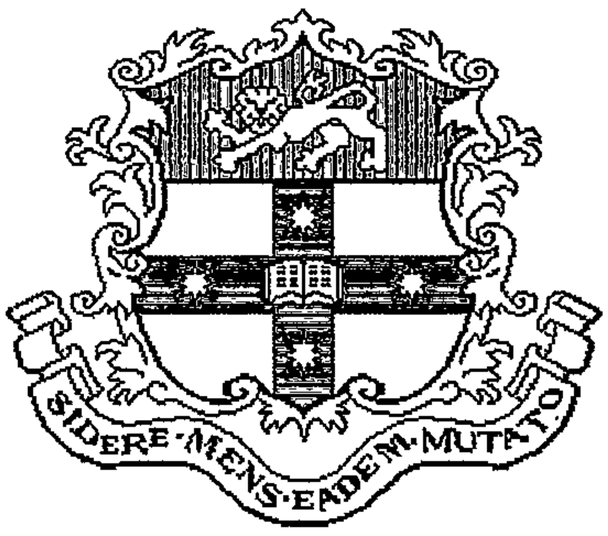
THE EFFECTS OF EXPOSURE TO ELECTROMAGNETIC FIELDS SIMILAR TO THOSE PRODUCED BY OVERHEAD POWERLINES UPON DNA FRAGMENTATION



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DEDICATION

This thesis is dedicated to my parents, for their unfailing love and constant support, and the encouragement to follow my dreams.

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DECLARATION OF AUTHORSHIP

This is to certify that the work presented in this thesis was carried out by the Master of Dental Science candidate, Annu Nangia, who was a postgraduate in the Discipline of Orthodontics, School of Dental Studies, Faculty of Dentistry, University of Sydney, and that the work has not been submitted to any other University or Institution for a higher degree.

August, 2000

Annu Nangia

TABLE OF CONTENTS

Dedication		i
Acknowledgments		ii
Declaration of Authorship		iii
Table of Contents		iv
List of Illustrations		viii
List of Tables		ix
Abbreviations		x
Summary		xi
Chapter 1 Literature Review		1
1.1	History of Therapeutic Uses of Electromagnetic Energy	1
	1.1.1 Magnetism	2
	1.1.2 Electric fields	2
	1.1.3 Pulsed Electromagnetic Fields	3
1.2	Electric, Magnetic and Electromagnetic Fields	4
	1.2.1 Electric Fields	4
	1.2.2 Magnetic Fields	5
	1.2.3 Electromagnetic Radiation	6
	1.2.4 Ionising vs Non-ionising fields	7
	1.2.5 Environmental Exposure to Electric, Magnetic and Electromagnetic fields	8
	1.2.6 Powerlines	10
	1.2.7 Electromagnetic Radiation Reduction	10
1.3	Effects of Radiation on the Cell	11
	1.3.1 Mechanism of Carcinogenesis	11
	1.3.2 The Multi-step Carcinogenesis Model	12
1.4	Community Anxiety with Regard to Electromagnetic Radiation	13
1.5	Health Effects of Electromagnetic Fields Reported in the Literature	14
	1.5.1 Electric Fields	14
	1.5.2 Magnetic Fields	15
	1.5.2.1 Background Radiation and the Influence of Powerlines	15
	1.5.2.2 Transportation	17

	1.5.2.3 Appliances	17
	1.5.2.4 Above Ground and Buried Powerlines	19
1.6	Methods of Exposure Assessment	20
	1.6.1 General Problems	20
1.7	Exposures in Epidemiological Studies	21
	1.7.1 Residential	21
	1.7.2 Wire Codes	21
	1.7.3 Distance	23
1.8	Exposure Systems in Laboratory Experiments	24
	1.8.1 *In vivo* Animal Studies	24
	1.8.2 *In vitro* Cellular Studies	26
1.9	Magnetic Field Exposure Studies	27
	1.9.1 Human Epidemiological Studies	28
	1.9.2 Genotoxic Assays	31
	1.9.3 Cell Culture Studies	31
	1.9.4 Animal Studies	33
1.10	Meta-analysis	34
1.11	Australian Exposure Guidelines	36
1.12	Comet assay	37
	1.12.1 Background	37
	1.12.2 Methodology	38
	1.12.3 Advantages	40
	1.12.4 Disadvantages	40
1.13	Aims and Objectives	41
Chapter 2 Materials and Methods		**42**
2.1	Development of a protocol for the Single Cell Gel Electrophoresis Assay	42
	2.1.1 Materials	42
	2.1.2 Cells	42
	2.1.3 Cell Culture	42
	2.1.4 Passaging of SAOS-2 Cells	43
	2.1.5 Storage of Cells	43
	2.1.6 Comet Assay Protocol	43

2.2 DNA Fragmentation Detection in SAOS-2 due to Electromagnetic Radiation Using the Single Cell Gel Electrophoresis Assay 47
2.2.1 Cell Culture 47
2.2.2 Exposure System 48
2.2.3 Arrangement of Flasks in the Exposure System 48
2.2.4 Collection of Cells 51
2.2.5 Cell Counting 51
2.2.6 Single Cell Gel Electrophoresis Assay 52
2.2.7 Microscopy 52
2.2.8 Statistical Analysis 52

Chapter 3 Results 53

3.1 Development of the SCGEA Protocol 53
3.1.1 Increased Gel Concentrations For The SCGEA 53
3.1.2 Frosted Slides Were Necessary For The SCGEA 53
3.1.3 Single Layer Of Agarose Is Sufficient For The SCGEA 54
3.1.4 Acid Treated Saos-2 Cells Inadequate As Positive Controls 54
3.1.5 Optimal Time And Temperature For The SCGEA 55
3.1.6 Short Exposures To Low Voltages 55
3.1.7 Storage Of Gels 56
3.1.8 DAPI As An Effective Stain For The SCGEA 56

3.2 Effects of Low Level Electromagnetic Radiation on the Appearance of Cells Displaying DNA Fragmentation in SOAS-2 cells 57
3.2.1 Experimental Design 57
3.2.2 Visual Classification of Damaged vs Undamaged cells 57
3.2.3 Expression of Results 60
3.2.4 Statistical Evaluation 61

Chapter 4 Discussion 62
Future research 66
Conclusions 67

Appendix 1	Cell Culture Techniques and Formulae	68
Appendix 2	Presentations	75
Literature Cited		76

LIST OF ILLUSTRATIONS

1.1	Electromagnetic Spectrum	8
1.2	The Multi-step Carcinogenesis Model	13
1.3	Different Wiring Codes (VHCC, OHCC, OLCC, VLCC)	22
2.1	Gaussmeter for Measuring AC magnetic fields	49
2.2	Diagrammatic Arrangement of Exposure Coils and SAOS-2 Cells	49
2.3	Photograph of Flasks in the CO_2 Incubator Within the Coil Apparatus	50
2.4	Schematic Diagram of Experimental Design	51
3.1a	Intact Cell Stained with Fluorescent Dye (DAPI) After Electrophoresis	58
3.1b	Comet Positive Cell Stained with Fluorescent Dye (DAPI) After Electrophoresis	58
3.1c	Apoptotic Cell Stained with Fluorescent Dye (DAPI) After Electrophoresis	58
3.2a	Comet Negative Cells (x 20 Magnification)	59
3.2b	Comet Positive Cells (x 20 Magnification)	59
3.3	Percentage of Comet Positive Cells (Irradiated vs Non irradiated)	60

LIST OF TABLES

1.1	Average Values for Magnetic Fields in Homes According to Room	15
1.2	Magnetic Fields as Function of Distance	16
1.3	Magnetic Field Strengths of Common Household Appliances	18
1.4	Measured Magnetic Fields and Wire Codes	23
1.5	Studies of Childhood Cancer and Residential Magnetic fields	28
1.6	Studies of Adult Cancer and Residential Magnetic fields	30
1.7	Laboratory Tests for Genotoxic Activity	31
1.8	*In vitro* Assessment of Genotoxicity of Magnetic Fields (Mutagenesis Assays)	32
1.9	*In vitro* Assessment of Genotoxicity of Magnetic Fields (Comet Assay)	32
1.10	*In vivo* Animal Studies of Magnetic Fields and Carcinogenesis	34
3.1	Wilcoxon Paired Signed Rank Test of SAOS-2 Cells Exposed to 10μT of Oscillating Magnetic Radiation for 7 Days in 9 Experiments	61

LIST OF ABBREVIATIONS

AC	Alternating Current
BCS	Bovine Calf Serum
CM	Complete Medium
CO_2	Carbon Dioxide
Da	Daltons
DAPI	4'6'-Diamidine-2'Phenylindole Dihydorchloride
DC	Direct Current
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diaminetetraacetic Acid
ELF	Extremely Low Frequency
EMF	Electromagnetic Field
EMR	Electromagnetic Radiation
HBSS	Hank's Balanced Salt Solution
HCl	Hydrochloric Acid
Hz	Hertz
MG	MilliGauss
mmol	Millimolar
µl	Microlitre
µT	MicroTesla
NaCl	Sodium Chloride
NHMRC	National Health and Medical Research Council
OHCC	Ordinary High Current Configuration
OLCC	Ordinary Low Current Configuration
RNA	Ribonucleic Acid
SAOS-2	Osteosarcoma cell line utilised
SCGEA	Single Cell Gel Electrophoresis Assay
T	Tesla
TAE	Tris Acetate Ethylene Diaminetraacetic Acid
V	Volts
VHCC	Very High Current Configuration
VLCC	Very Low Current Configuration

SUMMARY

Concern about the possible health effects of exposure to electromagnetic fields first arose when military personnel were exposed to fields of relatively high strength from high-frequency radar systems and video screens during World War II. Since then, claims have arisen of adverse health effects purportedly associated with high-frequency sources, such as radar units used by police, antenna systems used by the military, cellular phones used for communication, as well as microwave ovens and other appliances used in homes. Recently, attention has also focused on the potential for adverse health effects of low-frequency sources, such as transmission and distribution power lines. However, the effects of exposure to different sources of electric and magnetic fields can be quite different, depending on their frequency and strength so that the effects of fields generated by high voltage transmission lines operating at 50Hz may be different from those operating at mega or giga hertz frequencies.

Wertheimer and Leeper (1979) first raised questions of the possible adverse health effects of exposure to electric and magnetic fields from 60-Hz power lines. They reported epidemiological data suggesting an association between the configuration of power lines near homes and the incidence of leukemia and other types of childhood cancer. Similar studies have been published in succeeding years in the United States and in numerous other countries. The results of these studies have increased the scrutiny of the possible association between raised levels of electric and magnetic fields in residences and the incidence of cancer- the issue of most concern. Much of the early laboratory research on biologic effects of very low frequency electric and magnetic fields focused on the study of electric fields, but results of epidemiological and other studies have caused a gradual shift of interest toward magnetic fields as a possible cause of disease.

Extremely low frequency electric and magnetic fields are known or suspected to interact with biological systems in a number of ways. Some biological effects at high magnetic and electric field strengths, such as nerve stimulation and altered tissue healing have been used to set standards for occupational and public exposure to fields. Less widely accepted are other effects of such fields, particularly at low strengths, such as changes in cell metabolism and growth, gene expression, hormone levels, learning and behaviour, and promotion of tumours.

Summary

Several studies have investigated the health effects of magnetic fields and are mostly epidemiological. The number of investigations at the cellular and molecular level, although limited, are increasing in number as researchers become more interested in investigating the mechanisms by which damage may occur.

One of the unresolved issues in electromagnetic biophysics is whether low level electromagnetic fields cause DNA fragmentation. Previous studies dismiss this occurrence, but have not looked at DNA damage at the level of the individual cell. One of the more recently developed methods to investigate DNA damage is the single cell gel electrophoresis assay (SCGEA). First introduced in 1984 by Osterling and Johansson, this assay looks at DNA damage at the individual cell level. Unlike other methods, such as flow cytometry, which rely on a pooled sample of DNA to detect damage, this method does not require many cells to be damaged to enable detection. This assay is being used to detect DNA fragmentation by many workers investigating the effects of different forms of radiation including ultraviolet, x-ray and microwave radiation. Also, DNA damage by oxidative agents such as hydrogen peroxide; genotoxic agents such as nitrosamines in tobacco; organic mercury compounds; and insecticides, can be detected using this method. This thesis describes work using the SCGEA to detect DNA damage from low levels of electromagnetic fields similar to those generated by overhead power lines.

This project was to test the hypothesis that low level electromagnetic fields have an effect on DNA fragmentation detectable at the level of the individual cell. This hypothesis was addressed through pursuit of two experimental aims: one, to develop a simplified protocol for the single cell gel electrophoresis assay (also known as the Comet Assay) to screen for damaged DNA in cells, and two, to determine the effects of low level electromagnetic fields similar to those from overhead power lines on DNA fragmentation in an osteogenic sarcoma cell line using the developed assay as part of a pilot study.

Both aims were completed in this study and increased DNA fragmentation was observed when cells were exposed to low level electromagnetic fields. However, further analysis of the data coupled with a recent report demonstrating the detection of proliferating cells with the SCGEA (Wei et al., 2000), suggested that the low level electromagnetic fields studied may have increased cell proliferation as well as or, perhaps rather than, caused DNA fragmentation.

Unfortunately, time constraints prevented further work, which would have allowed resolution of this question.

CHAPTER 1 LITERATURE REVIEW

The notion that overhead power lines could cause cancer arose in 1979 when a report was published suggesting that an increased incidence in leukaemia among children living near power lines was related to magnetic fields (Wertheimer and Leeper, 1979). This work was followed by reports from Milham (1982), and Wright et al., (1982) showing that work in 'electrical occupations' was associated with a higher than expected incidence of leukaemia. Over 200 epidemiological studies have followed, and some of these report that cancers, particularly leukaemia and brain cancer, are weakly associated with power frequency electromagnetic fields (Moulder, 1998). Many of these studies have been greeted with scepticism as actual field measurements were often not performed (Linet et al., 1997) and even fewer studies have been coupled with laboratory evidence that these fields are indeed carcinogenic (Campion, 1997). Also, there seems to be an absence of plausible biophysical mechanisms through which these fields could affect biological systems (Blank,1995).

The public concern about power frequency fields is largely restricted to cancer, although some attention has been given to other possible health hazards, such as interference with pacemakers (Butous et al., 1983, Hayes and Vilestra, 1993), adverse pregnancy outcomes (Robert, 1996, Chernoff et al., 1992, Brent et al., 1993), allergic reactions (Liden 1996), and Alzheimer's disease (Sobel et al., 1996).

1.1 History of Therapeutic Uses of Electromagnetic Energy

Ever since their discovery, electricity and magnetism have been used in attempts to alleviate human ailments. Although it was about 600B.C. that Thales, a Greek philosopher of Miletus, discovered static electricity by rubbing amber with a cloth, it was the shock of an electric fish that was first prescribed by Aetius, a Greek physician, for the treatment of gout, which led to the beginning of electrotherapy.

Magnetism was discovered in Magnesia, a part of Asia Minor, in an iron ore known as magnes stone, magnetite, or lodestone. Circa 200B.C. the Greek physician Galen is reported to have used a magnet as a purgative (Frei, 1972).

1.1.1 Magnetism

Mesmer (1774) was probably the first notable person to experiment with magnetism as a therapeutic agent. His experiments interested other scientists of the day, but when he claimed "animal magnetism" through the imposition of his hands, he quickly lost favour with the scientific community and was denounced as a charlatan (Battocletti 1976).

About 100 years later, the French scientist, Durvel, presented the results of experiments on 100 men, two thirds of whom reported some kind of sensation or itching in response to a magnetic bracelet. As other scientists also felt the effects of this bracelet, it became difficult to discount Durvel's report completely (Battocletti 1976).

In 1966, Andreev in Moscow reported that the first stages of hypertension were relieved by the application of a magnetic bracelet, but that advanced disease was only slightly affected. He also reported that headaches were relieved by the application of a magnet to the back of the head for thirty minutes before sleeping.

The magnetic bracelet is still in popular use in some parts of Eastern Europe even though there is no theoretical basis for its therapeutic use (Battocletti, 1976).

1.1.2 Electric fields

The controlled use of electric fields in electro-therapeutic applications began in the latter part of the eighteenth century with the invention of various electrical generators and storage devices, such as the spark generator, the Leyden jar, the battery and electric dynamo. Most of the early applications involved the direct application of electricity to the human body to stimulate muscles and nerves, and in some cases, electroconvulsive therapy (Llauraldo et al., 1974). A study of the effects of electricity on the human body in the early 1930's led to the discovery that fibrillation is the lethal process in death from electric shock. This study led to the development of closed chest defibrillation and heart massage, procedures which have saved many lives (Carlisle and Carlisle, 1966).

Following a long period of disuse, electrical stimulation of nerves for the relief of pain once again became popular in the form of electro-acupuncture (Llauraldo et al., 1974). Sugiyama

(1974) in work on optical stimulation by means of DC and AC electric fields, found that the critical flicker frequency was increased by an AC electric field. From this study it was concluded that an electric field could potentially reduce human fatigue.

The use of electrical stimulation in bones dates back at least to 1812 at which time a patient with a successfully treated non union tibia was described (Brighton and Magnusson, 1985). However, the rationale for using electricity was not understood; electrical therapy was generally thought to be equivalent to a magic potion and electromagnetic related devices and concepts were staples of travelling "medicine men" or quacks.

Harsthorne (1841) and Lente (1850) used electrical energy to induce bone healing after fractures in non unions. After a century of neglect, interest in the use of electricity was revived in 1953 by a report by Yasuda and co-workers who induced osteogenesis by electrical stimulation around an implanted cathode in rabbit femurs. It appeared that electrical energy had the ability to affect both depository and resorptive bone and cartilage cells (Norton et al., 1977).

1.1.3 Pulsed Electromagnetic Fields

During the search for a surgically non invasive method to influence fracture healing, both time varying and magnetic fields were investigated (Basset et al., 1964). It was soon found that pulsing fields carried greater informational content than static DC (direct current) fields (Basset et al., 1974). It was found that such fields mimic the injury potentials generated in bone after fracture (Spadaro, 1982). These fields are known as pulsed electromagnetic fields (PEMF's). In the last three decades, considerable attention has been focused on the local biomechanisms of PEMF action as it has resulted in a claimed success rate equivalent to that of surgical repair (Yen-Patton, 1988). Exposure of non unions to PEMF's has been shown to convert a stalled healing process to active repair (Brighton et al., 1975). It has been suggested that the PEMF causes a change in membrane permeability, allowing increased flow of calcium, sodium and potassium ions across the cell membrane, thereby affecting the activity of intracellular cyclic adenosine monophosphate (Rodan et al., 1978). It is claimed that the bone repair stimulated by PEMF's is superior to DC electric fields (Basset et al., 1974).

1.2 Electric, Magnetic and Electromagnetic fields

1.2.1 Electric fields

Electric fields emerge as a property of atomic structure in which positively charged protons are attracted to, and balanced by, negatively charged electrons. The attraction of opposite charges and the repulsion of "like" charges can be readily detected experimentally. In the absence of any imbalance between particles with these equal and opposite charges, electric fields cannot be detected by humans at the macroscopic level. However, if an excess of either of these particles occurs, then it is possible to demonstrate the existence of these fields. The first such demonstrations were of static electric fields, in which the action of friction on different objects generated positive and negative charges respectively (Sears et al., 1987).

The difference in charge state between different objects, or points in space, establishes a potential difference which can be equilibrated by the movement of charged particles between these two points. This potential difference is quantitated and described in units of volts (V). The quantity of charged particles moving between these two points of potential difference is defined as a current and expressed in units of amperes (A). This is the basis for electrical circuitry in which charged particles being electrons, move between two points of potential difference via a wire or some other conductor (Giancoli, 1995). The property of conductivity varies for different types of charged particles, so that electrons, for example, travel readily through a copper wire, while positive ions fail to do so. It is the exchange of one type of charged particle for another under the influence of a potential difference which forms the basis of electroplating and electro-corrosion. Air is a poor conductor of electrons so that large potential differences are required before charge is able to flow in the form of lightning or an electrical spark.

Quite separate to electrical conductance, which is a property of both the material and the type of charged particle, is the property of electrical permittivity (Giancoli, 1995). Electrical permittivity represents a form of absorbency of electric fields, so that the electric field for a charged particle extends over a much greater volume of space in a vacuum as compared to the dimension of the electric field generated by the same particle in, for example, a lump of steel. It is possible to describe the properties of an electric field in terms of the strength of the field, E, in units of volts per metre (V/m), the displacement vector, D, in units of coulombs per

square metre (C/m^2) which represents the rate of change in field strength over distance; and the permittivity (ϵ) so that:

$$D= \epsilon E$$

For biologic materials the permittivity is a complex number consisting of a dielectric constant and a loss factor related to the conductivity. ϵ_0 is a constant, but for air and most biological materials, $\epsilon= 1$. In a vacuum, $\epsilon =\epsilon_0$. (Giancoli, 1995).

The electric field at power-line frequencies produced by specific voltages on high-voltage transmission lines can be accurately evaluated by analytic or numeric methods.

1.2.2 Magnetic Fields

Magnetic fields emerge as a property of moving charges. When a current travels along a wire, the actual movement through space of the charged particles generates another type of field separate to that of the electric field, which is defined as a magnetic field. Charged particles also interact with magnetic fields provided two conditions are met. Firstly, the charge must be moving, as no magnetic force acts on a stationary charge, and secondly, the velocity of the moving charge must have a component that is perpendicular to the direction of the magnetic field. If a charge moves parallel or anti-parallel to the field, the charge experiences no magnetic field (Sears et al., 1987).

Similar to electric permittivity, the penetration of magnetic fields varies with different media and this variability is expressed by a value described as medium permeability (μ). The strength of a magnetic field, B in units of Tesla (T), is related to the medium permeability, μ and magnetic flux density, H in units of amperes per metre (A/m) as described by the formula:

$$B=\mu H$$

For most biologic materials, except magnetite found in small quantities in some tissues, $\mu=1$. μ_0 is a constant. In a vacuum, $\mu=\mu_0$. (Giancoli, 1995).

An older unit of measurement for magnetic fields is Gauss (G), which may often be seen in the literature (Battocletti, 1974).

1 Tesla (T) = 10,000 Gauss (G)

1 Gauss = 1000 milliGauss (mG)

1 milliGauss = 90 milliAmperes/ metre (mA/m)

Magnetic fields from compact sources containing coils or magnets (transformers, appliances and televisions, for example) diminish rapidly with distance (d) in proportion with the distance cubed (National Research Council, 1997) ie.,

$$F=1/d^3$$

Where F= magnetic field (in Gauss or Tesla)

d= distance from the EMF source (in meters)

1.2.3 Electromagnetic Fields

The reciprocal induction of magnetic and electrical fields make it possible for these two types of fields to propagate one another and this self propagating pairing of electric and magnetic fields defines electromagnetic radiation (EMR) (Miller and Schroeer, 1987). This pattern of radiation arises when charged particles oscillate or accelerate, such as in an AC current. The changing electric current produces a magnetic field which in turn produces a changing electric field. Thus accelerating electric charges give rise to electromagnetic waves. The electric and magnetic components of the fields generated by moving charged particles are formally linked and mathematically described by a set of coupled differential equations called Maxwell's equations. In the mid 1800's James Clerk Maxwell recognised that a time varying magnetic field could produce a time varying electric field which in turn could produce a time varying magnetic field. Electric and magnetic fields could feed off each other in "empty" space, even in the absence of charges and currents (Giancoli, 1995). The details that Maxwell worked out were that the velocity, c, expressed in metres per second (m/s) of EMR is constant within a given medium, and that there was an inverse relationship between wavelength, λ, expressed in units of metres (m) and frequency, f, expressed in units of Hertz (Hz) such that:

$$\lambda \cong c/f$$

He worked out that the fields would propagate through space with a speed of $3x10^8$ m/s, which was the experimentally measured speed of light (Sears et al., 1987).

The frequency of EMR is determined by the frequency of oscillation or rate of acceleration of the particle generating the resultant radiation. An interesting property of EMR is that it behaves as a wave and also as a stream of particles. This duality is referred to as the wave-particle duality of electromagnetic radiation. The stream of particles are actually packets of energy and are called photons. The photon carries the electromagnetic force (Giancoli, 1995).

EMR occurs at all frequencies, but for slowly varying fields (ie. low frequencies), either electric or magnetic fields can predominate. Frequencies associated with power lines and their common harmonics are low enough for electric and magnetic fields generated by them to be considered separately (Kraus, 1992; Peck, 1953). At high frequencies, electric fields and magnetic fields travel together. The frequency determines the energy of the waves and the biological effects vary with the frequency (Blank, 1995). AC currents fall in the weaker range as their frequencies are low.

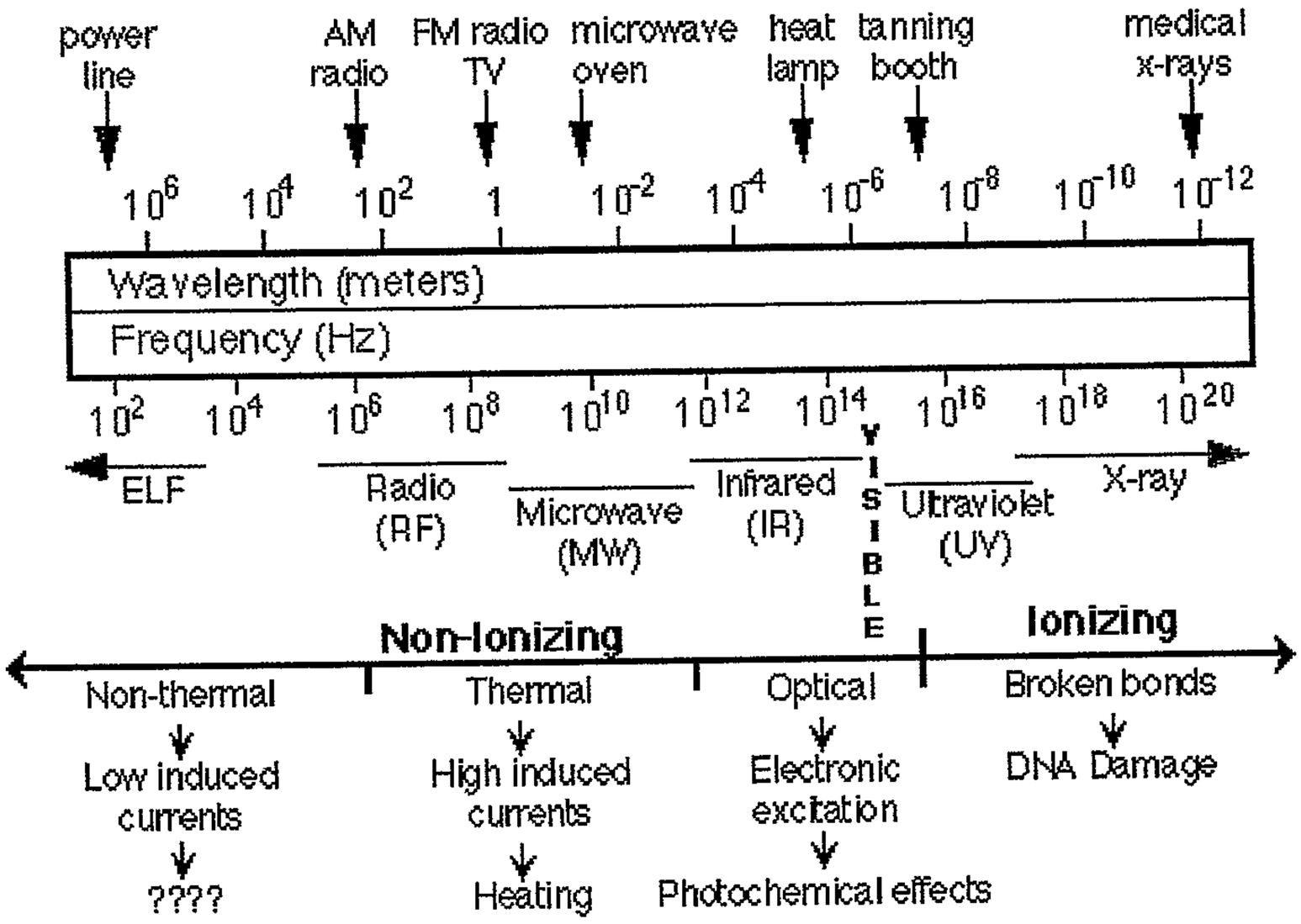
The extremely low frequency (ELF) designation is generally reserved for frequencies that range from 3 Hz to 3 kHz. Electric power in the United States is produced at 60 Hz, whereas power in Europe and other countries, including Australia, it is generally produced at 50 Hz (Australian Radiation Laboratory, 1997).

1.2.4 Ionising vs Non Ionising Radiation

The full range of EMR frequencies, both natural and anthropogenic, is described as the electromagnetic "spectrum" (Fig 1.1). This ranges from extremely low frequencies (ELF), associated with common household electric current (50-60 Hz), to radio waves (10^6-10^{10} Hz), microwaves (10^{10}-10^{12} Hz), infrared radiation (10^{12}- 10^{14} Hz), visible light (10^{14} Hz), ultraviolet radiation (10^{15} Hz), X-rays and gamma rays (>10^{17} Hz). Higher frequencies are associated with higher energy levels with only radiation of frequencies greater than about 10^{15} Hz capable of ionising atoms and molecules (ie. producing charged particles from the atoms and molecules with which it interacts) (National Research Council, 1997). EMR of wavelengths longer than ultraviolet radiation do not possess sufficient energy to cause ionisation. This is the

basis for the persistent view that non-ionising electromagnetic radiation is incapable of inducing biological effects other than heating (Foster and Guy, 1986). Ionising radiation is a well-understood cause of damage to biological systems through reactions of the products of ionisation with critical cellular components. ELF radiation, on the other hand, is non-ionising; it does not have sufficient quantal (photon) energy to produce ionisation in the manner of high-frequency radiation, and its mode of interaction, if any, with molecules and biological systems at low field strengths is speculative (National research Council, 1997).

Fig 1.1 Depiction of the Electromagnetic spectrum, showing the ionising and non ionising regions and the possible biological effects of differing frequencies of electromagnetic radiation. (taken from Moulder, 1998)



1.2.5 Environmental Exposure to Electric, Magnetic and Electromagnetic Fields

Most equipment used for the generation, transmission, and distribution of electric power in Australia generates ELF (50Hz) electric and magnetic fields. The components of the electric utility system that generate such fields include power plants (generating stations), which produce the electricity; high-voltage transmissions lines, which carry the electricity to major population centres; substations and their transformers, which reduce the voltage to levels suitable for distribution within a population centre; distribution lines (distribution primaries), which commonly carry power along residential streets; distribution transformers, which reduce

the voltage to amounts suitable for use in homes; and distribution secondaries (service drops), which carry electricity to individual residences. Transmission and distribution lines are commonly called "power lines," but the term can also include distribution secondaries (National Reseach Council, 1997).

Electric power that is used to operate devices in the home and workplace is also associated with the production of electric and magnetic fields. As electric charges move to produce a current, magnetic fields are created. An electric appliance connected to a source of electricity might have an electric field present even when it is turned off. When turned on and operating, a magnetic field is also present (MacDonald, 1975).

Since the range of magnetic fields encountered is usually quite small, the fields are generally described in units of microtesla (μT) or milligauss (mG). Although household alternating current in Australia has a frequency of 50Hz (Australian Radiation Laboratory, 1997), other relatively low-frequency electric and magnetic fields can be induced when the current is used to operate appliances, such as electric razors, hair dryers, video-display terminals, and dimmer switches.

Although most epidemiological studies are concerned with alternating current (AC) fields, direct current (DC) electric and magnetic fields also exist in the natural environment, and their magnitudes are much greater than the AC fields usually studied. The strength of the electric field at the earth's surface is about 130V/m while the magnetic field is around 50μT. Although birds, bacteria and migrating animals take advantage of the earth's natural magnetic field for orientation and navigation, these fields are not detected by humans (Blank, 1995).

Environmental AC fields are generally of a much lower strength than DC fields. In homes, electric fields are usually between 0 and 10 V/m. Electric fields do not penetrate the body effectively , and thus a field of 1V/m outside the body would measure 10^{-7}V/m beneath the skin (Blank, 1995).

For magnetic fields, the average values in the home are 0-0.2μT (Linet et al., 1997), although the fields around many appliances can be much higher (Table 1.3). Unlike electric fields, magnetic fields can pass through a body largely unattenuated, hence the magnetic field

outside and inside the body is of similar value. Magnetic fields directly under a power line can be up to 10μT (Sagan, 1996). The magnetic field decreases rapidly with distance, hence the field about 1 metre away from an electrical appliance in the home, such as a microwave of television, is almost zero (Wilson et al., 1990).

1.2.6 Powerlines

For electricity to be distributed economically over long distances, high voltages are used. Between the power station and suburban home, a series of transformers reduce the voltage along the way so that by the time it reaches the house the voltage has been effectively reduced to 240V (In Australia). As most transformers only work with alternating current, the direction of the current alternates back and forth. This establishes a frequency that is measured in Hertz (Hz), so that in a standard Australian 50Hz power line the direction of current is oscillating 50 cycles per second. In Europe and the United States, the frequency of electric power is 60Hz (National Research Council 1997).

The graph for AC current (voltage vs. time) forms a sine wave, with a positive voltage half of the time and a negative voltage for the other half (Giancoli, 1995). This is true of both electric and magnetic fields. The alternating oscillation of electrons generates electromagnetic radiation with a frequency of 50hz and a long wavelength, thus occupying the lower end of the electromagnetic spectrum. Higher voltages change the strength of the electromagnetic fields, but not the frequency. When living systems are exposed to high frequency radiation, the breaking apart of molecular bonds causes detrimental effects. High frequency EMR can contribute to carcinogenesis by fragmenting DNA. At ELF frequencies, electromagnetic radiation is non-ionising, suggesting that it cannot in theory, knock electrons away from atoms or alter molecular structures (Davis et al, 1992). However, low frequency electromagnetic radiation is nevertheless an energy force, and this energy is accepted as able to shake atoms and molecules back and forth to produce heat.

1.2.7 Electromagnetic Radiation Reduction

The wave characteristics of electromagnetic radiation from different sources can either add together or cancel each other out. If the radiation from two sources are in phase, then the peaks of each cycle will occur together, and the fields will be additive (Sears et al., 1987). On

the other hand, if the two sources are half a wavelength out of phase, then the propagating waves will cancel each other out, extinguishing further propagation. If the magnitude of the two fields is identical, then the fields will cancel each other out and the magnetic field measurement will be zero. "Neutral" and "hot wires" in household wiring need to be paired close together in part to produce a cancelling effect of fields generated by current flowing to and from appliances(National Research Council, 1987). This characteristic also provides a mechanism for configuring power lines so that EMF levels are reduced (Wertheimer and Leeper, 1979).

1.3 Effects of Radiation on the Cell

The process of changing the electron number in an atom or molecule is called ionisation and the product of this process is called an ion. When ionisation occurs in living cells, various kinds of damage occur. If the radiation dose is high, there will be immediate effects; if the dose is low there may be no apparent effect at all, or sometimes damage to only a single cell, the consequences of which may not become apparent for many years. Some damage of this kind to gametes can be passed on to future generations (Sumner, 1987).

1.3.1 Mechanism of Carcinogenesis

When growth of an organism is complete, the production of new cells by mitosis is very carefully controlled and just keeps pace with the continuous degeneration and loss of cells. In neoplasia the mechanism that controls cell growth fails to operate and cells continue to grow without limit. In time these cells outnumber the healthy cells, feeding at their expense and contributing nothing to the organism (Moulder, 1998).

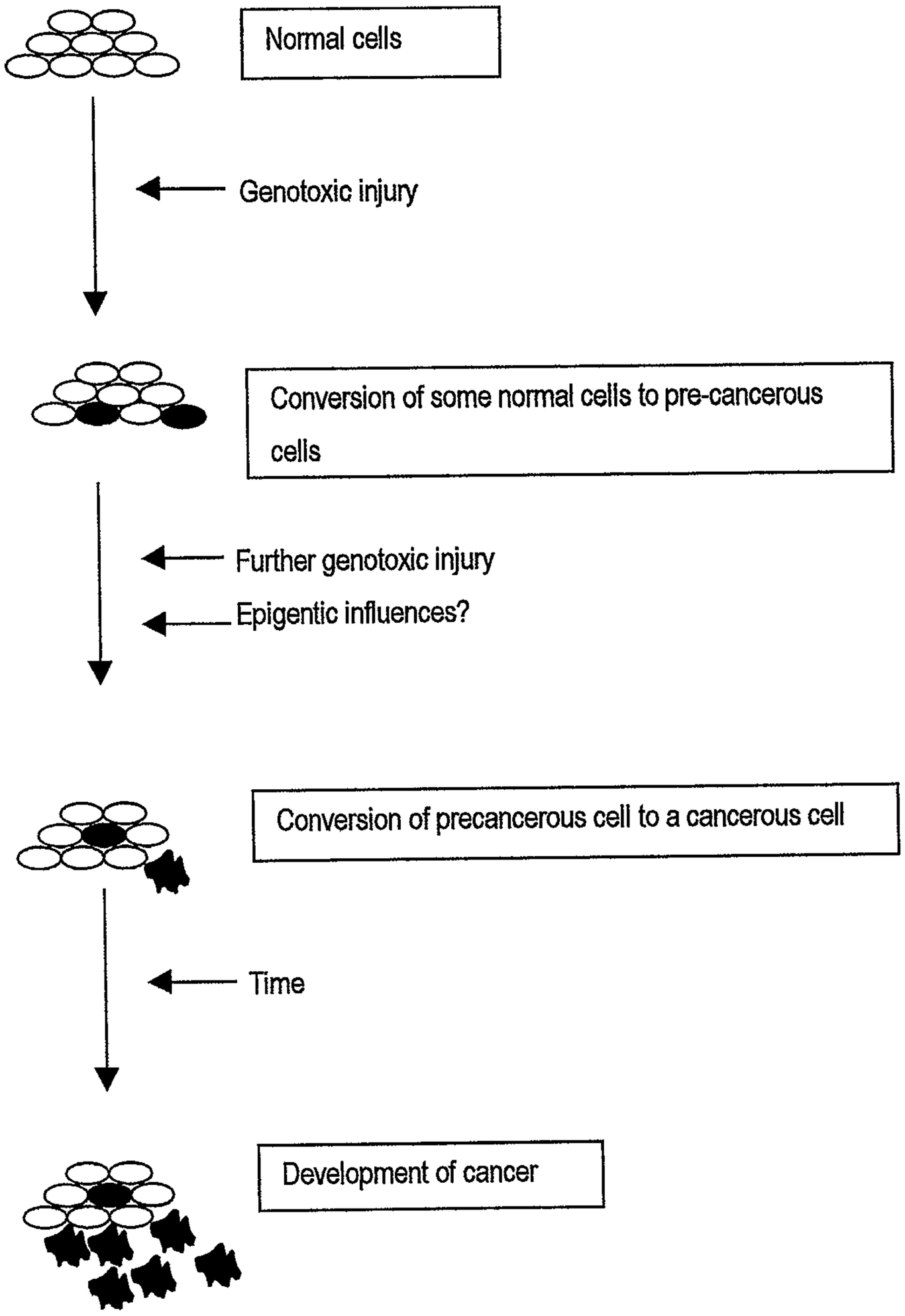
Our current understanding of carcinogenesis is that it is initiated by damage to DNA. Agents that cause such damage are called genotoxins. It is more likely that a series of genotoxic insults causes cancer, rather than a single event (Kheifets et al., 1997, Butterworth et al., 1995, Cohen and Ellwein, 1991). Such injuries occur to the human body all the time, but most have no effect due to repair mechanisms and injury in non critical sites. Only a very small fraction of genetic injuries that occur actually lead to carcinogenesis. On the other hand, some injuries may result in immediate death of the cell.

Since DNA damage is cumulative, there is a threshold level for genotoxins. As the dose of the carcinogen is lowered, so is the risk of cancer induction (Butterworth et al., 1995, Cohen and Ellwein, 1991). It also appears that non-genotoxic agents can also contribute to the development of cancer, even though these agents may not cause cancer by themselves. Such epigenetic carcinogens work indirectly by increasing the probability that other agents will cause genotoxic injury, which will lead to cancer (Kheifets et al., 1997). For example, an epigenetic agent may prevent repair of potentially genotoxic damage by the cell, or make it more vulnerable to genotoxic insults or may even stimulate cell division in a previously non-dividing cell that has suffered genotoxic injury.

1.3.2 The Multistep Carcinogenesis Model

It is currently believed that carcinogenesis is a multistep process driven by a series of injuries to the genetic material of cells (Fig 1.2) (Butterworth et al., 1995, Cohen and Ellwein, 1991, Moolgavkar, 1988). This current theory replaces the earlier two step model of "initiation – promotion" in the development of cancer. This model proposed that carcinogenesis began with an initiation process of genotoxic injury followed by a non genotoxic second step of promotion of the carcinogenesis. Although very effective at explaining carcinogenesis in some experimental models, several authors felt that this model was too simple (Butterworth et al., 1995, Cohen and Ellwein, 1991, Moolgavkar, 1988). This was largely because many researchers found that multiple genotoxic injuries are crucial in many types of cancer but that it was not clear if promotion was involved in all types of cancer (Moulder, 1998). In the current multistep model, promotion takes a minor role and even claims that cancer can occur from genotoxic insults alone.

Fig 1.2 The multistep carcinogenesis model (Modified from Moulder, 1998). Current research indicates that carcinogenesis may be a multistep process driven buy a series of injuries to the genetic material of cells



1.4 Community Anxiety with Regard to Electromagnetic Radiation

It is easy to see why so much attention has been given to the possibility that power-frequency electric and magnetic fields are associated with adverse effects. People who study how individuals respond to risk have learned that certain types of risks elicit stronger responses than others (Slovic 1987). One of the health effects that has been associated with exposure to electric and magnetic fields is an especially dreaded one, namely, cancer. Children have been

identified as a group of particular concern with regard to leukemia and possible long term reproductive and behavioural effects. The sources of the reported electric- and magnetic- field risks are largely imposed on people and not under their control. Furthermore, the fields that are the source of the reported risks are invisible and mysterious to many. All these factors cause many people to respond with concern and anxiety to potential risks associated with exposure to electric and magnetic fields (MacGregor et al. 1994).

1.5 Health Effects of Electromagnetic Fields Reported in the Literature

The health effects reported in epidemiological studies include cancer, primarily childhood leukemia; reproductive and developmental effects, primarily abnormalities and premature pregnancy termination; and neurobiological effects, primarily learning disabilities and behavioural modifications (Moulder, 1995). Each of these effects has been reported in epidemiological studies and associated with electromagnetic field exposure only by indirect estimates of the strength of fields. Childhood leukemia has attracted the most attention because of studies conducted in Denver, Los Angeles, and the Nordic countries that reported an increased risk of the disease in association with various indicators of exposure to electric or magnetic fields (Wertheimer and Leeper, 1979, London et al., 1991, Tomenius, 1986).

1.5.1 Electric Fields

Exposure to electric fields in the home and workplace is primarily from electric equipment used in those environments. Because of the ease with which electric fields are shielded or perturbed, electric fields in the home and workplace have not been characterised satisfactorily. Attempts have been made to measure personal exposure to electric fields (eg. by the Electrical Power Research Institute – (EPRI 1990)), but the measurements are heavily dependent on where the exposure meter is worn, the orientation of the exposure meter, and the presence of any conductors near the exposure meter. With that caveat, EPRI found that the mean personal exposure to 60-Hz electric fields in the home or office typically ranges from 5 to 10 V/m.

Power-line electric fields have been well characterised. Depending on line voltage, ground-level electric fields under a line might be as high as 10 kV/m, which is sufficient to cause fluorescent tubes to glow and to induce noticeable shock currents in a person who touches a

vehicle parked under a high-voltage line. Mean personal exposure to electric fields for substation, distribution-line, and transmission-line workers ranges from 50 to 5,000 V/m (EPRI 1990).

1.5.2 Magnetic Fields

1.5.2.1 Background Radiation and the Influence of Powerlines

The earth's natural static magnetic field of about 50 μT (range 30-70μT) is about 100 times stronger than the residential magnetic fields normally associated with the alternating current (AC) of power lines and electric appliances. It has been argued that because of this environmental exposure, artificial fields may not be relevant. However, exposure to the earth's field is constant, while man made fields are alternating and it is possible that the alternating fields affect health while static fields have no effect (National Research Council, 1997).

Background alternating magnetic fields in the centre of the rooms (away from most appliances) of a house are most likely caused by power lines and/ or grounding systems. In an EPRI study (1993) in which extensive magnetic-field measurements were taken in the centre of the rooms of 992 homes, only 5% of the homes had average alternating magnetic field strengths exceeding 0.29 μT.

The distribution of fields observed for selected rooms and the all-room averages are shown in Table 1.1. Household energy consumption and the presence of electric heating did not explain variations in the measured magnetic fields between homes (EPRI 1993).

Table 1.1 Average Values for the Magnetic Field in Homes According to Room (EPRI 1993)

Room centre n=992 homes	*Alternating Magnetic Field (μT)*
All rooms (average)	0.29
Kitchens	0.35
Bedrooms	0.29
Room with highest value	0.56

In the EPRI study, the power-line fields were found to be the dominant source of alternating magnetic fields. For most low-voltage power lines, the load current on the two wires is not always balanced. To analyse the effect of the imbalance, the load current can be mathematically divided into the balanced and unbalanced parts; the balanced part of the current produces a field that decreases approximately as the inverse square of the distance from the power line; the unbalanced part (the zero-sequence current) causes a field that decreases with the inverse of the distance. Therefore, at greater distances, the field associated with the zero-sequence current dominates. When the median field in a house is greater than 0.16 μT, the home in question is usually near a power line and the main field source is usually the balanced part of the power-line load current (EPRI, 1993).

The three most common sources of residential 50-Hz magnetic fields are electric appliances, the grounding system of the residences (most often, water pipes), and nearby power lines (most commonly, low-voltage distribution lines). Normally, unless there are wiring anomalies, internal residential wiring is not a significant source of personnel exposure. Although high-voltage transmission lines produce relatively high magnetic fields directly under them, they contribute relatively little to the residential and environmental levels at distances greater than 100 m, as illustrated in Table 1.2 (Environmental Protection Agency (EPA) 1992).

Exposure levels to alternating magnetic fields around the home are in the range of 0.01-0.025μT. For homes near powerlines, these levels may be as high as 0.1-0.5μT. Immediately under the powerline, alternating magnetic field levels of 6-10μT may be found (Sagan, 1996).

Table 1.2 Magnetic Fields as a Function of Distance from Power Lines (EPA 1992)

		Representative Magnetic Fields at Different Distances from Lines, μT		
Transmission lines, kV	Maximum alternating magnetic field under powerline (μT)	15metres	30metres	60metres
115	3.0	0.7	0.2	0.04
230	5.8	2.0	0.7	0.18
500	8.7	2.9	1.3	0.32

1.5.2..2 Transportation

Some transportation systems, including subways and intercity trains, operate on AC current and generate AC electric and magnetic fields. Measurements in Baltimore-Washington commuter trains indicate exposures to magnetic fields at 25 Hz with peak strengths as large as 50 μT in the passenger areas at seat height. The fields vary greatly with the position in the car as well as with the particular type and model of the car; detailed measurements indicate an average field strength of approximately 12.5 μT (National Research Council, 1997).

1.5.2..3 Appliances

The strongest alternating magnetic fields in homes are generally caused by appliances. However, the fields usually decrease rapidly with distance. As stated before, when the main source of a magnetic field in an appliance is a coil of wire, the field decreases approximately as the inverse cube of the distance (National Research Council, 1997). Some of the magnetic-field values measured near household and other appliances are shown in Table 1.3. The values show the range of all the measurements made (eg. 95% of the colour television sets measured emitted alternating magnetic fields of less than 0.33 μT at a distance of 56 cm). The values are based on measurements of the root-mean-square fields (taking a maximum magnetic field strength reading in three planes and extracting the square root of the sum of the squares of the individual readings) averaged over time from about I sec or more for the spot measurements to 24 hr for long-term and personal exposure measurements (Sears et al., 1987). Different appliances of the same type can produce different magnetic fields because of differences in their design. Important differences are the amount of current they use, the size and shape of conducting parts, the number of turns of wire in coils, and whether shielding or field-cancelling technology was used (George, 1998).

Table 1.3 Magnetic- Field Strengths of Common Household Appliances (EPA, 1992)

Sources	Magnetic Field at 0.15 m, μT	Magnetic Field at 0.3 m, μT
Bathroom sources		
Hair dryers	0.1-70.0	Bkg to 7
Electric shavers	0.4-60.0	Bkg to 10
Kitchen sources		
Blenders	3-10	0.5-2
Can openers	50-150	4-30
Coffee makers	0.4-1	Bkg to 0.1
Dishwashers	1-10	0.6-3
Food processors	2-13	0.5-2
Garbage disposals	6-10	0.8-2
Microwave ovens	10-30	0.1-20
Mixers	3.0-60	0.5-10
Electric ovens	0.4-2	0.1-0.5
Electric ranges	2.0-20	Bkg to 3
Refrigerators	Bkg to 4	Bkg to 2
Toasters	0.5-2	Bkg to 0.7
Laundry and utility-room sources		
Electric clothes dryers	0.2-1	Bkg to 0.3
Washing machines	0.4-10	0.1-3
Irons	0.6-2	0.1-0.3
Portable beaters	0.5-15	0.1-4
Vacuum cleaners	10-70	2-20
Office sources		
Air cleaners	11-25	2-5
Copy machines	0.4-20	0.2-4
Fax machines	0.4-0.9	Bkg to 0.2
Fluorescent lights	2-10	Bkg to 3
Electric pencil sharpeners	2-30	0.8-9
Video-display terminals	0.7-2	0.2-0.6
Workshop source		
Battery chargers	0.3-5	0.2-0.4
Drills	10-20	2-4
Power saws	5-100	0.9-30

(Bkg: Background)

In addition to the appliances listed in Table 1.3, alternating magnetic fields from electric blankets might contribute a large part to the alternating magnetic-field exposures in the home. When measured about 5 cm from the surface of the blanket, approximately the distance of internal organs, these fields average about 2.2 μT for conventional electric blankets and about 0.I μT for positive-temperature-coefficient blankets (National Research Council, 1997).

1.5.2..4 Above Ground and Buried Powerlines

For some homes power lines can be important sources of magnetic fields. Typical values for the magnetic fields from overhead powerlines lines are illustrated in Table 1.2 (EPA 1992). At peak usage, the magnetic fields could be double the average figures shown in the table. Note that the fields fall off roughly as the inverse cube of the distance from the line, and the magnetic field is greater for the higher-voltage lines. There are two reasons the magnetic field is greater for the higher-voltage lines: higher-voltage lines generally have thicker wires to carry more current (magnetic field is directly proportional to current); and higher-voltage lines have a greater separation between wires to avoid arcing. The magnetic fields produced by the three conductors of a transmission line tend to cancel one another. Greater wire spacings result in less cancellation; closer spacings result in more cancellation. Burying powerlines can greatly reduce their magnetic fields. This is because underground lines use rubber, plastic or foil to insulate the wires. This insulation does not shield the magnetic field, but it does allow the wires to be placed much closer to each other and therefore allows greater field cancellation (George, 1998).

There are two major types of underground power lines: direct burial (the individual wires are buried separately) and pipe-type cables (all the wires are placed in a single metal pipe). Direct-burial underground power lines can produce ground-level magnetic fields as large as equivalent capacity overhead lines (but over a more limited area). Although the underground wires might be closer together than overhead wires (tending to decrease the field), they are buried at a depth of only about 5 feet and, therefore, are much closer to the surface of the ground than overhead lines. In underground pipe-type transmission lines, the close spacing of the wires in the pipe and the metal pipe itself decrease the magnetic field, so that the resulting ground-level field is typically less than 0.1 μT (National Research Council, 1997).

Electric substations are installations where the voltages used with transmission lines are stepped down to lower voltages used with distribution lines (Giancoli, 1995). Electric and magnetic fields produced by substation equipment are generally not appreciable beyond the substation boundaries, but the fields can be somewhat stronger near them than in other parts of the neighbourhood, because the power lines converge at the substation and might be closer to the ground as they go in and out of the substation (Wilson et al., 1990).

1.6 Methods Of Exposure Assessment

1.6.1 General Problems

Electric and magnetic fields at 50 (or 60) Hz can be calculated or measured in practically any environment. Even their more complex characteristics (eg. harmonics and temporal and spatial changes) can be determined. Similarly, transients can be measured, albeit only with sophisticated instrumentation (National research Council, 1997). Determination of human exposure and, in particular, determination of human exposure as it relates to epidemiological studies is much more difficult. An average adult or child encounters a variety of environments of electric and magnetic fields in a day, not to mention in a month or a year (Wilson et al., 1990).

The original interest in possible health effects of power-line fields was precipitated by an epidemiological report (Wertheimer and Leeper 1979), which suggested that the strength of 60-Hz magnetic fields, as classified or estimated by a wire code, correlates with increased rates of childhood cancers, including leukemia.

Various characteristics of electric and magnetic fields might be responsible for their interaction with biological systems (eg., harmonics, transients, and temporal and spatial changes). Knowledge of which characteristic (if any) of the exposure fields is important in the interaction would permit reliable exposure assessment in epidemiological studies. Lack of knowledge of the relevant field characteristic makes comprehensive human exposure assessment nearly impossible. Nevertheless, many studies have been conducted with the tacit assumption that the 60Hz magnetic field is directly related to the exposure of interest. (Savitz and Baron, 1989).

Exposure can be assessed by direct measurements or by indirect modelling and estimation of the electric and magnetic fields present in the spaces occupied by humans or experimental animals. In most cases such evaluation have been made at 60 (or 50) Hz only Savitz and Baron, 1989).

1.7 Exposures In Epidemiological Studies

1.7.1 Residential

The primary hypothesis tested in most residential epidemiological studies of electric- or magnetic- field effects is that the presence of power lines near the home is related to occurrence of disease. With few exceptions, the studies have focused on indirect estimates of the magnetic fields rather than electric fields. At low frequencies, such as 50 or 60 Hz, electric fields are substantially shielded by the shell of a house and by surrounding trees, so that residential exposure to electric fields is difficult to describe and nearly impossible to model with any accuracy. In the study by London et al. (1991), nearby power lines appeared to have no influence on indoor electric fields.

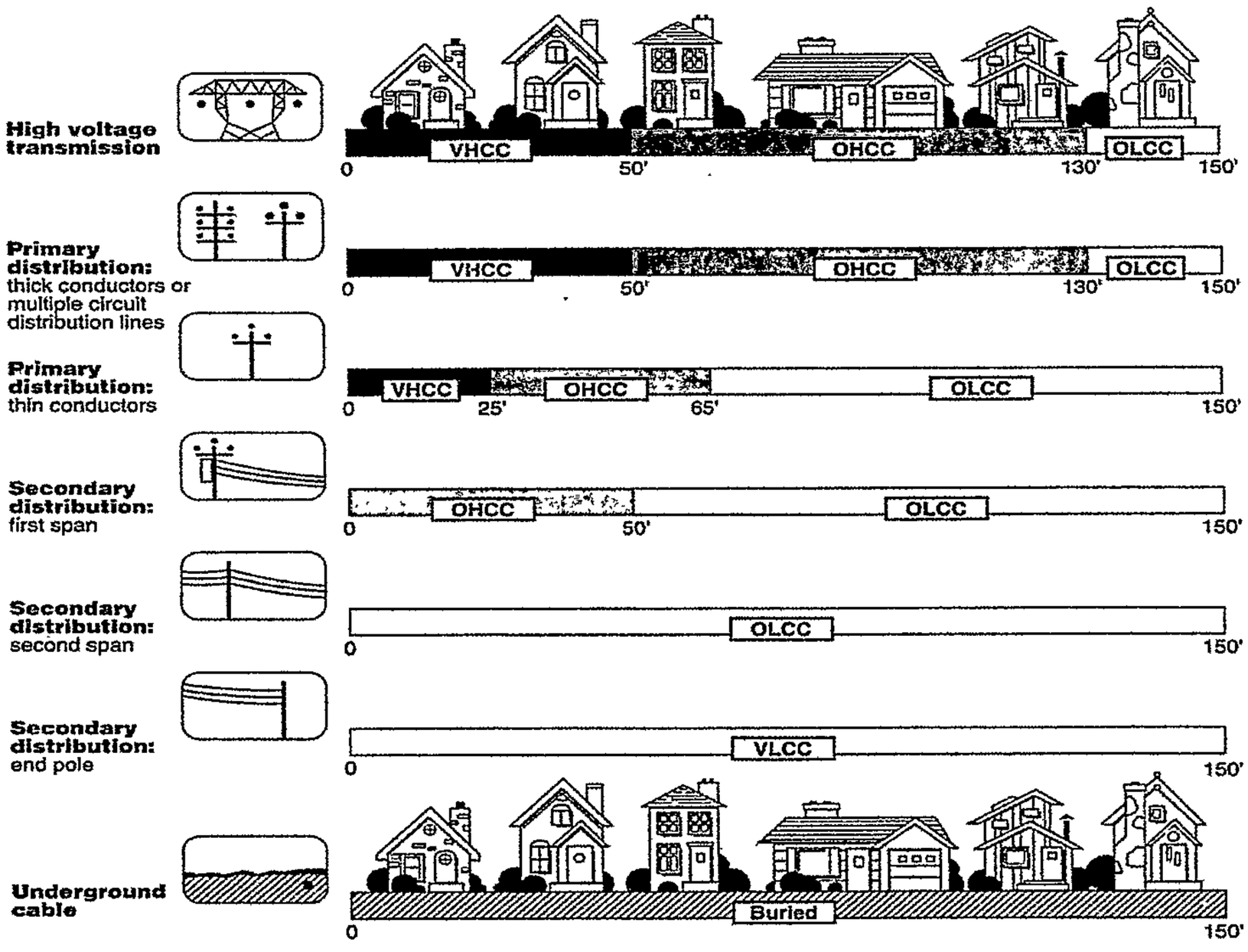
On the other hand, magnetic fields are largely unaffected by intervening structures and therefore might reflect more directly the operation of nearby power lines (Giancoli, 1995). Exposure assessments in the residential studies (eg. wire codes and distance to power lines) have been viewed as estimates or indirect measurements of some aspect of magnetic-field exposure experienced by the subjects before their disease was diagnosed (Myers et al., 1990). Because of limitations in instrumentation or available data, the magnetic-field characteristic examined is usually a short- or long-term root mean square average field (MacDonald, 1975). Each of the exposure assessment methods has its strengths and weaknesses. The following sections briefly describe the major types of exposure assessment used.

1.7.2 Wire Codes

This indirect measure was used in the first epidemiological study of presumed exposure to magnetic fields and cancer risk (Wertheimer and Leeper 1979). This means of exposure quantification was subsequently used in later studies with several refinements and modifications. Categories of wire codes and how they relate to the high-voltage transmission and distribution lines are illustrated in Figure 1.3. (EPRI, 1993).

The use of the wire code illustrated in Figure 1.3 and its modifications has a qualitative physical rationale but also significant quantitative limitations. Its use in previous epidemiological studies was based on the assumption that the wire code reflects the average exposure to 50-Hz magnetic fields. The rationale of this assumption is that larger power lines with thicker wires, which serve more residences and other consumers of electricity, carry more current and therefore provide a measure of exposure in the past and over a prolonged period. The merit of this rationale is that it considers (albeit in a qualitative manner) several of the factors used to calculate power-line magnetic fields; however, the reliability of the wire codes as a quantitative measure of exposure to 50-Hz magnetic fields is very limited. The following diagram is a summary of the characteristics of the wire codes as used in epidemiological studies (Wertheimer & Leeper, 1979, Savitz, et al., 1988, London et al., 1991).

Fig 1.3 A simplified schematic of the basic features of the differences in the wire codes as defined to support epidemiological studies. VHCC, OHCC, OLCC, and VLCC stand for very high, ordinary high, ordinary low, and very low current configurations. (EPRI, 1992).



Although the rank ordering of fields in homes is predicted reasonably well by wire codes, the wire code accounts for only 15-20% of the variance in magnetic-field measurements (National Research Council,1997). A large overlap exists between the estimated ranges of the alternating magnetic fields for various categories, for example, very high current configuration and ordinary high current configuration probably overlap significantly when actual measurements are made (National Research Council,1997 . There are large differences between the magnetic fields for different studies in different geographic locations for the same categories of the wire code. For instance, in a comparison between Los Angeles (Wertheimer and Leeper, 1982) and Denver (London et al, 1991), the differences between the cities within one category (VHCC) were greater than the differences between categories (Table 1.4). Also, total exposure to 50Hz magnetic fields in single-family residences can also be affected by other factors including appliance fields and grounding system fields. In addition, the field from a power line adjacent to the residence is a dominant factor for high wire codes but not for low wire codes.

Table 1.4 Measured magnetic fields (μT) and the associated wire code current configurations

Study	*VLCC*	*OLCC*	*OHCC*	*VHCC*
Wertheimer & Leeper, 1982	<0.05μT	<0.05μT	0.12μT	0.25μT
Savitz et al., 1988	0.03	0.051	0.09	0.216
London et al., 1991	0.043	0.058	0.066	0.107

The possibility that wire codes better represent a characteristic of magnetic fields other than their magnitude at 50 Hz is relatively unexplored so far. For instance, they might reflect the numbers and magnitude of transients. If laboratory studies had clearly indicated that 50-Hz magnetic fields were carcinogenic at levels as low as those considered in the epidemiological studies, then there might be less concern about the limitations of the wire codes as measures of exposure (Polk and Postow, 1996).

1.7.3 Distance

Since the magnetic field decreases with distance from an operating power line, distance can be used as a crude predictor of the field. The utility usually has records showing whether a particular line was operating during the pre-diagnosis period. Because the actual field also

varies with line load and the geometric configuration of the power line, the use of distance can result in significant misclassification, particularly if a study involves power lines of several different designs. Because of its simplicity, distance is frequently used to assign subjects to two or more exposure groups (National Research Council, 1997).

1.8 Exposure Systems In Laboratory Experiments

1.8.1 *In vivo* Animal Studies

A reasonably uniform field in an animal-exposure system can be significantly perturbed by two factors. One factor, which is unavoidable but controllable, is due to the presence of test animals and their cages. A considerable amount of information is available on proper spacing of animals to ensure the same exposure field for all test animals (Kaune 1981a) and to limit the mutual shielding of test animals (Kaune 198lb;). Animal cages, drinking bottles, food, and bedding cause additional perturbation to electromagnetic fields (Kaune 1981a,b). Particularly, any metallic or other highly conductive objects or substances (eg. animal excrement) must be eliminated or kept to a minimum. Typically, cages are constructed out of Lexan plastic or perspex (Creim et al. 1984). One of the most critical problems resulting in perturbed experimental fields of some studies is induction of currents in the nozzle of the drinking-water container. If the induced currents are sufficiently large, animals experience electric microshocks while drinking. Corrective measures have been developed to deal with this problem (Free et al. 1981; Kaune 1981b). Perturbation of the exposure field resulting from nearby metallic objects is easy to prevent (Sears et al., 1987).

Current flowing through an arrangement of coils produces a magnetic field in an animal-exposure apparatus. The apparatus can vary from a simple set of two Helmholtz coils (either round, square or rectangular because of the geometry of cages), to an arrangement of four coils (Merritt et al. 1983), to more complicated coil systems (Stuchly et al. 1991; Kirschvink 1992; Wilson et al. 1994; Caputa and Stuchly 1996). The main objectives in designing exposure apparatus for magnetic fields are (1) to ensure the maximal uniformity of the field within the largest volume encompassed by the Helmholtz coils, and (2) to minimise stray fields outside the coils, so that sham-exposure apparatus can be placed in the same room. Limiting the stray fields is a challenge, as shielding magnetic fields is much more complex than

shielding electric fields. Non-magnetic metal shields provide only a small reduction in the field strength. Only properly designed multi-layer shielding enclosures made of high-permeability materials, such as rubber or oil, are effective at shielding magnetic fields (George, 1988). An alternative solution relies on partial field cancellation. Two systems of coils placed side by side or one above the other form a quadrupole system that results in a substantially faster decrease of the magnetic field as distance from the system is increased (Harvey 1987; Stuchly et al. 1991). An even greater decrease is obtained with a doubly compensating arrangement of coils. Four coils (each consisting of four windings) are arranged side by side and up and down; coils placed diagonally are in the same direction as the field, and the neighbouring coils are in the opposite direction (Caputa and Stuchly 1996).

Common potential artefacts associated with magnetic-field-exposure systems are heating, vibrations, and audible or high frequency (non-audible for humans) noise. These factors can be minimised but not entirely eliminated with careful design and construction, which can be costly (Kirschvink 1992; Caputa and Stuchly 1996). To limit the vibration and noise, the coil windings should be restricted mechanically in their motion by the use of some sort of binding material.

Furthermore, the animal cage support system must be physically separate from the coils and their support system. Vibration and noise are inherently limited but not eliminated in a plate type of exposure system compared to a coil system (Miller et al. 1989). In general, the higher the desired magnetic flux density in the exposure system and the larger the required volume of the uniform field, the more severe the problems of heating and vibrations become.

Another important feature of a properly designed magnetic-field system is shielding against the electric field produced by the coils. Depending on the coil shape, the number of turns in the coil, and the diameter of the wire, a large voltage drop can occur between the ends of the coils. That produces exposure to the electric field in addition to the magnetic field. The electric-field strengths are different in the sham-exposure systems from those in the field-exposure systems. The electric field can be eliminated easily by electrostatic shielding of the coil windings (Giancoli, 1995). Shielding of the connecting wires is also recommended (Kaune, 1981b). Typical magnetic flux densities used in animal studies range from 10 μT to l mT, and densities up to 5 or 10 mT have been used in a few studies (See section 1.9.3). The

rationale for selecting a high flux density in some studies is given in terms of interspecies scaling of the induced current values from humans to rodents.

1.8.2 *In vitro* Cellular Studies

Similar types of coils to those used for animal studies can be used for *in vitro* studies (Misakian et al. 1993). However, illumination is generally not important in cellular experiments, so the investigator can use either solenoidal coils (which can interfere with illumination), or Helmholtz coils (which are more open and do not interfere with illumination).

In addition, *in vitro* exposures need to be characterised with respect to chambers, such as a petrie dish, flask, or tubes, holding the preparation and their placement in the field (orientation with respect to the direction of the applied magnetic field). This information is required for evaluation of induced electric fields and currents. These field and currents can be calculated for simple chamber geometries provided that the medium conductivity is known and the cell density is low (Misakian et al. 1993). The evaluation of the induced currents and fields is more complex for high-density cells in a monolayer, a confluent monolayer, or a tissue preparation.

In *in vitro* studies, special care has to be devoted to ambient levels of 50 Hz and to other magnetic fields. It is not uncommon that magnetic flux densities in incubators exceed the desired ambient level of approximately 0. I μT by 10-fold or more (Kaune 1981b). Similarly, some other laboratory equipment with electric motors might expose cells to high, but unaccounted for, magnetic flux densities. The potential problems with exposures that are unaccounted for or that are at incorrect levels, as well as the critical influences of temperature and carbon dioxide level on some cell preparations, can result in unreliable findings in laboratory experiments. The differences between the actual sham-exposure conditions and the assumed "no-exposure" conditions, as well as differences in cell density, suspending medium, and treatment of the cell preparation, quite likely explain the difficulties in reproduction of the test results by other laboratories and in corroboration of various experiments.

1.9 Magnetic Field Exposure Studies

1.9.1 Human Epidemiological Studies

1997 saw the publication of an important epidemiological study of childhood leukaemia and magnetic fields in America by Linet et al. This study represented a significant advance, both in terms of methodology and size. Whilst its findings have been interpreted in a variety of ways, they were largely negative and swung the balance of evidence further away from the suggestion that low frequency magnetic fields cause cancer.

Over the past 21 years, there have been 20 studies of children and adults evaluating residential exposures to magnetic fields and proximity to power lines in relation to the risk of cancer. The findings have been mixed for children and generally negative for adults, although correlations have not been observed between directly measured residential EMF exposures and risk. These are summarised in tables 1.5 and 1.6.

In 1995, Bowman et al., hypothesised that the risk of childhood leukemia might be related to specific combinations of static (geomagnetic) and power-frequency fields. Childhood leukemia data from the Los Angeles were analysed on the basis of these combinations. No correlation of cancer with measured static or power-frequency fields were found; but the authors claimed a positive trend for the combined power-frequency and static field data. An issue not addressed by the authors is that all resonance theories require a specific orientation between the power-frequency and the static field. Thus it should not be the total static field that matters, but only the component of the static field with the right orientation to the power-frequency field.

Table 1.5: Residential electric and magnetic field exposure and cancer: studies of childhood cancer

Study	*Geographic area of study*	*Time*	*Number of cases*	*Number of controls*	*Exposure assessment strategy*	*Findings*
Wertheimer & Leeper, 1979	USA (Denver, Colarado)	1950-1973	344 cases, 491 residences	344 cases, 472 residences	Wire codes	↑ incidence of leukaemia and brain cancer
Fulton et al., 1980	USA (Rhode Island)	1964-1978	119 cases, 209 residences	240 cases, 240 residences	Wire codes	No excess incidence of leukaemia
Tomenius 1986	Sweden (Stockholm County)	1958-1973	716 cases, 1,172 residences	716 cases, 1,015 residences	Wire codes, Spot field measurements	↑ incidence of total cancers if living close to 200kv powerlines No excess incidence of leukaemia or brain cancer for either type of exposure
Savitz et al., 1988	USA (Denver, Colarado)	1976-1983	356	278	Wire codes, Spot field measurements	↑ incidence of brain cancers for high current configurations wires but not associated with high magnetic fields
Coleman et al., 1989	UK (SE London)	1965-1980	811	1,614	Wire codes	No excess incidence of leukaemia
Myers et al., 1990	UK (Yorkshire Health Region)	1970-1979	419	656	Distance from overhead power lines	No excess incidence of leukaemia, solid tumours or total cancers
London et al., 1991	USA (Los Angeles County)	1980-1987	331	257	Wire codes, Spot field measurements, 24 hour field measurements, household appliance use	↑ incidence of leukaemia for high current configurations wire codes, but not for measured fields

Feychting & Ahlbom, 1993	Sweden	1960-1985	142	558	Wire codes, Spot field measurements	No ↑ incidence in cancer for measured fields; ↑ incidence of leukaemia for calculated wire code fields of 0.4μT
Olsen et al., 1993	Denmark	1968-1986	1,707	4,788	Wire codes	No significant ↑ for leukaemia, brain cancers or lymphoma
Verkasalo et al., 1993	Finland	1970-1989	140	129,800 in cohort	Wire codes	No significant ↑ for brain cancers in girls, or for leukaemia, lymphoma or other cancers in either gender
Petridou et al., 1993	Greece	-	-	-	Distance from substations and transmission lines	Living within 100m of a substation or 5m of a power line not associated with leukaemia
Fajardo – Gutierrez et al., 1993	Mexico City	-	81	77	Distance from power lines	No ↑ incidence of leukaemia
Linet et al., 1997	9 US states	1989	638 with Acute Lymphoblastic leukaemia	613 matched controls	Measured over 24 hour period by subjects wearing monitors	No association of measured fields or wire codes with leukaemia

Table 1.6 Residential Electric and Magnetic Field exposure and cancer: Adult Studies

Study	*Geographic area of Study*	*Time*	*Number of cases*	*Number of controls*	*Exposure assessment strategy*	*Findings*
Wertheimer & Leeper, 1982	USA (Denver, Boulder and Longmont, Colorado)	1967-1975 (Boulder and Longmont) 1977 (Denver)	1,179	1,179	Wire codes	Excess incidence of total cancer and brain cancer found, but not for leukaemia
McDowall, 1986	UK (East Anglia)	1971-1983	213	7,631 in cohort	Distances from substations and distribution lines	No overall ↑ in cancer, leukaemia or female breast cancer
Severson et al., 1988	USA (Washington)	1981-1984	164	204	Wire codes, 24 hr field measurements spot field measurements	No excess incidence of leukaemia for either wire codes or measured fields
Coleman et al., 1989	UK (SE London)	1965-1980	811	1,614 cancer controls, 254 population controls	Wire codes	No excess incidence of leukaemia found
Youngson et al., 1991	UK (NW and Yorkshire)	1983-1985 (NW) 1979-1985 (Yorkshire)	3,276 (1,491 in NW, 1,770 in Yorkshire)	3,144 (1,491 in NW, 1,653 in Yorkshire)	Distance from overhead power lines, calculated fields	No excess incidence of leukaemia or lymphoma found
Schreiber et al., 1993	Netherlands	1956-1987	431	3,549 in cohort	Wire codes	No cases of leukaemia or brain cancer found
Feychting & Ahlbom, 1994	Sweden	1960-1985	548	1,091	Wire codes, spot field measurements	No ↑ in leukaemia or brain cancer

1.9.2 Genotoxic Assays

Several cellular assay systems exist for detecting genotoxicity. Traditionally these have been mutagenesis tests. Other tests detect chromosome aberrations, sister chromatid exchanges, DNA strand breaks (comet assays), micronuclei formation, and cell transformation. These assays are listed in table 1.7. A replicated positive result in any of these genotoxicity assays would be evidence that an agent was a potential carcinogen.

Table 1.7 Laboratory Tests for Genotoxic Activity (Moulder, 1998)

Test	*Description*
Cancer induction (in vivo)	Test for increased cancer in animals. Animals are exposed to an agent for long periods of time (often for lifetime) and examined for an increase in cancer.
Mutagenesis (in vivo)	Test for changes in the genetic material of eggs or sperm than can be passed on to offspring. Animals are exposed to the agent and then mated, and their offspring are examined for inherited defects. Alternatively, the offspring are examined for changes in the sex ratio, since mutations are more likely to kill male than female offspring.
Mutagenesis (in vitro)	Test for changes in the genetic material of cells that can be passed on to their progeny (daughter cells). Cells are exposed to an agent, and their progeny are examined for inherited changes.
Sister chromatid exchanges, SCEs (in vivo or in vitro)	Test for the presence of breakage and rejoining of pieces of chromosomes. The test can be applied to white blood cells from exposed organisms (including humans) or to cells exposed in cell culture.
Micronucleus formation (in vivo or in vitro)	Test for the presence of pieces of chromosomes that have become detached as a result of damage to the genetic apparatus of the cell. The test can be applied to white blood cells from exposed organisms (including humans) or to cells exposed in cell culture.
DNA strand breaks (in vivo or in vitro)	Test for the presence of breaks in the genetic material of cells (the DNA), as opposed to breaks in the chromosomes.
Cell transformation (in vitro)	Tests for whether cells growing in cell culture undergo a set of changes when exposed to an agent that resemble their response to a carcinogen. These changes include loss of density-dependent inhibition of cell growth (loss of "contact inhibition") which causes cells to pile up ("focus formation"), and acquisition of the ability to grow in soft agar ("anchorage-independent cell growth").

1.9.3 Cell Culture Studies

Several studies testing the potentially carcinogenic effects of magnetic fields using cell cultures have been performed, with many of these studies adequately replicated. These have been performed using the assays listed in table 1.7. For mutagenesis assays, alternating magnetic field strengths of 0.012 to 1000μT have not been found to be mutagenic in

experimental work on bacteria or yeast. (table 1.8). Separate to this are reports on work with mammalian cells by Miyakoshi et al (1996,1997) which describe mutagenesis using levels of magnetic fields of 400,000μT. However, these high levels are not thought to be relevant to human health, as the levels are several hundred thousands higher than those humans would be exposed to. Other studies with mammalian cells using field strengths of 1-300μT found no effects (Nafziger et al., 1993, Suri et al., 1996) (table 1.8).

Table 1.8 In vitro assessment of genotoxicity of magnetic fields: mutagenesis assay

Reference	*Duration*	*Magnetic Field Exposure (AC)*	*Findings*
Juutilainen& Liimatianen, 1986	6.5 or 48 hrs	0.12,1.2,12.or 120μT	No excess mutations in bacteria
Ager and Radul, 1992	15 min on/off for 3hrs, then 3-6 days continuous	1000μT	No excess mutations in yeast
Nafziger et al, 1993	7 days	1μT	No excess mutations in mammalian cells
Suri et al., 1996	120 hrs	3000μT	No excess mutations in mammalian cells
Miyakoshi et al., 1996,1997	1-20 hrs	400000μT	Excess mutations in human tumour cells

Five studies assessing the ability of 0.2 to 5000μT magnetic fields to cause DNA strand breakage have found no effects either (table 1.9). The assay used in these studies is referred to as the Single Cell Gel Electrophoresis Assay (SCGEA), also known as the Comet Assay. This will be discussed in further detail in section 1.11.

Table 1.9: In vitro assessment of genotoxicity of magnetic fields: Comet assay

Reference	*Duration*	*Magnetic Field Exposure (AC)*	*Findings*
Reese et al., 1988	1hour	100 or 2000 μT	No ↑ in DNA strand breakage in mammalian cells
Novelli et al., 1991	1-24 hours	200μT	No↑ in DNA strand breakage in yeasts
Fiorani et al., 1992	1-24 hours	0.2,2,20,100 or 200 μT	No↑ in DNA strand breakage in human tumour cells
D'Agruma et al., 1993	48 hours	0.2-200 μT	No ↑ in strand breakage in bacterial DNA
Fairbairn and O'Neill, 1994	1 or 24 hours	5000μT	No ↑ in DNA strand breakage in mammalian cells

A potential relationship between cancer-cell growth and magnetic field exposure was described by Liburdy et al. (1993). In this study, human oestrogen responsive breast cancer cells (MCF-7 cell line) were used. These cells grow rapidly in the presence of normal concentrations of female sex hormones, but their growth rate is decreased by a hormone produced by the pineal gland, melatonin. Other studies have reported that melatonin synthesis is altered by exposure of whole animals to extremely-low-frequency (ELF) EMF (Wilson et al. 1990), and it has been proposed that disruptions of the normal daily cycles of melatonin synthesis are a risk factor for human breast cancer (Stevens 1987a,b).

Liburdy et al. (1993) confirmed that melatonin at normal physiological concentrations could decrease the growth rate of MCF-7 cells. However, application of a 1.2μT (12-mG) sinusoidal magnetic field at 60 Hz prevented the oncostatic action of melatonin on the breast cancer cells. A lower field of 0.2 μt (2 mG) did not have any significant effect, suggesting that a threshold might exist between 0.2 and 2 μT.

However, in June 1999, the US National Institute of Health (NIH) office of Research Integrity announced that Robert Liburdy had engaged in scientific misconduct by intentionally falsifying and fabricating data and claims about purported cellular effects of EMR reported in two scientific papers (NIH Guide, 1999). The papers (Liburdy, 1992 a,b) reported that EMR exerted a biological effect by altering the entry of calcium across a cell's surface membrane. These claims were potentially important as they linked EMR and calcium signalling, a fundamental cell process governing many important cellular functions. As a result, studies by this author would need to be replicated by other laboratories before further conclusions can be made.

1.9.4 Animal Studies

There are only a limited number of long term animal exposure studies looking at the genotoxicity of ELF and carcinogenesis. They are listed in table 1.10. Exposures have been variable, ranging between 20μT and 24,000μT with exposure times from 2 weeks to 2 years. Depending on the country in which the experiments were performed, the frequency was either 50 Hz or 60 Hz. Almost all of the studies found that there was no increased incidence of tumours in the animals, although Beniashvili et al., (1992) found an increase in mammary

tumours in rats and Fam and Mikhail (1996) found an increased incidence of lymphoma over 3 generations of mice. However, exposure levels were 24,000μT, which is several thousand times higher than a person would be exposed to if living close to a power line. Nonetheless, these studies did suggest a positive effect worthy of further investigation.

Table 1.10 in vivo Animal studies of magnetic fields and carcinogenesis (Moulder, 1998)

Reference	*Duration*	*Magnetic Field Exposure (AC)*	*Findings*
Bellossi, 1991	30min/day 2 weeks over 5 generations	6000μT	No ↑ in leukaemia in leukaemia prone mice
Beniashvili et al., 1992	30 min/day for 104 weeks	20μT	No ↑ in mammary tumours in rats
Beniashvili et al., 1992	3 hours /day for 104 weeks	20μT	↑ in mammary tumours in rats
Rannug et al., 1993	20hour/day for 103 weeks	50 or 500μT	No ↑ in skin tumours, leukaemia or lung adenomas in mice
Fam & Mikhail, 1996	3 generations	24,000μT	↑ in lymphoma incidence in mice
Yasui et al., 1997	23 hours/day for 104 weeks	500or5000μT	No↑ in incidence of any tumours in rats
Mandeville et al., 1997	20 hours /day for 104weeks	2,20,200 or 2000 μT	No↑ in incidence of any tumours in rats
Harris et al., 1997	20 hours /day for 78 weeks	1,100 or 1000μT	No↑ in lymphoma in lymphoma prone mice
Harris et al., 1997	15 min on, 15 min off , 20 hours/day for 78 weeks	1000μT	No↑ in lymphoma in lymphoma prone mice

1.10 Meta-Ananlysis

Since the publication of a seminal study by Wertheimer and Leeper (1979), scientists, policy makers, and the public have attempted to make sense of provocative and conflicting studies about the possible association between exposure to electric and magnetic fields and the incidence of disease. As this controversy continues, organising and reviewing the existing data can provide important insights into the reasons for inconsistent results, gaps in investigative strategies, and limitations in understanding. Toward that end, a meta-analysis was undertaken of the most compelling subset of these data: residential exposure to magnetic fields or their surrogates and the incidence of childhood leukemia (National Research Council, 1997). This analysis is an attempt to gain an understanding of the importance of individual studies in prompting the overall conclusions of a possible link between exposure to electric and magnetic fields and cancer and the importance of the constraints needed in any

successive study to assure that it would have sufficient statistical power to influence the present conclusions.

Meta-analysis is a statistical method used to provide a single risk estimate that summarises the results of a set of similar studies (Dickersin and Berlin 1992; Petitti 1994). In epidemiology, meta-analysis is applied most often to clinical trial data in which the major differences among studies are the differences in specific populations examined rather than in characteristics of the study designs. The validity of broadening the application of this method to environmental epidemiology is controversial because of the differences among studies that might include different methods of exposure assessment, techniques for identification of controls, ways of accounting for such factors as confounders, and manner of subject selection. These differences might result in substantial heterogeneity, calling into question the logic of a single summary statistic (Blair et al. 1995) particularly for case-control studies. However, meta-analysis methods also can be used as an aid to evaluate the strength and consistency of an exposure-disease relationship, to look for design factors that might explain any heterogeneity, to conduct sensitivity and influence analyses, and to evaluate the robustness of additional studies of similar design.

The characteristics of 20 studies that have examined the possible association between residential exposure to magnetic fields and leukemia are shown in Tables 1.5 and 1.6. Of these, 13 studies have addressed childhood leukemia. Some studies report positive results, and others report no association. There is disagreement about the quality, bias, accuracy, and uncertainties in these studies, resulting in differing interpretations of the likelihood of a possible association overall. Some who examine the evidence find that the positive results are sufficiently compelling to conclude that an overall association exists (Ahlbom et al. 1993). Others argue that individual study results are artefacts due to systematic or random bias and that proper adjustment has not been made for multiple comparisons. Most conclude that the results, although interesting, do not show a consistent pattern of association (ORAU 1992; Peach et al. 1992; NRPB 1992). Recognising the great cost of additional studies, government agencies are grappling with the development of policies in light of uncertainties and controversies (National Research Council, 1997).

Three sets of investigators have previously conducted meta-analyses of childhood cancer and residential exposure to magnetic fields. A report by Great Britain's Advisory Group on Non-

ionising Radiation of the National Radiation Protection Board summarised results of the 12 childhood residential studies (studies 1-12 in table 1.5), providing pooled odds-ratio estimates for each exposure metric (NRPB 1992). For wire codes, excluding the Wertheimer and Leeper (1979) study, the board found a statistically significant increased odds ratio. For data based on the distance from the source of electromagnetic fields and for measured magnetic fields, no statistically significant increase in the pooled odds ratios were found although a slight apparent increase was noted. They concluded that in spite of the increased odds ratios, the small sample sizes (three for each estimate) and methodologic problems in each of the studies precluded drawing definitive conclusions.

Ahlbom et al., (1993) combined the results from three studies conducted in the Nordic countries (Olsen et al. 1993; Verkasalo et al. 1993; Feychting and Ahlbom 1993) and argued that because they believed those studies were more similar to one another than to other studies (all used a population registry and estimates of historical exposure), they were appropriate for use in a meta-analysis. By combining the risk ratios of those studies and assigning them weighting factors proportional to the inverse of their variances, Ahlbom and colleagues (I993) found statistically significant increased risk ratios for childhood leukemia.

Washburn et al. (I994) conducted a set of meta-analyses for leukemia, lymphoma, and nervous-system cancers. For the combined results of 13 studies (table 1.5), they found increased risks for all three diseases; those for leukemia and nervous system tumours were statistically significant. Their sensitivity analyses showed that the inclusion or exclusion of data that overlap in the two Swedish studies and the choice of exposure metric had a limited effect on the results.

1.11 Australian Exposure Guidelines

There are currently no Australian standards regulating exposure to electric and magnetic fields. The National Health and Medical Research Council has issued *Interim Guidelines on Limits of Exposure to 50/60 Hz Electric and Magnetic Fields (1996)*. These guidelines are aimed at preventing immediate health effects resulting from acute exposure to these fields. The recommended magnetic field exposure limits for members of the public (24-hour exposure) are 1,000 mG (0.1 milliTesla) and for occupational exposure (whole working day) is 5,000 mG (0.5 milliTesla).

The NHMRC notes that "although there are limitations in the epidemiological studies that suggest an increased incidence of cancer among children and adults exposed to 50/60 Hz fields, the data cannot be dismissed. Additional studies will be required before this data can serve as a basis for risk assessment". In other words, because the research data does not indicate an exposure level at which a cancer risk exists, it is simply not possible to determine an exposure limit below which that risk would disappear. Hence, the above NHMRC limits do not apply to the avoidance of cancer risk resulting from chronic exposure to 50/60 Hz magnetic fields.

1.12 Comet Assay

1.12.1 Background

Several methods have been developed to measure DNA strand breakage produced in individual cells. Rydberg and Johanson (1978) described a technique for analysing single cells based on differential lysis of irradiated cells in alkali. Rydberg went on to adapt this technique for flow cytometry in 1984. For this process he mixed cells with agarose beads prior to irradiation and alkaline lysis treatment. Roti Roti and Wright (1987) developed an assay they called the halo assay in which DNA strand breakage allowed relaxation of supercoiled DNA.

It was in 1984 that Ostling and Johanson presented the idea of performing gel electrophoresis on individual cells, which had been embedded in agarose. Negatively charged DNA fragments were drawn towards the anode during electrophoresis. This technique later became known as the single cell microgel electrophoresis assay or simply the microgel electrophoresis assay. This microelectrophoretic technique enabled the direct visualisation of DNA damage in individual cells (Ostling and Johanson, 1984). Olive (1991) went on to name this the Comet Assay based on the appearance of the cells under fluorescent microscopy.

The original method set out by these authors was performed under neutral conditions and was found to be sensitive for detecting single stranded breaks of DNA. But such conditions were ineffective for removing all the proteins during lysis and subsequent authors developed more stringent lysis conditions which could allow for detection of double stranded breaks as well.

Two independent laboratories set about refining the comet assay for detecting single stranded breaks (Olive 1989, Singh et al., 1988).

Since then, these authors and several others have set about refining these techniques as the interest in the comet assay has increased. This is probably because of the technique's ability to display DNA damage in an individual cell within a population. The technique also allows for examination of DNA repair under a variety of conditions, hence proving valuable in the study of mechanisms of genotoxicity and repair (Fairbairn et al, 1995).

1.12.2 Methodology

The method described by Ostling and Johanson (1984) involved five basic steps. In the first step single cells were exposed to ionising radiation then embedded in agarose on a microscope slide. Next the cells were lysed in a neutral high salt and detergent buffer to remove proteins. This was followed by a low voltage electrophoresis step in which DNA fragments were pulled towards the anode. Next was the staining step using acridine orange, a fluorescent dye. Finally the stained DNA from individual cells were viewed using a fluorescent microscope. The amount of DNA removed from the original position in the cells was measured with a microscope photometer and was used to measure DNA damage.

While the five basic steps have remained constant, several protocols have been published since with modifications at every step. This has been to increase the sensitivity and reproducibility of the assay. After exposure to the potentially DNA damaging agent, cells are embedded in agarose. There are two methods for this, one being the single layer technique where cells are mixed with agarose and layered directly onto a microscope slide (Fairbairn and O'Neill, 1994, Olive, 1989) or the sandwich technique where cells in agarose are layered over a slide already covered in plain agarose, then covered by another layer of plain agarose (Pool Zobel et al., 1993, Arlett et al., 1993)

The second step of lysis has been modified depending on whether single stranded or double stranded DNA break detection is required. Singh et al. (1988) and Olive et al. (1990) found that alkaline lysis allowed denaturation of the duplex DNA and made it possible to specifically detect single stranded breaks as well as double stranded breaks. To detect only double stranded breaks, the assay only needs to be performed under neutral conditions (Olive et al,

1991, Marples et al., 1998, Hu and Hill, 1996). However this needs to be coupled with more extensive lysis.

The third step of electrophoresis has undergone probably the most modification. The timing and voltage used is largely empirical. Timing ranges from a few minutes to an hour, and voltages range from 0.5 volts per centimetre to 25 volts according to published literature (Fairbairn et al, 1995, Olive et al, 1990, 1991, Singh et al., 1988, 1994, 1995). DNA is really only required to migrate a fraction of a millimetre and thus very low voltages only are required for this technique. No consensus has been reached on this issue.

Several fluorescent dyes have been used for detecting the damaged DNA. Propidium iodide is a popular choice, mainly through its ease of availability (Olive et al., 1994) Other popular dyes include acridine orange (Ostling and Johanson, 1984,1987), DAPI (Olive et al., 1994), and ethidium bromide (Singh et al., 1988). Singh et al. (1992) claimed that a dye called YOYO-1 was an ideal dye for this technique as it could easily be detected under fluorescent microscopy when bound to even the smallest DNA fragment. There does not seem to be any other reports using this dye published in the literature.

The methods of image analysis are also as varied as the applications for which the comet assay has been proposed for (Fairbairn et al., 1995). Since comets are formed upon the principle of releasing damaged DNA from the core of the nucleus with electrophoresis, several different attempts have been made to evaluate and quantify comet formation. The simplest is to see whether or not damage has occurred by searching for the presence of a comet under fluorescent microscopy (Olive et al., 1990, Olive and Durand, 1992, Cerda et al 1997). This is because the assay produces a visual end point. It is also possible to grade the damage detected (Fairbairn et al., 1995), but this can be confounded by observer bias. More commonly the distance of DNA migration from the body of the core has been used to evaluate the extent of DNA damage (Fairbairn et al., 1995, Fairbairn and O'Neill 1994, Singh et al., 1990, 1994).

An increasingly popular method of comet evaluation is an endpoint referred to as the comet moment, which is defined as the product of tail length and the fraction of total DNA in the tail (Olive 1989, Olive et al., 1990). Even with this method there has been a lack of inter observer aggreement. Another problem is that this technique depends on the intensity of the fluorescent

comet, which may vary with the type of dye used, and also with the fading of the dye as it is exposed to light.

1.12.3 Advantages

There are several advantages of the comet assay, which is why it has become popular in the current literature. Firstly, only a small sample of a few thousand cells is required to perform the assay. The slides of the gels can be dried and stored for analysis up to a year later (Lai and Singh, 1996). It is a sensitive test that can detect extremely low levels of damage in DNA (Singh et al., 1994, Malyapa et al., 1993). This assay has been reported to be sensitive enough to detect one break per 2x10 10 Da of DNA in lymphocytes (Singh et al 1994). Another advantage of the technique is that virtually any cell can be analysed (Fairbairn et al., 1995).

1.12.4 Disadvantages

Unfortunately, the technique is not standardised and several protocols exist in the literature (Fairbairn et al., 1995), making it difficult for the technique to be widely used without thorough knowledge of the methodology. This means that anyone planning to use the assay has to run a series of trials so that they can establish a protocol that suits their laboratory and exposure conditions the best. Lysis and electrophoresis conditions need to be tailored to the expected level of damage. If DNA is damaged extensively, it may result in the loss of DNA or fragmented pieces that are too small to be detected by routine fluorescent microscopy (Olive, 1999). Another major problem is cells in the S phase of the cell cycle may appear as comets in the assay, thus confounding the results(Olive, 1999). This phenomenon was reported at a late stage of the work described in this thesis. Radiolabelling and cell sorting procedures may help minimise such errors so that replication forks and Okasaki fragments are not included in the measured response.

1.13 **Aims and Objectives**

To obtain a better indication of the effect of EMF upon DNA fragmentation in cells, the objectives were addressed through the pursuit of two specific aims:

1. To develop a simple protocol for the comet assay which can be used for rapid screening of DNA fragmentation in cells after exposure to a potentially genotoxic agent such as electromagnetic radiation.

2. To perform a pilot study using this protocol to screen for DNA fragmentation in SAOS-2 cell cultures exposed to 10μT of 50Hz electromagnetic radiation.

CHAPTER 2 MATERIALS AND METHODS

2.1 Development of A Protocol For The Single Cell Gel Electrophoresis Assay

2.1.1 Materials

Medium 199 (M199) was purchased from GIBCO (Grand Island, NY); bovine calf serum, L-glutamine and trypsin/EDTA were purchased from CSL (Victoria, Australia), proteinase K and DAPI was from Boehringer (GmBH, Germany) while Triton x-100 was from BDH chemicals (Victoria, Australia). All other chemicals were purchased from Sigma (St Louis, MO, USA).

2.1.2 Cells

The human osteogenic sarcoma cell line SAOS-2 was used in these studies. This cell line was chosen as it is a well established line which provides unlimited numbers of cells. The cells are quite hardy with a reported viability of 82% (Rodan et al., 1987) and are easily manipulated *in vitro*. The advantage in using such a cell line is that most of its characteristics are already well known (Fogh 1986).

The original cell line was derived from an 11 year old caucasian female (Fogh et al., 1977). The cells are immortal and the origins and the characteristics can be found in the text by the ATCC (American Type Culture Collection) Cell lines and Hybridomas, 8th ed. 1994. The cell line is catalogued as ATCC HTB-85 SAOS-2. The cells grow as a monolayer with a logarithmic growth and doubling time of 37 hours (Rodan et al., 1987).

2.1.3 Cell Culture

All cell culture work was performed in a laminar flow cabinet (Email Westinghouse, NSW, Australia) to reduce the possibility of cross contamination. No incidences of contamination were detected during the study. Standard laboratory aseptic techniques were employed throughout the procedures with all surfaces and gloves wiped down with 70% alcohol and all equipment being autoclaved before use. Media was filtered with a glass filter (Millipore) or with a Minisart filter (Sartorius, Germany) for smaller volumes. Cells were grown in sterile 75cm^2 flasks in an incubator (Nu Aire CO_2 water jacketed incubator, supplied by Quantum Scientific,

Australia) at 37°C in a 5% carbon dioxide atmosphere with a relative humidity of 100%. The purpose of the carbon dioxide is to prevent alkalinisation of the media. Cells were cultured at a physiologic pH of around 7 pH units. An environment that is either too acidic or basic would have been toxic to the cells. The temperature, humidity and pH conditions maintained throughout this experiment provided the cells with optimal conditions for growth.

2.1.4 Passaging of SAOS-2 Cells

Confluent SAOS-2 cell cultures were rinsed with culture medium M199 and then dispersed using trypsin-EDTA every fourth day. Following neutralisation with complete medium containing M199 and 10% bovine calf serum (BCS), the cells were centrifuged and resuspended in fresh medium containing 10% serum and seeded into three separate, new 75cm^2 culture flasks (Appendix 1).

2.1.5 Storage of Cells

In order to maintain a ready supply of available cells, SAOS-2 cells were regularly harvested and frozen in liquid nitrogen (Appendix 1). After trypsinisation, cells were centrifuged to form a cell pellet. The supernatant was removed and the pellet resuspended in complete medium containing 10% BCS and 10% dimethyl sulfoxide (DMSO). Cells were placed in 1.2ml cryogenic vials containing 10^6 cells/ml and cooled at the rate of -1°C /minute in a -70°C freezer overnight then stored in a liquid nitrogen tank.

2.1.6 Comet Assay Protocol

The core of this thesis was to develop a simple protocol for the single cell gel electrophoresis assay (SCGEA) which could be used for rapid screening of DNA fragmentation in cells after exposure to a genotoxic agent such as electromagnetic radiation. Several different variations of the protocol were tested before concluding with the one detailed below (See results for a description of variations tested in the development of this method).

Step 1: Preparation of the agarose-cell suspension

After exposing the cells to a known genotoxic agent such as hydrochloric acid or x-radiation, the cells were harvested and counted. Cells were resuspended in 5 ml HBSS to achieve a final concentration of 5,000 cells/ml. Counting of the cells was done using a haemocytometer (Appendix 1).

A 1% agarose gel was prepared by mixing 0.2g low melting point agarose (Sigma Chemical Company) with 10ml x50 TAE (0.04 M Tris-acetate, 0.001M EDTA, pH 8) buffer and transferring to a 15ml centrifuge tube after heating in a microwave to dissolve the powdered agarose (Appendix 1). The gel viscosity was kept constant by placing the tube in a 37°C water bath during preparation of experimental gels.

Several microscope slides (Mediglass Superfrost, 76x26mm, Nunc International) were wiped with 70 % alcohol and placed on a metal tray in a -20°C freezer for 10 minutes to cool the slides. 50µl of agarose gel was mixed rapidly with 50µl of cell suspension in a 1ml Eppendorf tube using a 200µl micropipette (Pipetman, Merck) to achieve an even distribution of cells in the agarose. Using the same pipette, the entire 100µl gel-cell suspension was pipetted on to the frosted section of a microscope slide to make a microgel. A clean coverslip (Mediglass-Microscope coverglass 22x50mm, Nunc International) was placed over the gel to reduce the gel thickness to about 0.5mm. Several gels were prepared at a time and the entire tray was placed back in the freezer for at least 15 minutes.

The tray was then removed from the freezer and the coverslips gently slid off the slides in a horizontal direction, making sure that the gel had not lifted off from the slide. If the gel lifted off, a new gel was prepared for the experiment as experience showed that these gels had a higher chance of being lost during latter stages of the procedure.

Step 2: Lysis of cells

Slides holding the gels were placed horizontally in a plastic multiple slide holder in ice cold lysis buffer (2.5mol NaCl, 1% N-lauroyl sacosinate, 100mmol EDTA, 10mmol Tris, pH 10) and also containing 1% Triton X-100 (Appendix 1). This is a high salt buffer which denatures the

duplex DNA, allowing individual strands to separate and migrate independently. The alkali also causes strand breakage at alkali labile sites (Singh et al., 1988, Olive et al., 1990). Both neutral and alkaline lysis conditions allow for the detection of double stranded DNA breaks; however the alkaline assay facilitates further denaturation, unwinding and expression of single stranded breaks in DNA (Olive 1999). The gels in this buffer were placed in a fridge overnight at 4°C.

Deproteination was carried out the next day by adding proteinase K to the gels. Briefly, the slides were removed from the lysis buffer and placed horizontally on a metal tray. 300µl of a solution containing lysis buffer and 1mg/ml of proteinase K was placed over each gel and quickly covered with a small square of Parafilm (American National Can Chicago, Illinois, USA). The entire tray was covered in foil and incubated at 37°C for 2 hours.

Following incubation, the tray was transferred to a –20°C freezer for 15 minutes and then the Parafilm removed. This step was essential, as after incubation, the gels were quite fragile and tended not only to break, but easily came away from the frosted microscope slide. Cooling the gel prior to handling prevented inadvertent loss of the gels.

Step 3: Electrophoresis

A low salt, no detergent electrophoresis buffer (300mmol sodium hydroxide, 1mmol EDTA, pH 13) in pyrogen free water from the MilliRO 12 Plus, MilliQ PF system (Millipore Industries)was freshly made for each experiment (Appendix 1 1). Prior to electrophoresis, the gels were rinsed with this buffer thoroughly as high salt levels have been found to retard migration of DNA during electrophoresis (Olive et al., 1992). The remaining buffer was added to a horizontal electrophoresis unit connected up to a power supply.

The slides were placed horizontally in the electrophoresis unit and covered with the electrophoresis buffer to a level of 5mm above the gels. The DNA was allowed to unwind in this solution for 40 minutes prior to electrophoresis.

Much of the variation in reported SCGEA protocols has been found in the electrophoresis step regarding the time and voltage supply. In this project a voltage of 0.75 V/cm over an 18cm

distance (total voltage 13.5V) over a 20 minute period was found to be optimal. Only small voltages were required as the DNA fragments only need to migrate a fraction of a millimetre for microscopic detection.

At the end of electrophoresis, the power supply was disconnected and the buffer overlying the slides carefully removed, making sure not to create "waves" as the gels were fragile due to heating from the electrophoretic action. Slides were handled so that they were always horizontal and placed on a tray.

Step 4: Neutralization

Round coverslips of 15mm diameter were placed in each well of a 12 well plate (CoStar, Cambridge, MA, USA). The coverslip made it easy to transfer the gels out of the wells on to another microscope slide for viewing under a fluorescent microscope later on. The size of the wells corresponded well with the size of the gels and the small size of the wells allowed their easy detection as the gels were transparent. Each warm gel was carefully slid off the slide into a well with the assistance of 1ml 400mmol Tris-HCl (pH 8) solution (Appendix 1). Tris-HCl neutralised the effects of the electrophoresis buffer. After gentle rinsing the Tris-HCl was aspirated with a 1ml pipette with a narrow bore plastic pipette tip so that the gel would not be aspirated into the pipette. 2ml of 400mmol Tris-HCl was added to the well and replaced twice more at 30 minute intervals.

Step 5: Dehydration

After removal of the Tris-HCl solution, 1ml of absolute alcohol was added to the well and left for one hour. Excess alcohol was removed and the gels allowed to dry at room temperature for 3 days. This was done to dehydrate the gel as it is reported in the literature that this procedure will allow storage of the gels for up to a year (Singh et al., 1995). This procedure meant that gels did not have to be immediately viewed under fluorescent microscopy and that imaging could be done at a single setting at a later date.

Step 6: Staining

Gels were stained with 1ml of 1μg/ml 4'6,-Diamidine-2'-phenylindole dihydrochloride (DAPI) solution. After staining for 10 minutes the stain was washed with copius amounts of MilliQ water.

Step 7: Fluorescent imaging

The coverslips containing the gels were carefully lifted with a pair of fine tipped college tweezers, placed on a microscope slide and viewed under a fluorescent microscope. Cells with the appearance of a comet with a head containing most of the DNA and a tail with fragmented DNA of various sizes were considered "comet positive" cells. Intact cells appeared as only the head of the comet. Cells were viewed using a Zeiss KS-400 epifluorescence microscope illuminated by a 100W mercury light and equipped with a DAPI filter (excitation at 330-380nm). This was attached to a 12 bit cooled CCD colour camera. For each experiment 100 cells per slide were recorded using a x20 objective. Cells were visually classified as either comet positive (comet tails visible) or comet negative cells (no tail visible).

2.2 DNA Fragmentation Detection In SAOS-2 Due To Electromagnetic Radiation Using The Single Cell Gel Electrophoresis Assay

Cell cultures were exposed to electromagnetic radiation at levels similar to those found directly under overhead powerlines. SAOS-2 cells used in the development of the assay were once again employed in a series of 9 experiments which were performed in triplicate. Cells that detached from the culture monolayer in the flask were apoptotic and were used as positive controls in these experiments. This is because apoptotic cells have significant amounts of DNA fragmentation and show up as comets under fluorescent analysis (Fairbairn et al., 1995).

2.2.1 Cell Culture

Stored SAOS-2 cells were thawed and cultured in 75 cm^2 culture flasks (Appendix 1). Once cultures became confluent, the cells were passaged into three 75cm^2 flasks as described

previously. One flask served as a source of cells for further experiments, while the other two were used in individual experiments, one as a control, and the other as the experimental flask, exposed to electromagnetic radiation. Floating cells that became detached from the monolayer of confluent cells due to apoptosis served as the positive control in these experiments.

2.2.2 Exposure System

Magnetic field exposure system

Each unit was designed to expose up to three 75cm^2 flasks to a homogenous oscillating magnetic field of 10μT at 50Hz.

Helmholtz coils

The units were constructed out of 0.5 mm copper coil, double coated with polyester amide insulation. Two coils, each of 100 turns and 40cm in diameter were attached to perspex sheets opposing one another and were separated by a distance of 20cm, which was equal to the radius of the coils. This arrangement is known as a Helmholtz configuration (Basset et al., 1981) and produces a homogenous magnetic field between the two coils. All bindings and frameworks were made out of non conducting materials (perspex) to avoid interference with the magnetic field. The coils were connected to a 240V power supply at 50Hz which was regulated to 9V via a power adaptor to provide a homogenous alternating magnetic field of 10μT (fig 2.2). This level was chosen as it is the maximum a person would be exposed to if living directly under, or within 100 metres, from an overhead power line (Sagan, 1996).

2.2.3 Arrangement Of Flasks In The Exposure System

The magnetic field measurements were performed using a Gaussmeter (RFI Industries, Australia) (fig 2.1) which consists of a probe that could be oriented to detect magnetic fields in all three planes of space. Flasks were arranged within the exposure apparatus to obtain a homogenous magnetic field. Up to two flasks at a time were placed between the Helmholtz coils in the incubator such that the magnetic field lines were perpendicular to the monolayer of cells (fig 2.3). The flasks were placed on a polystyrene platform to raise the cells to the centre

of the magnetic field. It is emphasized that it is the magnetic field which is of greatest interest with regard to potential health effects (National Research Council, 1997).

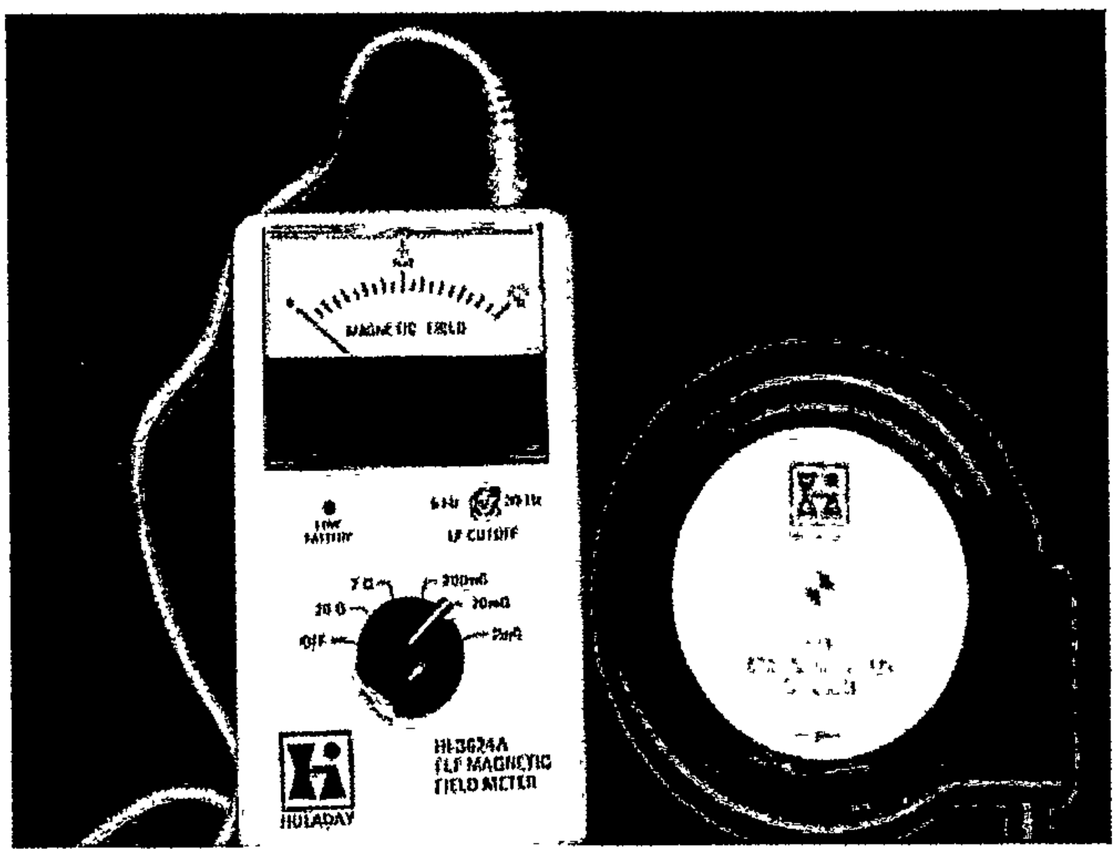


Fig 2.1: Gaussmeter used for measuring magnetic fields. The round probe on the right can be used to measure the field in all three planes of space.

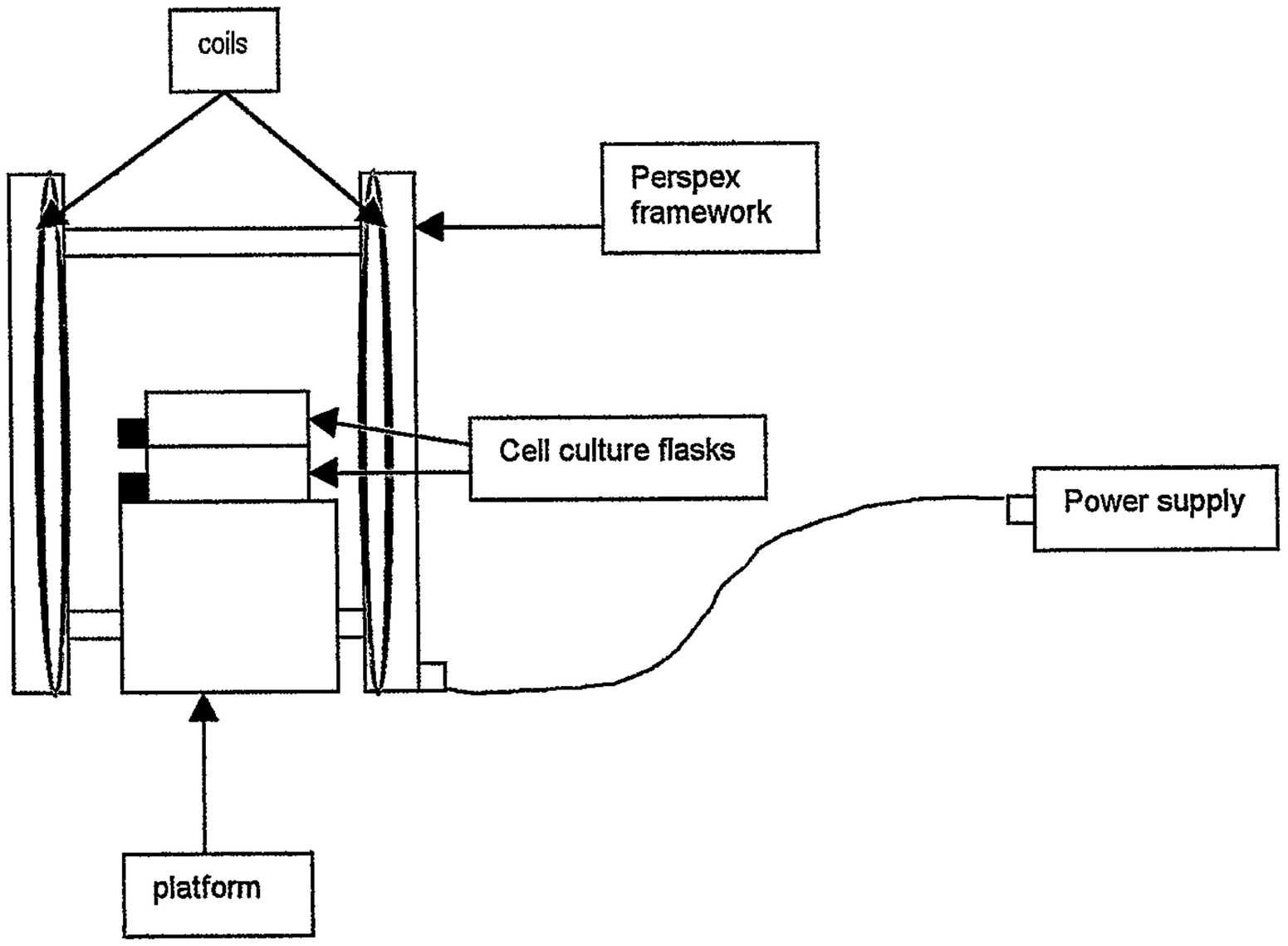


Fig 2.2: Diagram showing the arrangement of coils and cells in culture flasks in these experiments

The entire apparatus was transferred to an incubator (37°C, 5% CO_2, 100% relative humidity) and exposed to electromagnetic radiation for 7 days, with the complete medium being changed once at day 4.

One problem encountered was a rise in temperature in the incubator due to the energy generated from the coil exposure system. This was solved by moving the cells to an incubator (Labmaster, supplied by Quantum Scientific, Australia) with an automatically controlled thermostat which allowed for constant temperature regulation. As this incubator was not aerated with carbon dioxide, the flasks had to be filled with CO_2 enriched air then sealed prior to placement in the incubator.

The control cell culture was housed in similar incubation conditions, but without exposure to any detectable magnetic field. This was measured using a Gaussmeter (RFI industries, NSW, Australia) and found to be zero. The incubator itself did not seem to impart any magnetic field within the chamber.

The basis for this protection from extraneous fields in incubators is the low level of permeability to magnetic fields of the incubator, which is acting as a Faraday cage, preventing the entry of stray radiation (Giancoli, 1995).

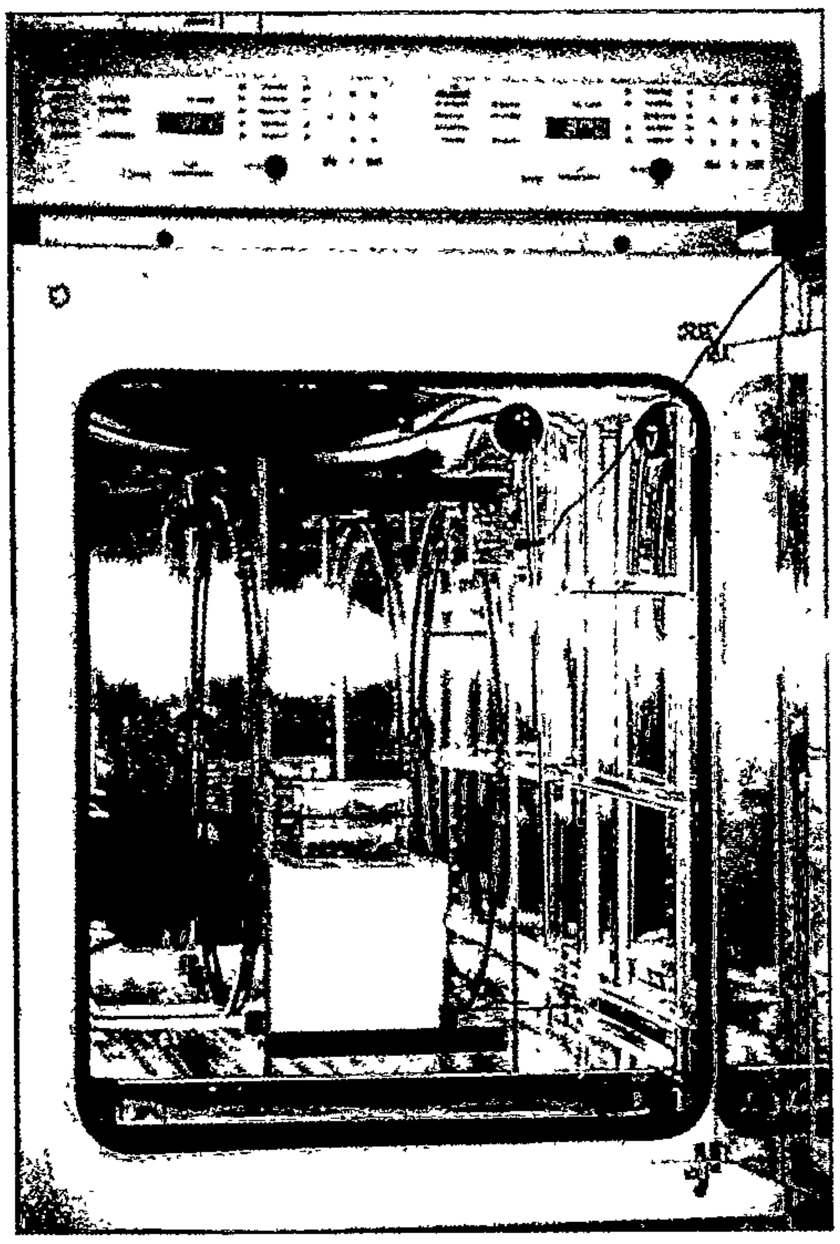


Fig 2.3 Photograph of flasks in the CO_2 incubator within the coil apparatus

2.2.4 Collection Of Cells

Both the exposed flask and the control flask were removed from the incubator and placed in a laminar flow cabinet that had been wiped down with 70% alcohol to reduce the risk of contamination. The medium from each flask was poured in to a centrifuge tube and labelled either positive control, irradiated, or positive control, not irradiated and centrifuged to form a pellet of cells. The supernatant was aspirated, taking care not to disturb the pellet which was then resuspended in 5ml HBSS and stored at 4°C until ready to use for the preparation of gels. Adherent SAOS-2 cells were washed with M199, trypsinized, harvested and centrifuged in 5ml of serum to form a pellet, and then resuspended in 5ml HBSS for the preparation of the gels.

2.2.5 Cell Counting

Cells from each of the four samples (detached, non irradiated (A1), adherent, non irradiated (B1), detached, irradiated (A2), adherent, irradiated (B2)) (fig 2.4) were counted using a haemocytometer (Neubauer, Germany), whereby cells in the centre and corner square on the counting chamber grid were counted and the number of cells per millilitre calculated. Cells were only counted in a particular square if they were wholly in the square or on the upper and left hand border of the grid. Each square of the haemocytometer represented a total volume of 10^{-4}ml. The average cell count per square multiplied by 10^4 provided the cell count per ml.

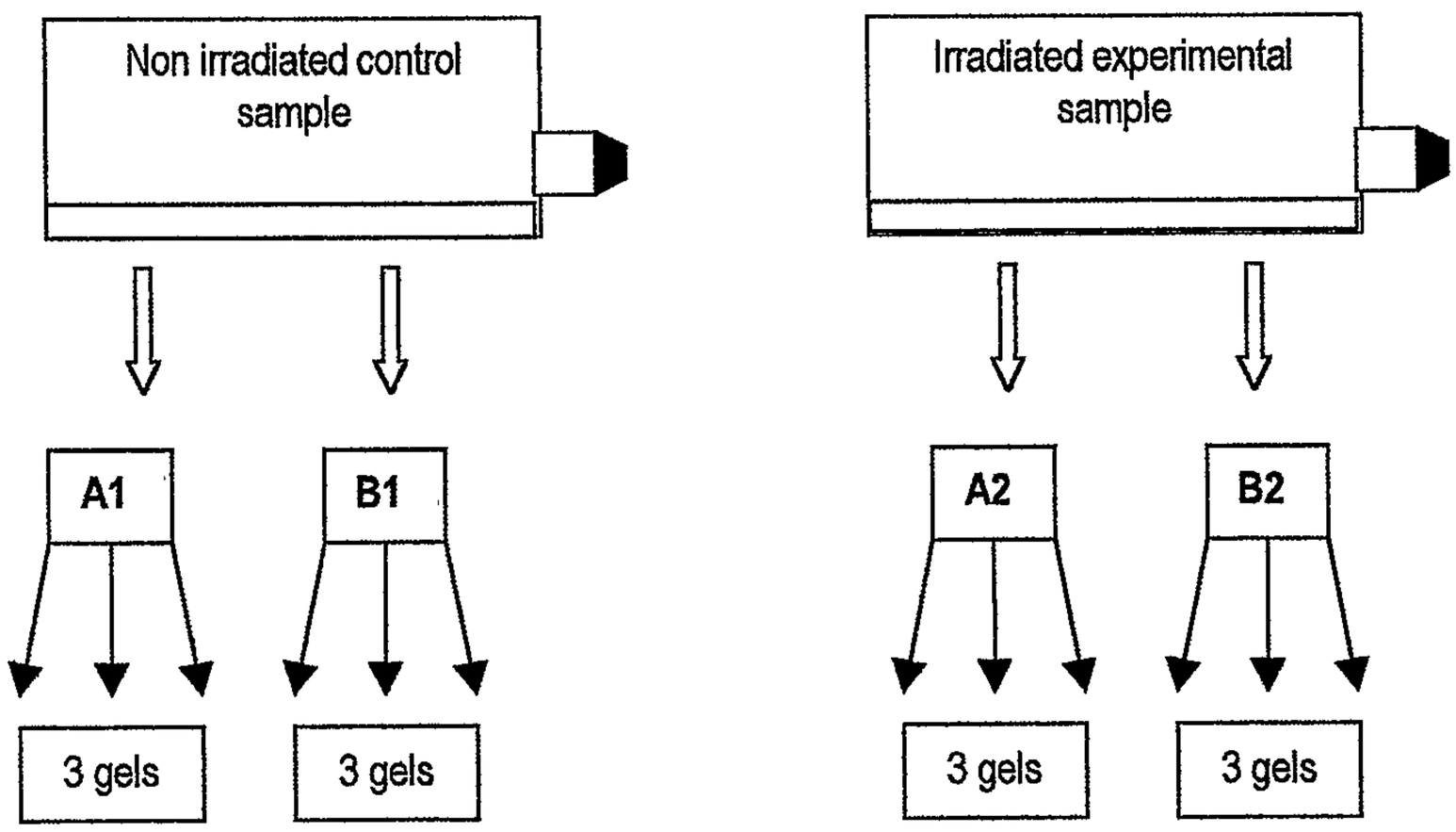


Fig 2.4: Apoptotic and viable cells were collected from each flask and mixed with 1% agarose gel to make 3 gels per sample, resulting in a total of 12 gels per experiment.

A single cell suspension of 1ml at a concentration of 100 cells per μL in HBSS was prepared to create gels with 5,000 cells per gel.

2.2.6 Single Cell Gel Electrophoresis Assay

Cells were embedded in low melting point agarose gel and subjected to the SCGEA as described in section 2.1. Gels for each sample (A1, B1, A2, B2) were made in triplicate and the experiment repeated 9 times.

2.2.7 Microscopy

Gels were placed on microscope slides after staining and viewed under a fluorescent microscope as described in the protocol. 100 cells per gel were counted, visually classifying the cells as either damaged (comet appearance of the cell) or undamaged.

2.2.8 Statistical Analysis

All experiments were performed in triplicate and the final experiment repeated 9 times. Results were analysed for statistical significance using the Wilcoxon matched pairs ranked sign test.

CHAPTER 3 RESULTS

3.1 A Modified SCGEA Protocol Was Used To Detect DNA Fragmentation In SAOS-2 Cells

A set of trial experiments were carried out using SAOS-2 cell cultures in order to establish the protocol. The protocol was based on the previously published methods of Singh et al. (1988,1991,1994,1995) and Olive et al. (1989,1991,1992,1998). These previous methods vary in detail but were too complicated for the purpose of this thesis, as our basic aim was to develop a simple protocol for the rapid screening of DNA fragmentation. The new protocol is detailed in the materials and methods.

Despite several protocols being published in the literature, a new protocol for use on SAOS-2 cells exposed to EMF needed to be set up for this thesis, suited to the available departmental conditions.

3.1.1 Increased gel concentrations were required for the SCGEA

Several setbacks of varying degrees were encountered in almost all of the steps outlined in the protocol. Various alternatives needed to be tested. In the initial stages of preparing the agarose gel-cell suspension, it was found that although providing further migration of DNA fragments through the gel after electrophoresis, the 0.5% gels were too fragile to cope with repeated handling through the experiments. A 1% gel was found to be optimal, both in handling characteristics and for adequate detection of DNA migration.

3.1.2 Frosted slides were necessary for gel adherence in the SCGEA

The gels had a tendency to slide off when the microscope slides were no longer horizontal. Several methods to encourage gel adherence to the slides were tested, including etching the slide surface with various grades of sandpaper, as well as phosphoric and hydrofluoric acid. L-lysine and positively charged slides were also tested. Slides with chambers (Lab Tek II, Nunc International) were also trialed, but during the electrophoresis stage when it became necessary to remove the chamber surrounding the gel, there was nothing left to secure the gels to the slides and they became dislodged.

The only way to secure the gels to the slides was to pipette the cell-gel suspension on to the frosted section of a Mediglass brand (Nunc International) microscope slide. Fully frosted slides were not available for purchase in small quantities, but it was found that the size of the gel created (0.8-1.0 cm diameter) was more than sufficient for embedding 5,000 cells as well as for stability in handling.

3.1.3 A single layer of agarose was sufficient for the SCGEA

A 'sandwich' agarose layering technique (Singh et al., 1995), where a layer of plain gel is layered on to a slide and then another layer of agarose gel containing cells layered on top of this was found to distort the image when viewed under fluorescent microscopy. This layering technique was not found to be useful in improving gel adherence to the slide. Hence a single cell-gel layer was used in this thesis.

Gels that were too thin after coverslips were placed over them were also found to be very fragile. An optimal thickness of about 0.5mm of the gel was necessary to cope with handling. Gels that were too thick provided problems with visualisation of the cells as cells appeared to be in several layers, making counting of cells and visualisation of comet tails difficult.

Rough handling during gel preparation was also found to be a critical factor in gel loss. If cover slips were not removed horizontally and the gels were even slightly lifted off, then it was quite likely that this gel would be lost at some stage of the experiment. Hence if this was noted, it was necessary to discard this gel and make a new one to replace it.

Even if gels seemed quite adherent to the frosted section of the slides, they still needed to be placed horizontally in the lysis buffer, as placing the slides vertically in the buffer was a high risk method for losing the gels.

3.1.4 Acid treated SAOS-2 cells were inadequate for use as positive controls in the SCGEA

Prior to using apoptotic cells as the positive control to test if the experiments had worked, there was an attempt to fragment the DNA in cells using high concentrations of hydrochloric

acid on harvested SAOS-2 cells. However, it was found that the presence of residual acid made the gels quite fragile, each of the gels being lost during the lysis phase of the protocol.

3.1.5 Optimal time and temperature conditions for lysis and deproteination were required

An overnight lysis was found to be necessary to denature the cellular DNA and to allow strands of DNA to migrate, thus providing for detection of single and double stranded DNA breaks. Shorter lysis durations would probably be better suited to double strand break detection only.

In the deproteination step, cooling of the gels prior to removal of the Parafilm was again important, as incubation at 37°C for 2 hours softened the gels, making them fragile. Freezing the gels for 15 minutes improved their handling characteristics, preventing unnecessary 'lift off' of gels.

3.1.6 Short exposures to low voltages were required in the SCGEA

The optimal voltage and duration of the electrophoresis step took the longest to establish. Several variations were listed in the literature, with each author claiming superior characteristics of their own protocol. It remained for each variation to be tested in the laboratory conditions being used in this thesis, as well as the cell culture and exposure type being used. Times and voltages that were too short were found to give inadequate visual distinction between the head and tail of the comet, often resulting in false positives. Duration and voltages that were too long resulted in excessive migration of DNA fragments, once again making it difficult to see the tail of the comet as the stained sections were spread out rather than being concentrated in a smaller area. The optimal time and voltage used in this thesis (13.5 V / 20 min) resulted in a comet tail about 3-4 times the length of the diameter of the head. This allowed for adequate DNA material to be stained and visualised with basic DNA stains like DAPI, without the need to resort to computerised, digital imaging systems, which are not only expensive, but time consuming as well.

Neutralisation with Tris-HCl for at least one hour, with three rinses, removed any remaining salts acquired from the lysis and electrophoresis buffers. This resulted in a gel that was easier to stain, with minimal debris detected on microscopy.

3.1.7 Storage of gels required alcohol dehydration

Some protocols do not indicate the use of an alcohol dehydration step. This is because fresh gels were stained and viewed immediately. On the other hand, dehydration of the gels meant that they could be stored for up to a year (Lai and Singh, 1996) and viewed at a single setting.

3.1.8 DAPI was an effective stain for use in the SCGEA

A variety of stains provided clear distinction of the head and the tail of the comet positive cells under fluorescent microscopy. There was no need to use elaborately sensitive stains such as YOYO-1 (Lai and Singh, 1996) as the aim was to only view the cells, not measure their tail lengths for DNA content analysis. The protocol in this thesis was intended for screening purposes only. Hence, fluorescent stains such as DAPI, propidium iodide and ethidium bromide were all adequate, with DAPI providing the clearest image of SAOS-2 cells .

3.2 Effects Of Low Level Electromagnetic Radiation On The Appearance Of Cells Displaying DNA Fragmentation In SAOS-2 Cells

3.2.1 Experimental design

The response of SAOS-2 cells was examined after seven days of exposure to a 10μT magnetic field generated by a 9V power source connected to a 240V standard wall power supply operating at 50Hz. Cells were counted prior to embedding in agarose gel to obtain 5,000 cells per gel. Three gels per sample were made and 100 cells examined per gel. The unwound, relaxed DNA was able to migrate out of the cell during electrophoresis and was visualised by staining with DAPI. The cells containing small DNA fragments appeared as fluorescent comets with tails of DNA fragments, whereas normal, undamaged DNA did not migrate far from their origin in the nucleus. Apoptotic SAOS-2 cells were used as the positive control for each experiment. This was performed for each experiment to ensure that the assay had worked.

3.2.2 Visual Classification Of Damaged Vs Undamaged Cells

Figure 3.1 shows the appearance of typical comet negative (fig 3.1a), comet positive (fig 3.1b) and apoptotic (fig 3.1c) SAOS-2 cell as visualised under a x40 fluorescent microscope after staining with DAPI. These images were used as the basis for visual scoring of damaged cells in the experimental population. Figure 3.2 shows the comet positive and comet negative cells under x20 magnification using the same microscope.

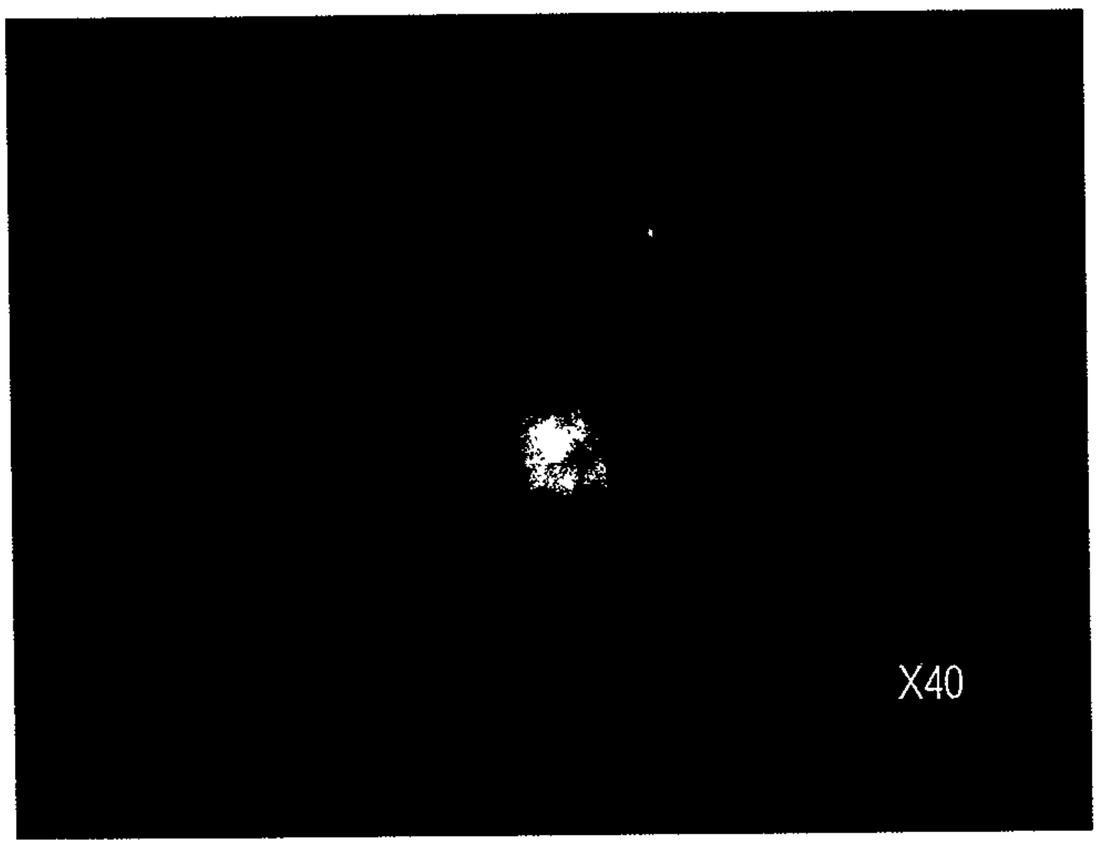


Fig 3.1a: Intact SAOS-2 cell stained with DAPI as seen under fluorescent microscopy

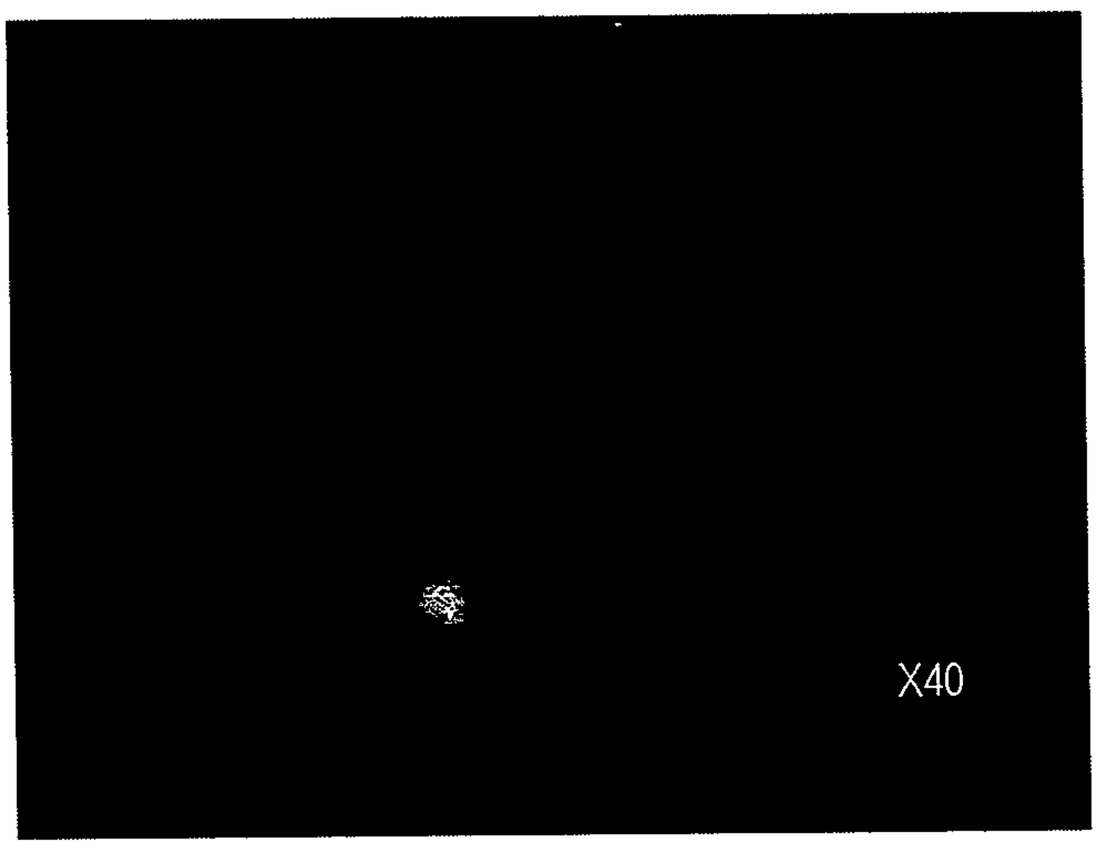


Fig 3.1b: Comet positive SAOS-2 cell with its distinctive tail as seen under fluorescent microscopy

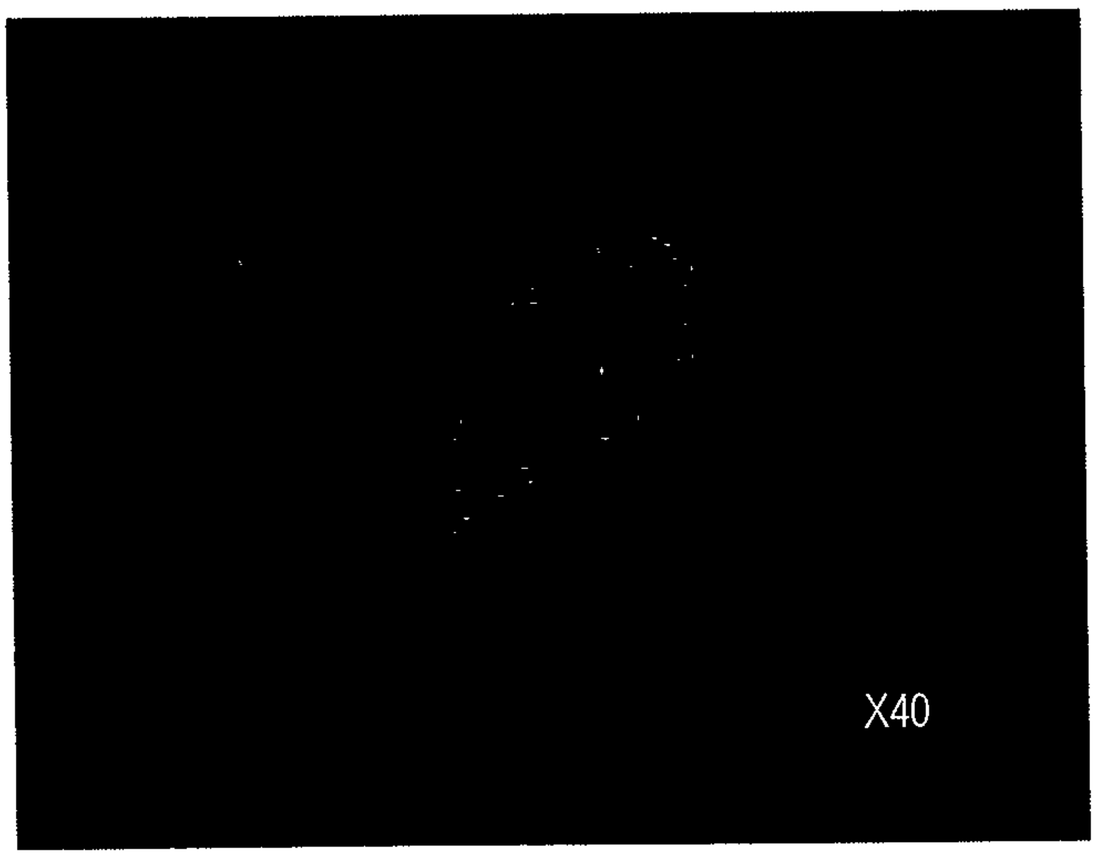


Fig 3.1c: Apoptotic SAOS-2 cell . Note the long and wide tail of the comet, indicating significant fragmentation of the DNA

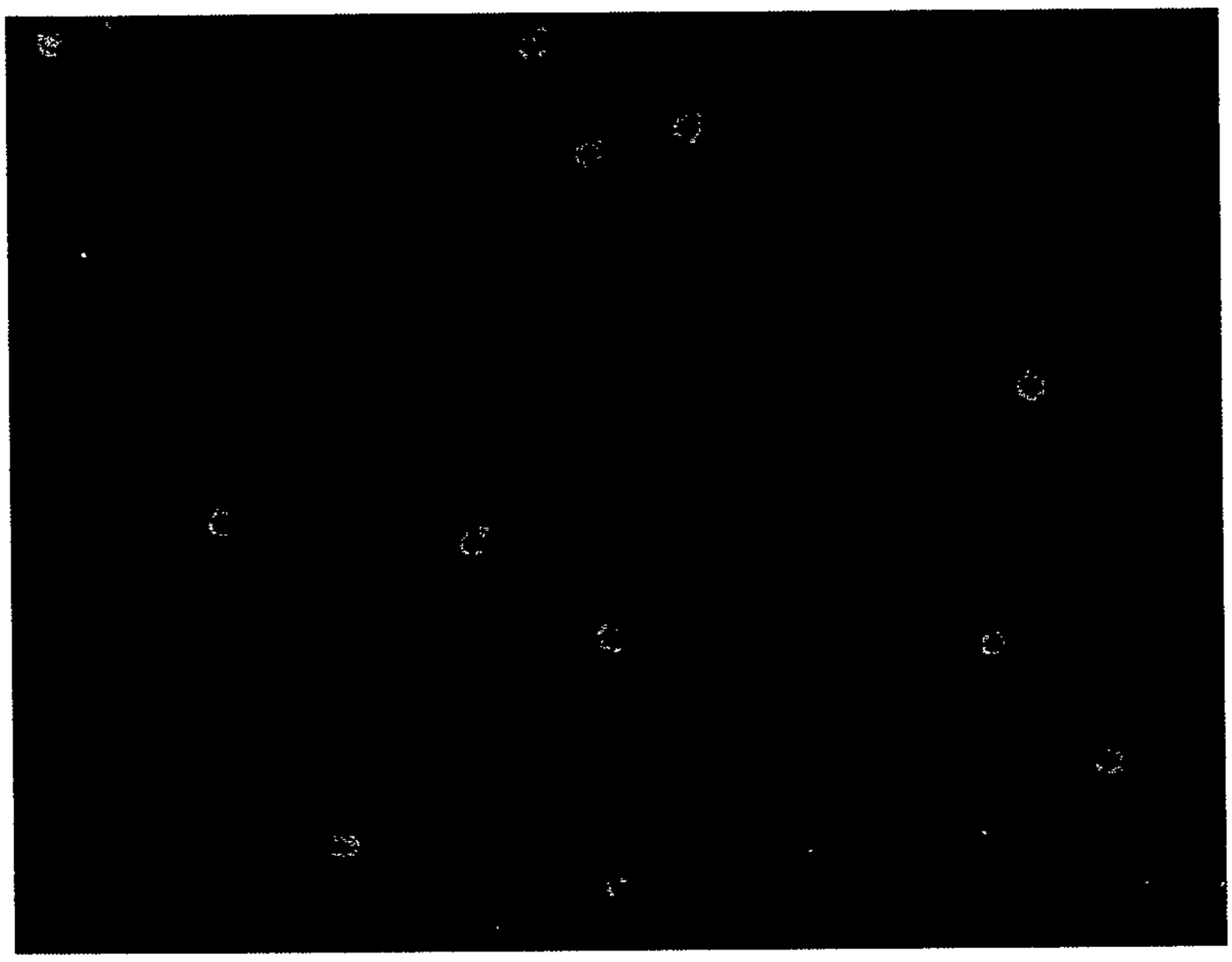


Fig 3.2a: Comet negative SAOS-2 cells under x20 magnification

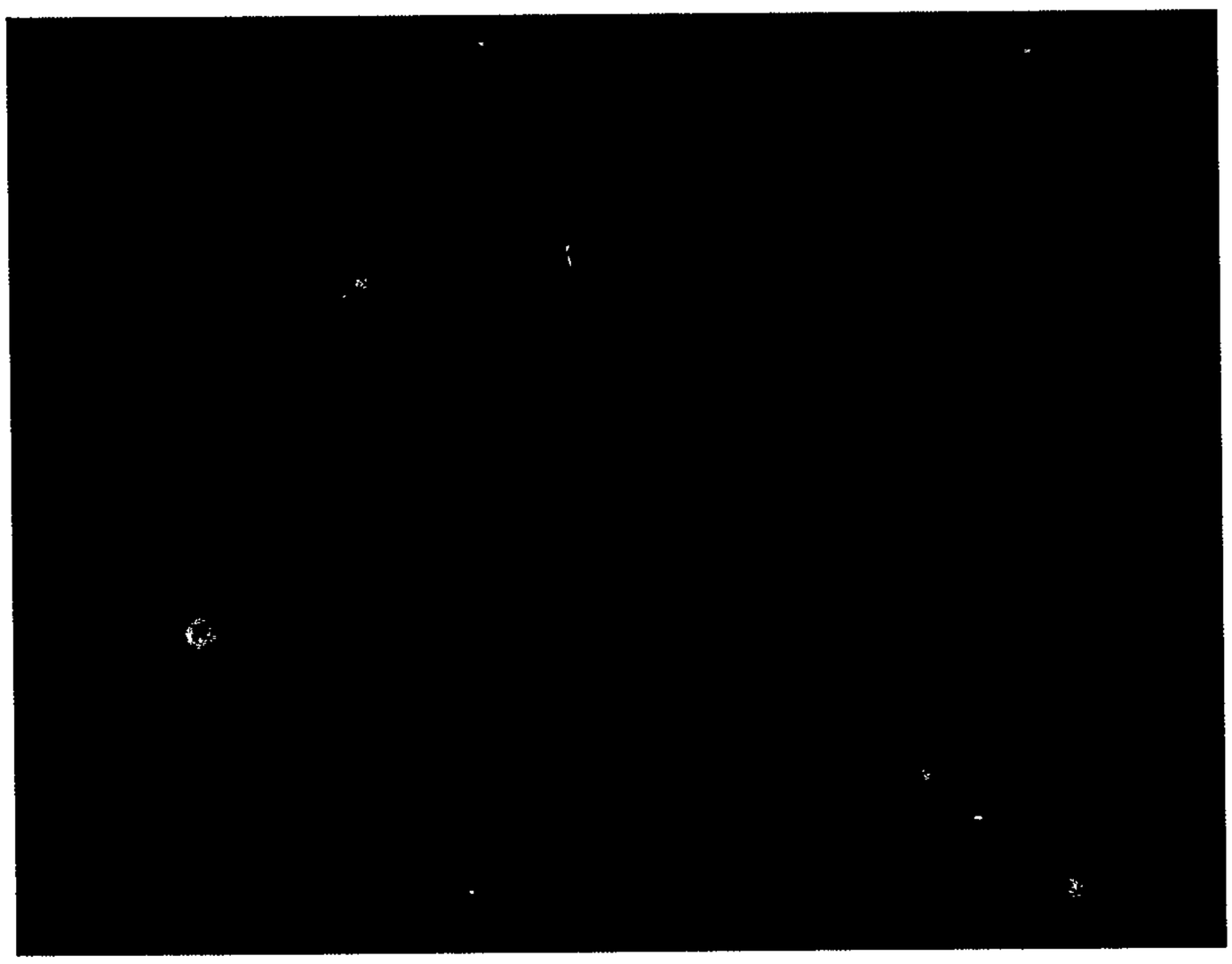


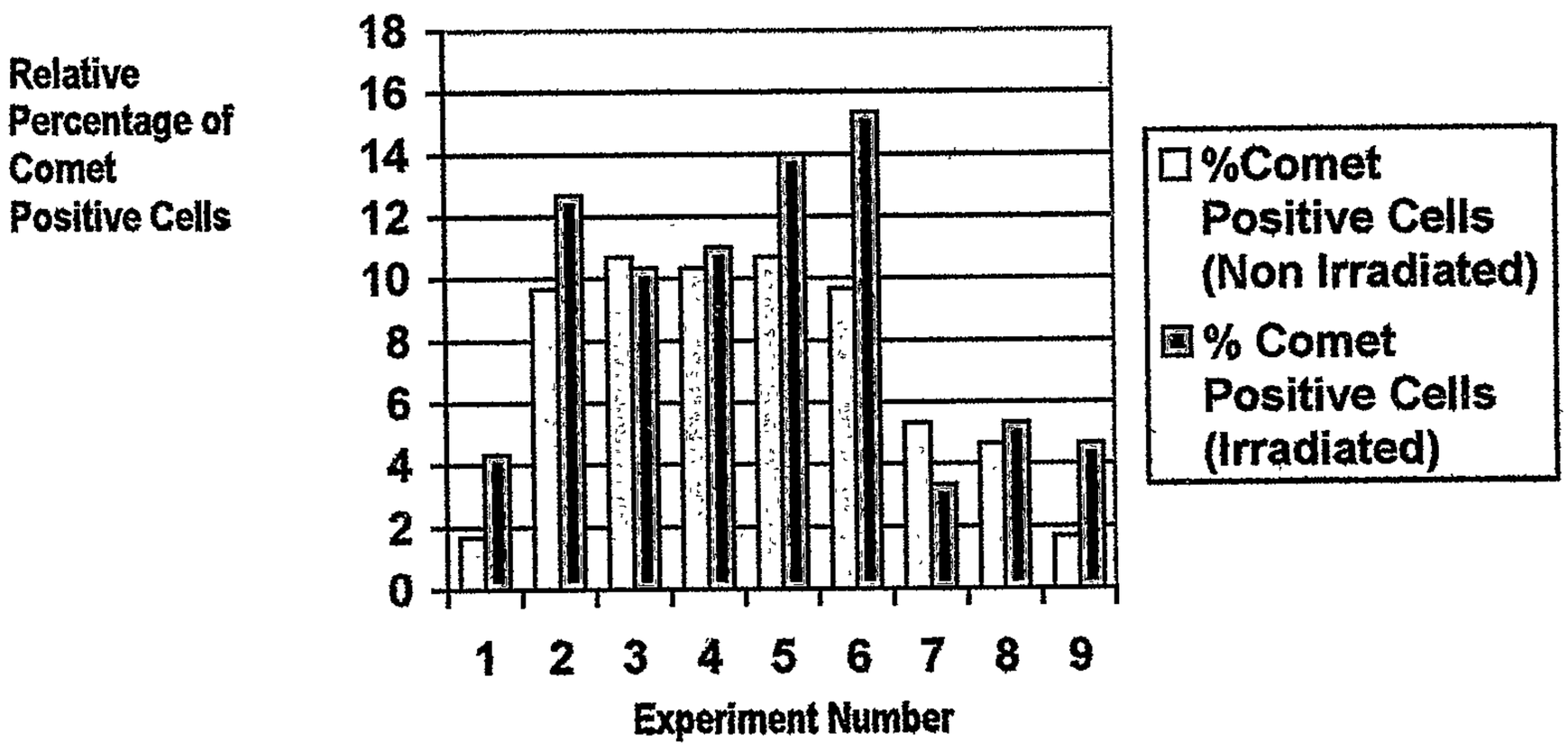
Fig 3.2b: Comet positive SAOS-2 cells under x20 magnification

3.2.3 Expression of Results

The number of comet positive and negative cells were determined in counts and the Wilcoxon paired signed rank test used to statistically evaluate the experiments. The average percentage of damaged cells detected for the three replicate gels of each sample of irradiated and non irradiated cells for all 9 experiments is indicated in figure 3.3.

It is seen that in 7 out of 9 experiments, there was a modest increase in the relative percentage of comet positive cells when 300 cells over 3 separate slides were counted.

Figure 3.3: Percentage of comet positive SAOS-2 cells in cultures exposed to a 10μT magnetic field compared with unexposed cells in 9 separate experiments



3.2.4 Statistical Evaluation

The Wilcoxon paired signed rank test indicates that the results are statistically significant at the 5% level (ie $p < 0.05$). Table 3.1 shows the ranking of the Wilcoxon Ranked Sign test used to assess the statistical significance of data shown in figure 3.3.

Table 3.1: Wilcoxon paired signed rank test for SAOS-2 cells exposed to 10μT of magnetic radiation for 7 days in a series of 9 experiments

N=9

Plus ranks=5 **Minus ranks=40**

N (pairs)	Control comets %	Experimental comets %	Difference	Rank	Signed Rank
1	1.67	4.33	-2.66	5	-5
2	9.67	12.67	-3.00	6.5	-6.5
3	10.67	10.33	0.34	1	1
4	10.33	11.00	-0.77	3	-3
5	10.67	14.00	-3.33	8	-8
6	9.67	15.33	-5.66	9	-9
7	5.33	3.33	2.00	4	4
8	4.67	5.33	-0.67	2	-2
9	1.67	4.67	-3.01	6.5	-6.5

CHAPTER 4 DISCUSSION

For several years there has been a view in the community that exposure to EMR from overhead power lines has lead to an increased risk of certain types of cancers. Such reports have often saturated the lay media but do not accurately convey the results of many years of research: that there is no clear scientific evidence to indicate that exposure to a 50 or 60Hz EMR around the home, office or environment is a hazard to human health (Australian Radiation Laboratory, 1999).

All electric currents generate EMF in which the electric field is proportional to the voltage and the magnetic field is proportional to the current. Separate to this is EMR, generated by the accelerating and oscillating current. The direction of the current and therefore the magnetic field changes 100 (or 120) times per second, to give a frequency of 50Hz (or 60 Hz) (ie, 50 or 60 cycles per second).

Electric fields can be easily shielded, but the shielding of magnetic fields is technically difficult and therefore very expensive. Buried power lines generate lower magnetic fields, not because of the earth eliminating their field, but due to their design. The easiest way to reduce exposure to magnetic fields is to increase one's distance from the source (Wilson et al., 1990).

The power frequency range is very close to the frequencies of many natural processes in the body. This range includes rates of physiological reactions that involve charge transfers between molecules. For example, the duration of action potentials in excitable tissues (nerves and muscles) are in the1-10ms range (ie 100-1000Hz), and the turnover in numbers of many enzymes are in the 50Hz power frequency range (Blank, 1995). *In vitro* studies by Basset and co-workers (1962, 1964, 1989) have shown changes in the activity of enzymes as well as stimulation of biosynthetic processes that involve RNA polymerase. The mechanism of these effects on physiological and biochemical processes may be directly linked to the power frequency range of the electromagnetic spectrum. Streaming potentials due to the flow of blood, and piezoelectric potentials due to the stressing of bones, generally occur at lower frequencies (Blank, 1995).

An important aim of this thesis was to develop a protocol for a single cell gel electrophoresis assay (SCGEA) that could be used for rapid screening of DNA damage due to exposure to EMF. According to the literature, the assay has multiple applications, such as evaluation of cell DNA damage, monitoring of DNA repair kinetics , studying the induction of apoptosis by certain drugs and biomonitoring populations (Tice, 1995, Faibairn et al., 1995).

The original method set out by Ostling and Johansson (1984) was performed under neutral conditions and was found to be sensitive for detecting double stranded breaks on DNA. But such conditions were ineffective for removing all the proteins during lysis and subsequent authors developed more stringent lysis conditions which could allow for detection of single stranded breaks. Two independent laboratories set about refining the techniques of the comet assay for detecting single stranded breaks, one by Olive et al., (1990, 1992, 1999), the other by Singh et al., (1988, 1994, 1995).

This assay has been reported to be very sensitive and can detect one break per $2x10^{10}$ Daltons of DNA in lymphocytes (Singh et al, 1994). The popularity of the comet assay in clinical analysis and biomonitoring owes much to the relative simplicity and rapidity of the technique, combined with the important practical factor that only relatively few cells are required for analysis (Olive 1999). With all the papers published so far, the protocols for the assays are highly variable (Fairbairn et al., 1995). Each step of the assay has been modified by various operators to suit their experimental and laboroatory conditions, as well as to extract the maximum amount of information from an experiment. Differences range from the concentration and number of layers of agarose, salt concentrations of the lysis solution, addition of a deproteination step, electrophoresis time and voltage, staining materials and image analysis techniques.

With no standardised methodology, this thesis set about developing one that would suit departmental laboratory conditions and would serve the purpose of a rapid screening test without becoming overloaded with excessively sensitive steps for the purpose of detecting the type of DNA damage. Only an alkaline assay was required as both single and double stranded break detection was of interest. As only weak magnetic fields were used, there was no interest in the first instance, in quantitating the degree of DNA damage, only whether it had occurred or not.

It was found that it was possible to perform the assay with only a single layer of agarose with suspended cells. Sandwich or multiple layers were not required, as long as the slides were kept horizontal throughout most of the experiment. Lysing overnight provided the best results as did allowing for 40 minutes of DNA unwinding before electrophoresis. This was a crucial step, since residual salt can affect DNA unwinding and can also inhibit DNA migration (Fairbairn et al., 1995, Olive et al., 1992). A need to recirculate the buffer for the experiments was not found necessary as indicated by Singh et al., 1995. The use of 300mmol NaOH during electrophoresis resulted in longer tails which could be clearly distinguished from the heads, thus making analysis easier. All solutions were kept alkaline as we wanted to detect single stranded breakage of DNA, as well as double stranded breaks. Another step eliminated was the use of any image analysis to measure comet length or DNA content in the tail. Since the test developed was for screening purposes, only visual scoring for the presence or absence of a comet tail was necessary. Finally, in the staining step it was found that stains such as ethidium bromide, propidium iodide or DAPI provided adequate visualisation of the comets, with DAPI providing the best result and also being the cheapest stain available.

The next stage was to use this protocol to screen for DNA fragmentation in an SAOS-2 cells exposed to 10μT of 50 Hz electromagnetic radiation. There have been only a handful of studies that have looked at DNA damage due to electromagnetic fields with an assay as sensitive as the comet assay, and most of these have looked at higher frequency radiation such as those emitted from mobile phones (Singh et al., 1995, Malyapa et al., 1997). Only one study has investigated the effects of low oscillating electric currents generated from electromagnetic fields and assessed this effect with the comet assay (Fairbairn and O'Neill, 1996). However, this study exposed cell cultures to much higher levels of magnetic radiation than would be expected if someone were living close to high tension power lines, the second aim of this thesis. Another study investigating the effect of electrically generated magnetic fields on cell lines used flow cytometry (Wei et al., 2000) to detect DNA damage. That study did not find significant amounts of DNA damage being induced, in spite of using fields which were 8-90 times greater than the amount used in this thesis.

The magnetic field used in this thesis was limited to 10μT, which is the maximum magnetic field exposure levels a person would receive if living within 100 metres on an overhead power line (Sagan, 1996). Although the literature reports on studies using a wide range of magnetic fields generated by EMR's in experiments, there is often no rationale stated for using the

levels chosen. Most epidemiological studies report that magnetic fields induced by power lines as measured in the centre of a room within a house would normally generate a maximum field of 0.1-0.3μT (Linet et al., 1997, Feychting and Ahlbom, 1993, Kaune et al., 1987). EPRI (1993) also states that the maximum strength of the magnetic field for 95% of homes measured in a study they conducted was under 0.29μT. Yet some reports in the literature are still using fields of 400,000μT (Miyakoshi et al., 1996, 1997) and 24,000μT (Yasul et al., 1997) to "simulate the effects" of power line magnetic fields and look at their association with cancer, without explaining why those levels were chosen.

Experiments in this thesis demonstrated a statistically significant increase in the number of comet positive cells after 7 days exposure to EMF of 10μT. This is in contrast to Fairbairn and O'Neill (1994) who performed a similar study using Raji and HeLa cell lines and found no association, despite using fields in the order of 5mT which was 500 times greater than the exposure levels used in this thesis. It seems likely, however, that this was because of the very short, one hour exposure time these authors used. They also only used a 10 minute electrophoresis time, which may not have been long enough for adequate DNA migration through the gel to be detected during analysis.

However, despite repeating the experiments over nine weeks to improve the statistical power of the study, the actual number of comet positive cells detected in both the irradiated and non-irradiated group decreased at the 7th, 8th and 9th week of the experiment. It is possible that SAOS-2 cells undergo phenotypic change with repeated culturing, despite being classified as an 'immortal cell line', and it may have been more appropriate to thaw new cells from a stored aliquot of initial cells, rather than continue passaging the same group of cells over the nine week experimentation period.

A recent publication, appearing only late during this thesis, raises the alternative possibility that rather than causing DNA fragmentation, 50Hz EMF may actually stimulate cell proliferation. Tuck et al., (2000) demonstrated that Okazaki fragments, generated during DNA synthesis, are detected as comets in the SCGEA. This suggests that fields studied in this thesis may not have caused DNA fragmentation, but rather these fields may have increased the number of cells replicating DNA.

This interpretation raises the possibility that 50Hz EMF are not directly carcinogenic, but act as a promoter for cells already carrying genetic lesions (see section 1.3.2). It also means that the use of SAOS-2 cells, an osteosarcoma cell line, was an appropriate choice of cell culture, if promotor, rather than initiator effects are to be investigated with respect to EMF. Further investigation of this possibility requires the combined use of proliferation assays, flow cytometry (FACS) analyses and bromodeoxyuridine (Brd-Urd) labelling. This would allow for separation of cells in to their various phases of the cell cycle as it is known that during S phase there are several thousand DNA fragments being replicated, which could be interpreted as DNA fragmentation by our assay (Olive, 1999). Unfortunately, time limitations precluded pursuit of this possibility in this thesis.

Future Research

The development of a sensitive screening assay which can detect DNA damage at the individual cell level brings with it the possibility of detecting the effects of genotoxic agents that would not otherwise be seen by an assay that requires a large number of cells to be damaged. With the methodology developed in this thesis it may be possible to detect the effects of a range of magnetic fields on not just cell culture lines, but also on an animal model exposed to electromagnetic radiation. This thesis also alludes to the possibility that 50HZ EMR may not be directly carcinogenic, but may actually promote proliferation of cells that have been damaged by another genotoxic agent. It also seems necessary to test this assay on cells in different phases of the cell cycle, as cells in S phase may confound the results found with respect to DNA fragmentation analysis. Investigation of these areas would be needed before the final significance of the results in this thesis can be interpreted.

Conclusions

1. A rapid, yet simple protocol for the comet assay was developed that proved to be a cost effective screening tool for DNA fragmentation detection in individual cells.

2. A statistically significant amount of comet positive cells were detected in a population of SAOS-2 cells exposed to 10μT of 50Hz electromagnetic radiation in a pilot study. However, it is appreciated that this effect may have been due to confounding factors, such as cell proliferation.

3. Further investigation of these changes is required to define the basis for the comet positive cells detected.

APPENDIX 1

CELL CULTURE TECHNIQUES

Subculturing of SAOS-2 cells

1. Wipe down the working area of the laminar flow hood with 70% alcohol.
2. Tighten lid on flask to prevent spillage of contents, then remove from the incubator an place in the laminar flow hood.
3. Aspirate medium from flask.
4. Gently wash monolayer of cells with 3-4 ml of fresh M199 and aspirate.
5. Add 5ml of 0.025% Trypsin-0.29% EDTA to the flask and replace the flask horizontally in the 37°C/5% CO_2 incubator for 5 minutes.
6. Remove the flask from the incubator and tap the flask on the side to dislodge the cells.
7. Neutralise the reaction by adding 10ml of M199 with 10% BCS to the flask and transfer the contents by pipette to a 50ml centrifuge tube.
8. Centrifuge the cell supension at 1000 revolutions per minute for 5 minutes.
9. Remove the supernatant from the centrifuge tube, being careful not to dislodge the cell pellet.
10. Add 45ml of fresh M199 containing 10% BCS to the centrifuge tube and resuspend the cells by pipetting.
11. Place 15ml of the cell suspension in three sterile 75cm^2 flasks and place horizontally in the 37°C/ 5% CO_2/ 100% relative humidity incubator with the lids loosened. Label the flasks with the date, cell type and passage number.
12. Passage the cells every four days to maintain optimum cell growth.

Freezing down and thawing stored cells

The usual practice is to freeze down cells at the 4^{th} or 5^{th} passage.

Materials

1. Complete medium (CM) (M199 containing 10% BCS)
2. 0.025% Trypsin-0.29% EDTA

3. Dimethyl Sulfoxide (DMSO) C_2H_6SO 99.5% (Sigma Chemical co.)
4. 1.2ml cryogenic vial
5. Confluent cells in 75cm_2 flask
6. M199 solution

Method

1. Prepare 10% DMSO in complete medium (1mL DNSO in 9ml CM)
2. Trypsinize confluent flask of cells as described previously.
3. Label a cryogenic vial and record details in a cell culture record book
4. Resuspend pellet of cells with 1ml DMSO-CM. Transfer the supsension to a sterile cryogenic vial.
5. Place the vial in a polystyrene box and let cool at -1°C/minute in a -70°C freezer overnight.
6. Transfer vial to a cane in a -196°C liquid nitrogen tank. Cells can be stored with good viability for several months.

Thawing the stored cells

1. Thaw cryogenic vial in a hotwater bath at 37°C. Wipe the outside of the flask with 70% alcohol.
2. Transfer cells to a centrifuge tube containing 10ml CM and resuspend the cells, then centrifuge at 1000RPM for 5 minutes
3. Remove supernatant without disturbing the pellet and resuspend in 15ml CM. Transfer to a labelled 75cm^2 flask and incubate.

Staining technique with DAPI

0.5ml of DAPI slution (1μg/ml) was added to each gel and left for 10 minutes, before being aspirated and the gel rinsed with 3-4ml of MilliQ water once. Gels could be viewed immediately or several months later, provided they were stored away from direct light.

CELL CULTURE FORMULAE

Media M199

Materials

1. 1 sachet M199
2. 29mL of 7.5% sodium bicarbonate solution
3. Water to make up 1 litre

Method

Dissolve contents in water to make 1 litre. Adjust pH to 7.2. Filter the solution and then store at 2°-7°C.

Complete medium preparation

Materials

1. 10mL penicillin / streptomycin antibiotic solution
2. 2ml L-glutamine solution
3. 500mL Sterile M199 solution
4. 10% Bovine Calf Serum (BCS)

Method

1. Prepare M199 as above
2. Add the penicillin / streptomycin antibiotic solution as well as L- glutamine solution. L-glutamine needs to be re added to the medium every two weeks (2ml for every 500ml M199).
3. Add BCS to make a 10% final concentration in the medium just prior to passaging of cells

1% Agarose gel

Materials

1. 0.2gm low melting point (37°C) agarose powder
2. 10ml TAE buffer

Method

1. Add agarose to TAE buffer and heat in a microwave on medium for 20 seconds. Stir the solution and heat for a further 10 seconds. This makes a 2% gel. The final concentration of 1% is achieved when 50µl of gel is mixed with 50µl of cell suspension.

Lysis buffer

Materials

1. 2.5mol/l 1% sodium chloride
2. 1% sodium N-lauroyl sacosinate
3. 100mmol/l disodium EDTA
4. 10mmol/l Tris base
5. 1% Triton X-100
6. MilliQ water to make a final concentration of 1000ml
7. 10Mol Sodium hydroxide
8. 10Mol Hydrochloric acid

Methods

1. Add EDTA and Tris base to 500ml of millicule water and place on a magnetic stirer. Do not heat the solution as organic salts degrade with temperatures above 50°C. Add NaOH to make the solution alkaline as EDTA needs a pH of greater than 8 to dissolve.
2. Once dissolved, add NaCl and sodium N-Luaroyl sacosinate with an extra 200 mL of water Check that the pH is over 10; if not add NaOH or HCl as required.
3. Make to the final volume.
4. Autoclave the buffer
5. One hour before using the buffer add 1% Triton X-100.

Electrophoresis buffer

Materials

1. 300mmol NaCl (12gm in 1 litre solution)
2. 1 mmol tetra sodium EDTA (3.72gm in 1litre solution)
3. MiiliQ water

Methods

1. Dissolve EDTA and NaCl in Millicule water and make to a final volume of 1 litre. Adjust to pH 8 and use immediately.

Neutralising solution

Materials

1. 400mmol Tris (48.46gm in 1 litre water)
2. MilliQ water

Method

1. Dissolve Tris in MilliQ water and make to a final volume of 1 litre. Adjust pH to 8. Autoclave buffer and store at 4°C.

DAPI

Dissolve 1mg in MilliQ water to a final concentration of 1μg/ml. Prepare 10ml aliquots and store the stock solution at -20°C

L Glutamine

Materials

1. 1.5gm L Glutamine
2. 100ml M199

Methods

1. Dissolve L glutamine in M199 to make 100ml.
2. Sterile filter
3. Store in 2ml aliquots at -20°C

Phosphate Buffered Saline

Take 10 PBS tablets

Add to 1 L millicule water

Adjust pH to 7.2

Autoclave

Store at 2-8°C

Antibiotics

Combine in equal amounts

Penicillin at 5000IU / mL

Streptomycin at 5000IU / mL

Trypsin- EDTA (Trypsin- ethylene diaminetetraaceteic acid)

Make up 0.02% EDTA in phosphate buffered saline (PBS)

EDTA 200mg

PBS 100mL

Dissolve 50mg Trypsin in 90 mL PBS

Add 10mL 0.2% EDTA/PBS to the trypsin solution

Filter and sterilise

Store at 2-8°C.

HBSS

Materials

1. 1 sachet Hank's salts
2. 0.35gm $NaHCO_3$
3. MilliQ water

Method

1. Dissolve contents in Mwater and make to a final volume of 1 litre.
2. Adjust pH to 7.2
3. Sterile filter and store at 4°C

APPENDIX 2

POSTER PRESENTATIONS OF THIS RESEARCH

2000	Australian Society of Orthodontists Clinical Day - Oral Presentation
2000	Australian Society of Orthodontics Clinical Day – Best Poster Presentation by a Postgraduate

LITERATURE CITED

Ager DD. Radul JA. (1992). Effect of 60-Hz magnetic fields on ultraviolet light-induced mutation and mitotic recombination in Saccharomyces cerevisiae. Mut Res 283:279-286.

Ahlbom A. Feychting M. Koskenvuo M. Olsen J.H. Pukkala E. Schulcen G. and Verkasalo P. (1993). Electromagnetic fields and childhood cancer [letter]. Lancet 342(8882):1295-1296.

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 Research. 53(3):609-14.

Australian Radiation Laboratory (1999). Radiation health series / National Health and Medical Research Council. Canberra : Australian Govt. Pub. Service.

Bassett CAL. Becker RO (1962). Generation of Electric Potentials in bone in response to mechanical stress. Science 137: 1063-1064.

Bassett CAL. Pawluk RJ. Becker RO. (1964). Effect of electric currents on bone in vivo. Nature 204: 652-654.

Bassett CA. Pawluk RJ. Pilla AA (1974). Acceleration of fracture repair by electromagnetic fields. A surgically noninvasive method. Annals of the New York Academy of Sciences. 238:242-62.

Bassett C.A.L. (1989). Fundamental and practical aspects of therapeutic uses of pulsed electromagnetic fields (PEMFs). CRC Crit. Rev. Biomed. Enc 17:451-529.

Battocletti J. H. (1976). Electromagnetism man and the environment. London : P. Elek.

Bellossi A (1991). Effect of pulsed magnetic fields on leukemia-prone AKR mice. No effect on mortality through five generations. Leuk Res 15:899-902.

Beniashvili DS. Bilanishvili VG. Menabde MZ (1991). Low-frequency electromagnetic radiation enhances the induction of rat mammary tumors by nitrosomethyl urea. Cancer Letters. 61(1):75-9

Blair A. Burg J. Foran J. Gibb H. Greenland S. Morris R. Raabe G. Savitz D. Teta J. Wartenberg D. (1995). Guidelinesfor application of meta-analysis in environmental epidemiology. ISLI Risk Science Institute. Regulatory Toxicology & Pharmacology. 22(2):189-97.

Blank M. (1995). (Editor) Electromagnetic Fields: Biological interactions and mechanisms. American Chemical Society Washington DC.

Bowman JD. Thomas DC. London SJ. Peters JM (1995). Hypothesis: the risk of childhood leukemia is related to combinations of power-frequency and static magnetic fields. Bioelectromagnetics. 16(1):48-59.

Brent RL. Gordon WE. Bennett WR. Beckman DA (1993). Reproductive and teratologic effects of electromagnetic fieldsReproductive Toxicology. 7(6):535-80.

Brighton CT. Friedenberg ZB. Zemsky LM. Pollis PR. (1975). Direct-current stimulation of non-union and congenital pseudarthrosis. Exploration of its clinical application. Journal of Bone & Joint Surgery - American Volume. 57(3):368-77.

Butterworth BE. Conolly RB. Morgan KT (1995). A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments. Cancer Letters.93(1):129-46.

Butrous GS. Male JC. Webber RS. Barton DG. Meldrum SJ. Bonnell JA. Camm AJ (1983). The effect of power frequency high intensity electric fields on implanted cardiac pacemakers.Pacing&Clinical-Electrophysiology.6(6):1282-92.

Campion EW (1997) Power lines, cancer and fear. The New England Journal of Medicine. 337:44-46.

Caputa K Stuchly MA. (1996). Computer controlled systems for producing uniform magnetic fields and its application in biomedical research. IEEE Trans. Instrum. Meas. 45 (3) :701-709.

Carlisle N. Carlisle J (1966). Marvels of Medical Engineering. The Oak Tree Press London.

Cerda H. Delincee H. Haine H. Rupp H (1997). The DNA 'comet assay' as a rapid screening technique to control irradiated food. Mutation Research. 375(2):167-81.

Chernoff N. Rogers JM. Kavet R (1992). A review of the literature on potential reproductive and developmental toxicity of electric and magnetic fields. Toxicology. 74(2-3):91-126.

Cohen SM. Ellwein LB (1991). Genetic errors cell proliferation and carcinogenesis. Cancer Research. 51(24):6493-505

Coleman MP. Bell CM. Taylor HL. Primic-Zakelj M (1989). Leukaemia and residence near electricity transmission equipment: a case-control study British Journal of Cancer. 60(5):793-8.

Cooper W J Curry R D O'Shea K (1998). Environmental applications of ionizing radiation. John Wiley New York.

Creim JA. Lovely RH. Kaune WT. Phillips RD (1984). Attempts to produce taste-aversion learning in rats exposed to 60-Hz electric fields. Bioelectromagnetics. 5(2):271-82.

D'Agruma L. Colosimo A. Angeloni U. Novelli G. Dallapiccola B (1993). Plasmid DNA and low-frequency electromagnetic fields. Biomedicine & Pharmacotherapy. 47(2-3):101-5.

Davis CC. Czerska EM. Elson EC.. Swicord ML. Czerski P (1992). Effects of continuous and pulsed 2450-MHz radiation on spontaneous lymphoblastoid transformation of human lymphocytes in vitro. Bioelectromagnetics. 13(4):247-59.

Dickersin K Berlin J (1992). Meta analysis: state of the science. Epidemiol. Rev. 14:154-176.

EPA (US Environmental Protection Agency) (1992). EMF in your environment: magnetic field measurements of everyday electrical devices. EPA/402/R-92/008. Office of radiation and indoor air US EP Washington DC.

EPRI (Electric Power Research Institute) (1990). The EMDEX project: Technology transfer and occupational measurements. Project RP2966-1. Prepared by T Dan Bracken for EPRI Palo Alto Calif.

EPRI (Electric Power Research Institute) (1993). Survey of residential magnetic field sources: goals results and conclusions. Vol 1. Prepared by High Voltage Transmission Research Centre for EPRI Palo Alto Calif.

Fairbairn DW O'Neill KL (1994). The effect of electromagnetic field exposure on the formation of DNA single strand breaks in human cells. Cell Molecular Biology 4:561-567.

Fairbairn DW. Olive PL. O'Neill KL (1995). The comet assay: a comprehensive review. Mutation Research. 339(1):37-59.

Fajardo-Gutieerez AJ Garduno-Espinosa J. Yamamoto-Kimura L. Hernandez-Hernandez DM. Gomez-Delgado A. Mejia-Arangure M. Cartagena-Sandoval A. Martinez-Garcia MC. (1993) Residence close to high tension electric power lines and its association with leukaemia in children (in Spanish). Biol. Med. Hosp. Infant Mex. 50: 32-38.

Fam WZ Mikhail EL (1996). Lymphoma induced in mice chronically exposed to very strong low-frequency electromagnetic field. Cancer Letters 105:257-269.

Feychting M and Ahlbom A (1993). Magnetic fields and cancer in children residing near Swedish high-voltage Power Lines. Am J Epidem 7:467-481.

Feychting M and Ahlbom A 1994: Magnetic fields leukemia and central nervous system tumors in Swedish adults residing near high-voltage power lines Epidemiology 5:501-509.

Fiorani M. Cantoni O. Sestili P. Conti R. Nicolini P. Vetrano F. Dacha M (1992). Electric and/or magnetic field effects on DNA structure and function in cultured human cells. Mutation Research. 282(1):25-9.

Fogh J. Fogh JM. Orfeo T (1977). One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. Journal of the National Cancer Institute. 59(1):221-6

Fogh J. (1986). Human tumor lines for cancer research. [Journal Article] Cancer Investigation. 4(2):157-84.

Foster KR. Guy AW (1986). The microwave problem. [Journal Article] Scientific American. 255(3):32-9.

Free MJ. Kaune WT. Phillips RD. Cheng HC (1981). Endocrinological effects of strong 60-Hz electric fields on rats Bioelectromagnetics. 2(2):105-21.

Frei EH (1972). Biomagnetics. IEEE trans on magnetics MAG-8 407-413.

Fulton JP. Cobb S. Preble L. Leone L. Forman E (1980). Electrical wiring configurations and childhood leukemia in Rhode Island. American Journal of Epidemiology. 111(3):292-6.

George DG. (1998). International Radiation Protection Association Guidelines on Protection Against Non Ionising Radiation. Pergamon New York.

Giancoli D C (1995). Physics : principles with applications: Prentice Hall Englewood Cliffs N.J. 4th ed.

Harris AW. Basten A. Gebski V. Noonan D. Finnie J. Bath ML. Bangay MJ. Repacholi MH (1998). A test of lymphoma induction by long-term exposure of E mu-Pim1 transgenic mice to 50 Hz magnetic fields. Radiation Research.149(3):300-7.

Harsthorne AM (1841). On the cause and treatment of pseudoarthorosis and especially of that form of it sometimes called supernumery joint. American Journal of Medical Science. 1: 143.

Harvey SM (1987). Magnetic field exposure apparatus for small animal studies. Phase II Rep. No. 87-118-K. Ontario Hydro research Division.

Hay R. Caputo J. Chen TR. Macy Marvin McClintock P: (Editors). ATCC Cell Lines and Hybridomas 8th Edition (1994). American Type Culture Collection, Rockville Maryland.

Hayes DL. Vlietstra RE (1993). Pacemaker malfunction. Annals of Internal Medicine 119:828-835.

Hu Q. Hill RP(1996): Radiosensitivity apoptosis and repair of DNA double-strand breaks in radiation-sensitive Chinese hamster ovary cell mutants treated at different dose rates. Radiation Research. 146(6):636-45.

Juutilainen J Liimatainen A (1986). Mutation frequency in Salmonella exposed to weak 100-Hz magnetic fields. Hereditas 104:145-147.

Kaune WT (1981a). Interactive effects of 60Hz electric field exposure systems. Bioelectromagnetics 9:1-24.

Kaune WT (1981b). Power frequency fields averaged over the body surfaces of grounded animals and humans. Bioelectromagnetics 2: 13-32.

Kheifets LI. London SJ. Peters JM (1997). Leukemia risk and occupational electric field exposure in Los Angeles County California. American Journal of Epidemiology. 146(1):87-90.

Kirschvink JL Kobayashi-Kirschvink A. Diaz-Ricci JC. Kirschvink SJ (1992): Magnetite in human tissues: A mechanism for the biological effects of weak ELF magnetic fields. BEM Suppl 1:101-113.

Kraus JD (1992). Electromagnetics. 4th edition. New York: McGraw-Hill

Lai H Singh NP.. (1996). Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. International Journal of Radiation Biology. 69(4):513-21.

Lente RW (1850). Cases of un-united fracture treated by electricity. New York State medical Journal. 5: 317.

Liburdy RP (1992a). Biological interactions of cellular systems with time-varying magnetic fields. Annals of the New York Academy of Sciences 649:74-95.

Liburdy RP (1992b). Calcium signaling in lymphocytes and ELF fields. Evidence for an electric field metric and a site of interaction involving the calcium ion channel. FEBS Letters 301:53-59.

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.14:89-97.

Liden S (1996). "Sensitivity to electricity" - a new environmental epidemic. Allergy 51:519-524.

Linet MS. Hatch EE. Kleinerman RA. Robison LL. Kaune WT. Friedman DR. Severson RK. Haines CM. Hartsock CT. Niwa S. Wacholder S. Tarone RE (1997). Residential exposure to magnetic fields and acute lymphoblastic leukemia in children New England Journal of Medicine. 337(1):1-7.

Llauraldo JG Sances A Jnr Battocletti JH (1974) Editors. Biologic and Clinical Effects of Low Frequency Magnetic and Electrical fields. CC Thomas Springfield Illinois Part 3 and workshop 3.

London SJ. Thomas DC. Bowman JD. Sobel E. Cheng TC. Peters JM (1991). Exposure to residential electric and magnetic fields and risk of childhood leukemia American Journal of Epidemiology. 134(9):923-37.

Malyapa RS. Ahern EW. Straube WL. Moros EG. Pickard WF. Roti Roti JL (1997). Measurement of DNA damage afterexposure to 2450 MHz electromagnetic radiation. Radiation Research. 148(6):608-17

Mandeville R. Franco E. Sidrac-Ghali S. Paris-Nadon L. Rocheleau N. Mercier G. Desy M. Gaboury L (1997). Evaluation of the potential carcinogenicity of 60 Hz linear sinusoidal continuous-wave magnetic fields in Fischer F344 rats. FASEB Journal. 11(13):1127-36

MacDonald Simon GG (1975). Physics for the life and health sciences . Editors Simon G. G. MacDonald, Desmond M. Burns.: Addison-Wesley Pub. Co. Reading Mass.

MacGregor DG. Slovic P. Morgan MG (1994). Perception of risks from electromagnetic fields: a psychometric evaluation of a risk-communication approach. Risk Analysis. 14(5):815-28.

Marples B. Longhurst D. Eastham AM. West CM (1998). The ratio of initial/residual DNA damage predicts intrinsic radiosensitivity in seven cervix carcinoma cell lines. British Journal of Cancer. 77(7):1108-14.

Maxwell JC (1873). Treatise on Electricity and Magnetism. Oxford University Press.

McDowall ME (1986). Mortality of persons resident in the vicinity of electrical transmission facilities. Br J Cancer 53:271-279.

Merritt RC Purcell C Stroink G. (1983). Uniform magnetic fields produced by three four and five square coils. Rev. Sci. Instrum. 54: 879-882.

Milham S(1982). Mortality from leukemia in workers exposed to electrical and magnetic fields (letter). New England Journal of Medicine 307:249.

Miller DL. Miller MC. Kaune WT (1989). Addition of magnetic field capability to existing extremely-low-frequency electric field exposure systems. Bioelectromagnetics. 10(1):85-98.

Miller F Schroeer D. (1987). College physics: Harcourt Brace Jovanovich San Diego. 6th ed.

Misakian M Sheppard AR. Krause D. Frazier ME. Miller DL (1993). Biological physical and electrical parameters for in vitro studies with ELF magnetic and electric fields: A primer. Bioelectromagnetics suppl. 2: 1-73.

Miyakoshi J Yamagishi N. Ohtsu S. Mohri K. Takebe H (1996). Increase in hypoxanthine-guanine phosphoribosyl transferase gene mutations by exposure to high-density 50-Hz magnetic fields. Mutation Research 349:1109-1114 .

Miyakoshi J. Kitagawa K. Takebe H (1997). Mutation induction by high-density 50-Hz magnetic fields in human MeWo cells exposed in the DNA synthesis phase. [Journal Article] International Journal of Radiation Biology. 71(1):75-9.

Moolgavkar SH (1988). Biologically motivated two-stage model for cancer risk assessment. ToxicologyLetters. 43(1-3):139-50.

Moulder JE Foster KR (1995). Biological effects of power-frequency fields as they relate to carcinogenesis. Proceedings of the Society of Experimental Medicine and Biology 209:309-324.

Moulder JE (1998). Power-frequency fields and cancer. Critical Reviews in Biomedical Engineering 26:1-116.

Myers A Clayden AD. Cartwright RA. Cartwright SC (1990). Childhood cancer and overhead powerlines: a case-control study. British Journal of Cancer 62:1008-1014.

Nafziger J. (1993). DNA mutations and 50 Hz EM fields. Bioelec Bioenerg 30:133-141.

National Health and Medical Research Council (NHMRC). (1999). Radiation health series / Australian Radiation Laboratory Canberra : Australian Govt. Pub. Service.

National Research Council (U.S.) (1997). Possible health effects of exposure to residential electric and magnetic fields National Academy Press Washington DC.

NIH Guide (1999). Findings of Scientific Misconduct. U.S. Department of Health and Human Services. June 18

Norton LA. Rodan GA. Bourret LA (1977). Epiphyseal cartilage cAMP changes produced by electrical and mechanicalperturbations. Clinical Orthopaedics & Related Research. (124):59-68.

Novelli G. Gennarelli M. Potenza L. Angeloni P. Dallapiccola B (1991). Study of the effects on DNA of electromagnetic fields using clamped homogeneous electric field gel electrophoresis. Biomedicine & Pharmacotherapy. 45(10):451-4.

NRPB (National Radiological Protection Board) (1992). Electromagnetic fields and the risk of cancer Vol 3 pp1-138. NRPB Chilton Didcot UK.

Olive PL. (1989). Cell proliferation as a requirement for development of the contact effect in Chinese hamster V79 spheroids. Radiation Research. 117(1):79-92.

Olive PL Banath JP Durand RE (1990). Heterogeneity in radiation-induced DNA damage and repair in tumour and normal cells measured using the 'comet' assay. Radiation Research 122:69–72.

Olive PL. Wlodek D. Banath JP (1991). DNA double-strand breaks measured in individual cells subjected to gel electrophoresis. Cancer Research. 51(17):4671-6.

Olive PL (1992). Factors influencing DNA migration from individual cells subjected to gel electrophoresis. Experimental Cell Research 198: 259-267.

Olive PL. Durand RE (1992). Detection of hypoxic cells in a murine tumor with the use of the comet assay. Journal of the National Cancer Institute. 84(9):707-11.

Olive PL. Banath JP. Fjell CD (1994). DNA strand breakage and DNA structure influence staining with propidium iodide using the alkaline comet assay. Cytometry. 16(4):305-12.

Olive PL Johnston PJ Banath JP Durand RE (1998). The comet assay: a new method to examine heterogeneity associated with solid tumours. Nature Med; 4: 103–5.

Olive PL (1999). DNA damage and repair in individual cells: applications of the comet assay in radiobiology. International Journal of Radiation Biology. 75(4):395-405.

Olive PL. Durand RE. Jackson SM. Le Riche JC. Luo C. Ma R. McLaren DB. Aquino-Parsons C. Thomson TA. Trotter T (1999). The comet assay in clinical practice. Acta Oncologica. 38(7):839-44.

Olsen JH Nielsen A. Schulgen G (1993). Residence near high voltage facilities and risk of cancer in children. British Medical Journal 307:891-895.

ORAU (Oak Ridge Associated Universities) (1992). Helath effects of low frequency electric and magnetic fields. ORAU Publication No. 92/F8. ORAU Oak Ridge Tenn.

Ostling O Johanson KJ. (1984). Microelectrophoretic study of radiation induced DNA damages in individual mammalian cells. Biochem. Biophys. Res. Commun. 123: 291-298.

Ostling O. Johanson KJ. Blomquist E. Hagelqvist E (1987). DNA damage in clinical radiation therapy studied by microelectrophoresis in single tumour cells. A preliminary report. Acta Oncologica. 26(1):45-8.

Peach HG. Bonwick WJ. Wyse T. (1992). Report of the panel on electromagnetic fields and health. The Victorian Government Melbourne Australia.

Peck ER (1953). Electricity and magnetism. New York: McGraw-Hill.

Petitti D (1994). Meta analysis decision analysis and cost effectiveness analysis. New York: Oxford University Press pp246.

Petridou E Kassimos D. Kalmanti M. Kosmidis H. Haidas S. Flytzani V. Tong D. Trichopoulos D (1993). Age of exposure to infections and risk of childhood leukemia British Medical Journal 307:774.

Polk C. Postow E (1986). CRC handbook of biological effects of electromagnetic fields. editors Charles Polk Elliot Postow. Boca Raton Fla. : CRC Press

Pool-Zobel BL. Munzner R. Holzapfel WH (1993). Antigenotoxic properties of lactic acid bacteria in the S. typhimurium mutagenicity assay. Nutrition & Cancer. 20(3):261-70

Rannug A. Holmberg B. Ekstrom T. Mild KH (1993). Rat liver foci study on coexposure with 50 Hz magnetic fields and known carcinogens. Bioelectromagnetics. 14(1):17-27.

Reese JA. Jostes RF. Frazier ME (1988). Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis for DNA single-strand breaks. Bioelectromagnetics. 9(3):237-47.

Robert E (1996). Teratogen update: electromagnetic fields. Teratology. 54(6):305-13.

Rodan GA. Bourret LA. Norton LA (1978). DNA synthesis in cartilage cells is stimulated by oscillating electric fields. Science. 199(4329):690-2.

Rodan SB. Imai Y. Thiede MA. Wesolowski G. Thompson D. Bar-Shavit Z. Shull S. Mann K. Rodan GA (1987). Characterization of a human osteosarcoma cell line (SAOS-2) with osteoblastic properties. [Journal Article] Cancer Research.47(18):4961-6.

Roti Roti JL. Wright WD (1987). Visualization of DNA loops in nucleoids from HeLa cells: assays for DNA damage and repair. Cytometry. 8(5):461-7.

Rydberg B (1984). Detection of DNA strand breaks in single cells using flow cytometry. International Journal of Radiation Biology & Related Studies in Physics Chemistry & Medicine. 46(5):521-7.

Rydberg B. Johanson KJ (1975). Radiation-induced DNA strand breaks and their rejoining in crypt and villous cells of the small intestine of the mouse. Radiation Research. 64(2):281-92.

Sagan LA. (1996). Electrical and magnetic fields: Invisible risks? Gordon and Breach Publishers

Savitz D (1988). Case-Control Study of Childhood Cancer and Residential Exposure to Electric and Magnetic Fields. American Journal of Epidemiology; 128: 21-38

Savitz DA. Baron AE (1989). Estimating and correcting for confounder misclassification American Journal of Epidemiology. 129(5):1062-71.

Schreiber GH. Swaen GM. Meijers JM. Slangen JJ. Sturmans F (1993). Cancer mortality and residence near electricity transmission equipment: a retrospective cohort study International Journal of Epidemiology. 22(1):9-15

Sears F W Zemansky MW Young H D. (1987) University physics: Addison-Wesley Pub. Co. Reading Mass. 7th ed.

Severson RK. Stevens RG. Kaune WT. Thomas DB. Heuser L. Davis S. Sever LE (1988). Acute nonlymphocytic leukemia and residential exposure to power frequency magnetic fields. American Journal of Epidemiology. 128(1):10-20.

Singh NP. McCoy MT. Tice RR. Schneider EL (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research. 175(1);184-91.

Singh NP. Danner DB. Tice RR. Brant L. Schneider EL (1990). DNA damage and repair with age in individual human lymphocytes. Mutation Research. 237(3-4):123-30.

Singh NP. Stephens RE. Schneider EL. (1994). Modifications of alkaline microgel electrophoresis for sensitive detection of DNA damage. International Journal of Radiation Biology. 66(1): 23-8.

Singh NP. Graham MM. Singh V. Khan A. (1995). Induction of DNA single-strand breaks in human lymphocytes by low doses of gamma-rays. International Journal of Radiation Biology. 68(5):563-9.

Slovic P (1987). Perception of risk. Science 236: 280-285.

Sobel E. Dunn M. Davanipour Z. Qian Z. Chui HC (1996): Elevated risk of Alzheimer's disease among workers with likely electromagnetic field exposure. Neurology 47:1477-1481.

Spadaro JA (1982). Electrically enhanced osteogenesis at various metal cathodes. Journal of Biomedical Materials Research. 16(6):861-73.

Stevens RG (1987a). Electric power use and breast cancer: a hypothesis. American Journal of Epidemiology. 125: 294-300.

Stevens RG (1987b). Electric power use and breast cancer. American Journal of Epidemiology. 125: 556-561.

Stuchly MA McLean JR. Burnett R. Goddard M. Lecuyer DW. Mitchel RE (1992). Modification of tumor promotion in the mouse skin by exposure to an alternating magnetic field. Cancer Letters 65:1-7.

Sugiyama S (1974). Control of visual fatigue by means of DC and AC electric fields. In "Biologic and clinical effects of low frequency magnetic and electrical fields. CC Thomas Springfield Illinois chapter 5.

Sumner D (1987). Radiation Risks: An evaluation. Taragon Press Glasgow.

Suri A. deBoer J. Kusser W. Glickman BW. (1996). A 3 milliTesla 60 Hz magnetic field is neither mutagenic nor co-mutagenic in the presence of menadione and MNU in a transgenic rat cell line. [Journal Article] Mutation Research. 372(1):23-31.

Tice RR. Strauss GH. (1995). The single cell gel electrophoresis/comet assay: a potential tool for detecting radiation-induced DNA damage in humans. Stem Cells. 13 Suppl 1:207-14.

Tomenius L (1986). 50-Hz electromagnetic environment and the incidence of childhood tumors in Stockholm County. Bioelectromagnetics 7:191-207.

Tuck A. Smith S. Larcom L (2000). Chronic lymphocytic leukemia lymphocytes lack the capacity to repair UVC-induced lesions. Mutation Research. 459(1):73-80.

Verkasalo PJ Pukkala MY Hongisto JE (1993). Risk of cancer in Finnish children living close to power lines. British Medical Journal 307:895-899.

Washburn EP Orza M Berlin J (1994). Residential proximity to electrical transmission and distribution equipment and the risk of childhood leukemia childhood lymphoma and childhood nervous system tumours: Systematic review evaluation and meta-analysis. Cancer Causes Control 5:229-309.

Wei M. Guizzetti M. Yost M. Costa LG (2000). Exposure to 60-Hz magnetic fields and proliferation of human astrocytoma cells in vitroToxicology & Applied Pharmacology. 162(3):166-76.

Wertheimer N. Leeper E (1979). Electrical wiring Configurations and Childhood Cancer. American Journal of Epidemiology ; 109: 273-284

Wertheimer N and Leeper E (1982). Adult cancer related to electrical wires near the home. International Journal of Epidemiology 11:345-355.

Wilson BW Wright CW Morris JE (1990). Evidence for an effect of ELF electromagnetic fields on human pineal gland function. Journal Pineal Research. 9:259-269.

Wilson BW Caputa K. Stuchly JD (1994). Design and fabrication of well confined uniform magnetic field exposure systems. Bioelectromagnetics. 15: 563-577.

Wright WE Peters JM. Mack TM (1982). Leukaemia in workers exposed to electrical and magnetic fields (letter). Lancet 8308 (Vol II):1160-1161.

Yasuda I (1953). Fundamental aspects of fracture treatment. Journal of the Kyoto Medical Society. 4: 395-406.

Yasui M. Kikuchi T. Ogawa M. Otaka Y. Tsuchitani M. Iwata H (1997). Carcinogenicity test of 50 Hz sinusoidal magnetic fields in rats.z Bioelectromagnetics. 18(8):531-40.

Yen-Patton GP. Patton WF. Beer DM. Jacobson BS (1988). Endothelial cell response to pulsed electromagnetic fields:stimulation of growth rate and angiogenesis in vitro. Journal of Cellular Physiology. 134(1):37-46.

Youngson JHAM. Clayden AD. Myers A(1991). A case/control study of adult haematological malignancies in relation to overhead powerlines. British Journal of Cancer 63:977-985.