nestmann ER. 1991. Recommended protocols based on survey on current practice in genetoxicity testing labs. IV

Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures. *Mutation Res.* 246:301-322.

Takahashi K, Kaneko I, Date M, & Fukuda E. 1987. Induce of pulsing electromagnetic field on the frequency of sister chromatid exchanges in cultured mammalian cells. *Experimentia* 43(3):331-332.

Takehisa S, & Wolff S. 1978. The induction of sister-chromatid exchanges in Chinese hamster ovary cells by prolonged exposure to 2-acetylaminofluorene and S-9 mix. *Mutation Res.* 58(1):103-106.

Tan DX, Poeggeler B, Reiter RJ, Chen LD, Chen S, Manchester LC, & Barlow-Walden LR. 1993. The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole *in vivo. Cancer Lett.* 70(1-2):65-71.

Taylor JH. 1958. Sister chromatid exchanges in tritium labeled chromosomes. *Genetics* 43:515-529.

Taylor JH, Woods PS, & Hughes WL. 1957. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. *Proc. Natl. Acad. Sci. USA* 43:122-138.

Teare MD, Wallace SA, Harris M, Howell A, & Birch JM. 1994. Cancer experience in the relatives of an unselected series of breast cancer patients. *British J. Cancer* 70(1):102-111.

Télez M, Martínez B, Criado B, Ortega B, Peñagarikano O, Flores P, Ortiz-Lastra E, & Arrieta I. 2001. Evaluation of the cytogenetic damage induced by the antihypertensive drug nimodipine in human lymphocytes. *Mutagenesis* 16(4):345-351.

Tenforde TS. 1989. Biological responses to static and time-varying magnetic fields. *In* Interaction of electromagnetic waves with biological systems. ed. Lin, J. C. pp. 83-107. New York: Plenum.

Tenforde TS. 1992. Biological interactions and potential health effects of extremely-low-frequency magnetic fields from power lines and other common sources. *Annu. Rev. Pbl. Health* 13:173-196.

Tenforde TS. 1993. Cellular and molecular pathways of extremely low frequeny electromagnetic field interactions with living systems, *In* Martin Blank, (Ed), Electricity and Magnetism in Biology and Medicine. San Francisco Press, Inc., pp.936.

Tenforde TS. 1996. Biological interaction of extremely low frequency electromagnetic fields. Biological effects of magnetic and electromagnetic fields, Plenum Press, New York: 23-35.

Tenforde TS, & Kaune WT. 1987. Interaction of extremely low frequency electric and magnetic fields with humans. *Health Phys.* 53(6):585-606.

Theriault G, Goldberg M, Miller AB, Armstrong B, Guenel P, Deadman J, Imbernon E, To T, Chevalier A, Cyr D, & Wall C. 1994. Cancer risks associated with occupational exposure to magnetic field among electric utility workers in Ontario and Quebec, Canada and France:1970-1989. *Am. J. Epidemiol.* 139:550-572.

Thomson EJ, & Perry PE. 1988. The identification of micronucleated chromosomes: a possible assay for aneuploidy. *Mutagenesis* 3:415-418.

Tice RR. 1995. Applications of the single cell gel assay to environmental biomonitoring for genotoxic pollutants. *In* Butterworth, F.M.. (Ed) Biomonitors and Biomerkers as indicators of environmental change, Plenum Press, New York, pp. 69-79.

Tice RR, Chaillet J, & Schneider EL. 1975. Evidence derived from sister chromatid exchanges of restricted rejoining of chromatid subunits. *Nature* 256:642-644.

Tice RR, Chaillet J, & Schneider EL. 1976. Desmonstration of spontaneous sister chromatid exchanges *in vivo*, experiments. *Cell Res.* 102:426-429.

Tice RR, Strauss GHS, & Peters WP. 1992. High dose combination alkylating agents with autologous bone marrow support in patients with breast cancer: preliminary assessment of DNA damage in individual peripheral blood lymphocytes using the single cell gel electrophoresis assay. *Mutation Res.* 271:101-113.

Tice RR, Hook GG, Donner M, McRee DI, & Guy AW. 2002. Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. *Bioelectromagnetics* 23:113-126.

Tofani S, Ferrara A, Anglesio L, & Gilli G. 1995. Evidence for genotoxic effects of resonant ELF magnetic fields. *Bioelectrochem. Bioenerg.*36:9-13

Torres de Araujo FF, Pires MA, Frankel RB, & Bicudo CEM. 1986. Magnetite and Magnetotaxis in Algae. *Biophys. J.* 50:375-378.

Tounekti O, Belehradek J, & Mir LM. 1995. Relationship between DNA fragmentation, chromatin condensation, and changes in flow cytometry profiles detected during apoptosis. Exp. *Cell Res.* 217:506-516.

Trillo MA, Ubeda A, & Wagner P. 1990. Development of chickembryos in a pulsed magnetic field. *Bioelectromagnetics* (N.Y.) 11:169-187.

Tsun-Jen C, Pei-Yi H, Mei-Lan Du, Chung-Li, Ruey-Hong W & Pau-Chung C. 2000. Increased lymphocyte sister chromatid exchange frequency in workers with exposure to low level of ethylene dichloride. *Mutation Res.* 470(2):109-114.

Tucker JD, & Preston RJ. 1996. Chromosome aberrations, micronuclei, aneuploidy, sister chromatid exchanges and cancer risk assessment. *Mutation Res.* 365:147-159.

Tucker JD, Eastmond DA, & Littlefield LG. 1997. Cytogenetic end-points as biological dosimeters and predictor of risk in epidemiological studies, *In* P. Toniolo, P. Boffetta, D.E.G. Shukr, N. Rothman, B. Hulka, N. Pearce (Eds.), Application of Biomarkers in Cancer Epidemiology, IARC Scientific publication No. 142, pp. 185-200.

Tucker JD, Christensen ML, Strout CL, & Carrano AV. 1986. Determination of the baseline sister chromatid exchange frequency in human and mouse peripheral lymphocytes using monoclonal antibodies and very low doses of bromodeoxyuridine. *Cytogenet. Cell Genet.* 43 :38-42.

Tynes T, Anderson A, & Langmark F. 1992. Incidence of cancer in Norwegian workers potentially exposed to electromagnetic fields. *Am. J. Epidemiol.* 136:81-88.

Uckun FM, Kurosaki T, Jin J, Jun X, Bolen J, & Luben RA. 1995. Exposure of B-lineage lymphoid cells to low energy electromagnetic fields stimulates lyn kinase. *J. Biol. Chem.* 270: 27666-27670.

Verkasalo PK, Pukkala E, Stevens RG, Ojamo M, & Rudanko SL. 1999. Inverse association between breast cancer incidence and degree of visual impairment in Finland. *British J. Cancer* 80(9):1459-1460.

Vijaylaxmi MN, Meltz ML, & Wittler MA. 1997. Proliferation and cytogenetic studies in human blood lymphocytes exposed *in vitro* to 2450 MHz radio-frequency radiation. *Int. J. Radiat. Biol.* 72:751-757.

Vijayalaxmi MS, Newton C, Steel M, Evans HJ & Pentland B. 1983. Spontaneous and mutagen induced sister chromatid exchange in multiple sclerosis. *J. Med. Genet.* 20:372-376.

Vijayalaxmi, Reiter RJ, & Meltz ML. 1995. Melatonin protects human blood lymphocytes from radiation-induced chromosome damage. *Mutation Res.* 346(1):23-31.

Vijayalaxmi, & Strauss GHS, & Tice RR. 1993. An analysis of γ-ray-induced DNA damage in human blood leukocytes, lymphocytes and granulocytes. *Mutation Res.* 292:123-128.

Vijayalaxmi, Tice RR, & Strauss GHS. 1992. Assessment of radiation-induced DNA damage in human blood lymphocytes using the single-cell gel electrophoresis tchnique. *Mutation Res.* 271:243-252.

Vijayalaxmi, Leal BZ, Szilagyi M, Prihoda TJ, & Meltz ML. 2000. Primary DNA damage in human blood lymphocytes exposed *in vitro* to 2450 MHz radiofrequency radiation. *Radiat. Res.* 153(4):479-86.

Vijayalaxmi, Bisht KS, Pickard WF, Meltz ML, Roti Roti JL, & Moros EG. 2001. 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, Bisht KS, Pickard WF, Meltz ML, Roti Roti JL, & Moros EG. 2001a. 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 MN, Leal BZ, Meltz ML, Pickard WF, Bisht KS, Roti Roti JL, Straube WL, & Moros EG. 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. Frei MR, Dusch SJ, Guel V, Meltz ML, & Jauchem JR. 1998. Correction of an error in calculation in the article "Frequency of micronuclei in the peripheral blood and bone marrow of cancer-prone mice chronically exposed to 2450 MHz radiofrequency radiation. *Radiat. Res.* 149(3):308.

Visvardis EE, Tassiou AM, & Piperakis SM. 1994. Study of DNA damage induction and repair capacity of fresh and cryopreserved lymphocytes exposed to H_2O_2 and gamma-irradiation with the alkaline COMET assay. *Mutation Res.* 383: 71-80.

Vogel W, & Bauknecht T. 1978. Differential chromosome staining by *in vivo* treatment as a mutagenicity test system. *Nature* 260:448-449.

Vorobtsova I, Darroudi F, Semyonov A, Kanayeva A, Timofeyeva N, Yakovleva T, Zharinov G, & Natarajan AT. 2001. Analysis of chromosome aberrations by FISH and Giemsa assays in lymphocytes of cancer patients undergoing whole-body irradiation: comparison of *in vivo* and *in vitro* irradiation. *Int. J. Radiat. Biol.* 77(11):1123-1131.

Waksvik H, Brogger A, & Stene J. 1977. Psoralen/ UVA treatment and chromosomes. 1. Aberrations and sister chromatid exchange in human lymphocytes *in vitro* and synergism with caffeine. *Hum. Genet.* 38:195-207.

Walcott C, Gould JL, & Kirschvink JL. 1979. Pigeons have magnets. *Science* 205:1027-1029.

Walcott C. 1991. Magnetic maps in pigeons: Orientation in birds. Ed. Berthold P, & Basel B. 38-51.

Walker MM, & Bitterman ME. 1989. Conditioned responding to magnetic fields by honeybees. *J. Expt. Biol.* 157:67-73.

Walker MM, & Bitterman ME. 1989a. Attached magnets impair magnetic field discrimination by honeybees. *J. Expt. Biol.* 141:447-451.

Walker MM, & Bitterman ME. 1989b. Honeybees can be trained to respond to very small changes in geomagnetic field intensity. *J. Expt. Biol.* 145:489-494.

Walker MM, Diebel CE, Haugh CV, Pankhurst PM, Montgomery JC & Green CR. 1997. Structure and function of the vertebrate magnetic sense. *Nature* 390:371-376.

Walter J, & Crinnion ND. 2000. Environmental Medicine: Excerpts from Articles on Current Toxicity, Solvents, Pesticides and Heavy Metals. Originally published as multi-part review article *In* Alternative Medicine Review, Volume 5, Dover, Idaho 83825 U.S.A.

Washburn EP, Orza MJ, Berlin JA, Nicholson WJ, Todd AC, Frumkin H, & Chalmers TC. 1994. Residential proximity to electricity transmission and distribution equipment and risk of childhood leukemia, childhood lymphoma, and childhood nervous system tumors: Systematic review, evaluation, and meta-analysis. *Cancer Causes and Cont.* 5:299-309.

Weaver JC, & Astumian RD. 1990. The response of living cells to very weak electric fields: The thermal noise limit. *Science* (Washington, D. C.) 247:459-462.

Wedrychowski A, Schmidt WN, & Hinilica LS. 1986. DNA-protein crosslinking by heavy metals in Novikoff hepatoma. *Arch. Biochem. Biophys.* 251:387-402.

Wei Q, Cheng L, Hong WK, & Spitz MR. 1996. Reduced DNA repair capacity in lung cancer patients. *Cancer Res.* 56: 4103-4107.

Welcsh PL, Owens KN, & King MC. 2000. Insights into the functions of BRCA1 and RCA2. *Trends in Genetics* 16(2):69-74.

Welker HA, Semm P, Willig RP, Commentz JC, Wiltschko W, & Vollrath L. 1983. Effects of an artificial magnetic field on serotonin N-acetyltransferase activity and melatonin content of the rat pineal gland. *Expt. Brain Res.* 50(2-3):426-432.

Wertheimer N, & Leeper E. 1979. Electrical wiring configurations and childhood cancer. *Am. J. Epidemiol.* 109 (3): 273-284.

West RW. 1994. Enhancement of anchorage-independent growth in JB6 cells exposed to 60 hertz magnetic fields. *Bioelectrochem. Bioenerg.* 34:39-43.

Whitehouse HLK. 1963. A theory of crossing-over by means of hybrid deoxyribonucleic acid. *Nature* (Lond.) 199:1034-1040.

Wiesenfeld K, & Moss F. 1995. Stochastic resonance and the benefits of noise: from ice ages to crayfish and squids. *Nature* 373:33-36.

Wiesner G, Hoerauf K, Schroegendorfer K, Sobczynski P, Harth M, & Ruediger HW. 2001. High-level, but not low-level, occupational exposure to inhaled anesthetics is associated with genotoxicity in the micronucleus assay. *Anesthesia & Analgesia* 92(1):118-122.

William FM, & Peter EC. 1977. The incidence of sister chromatid exchanges in cultured human lymphocytes. *Mutation Res.* 42:305-312.

Wilson BW, Anderson LE, & Hilton DI. 1981. Chronic exposure to 60-Hz electric fields: effects on pineal function in the rat. *Bioelectromagnetics* 2:371-380.

Wilson BW, Wright CW, Morris JE, Buschbom RL, Brown DP, Miller DL, Sommers-Flannigan R, & Anderson LE. 1990. Evidence of an effect of ELF electromagnetic fields on human pineal gland function. *J Pineal Res.* 9(4):259-269.

Wolff S. 1983. Sister chromatid exchange as a test for mutagenic carcinogens. *Ann. NY. Acad. Sci.* 407:142-153.

Wolff S. 1977. Sister-chromatid exchange. *Ann. Rev. Genet.* 11:183-201.

Wolff S. 1979. Sister chromatid exchange: the most sensitive mammalian system for determining the agents. Academic Press, New York, pp. 229-246.

Wolff S, & Carrano AV. 1979. Report on the workshop on the utility of sister chromatid exchange. *Mutation Res.* 64:53-56.

Wolff S, & Perry P. 1974. Differential Giemsa staining of sister chromatid and the study of sister chromatid exchanges without autoradiography. *Chromosoma* 48:341-353.

Wolff S, & Perry P. 1975. Insights on chromosome structure from sister chromatid exchanges and the lack of both isolabelling and heterolabelling as determined by the FPG technique. *Exp. Cell Res.* 93:23-30.

Wolff S, Bodycote J, & Painter RB. 1974. Sister-chromatid exchanges induced in Chinese hamster cells by UV irradiation of different stages of the cell cycle: The necessity for cells to pass through S. *Mutation Res.* 25:73-81.

Wolff S, Rodin B, & Cleaver JE. 1977. Sister chromatid exchanges induced by mutagenic carcinogens in normal and xeroderma pigmentosum cells. *Nature* 265:347-349.

Wolff S, Bodycote J, Thomas GH, & Cleaver JE. 1975. Sister chromatid exchange in xeroderma pigmentosum cells that are defective in DNA excision repair or post-replication repair. *Genetics* 81(2):349-355.

Wood AW, Armstrong SM, Sait ML, Devine L, & Martin MJ. 1998. Changes in human plasma melatonin profiles in response to 50 Hz magnetic field exposure. *J. Pineal Res.* 25(2):116-127.

Wu YQ. 1986. Genetic etiology of esophageal cancer--III. The spontaneous and mitomycin-C (MMC) induced sister chromatid exchanges (SCE) and chromosome aberrations in cultured lymphocytes from members of "high risk" and "low risk" esophageal cancer families in Linxian County (Chinese). Chung-Hua Chung Liu Tsa Chih (*Chinese Journal of Oncology*) 8(1):23-25.

Yager JW, Sorsa M, & Selvin S. 1988. Micronuclei in cytokinesis-blocked lymphocytes as an index of occupational exposure to alkylating cytostatic drugs. *IARC Scientific Publications* 89: 213-216.

Yaguchi H, Yoshida M, Yjima Y, & Miyakoshi J. 1999. Effect of high-density extremely low frequency magnetic field on sister chromatid exchanges in mouse m5S cells. *Mutation Res.* 440(2):189-194.

Yellon SM. 1991. An acute 60-Hz magnetic field exposure suppresses the nighttime melatonin rise in the pineal and circulation of the adult Djungarian hamster. Abstracts of the Annual Review of Research on Biological Effects of 50 and 60 Hz Electric and Magnetic Fields, Milwaukee, WI, November 3-7, A-25.

Yellon SM. 1994. Acute 60 Hz magnetic field exposure effects on the melatonin rhythm in the pineal gland and circulation of the adult Djungarian hamster. *J. Pineal Res.* 16:136-144

Yellon SM. 1996. Acute and dailey 60 Hz magnetic field exposure effects on the melatonin rhythm and photoperiod control of reproduction in the adult Djungarian hamster. *Am. J. Physiol.* 270:816-821.

Yokota K, Ueda K, Takenaka M, Fujiwara A, & Ohama K. 1987. Sister chromatid exchange (SCE) in lymphocytes of patients with cancer of the cervix uteri (Japanese). *Nippon Sanka Fujinka Gakkai Zasshi-Acta Obstetrica et Gynaecologica Japonica.* 39(2):249-255.

Zamansky GB, Latt SA, Kaplan JC, Kkleinman LF, Dougherty CP, & Black PH. 1980. The co-induction of sister chromatid exchanges and virus synthesis in mammalian cells. *Exp. Cell Res.* 126:473-476.

Zhadin MN, & Fesenko EE. 1990. Ionic cyclotron resonance in biomolecules. *Biomrd. Sci.* 1:245.

Zimmerman AM, Winters WD, & Cameron IL. 1990. Influence of 60 Hz magnetic fields on sea urchin development. *Bioelectromagnetics* 11:37-45.

Zhou J, Li C, Yao G, Chiang H, & Chang Z. 2002. Gene expression of cytokine receptors in HL60 cells exposed to a 50 Hz magnetic field. *Bioelectromagnetics* 23:339-346.

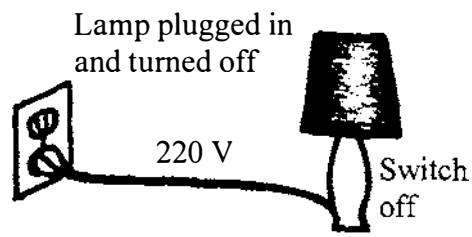
Zotti-Martelli L, Peccatori M, Scarpato R, & Migliore L. 2000. Induction of micronuclei in human lymphocytes exposed *in vitro* to microwave radiation. *Mutation Res.* 472(1-2):51-58.

Zwingelberg R, Obe G, Rosenthal M, Mevissen M, Buntenkötter S, & Löcher W. 1993. Exposure of rats to a 50-Hz, 30-mT magnetic field influences neither the frequencies of sister chromatid exchanges nor proliferation characteristics of cultured peripheral lymphocytes. *Mutation Res.* 302:39-44.

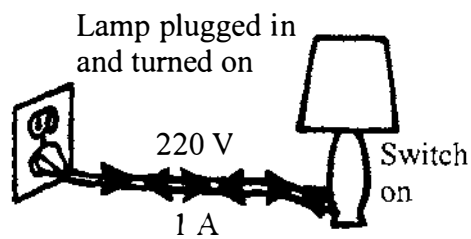
Appendix One (a): A simplified comparison between electrical pressure and water pressure.

Electrical pressure

Voltage: Electrical pressure-the potential to do work. Measured in volts (V) or in kilovolts (kV).
1 kV=1000 volts.

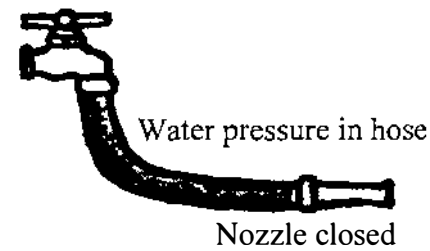


Current: The movement of electric charge (e.g., electrons). Measured in amperes (A).

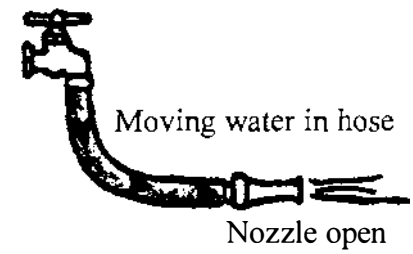


Water Pressure

Hose connected to an open faucet but with the nozzle turned off

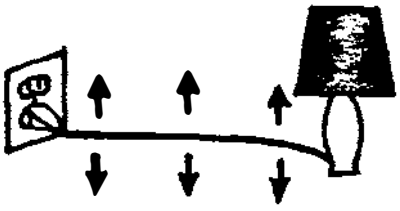
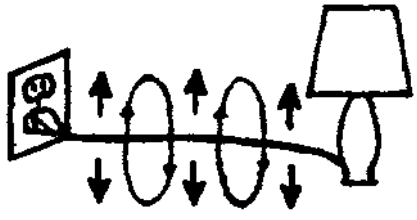


Hose connected to an open faucet and with the nozzle turned on.



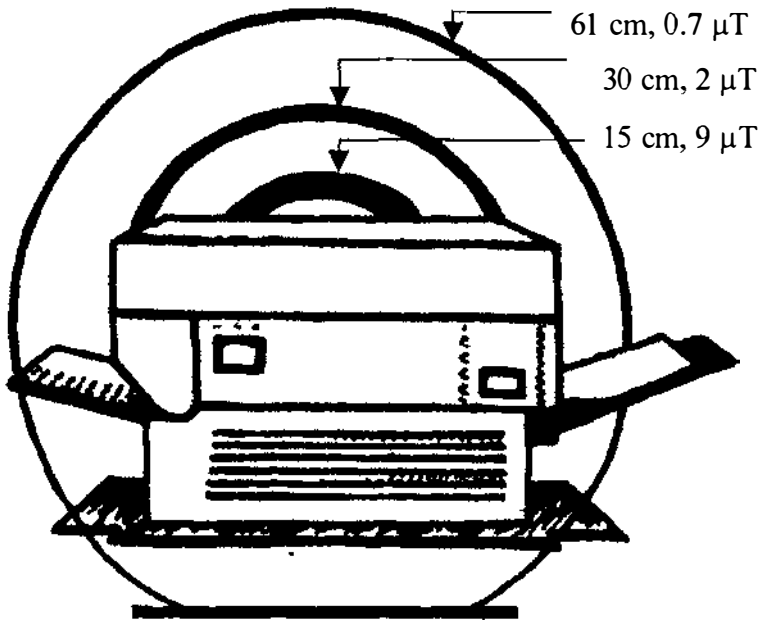
Source: NIOSH Facts: EMFs in the workplace, 1995.

Appendix One (b): A simplified comparison between electrical fields and magnetic fields.

Electric fields	**Magnetic fields**
• Produced by voltage.	Produced by current.
	
Lamp plugged in but turned off voltage produces an electric field.	Lamp plugged in but turned on voltage produces a magnetic field also.
• Measured in volts per meter (V/m) or in kilovolts per meter (kV/m).	Measured in tesla (T) or gauss (G). 1T = 10,000 G.
• Easily shielded (weakened) by conducting objects like trees and buildings	Not easily shielded (weakened) by most material.
• Strength decreases with increasing distance from the source.	Strength decreases with increasing distance from the source.

Source: NIOSH Facts: EMFs in the workplace, 1995.

Appendix One (c): Magnetic field decreases with increasing distance from the source and measured in tesla (T). This magnetic field is a 60 Hz power frequency field.



Source: EMF in your environment, EPA, 1992.

Appendix 2: PERSONAL QUESTIONNAIRE

Please read the following questions carefully and answer them as thoroughly and accurately as possible. The information you give will not be associated with your name in any public document and will be known only to the principal investigator of this study. The answers you provide may have a direct bearing on the interpretation of our results. Therefore, we ask that you kindly cooperate fully in providing correct information. Thank you for your interest.

Name: .....

Address:.....

.....

.....

Contact Phone: .....(Home) .....(Work)

To be filled in by principal investigator:

Code Number: .....

Date: ...../...../.....

This sheet is to be detached from the remainder of the questionnaire and filed by the principal investigator. Only the code number will be used as an identifier in subsequent pages. If additional space is needed for the completion of an answer, please write on the back of the page and identify the remaining part of the answer with the question's number.

Code No. .....

Personal History

1. Date: ...../...../.....

2. What is your birth date? ...../...../.....
(Month) (Day) (Year)

Present and Past Occupational History

3. Are you currently employed? ❒ YES ❒ NO

4. What is the name of the Company/Institution for which you now work or if unemployed, last worked?

.....

5. For how long have you worked for this Company or Institution?

.....

6. What type of work do / did you do?

.....

.....

.....

.....

.....

.....

.....

Code No. .....

Exposure History (work and non-work related)

7. Have you ever been exposed to any of the following in your job?

		When were you first exposed? (month , year)	When were you last exposed? (Month, Year)	How long in terms of days, months, or years in total were you exposed?
Asbestos	❒ YES→ ..... ❒ NO			
Radiation	❒ YES → ..... ❒ NO			
Coal Products	❒ YES→ ..... ❒ NO			
Dust (such as wood, leather)	❒ YES → ..... ❒ NO			
Pesticides, Herbicides	❒ YES → ..... ❒ NO			
Petroleum Products	❒ YES → ..... ❒ NO			
Dyes	❒ YES → ..... ❒ NO			
Solvents	❒ YES → ..... ❒ NO			
Other Chemicals (specify in question No. 8)	❒ YES → ..... ❒ NO			

Code No. .....

8. List the names of any specific substances to which you know you were exposed by either breathing or direct skin contact at work either in the last year or within the past ten years.

In the last year (12 months)	How frequently exposed on a monthly average	Within the past 10 years	How frequently exposed on a monthly average
.....			
.....			
.....			
.....			

9. Please list any chemical or physical exposure you experienced in the last year while practicing a hobby or other activities either at home or in other non-occupational settings. Refer back to the list in question No. 7 but do not limit your answers to only these substances.

In the last year (12 months)	How frequently exposed on a monthly average	Within the past 10 years	How frequently exposed on a monthly average
.....			
.....			
.....			
.....			

Code No. .....

Medical History

10. Have you taken any medication prescribed by a doctor in the past 1year (for example, blood pressure pills, antibiotics, insulin, tranquilizers, muscle relaxants, etc.)? ❒ YES ❒ NO

If yes, please indicate below:

Type of medication	Dose	Frequency?	Time period: Began (month)	Ended (month)
.....				
.....				
.....				
.....				

11. Have you taken any non-prescription medication in the past 1 year (for example, aspirin, antacid, anti-histamines, sedatives, or other drugs)?

❒ YES ❒ NO

If yes, please indicate below:

Type of medication	Dose	Frequency?	Time period: Began (month)	Ended (month)
.....				
.....				
.....				
.....				

Code No. .....

12. Do you take any vitamins currently or have you in the past 6 months?

❒ YES ❒ NO

If yes, please indicate below:

What kind of vitamins:	Dose	Frequency
.....		
.....		
.....		
.....		

13. Have you ever had any of the following illnesses?

Cancer	❒ YES	❒ NO
Hepatitis	❒ YES	❒ NO
Mononucleosis	❒ YES	❒ NO
Herpes	❒ YES	❒ NO
AIDS	❒ YES	❒ NO
Meningitis	❒ YES	❒ NO
Bacterial or viral infection	❒ YES	❒ NO
Cardiovascular disease	❒ YES	❒ NO
Diabetes	❒ YES	❒ NO
Other major illness	❒ YES	❒ NO

If yes, please specify what illnesses, when you were ill, and indicate treatment.

Illness	Period of illness (month, year to month, year)	Treatment
.....		
.....		
.....		
.....		

Code No. .....

14. List any other illnesses and their treatments you have experienced in the past 12 months (these should include colds, flu, etc.)

Illness	Period of illness (month, year to month, year)	Treatment
.....		
.....		
.....		
.....		

15. List any vaccination which you have received in the past 12 months .

Type of vaccination	Date administered
.....	
.....	
.....	
.....	

16. List any diagnostic or therapeutic X-rays other than dental you have received in the past 10 years .

Reason for X-rays	year received
.....	
.....	
.....	
.....	

Code No. .....

17. Have you ever had any dental X-ray? ❒ YES ❒ NO

If yes, within: ❒ the last month
❒ the last 6 months
❒ the last 6-12 months
❒ over one year

18. Have you had any surgery during the past year?

Date Reason

.....

.....

.....

.....

19. Give dates you have had any high fevers during the past year.

Date Associated illness Medication taken

.....

.....

.....

.....

Code No. .....

Smoking History

20. If you previously smoked cigarettes, cigars or a pipe, but have now given up, when did you last have a smoke? .....

21. Do you currently smoke cigarettes? ❒ YES ❒ NO

If yes, how many packs do you smoke each day?

- ❒ less than half a pack
- ❒ half-1 pack
- ❒ more than 1 pack

If you smoke more than one pack a day please state how many:

.....

22. Do you currently smoke cigars? ❒ YES ❒ NO

If yes, how many cigars do you smoke each day?

❒ 1 cigar ❒ 2-3 cigars ❒ 4 or more cigars

23. Do you currently smoke a pipe? ❒ YES ❒ NO

If yes, how many pipesful do you smoke each day?

- ❒ 1 pipeful
- ❒ 2-3 pipesful
- ❒ 4 or more pipesful

24. Do you smoke any substance other than tobacco ? ❒ YES ❒ NO

Code No.....

Diet History (should only reflect current habits)

25. Do you eat vegetables? ❒ YES ❒ NO

26. Do you eat meat? ❒ YES ❒ NO

27. Do you use diet sweeteners? ❒ YES ❒ NO

How much per day or week? .....

28. Do you use diet drinks? ❒ YES ❒ NO

How many per day or week? .....

29. Pertinent comments concerning diet not covered by above questions, e.g., special diet such as high protein, low carbohydrate, etc.

.....

.....

.....

.....

30. Do you drink coffee? ❒ YES ❒ NO

If yes, how much / day? .....

Decaffeinated? ❒ YES ❒ NO

31. Do you drink tea? ❒ YES ❒ NO

If yes, how much/day? .....

.....

.....

.....

Code No. .....

32. Do you drink beer? ❒ YES ❒ NO

If yes, please indicate your average weekly beer consumption:

❒ 1-6 cans (375 ml) a week or less
❒ 7-12 cans a week
❒ 13-24 cans a week
❒ more than 24 bottles a week.

33. Do you drink wine? ❒ YES ❒ NO

If yes, please indicate your average weekly wine consumption:

❒ 1-4 glasses a week or less
❒ 5-8 glasses a week
❒ 9-16 glasses a week
❒ more than 16 glasses a week.

34. Do you drink other alcoholic beverages (excluding beers and wines)?

❒ YES ❒ NO

If yes, please indicate type and average weekly consumption:

❒ 1-4 glasses (15 ml liquor) a week or less.
❒ 5-8 glasses a week
❒ 9-16 glasses a week
❒ more than 16 glasses a week.

Code No. .....

Genetic History

35. Are you aware of any birth defects or other genetic disorders or inherited diseases which affect your parents, brothers, sisters, or their children?

❒ YES ❒ NO

If yes, please specify .....

Thank you for your time.

Appendix 3: Consent Form

I,..... (print name), agree to have approximately 10ml of blood withdrawn from my body by a qualified person for the sole purpose of culturing my blood lymphocytes for chromosome examination.

I have read the accompanying Invitation Letter and Personal Questionnaire and fully understand the issues outlined in them. I further acknowledge that the withdrawal of my blood and the subsequent examination of my chromosomes by Mr. Wahab at Massey University will be for the express purpose of research into the effects of low frequency electromagnetic fields (EMFs) on human chromosomes.

I agree to participate in this experiment on the understanding that anonymity is preserved and that any results obtained from the analysis of my chromosomes will remain confidential except for public disclosure of possible EMF effects on my genetic material. I further understand that I will be able to have access to all results concerning me at any time.

I am also aware that I can withdraw from this research programme at any time.

Signature: .....

Date: .....

Appendix 4: Showing 50 (1-50) random microscope coordinates at (X and Y positions) for each of the 2 gels per slide.

	Slide Area 1			Slide Area 2	
Cell #	X Direction	Y Direction	Cell #	X Direction	Y Direction
01	170	13	**01**	117	13
02	169	10	**02**	117	17
03	171	06	**03**	120	13
04	171	13	**04**	116	10
05	164	10	**05**	106	16
06	162	14	**06**	114	10
07	170	18	**07**	117	14
08	171	10	**08**	113	21
09	158	21	**09**	118	07
10	156	08	**10**	110	19
11	164	07	**11**	109	10
12	154	08	**12**	110	21
13	167	05	**13**	107	10
14	165	07	**14**	119	16
15	156	09	**15**	111	18
16	165	10	**16**	109	18
17	165	21	**17**	119	23
18	167	14	**18**	104	21
19	159	10	**19**	108	21
20	160	13	**20**	112	08
21	158	20	**21**	108	16
22	155	16	**22**	104	09
23	163	17	**23**	118	03
24	156	22	**24**	120	11
25	171	19	**25**	105	21

26	154	17	**26**	113	11
27	153	15	**27**	121	05
28	165	08	**28**	107	09
29	154	08	**29**	114	07
30	169	14	**30**	111	15
31	169	22	**31**	122	03
32	166	11	**32**	116	06
33	158	14	**33**	119	08
34	158	23	**34**	115	12
35	165	09	**35**	115	03
36	171	21	**36**	102	15
37	172	14	**37**	111	07
38	157	03	**38**	118	19
39	161	19	**39**	117	03
40	164	13	**40**	102	08
41	155	07	**41**	105	12
42	167	08	**42**	116	17
43	152	10	**43**	109	18
44	157	03	**44**	110	13
45	154	02	**45**	109	08
46	172	17	**46**	107	10
47	167	21	**47**	112	10
48	167	09	**48**	103	04
49	158	14	**49**	111	12
50	171	22	**50**	103	14

Appendix 5:

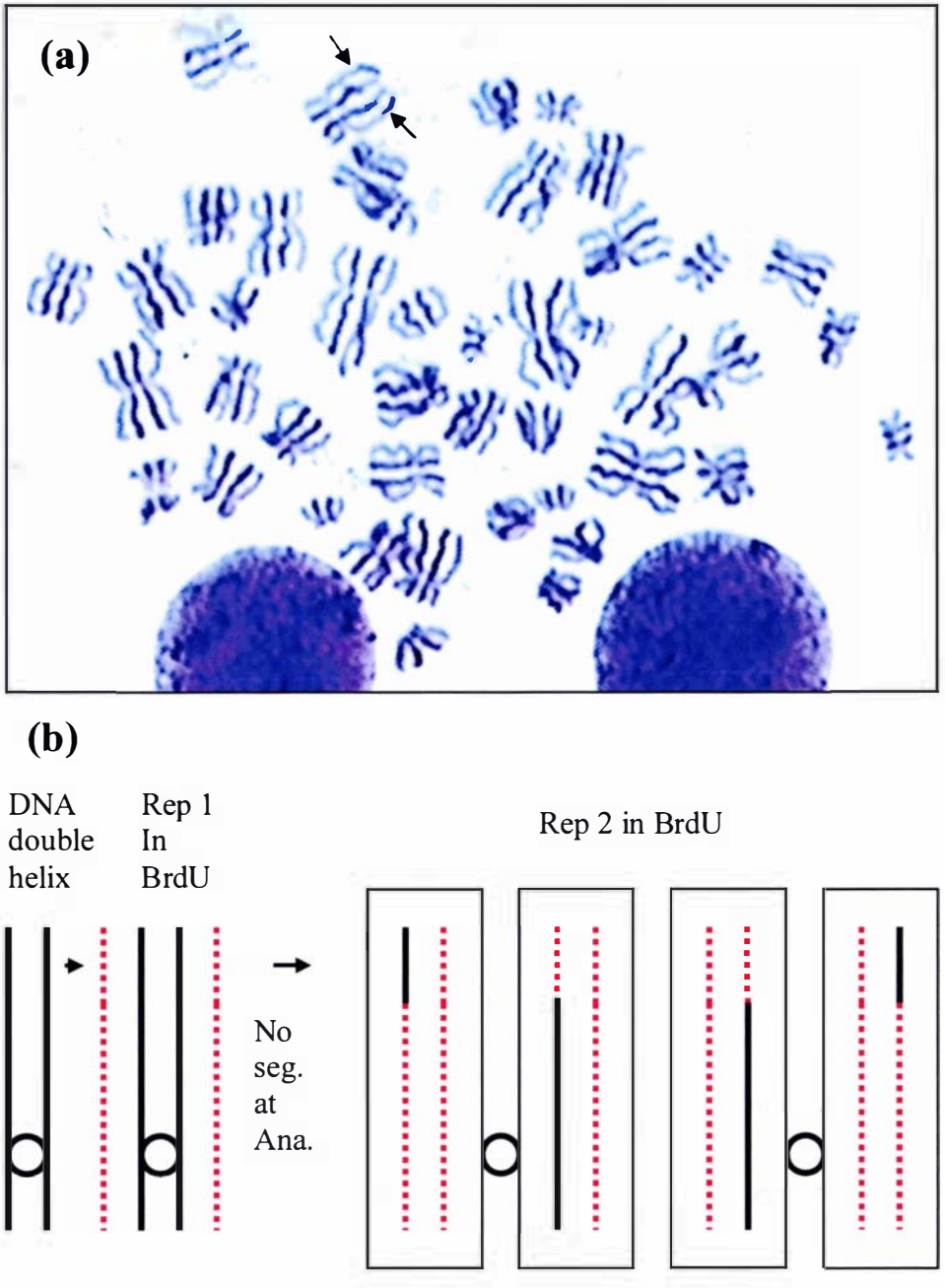


Figure 1: (a) A c-metaphase chromosome spread of a dividing peripheral blood lymphocyte that has undergone two rounds of DNA replication in the presence BrdU, showing failure of chromosome to segregate after the first round of replication. Arrows = sister chromatid exchange. **(b)** A diagrammatic representation of DNA replication and sister chromatid exchange as has occurred in the arrowed chromosome pair. Solid line = unlabelled; dotted line = DNA labelled with BrdU.

Appendix 5:

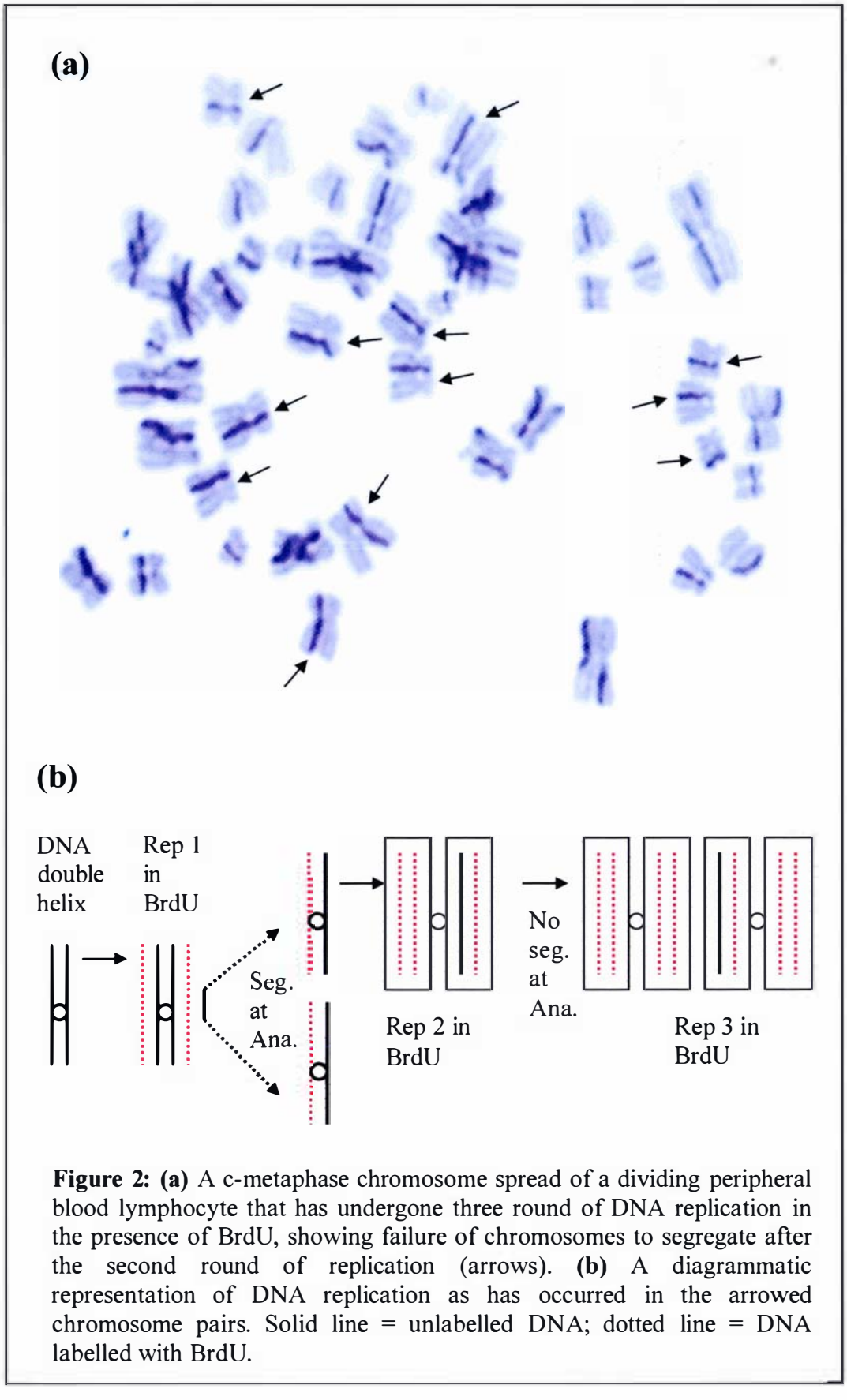


Figure 2: (a) A c-metaphase chromosome spread of a dividing peripheral blood lymphocyte that has undergone three round of DNA replication in the presence of BrdU, showing failure of chromosomes to segregate after the second round of replication (arrows). **(b)** A diagrammatic representation of DNA replication as has occurred in the arrowed chromosome pairs. Solid line = unlabelled DNA; dotted line = DNA labelled with BrdU.

Appendix 5:

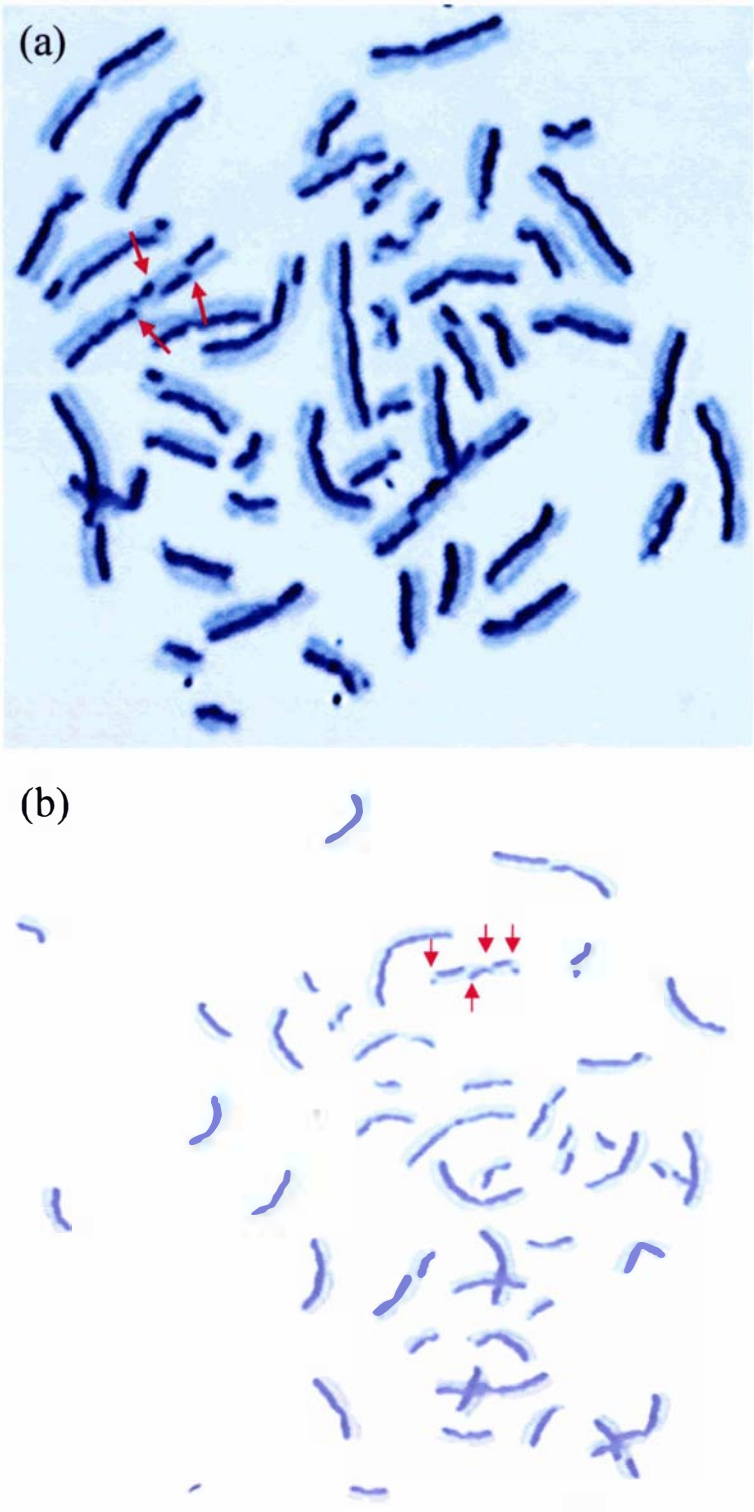


Figure 3: (a & b) Two c-metaphase spreads of a dividing peripheral blood lymphocyte subjected to EMF exposure which has undergone two rounds of DNA replication in the presence of BrdU, showing 3 and 4 SCEs (arrows), respectively.

Appendix 5:

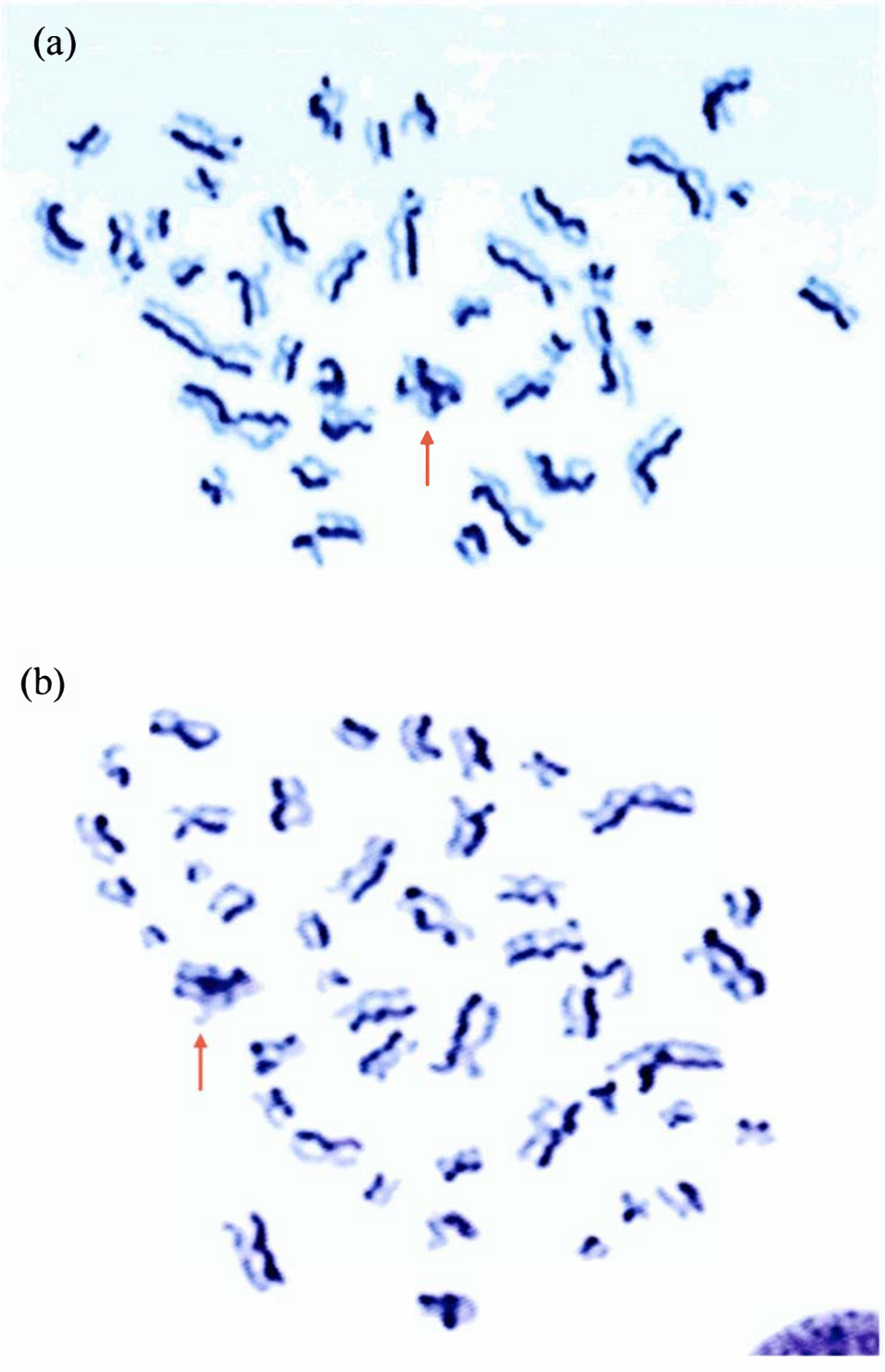
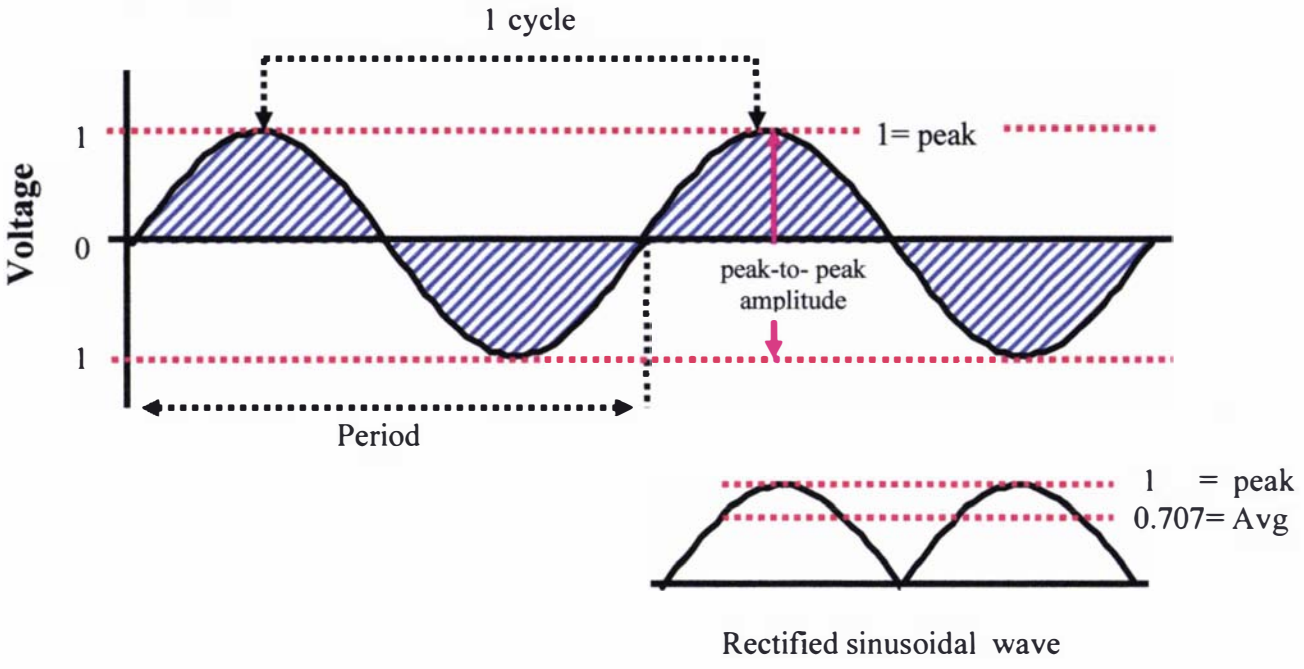


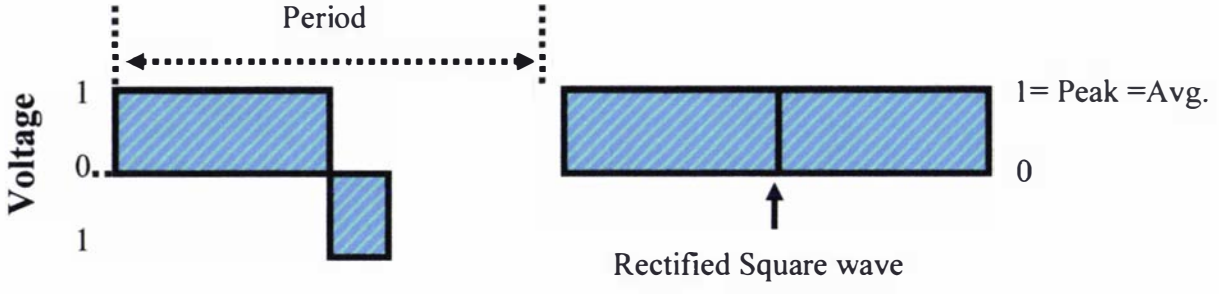
Figure 4: (a & b) Two c-metaphase spreads of a dividing peripheral blood lymphocyte subjected to EMF exposure which has undergone two rounds of DNA replication in the presence of BrdU, arrows showing complexes formed by 2-pairs of chromosomes.

Appendix 6:

Sinusoidal wave



Square wave



Appendix 7:

Table 1a: Showing the raw data of recorded temperatures (Control condition: coil off & no MF) inside the culture tube, near the coil, inside the cabinet, and the room where the incubator was placed over a 24 h period.

Time (h)	Culture Tube	Coil	Cabinet	Room	Real Time
0	37.0	37.0	37.0	22.0	5
1	37.0	37.0	37.0	21.7	6
2	37.0	37.0	37.0	21.5	7
3	37.0	37.0	37.0	21.2	8
4	37.0	37.0	37.0	20.8	9
5	37.0	37.0	37.0	20.7	10
6	37.0	37.0	37.0	20.3	11
7	37.0	37.0	37.0	20.1	mid night
8	37.0	37.0	37.0	20.0	1
9	37.0	37.0	37.0	19.9	2
10	37.0	37.0	37.0	20.0	3
11	37.0	37.0	37.0	20.0	4
12	37.1	37.0	37.0	19.9	5
13	37.0	37.0	37.0	20.6	6
14	37.0	37.0	37.0	20.5	7
15	37.0	37.0	37.0	21.2	8
16	37.0	37.0	37.0	21.6	9
17	37.0	37.0	37.0	21.9	10
18	37.0	37.0	37.0	22.0	11
19	37.0	37.0	37.0	22.0	noon
20	37.0	37.0	37.0	22.0	1
21	37.0	37.0	37.0	22.0	2
22	37.0	37.0	37.0	22.0	3
23	37.0	37.0	37.0	22.0	4
24	36.9	37.0	37.0	21.2	5
23	37.0	37.0	37.0	22.0	4
24	36.9	37.0	37.0	21.2	5

Table 1b: Showing the raw data of recorded temperatures (Coil on @ 1mT MF) inside the culture tube, near the coil, inside the cabinet, and the room where the incubator was placed over a 24 h period.

Time (h)	Culture Tube	Coil	Cabinet	Room	Real Time
0	37.0	37.0	37.0	22.0	5
1	37.0	37.0	37.0	21.5	6
2	37.0	37.0	37.0	21.1	7
3	37.0	37.0	37.0	20.8	8
4	37.0	37.0	37.0	20.4	9
5	37.0	37.0	37.0	20.1	10
6	37.0	37.0	37.0	20.1	11
7	37.0	37.0	37.0	19.8	mid night
8	37.0	37.0	37.0	19.8	1
9	37.0	37.0	37.0	19.6	2
10	37.0	37.0	37.0	19.7	3
11	37.0	37.0	37.0	19.5	4
12	37.0	37.0	37.0	19.0	5
13	37.0	37.0	37.0	19.1	6
14	37.0	37.0	37.0	20.0	7
15	37.0	37.0	37.0	20.5	8
16	37.0	37.0	37.0	21.2	9
17	37.0	37.0	37.0	21.9	10
18	37.0	37.0	37.0	21.9	11
19	37.0	37.0	37.0	21.9	noon
20	37.0	37.0	37.0	21.8	1
21	37.0	37.0	37.0	21.9	2
22	37.0	37.0	37.0	21.9	3
23	37.0	37.0	37.0	21.9	4
24	37.0	37.0	37.0	21.5	5

Appendix 7:

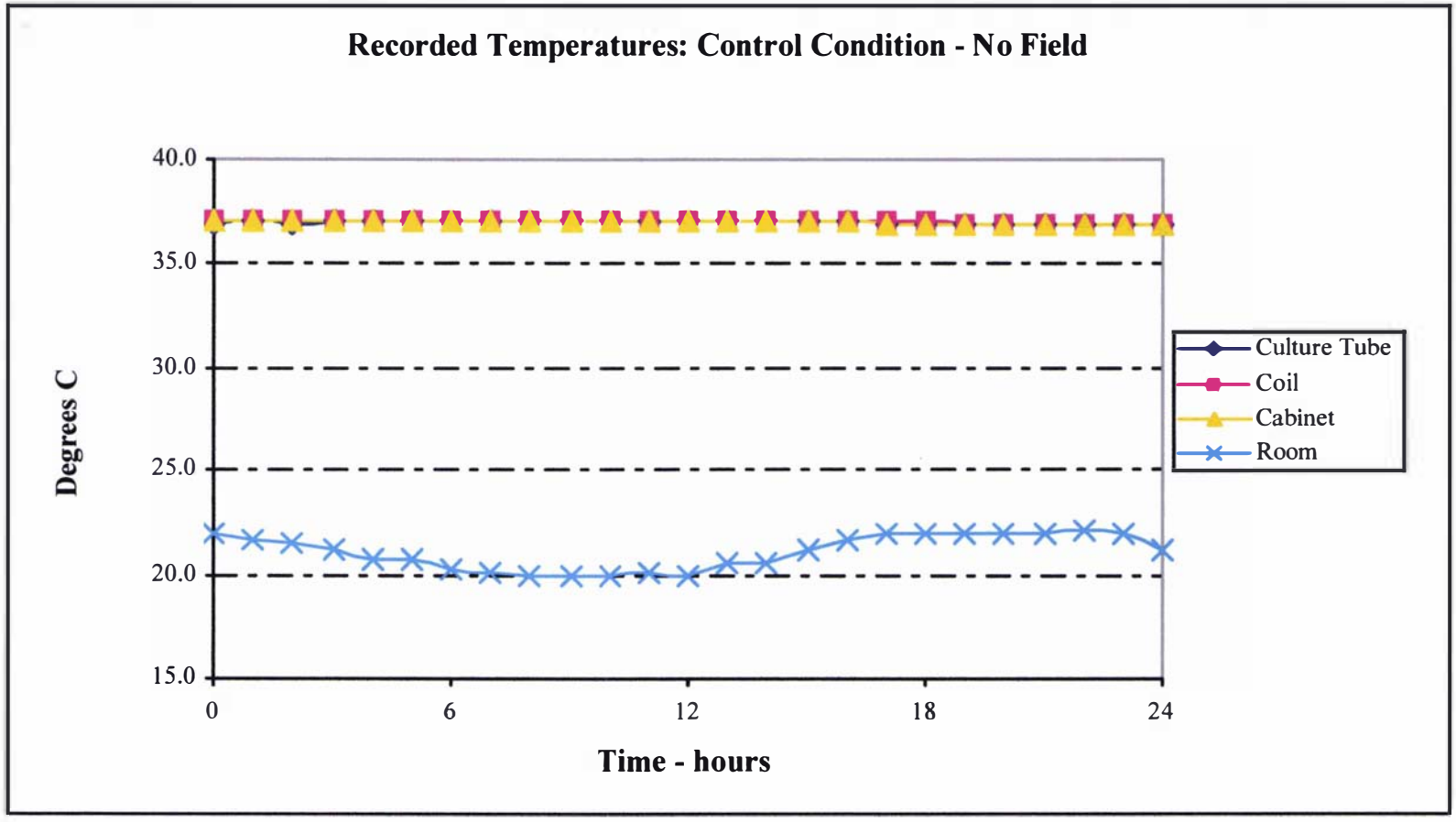


Figure 1a: Graph of recorded temperatures (Control condition: coil off & no MF) inside the culture tube, near the coil, inside the cabinet, and the room where the incubator was placed over a 24 h period.

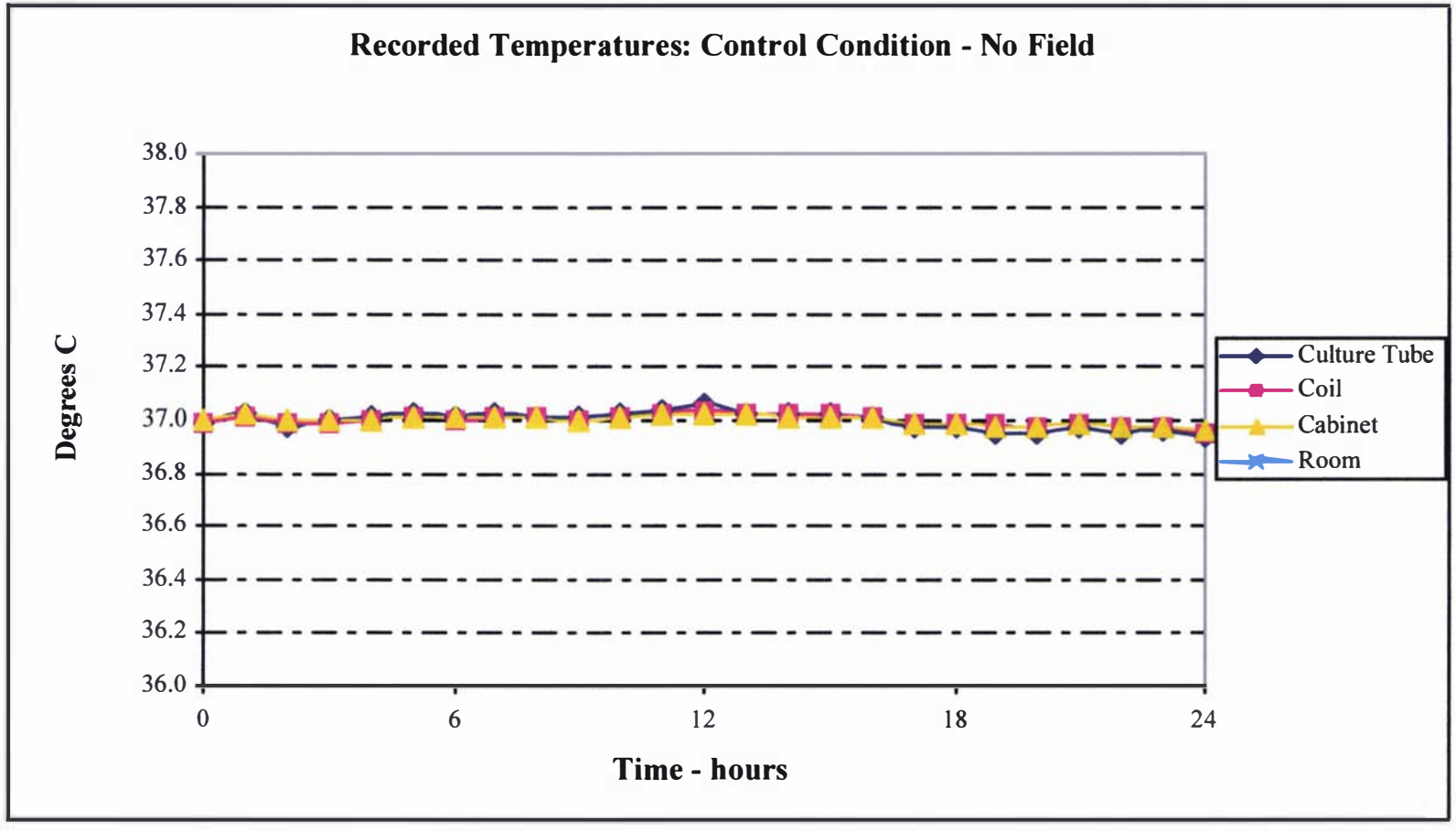


Figure 1b: Graph of recorded temperatures (Control condition: coil off & no MF) inside the culture tube, near the coil, and inside the cabinet over a 24 h period.

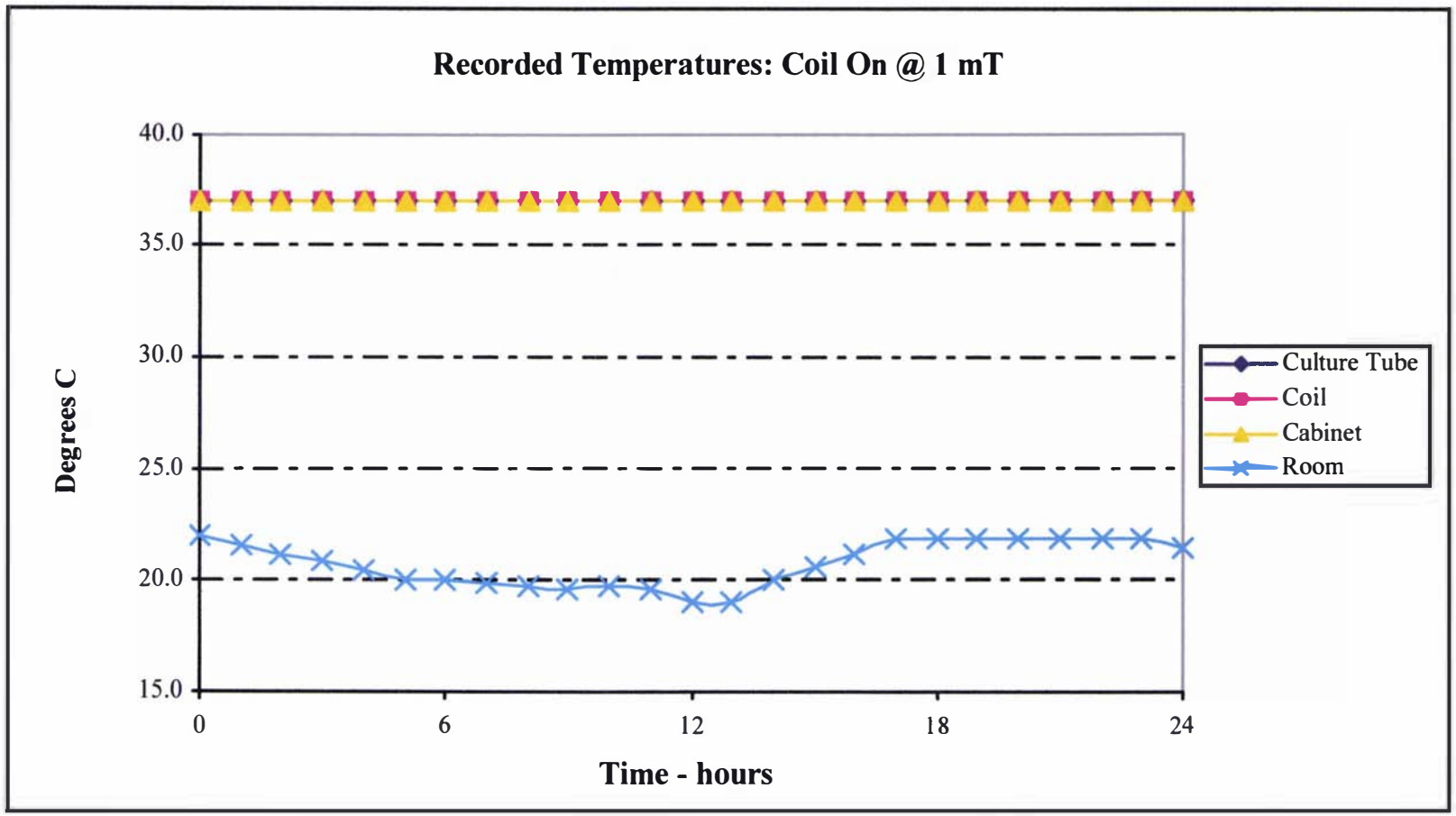


Figure 1c: Graph of recorded temperatures (Coil on @ 1mT MF) inside the culture tube, near the coil, inside the cabinet, and the room where the incubator was placed over a 24 h period.

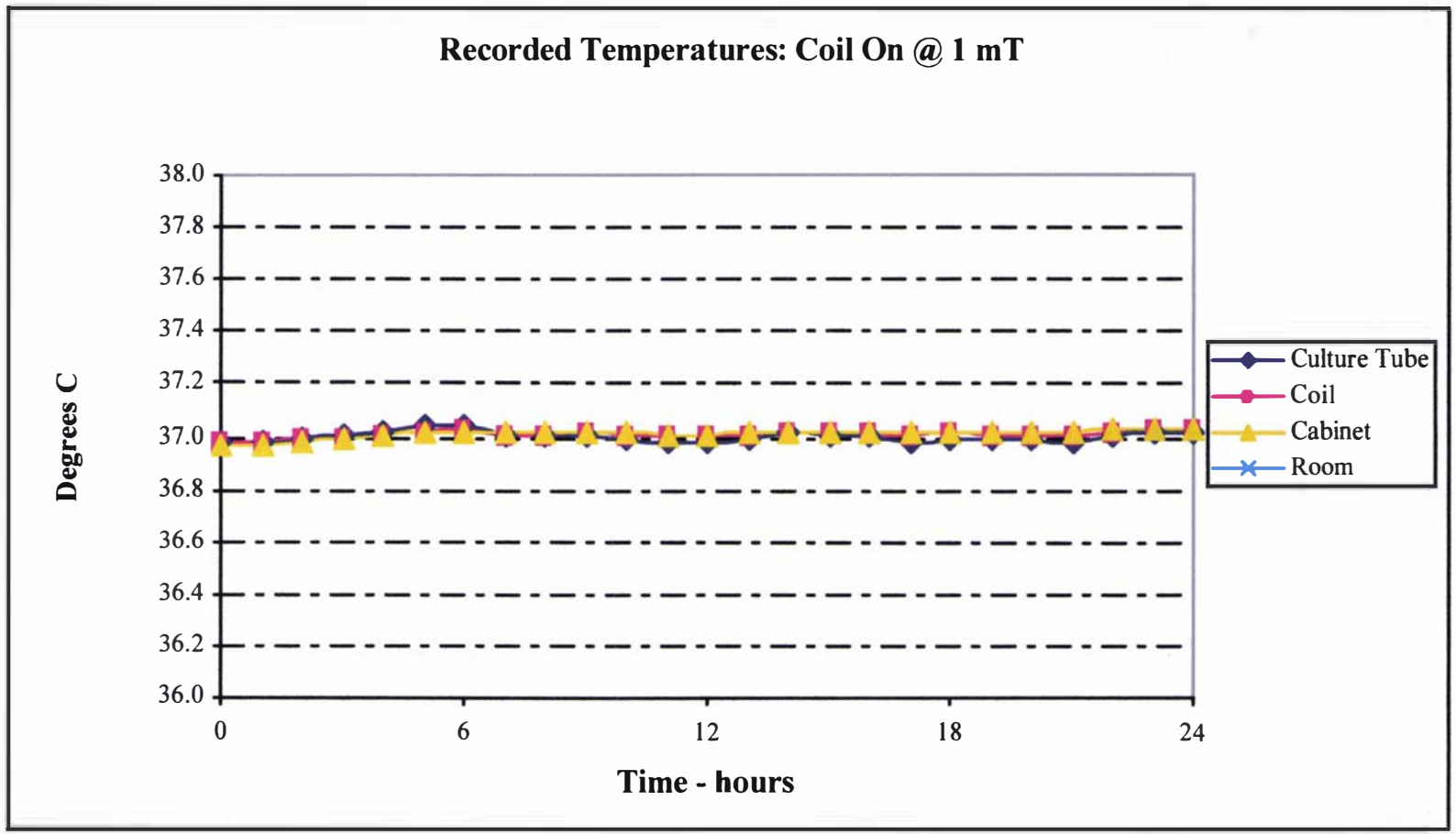


Figure 1d: Graph of recorded temperatures (Coil on @ 1mT MF) inside the culture tube, near the coil, and inside the cabinet over a 24 h period.