IMMUNE STIMULATION WITH SHORT-TERM EXPOSURE TO EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS IN MICE (*Mus. musculus*)

By

MICHELLE KIM WIESE

N.D.: Medical Technology (Cytology)

N.H.D.: Medical Technology (Cytology)

Dissertation submitted in fulfilment of the requirements for the degree:

MAGISTER TECHNOLOGIAE: BIOMEDICAL TECHNOLOGY

in the

DEPARTMENT OF HEALTH SCIENCES

at the

CENTRAL UNIVERSITY OF TECHNOLOGY, FREE STATE

BLOEMFONTEIN

2013

Study Leader: Dr CE Brand DTech (Biomedical Technology)

Co-study leader: Prof L de Jager PhD (Anatomy)

DECLARATION OF INDEPENDENT WORK

I, MICHELLE KIM WIESE, identity number [redacted] and student number 8707895, do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree MAGISTER TECHNOLOGIAE: BIOMEDICAL TECHNOLOGY, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

SIGNATURE OF STUDENT **DATE**

SUMMARY

Electromagnetic fields are present wherever electricity is created. The frequency range of these electromagnetic fields is from extremely low to extremely high. The fields present in domestic areas fall within the extremely low frequency range. These fields are created by domestic electrical appliances and telecommunication.

There has been much debate on the effect of exposure to these fields on human health. Research has not yet been able to prove adverse effect of these fields on human health. In fact, the benefits of magneto therapy has been recognized and used for several decades.

Recently a specific electromagnetic signal has been under investigation for its ability to stimulate the immune response. This signal is produced by a patented generator, called Immunent Activator. Studies performed with the Immunent Activator signal on farm animals revealed increased feed conversion and decreased intestinal lesions of animals with intestinal infections. Most of the research was performed on fish and fowls and evidence of similar findings in mammals is lacking.

In the current study, mice were exposed to the Immunent BV signal for seven days, after which immune cell counts were performed and compared to the immune cell counts of a control group of mice which received no electromagnetic exposure.

It was found that the T-lymphocyte population of immune cells in the exposed group of mice was statistically significantly higher than that of the control group. The neutrophil count was statistically significantly lower in the exposed group compared to the control group.

These findings revealed evidence of immune stimulation in the mice which were exposed to the Immunent Activator signal. Suggestions for further research could be made with regard to specific mechanisms of immune stimulation. The findings of this and other related studies hold benefits for the farming and health industry.

DEDICATION

I dedicate this work to the Lord God Almighty for blessing me with more than I could have ever wished for.

"*A good name is rather to be chosen than great riches, and loving favour rather than silver and gold. The rich and poor meet together: the Lord is maker of them all*".

Proverbs 22:1-2

"If we are to better the future, we must disturb the present".

Catherine Booth

ACKNOWLEDGEMENTS

I would like to express my thanks and appreciation to the following:

- God, for the wisdom and ability to achieve that which He has gifted me with
- My husband, Andre; and my children, Abigail and Tristan, for their support and understanding during the course of my study
- My friend, Dr Carien Weyers, for your advice and support
- The National Research Foundation for the financial support for the research project
- Dr CE Brand and Prof L. De Jager for their guidance during the completion of my study
- Linda Coertze for the editorial assistance provided
- Maryn Viljoen for the statistical analysis
- The Central University of Technology for providing the facilities to conduct the research and providing funds through the Innovation Fund to complete the study
- The Free State University Animal Unit for their help with the facilities to conduct the exposure of EMFs to the animals
- The Biomedical Technology programme colleagues and manager for their support and accommodation of class relief to complete the study
- My dearest colleagues and friends of CUT for their professional inputs and advice
- Immunent b.v. Werfberg 12, Veldhoven, The Netherlands for the funding of the research project

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

%	Percentage
µT	Microtesla
A/m	Ampere per meter
AC	Alternating current
ANOVA	Analysis of variance
APC	Antigen-presenting cells
Ca^2	Calcium
CD	Cluster of differentiation
CRP	C-reactive protein
DC	Direct current
DNA	Deoxyribonucleic acid
DOE	Department of energy
DxBxL	Depth x breadth x length
EDTA	Ethylenediaminetetraacetic acid
EF	Electric fields
ELF	Extremely low frequency
EMF	Electromagnetic field
FACS	Flourescence activated cell sorting
FBC	Full blood count
FDA	Food and drug administration
fl	Femtolitre
G	Gauss
g/dl	Grams per decilitre
G-CSF	Granulocyte colony-stimulating factor
GHz	Gigahertz
GM-CSF	Granulocyte-macrophage colony-stimulating factor
Hb	Haemoglobin
Hct	Haematocrit
HF	High frequency

Hz	Hertz
ICNIRP	International Commission on Non-Ionizing Radiation Protection
IFN	Interferon
IL	Interleukin
IM	Immunent
kD	Kilodalton
Kv/m	Kilovolt per meter
Lab	Laboratory
LF	Low frequency
M-CSF	Macrophage colony-stimulating factor
MCV	Mean corpuscular volume
MF	Magnetic fields
mG	Milligauss
MRI	Magnetic resonance imaging
mT	Millitesla
Na	Sodium
NHLS	National Health Laboratory Service
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institute of Health
NK	Natural Killer
NRC	Nuclear Regulatory Commission
O_2	Oxygen
PHA	Phytohaemagglutinin
Plt	Platelet
PMA	Phorbol 12-myristate 13-acetate
RBC	Red blood cell
RF	Radio frequency
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SANS	South African National Standards
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SD	Standard deviation
SI	Standard international

T	Tesla
T_C cells	Cytotoxic T-cells
TGF	Transforming growth factor
T_H cells	Helper T-cells
TNF	Tumor necrosis factor
UPE	Ultra-weak photon emission
US	United States
UV	Ultraviolet
V/m	Volt per meter
WBC	White blood cell

TABLE OF CONTENTS

CHAPTER 4:
RESULTS AND DISCUSSION

CHAPTER 1

INTRODUCTION

1.1 Introduction

Advanced electrical technology has created an electromagnetic environment in which people live, work and play, resulting in constant concern about the environment and human health. Life without electricity in the 21st century is unthinkable since almost our entire existence has become dependent on electricity and wireless telecommunication (Havas, 2000). However, where electricity is generated, electric fields (EF) and magnetic fields (MF) are created (Boorman *et al.*, 1999). The MFs created in this instance are known as electromagnetic fields (EMFs). EMFs constitute a wide spectrum, ranging from extremely low frequencies (ELF) to high frequencies (HF). Exposure to EMFs in the HF ranges is extremely harmful to animals and humans; hence these fields are strictly monitored within controlled environmental limits (Blank, 1995; Ahlbom & Feychting, 2003). The fields in the intermediate range are under constant investigation as they have yielded extremely controversial results. It is for this reason that ELF-EMFs have recently become a new focus of investigation.

People are exposed to EMFs in the ELF spectrum on a daily basis, since household appliances, computers and telecommunication systems fall within this range (Boorman *et al.*, 1999; Cifra *et al.*, 2011). With constant exposure of people to the EMFs created in our homes and work environment, it is inevitable that questions have been raised as to whether exposure to ELF-EMF has any influence on the human body or human health. Consequently, extensive research has been conducted in the field of electromagnetism and the possible health effects due to exposure to ELF-EMFs.

Initially, research concentrated on the adverse effects that ELF-EMFs have on the human biological system (Wertheimer & Leeper, 1979; Ahlbom & Feychting, 2003). Some scientists have claimed that exposure to ELF-EMFs can hold health risks, whereas others have claimed that these EMFs cannot interact with the

human body (de Kleijn *et al.*, 2011). It is well documented that exposure to higher field strength EMFs can be detrimental to human health (Wertheimer & Leeper, 1979). However, it was also found that short-term exposure to ELF-EMF does not have any detrimental effect on human biological systems (Hashish *et al.*, 2008), but that long-term exposure can cause chronic stress, resulting in tissue damage (de Bruyn & de Jager, 1994; Ahlbom & Feychting, 2003; Bonhomme-Faivre *et al.*, 2003).

The finding that short-term exposure to ELF-EMF is not detrimental to the human body redirected the research interest to EMFs in the ELF range. These fields are produced by power lines and day-to-day domestic electrical devices (Boorman *et al.*, 1999; de Kleijn *et al.,* 2011). The National Institute of Environmental Health Sciences (NIEHS) could not report substantial evidence that ELF-EMF adversely affects human health (NIEHS, online). Several studies were conducted to determine the effect of ELF-EMF on the immune system, but the findings were negative. An opinion was adopted in Europe by the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) that there is no conclusive evidence of adverse health effects as a result of exposure to ELF-EMF; hence further investigations are warranted (SCENIHR, 2009). Other research efforts also indicated that there is no conclusive evidence that exposure to low field strength EMFs poses a health risk (Boorman *et al.*, 1999; Repacholi & Greenbaum, 1999; Ahlbom & Feychting, 2003; Cifra *et al.*, 2011).

The possibility of potential therapeutic benefits of exposure to ELF-EMF has opened a new area of interest in this field. Various studies have shown the possibility of therapeutic benefits from short-term exposure to ELF-EMFs (Blank & Goodman, 2000; Simko & Mattson, 2004; Markov *et al.*, 2006; Goraca *et al.*, 2010; Waite *et al.*, 2011). With extensive research performed in the field of health effects due to exposure to ELF-EMF, an area of specific interest arose in the role of the immune response. There appears to be a notion that short-term exposure to ELF-EMF could beneficially affect the immune system. It was found that short-term exposure to fields in the ELF range affects the blood cell concentrations and results in lymphocyte proliferation (de Kleijn *et al.*, 2011). This same research group indicated an increased phagocytic activity in

macrophages, hence implicating an effect on the innate immune response. Substantial *in vitro* studies were performed on chickens and fish (Cuppen *et al.*, 2007; Elmusharaf *et al.*, 2007). Cuppen *et al.* (2006) hypothesized that ELF-EMF could boost or stimulate the immune system by "putting it into a state of alert due to cytokine production". A similar suggestion was proposed by Aldinucci & Pessina (1998). Several authors have suggested that short-term exposure to ELF-EMF can stimulate the immune response, specifically on a cellular level, leading to the production of cytokines (Blank & Soo, 1992; Simko & Mattson, 2004; Cuppen *et al.*, 2007; Elmusharaf *et al.*, 2007; de Kleijn *et al.*, 2011; Waite *et al.*, 2011).

If shown to be correct, the hypothesis by Cuppen *et al.* (2007) could be a breakthrough in the medical field. It could possibly imply toward boosting an early immune response (de Kleijn *et al.*, 2011). Since the hypothesis was based on results from studies performed on chickens, fish and *in vitro* studies, it seems necessary to expand the investigations to *in vivo* studies on mammals and, ultimately, on humans. However, before embarking on such studies there is a need for duplication of existing evidence to show that short-term exposure to ELF-EMF can promote innate immunity and for finding new evidence to support this theory. With limited evidence of these findings in mammals, supportive research can strengthen the suggestions made by Cuppen *et al*. (2007).

Recent studies indicated that exposure to a specific EMF signal (the Immunent Activator signal with multiple waveforms ranging from 20 - 5000 Hz) decreased the mortality in fish (Cuppen *et al.,* 2007) and also improved the feed conversion in chickens (Elmusharaf *et al.*, 2007). In addition, this same signal revealed enhancement of the immune system when a study on chickens, infected with coccidiosis, indicated reduced intestinal lesions after exposure to this specific signal (Elmusharaf *et al.,* 2007).

The Immunent Activator signal is created by a device designed and built by Immunent BV (Werfberg 12, Veldhoven, The Netherlands) which emits a specific ELF-EMF signal consisting of multiple, highly complex waveforms and intensity. These waveforms and intensity differ from those standard therapeutic signals one would expect them to conform to (Waite *et al.,* 2011). Using this patented signal,

several research studies have shown that short-term exposure to the signal enhances the immune system (Blank & Soo, 1992; Simko & Mattson, 2004; Cuppen *et al.*, 2007; Elmusharaf *et al.*, 2007; de Kleijn *et al.*, 2011).

Studies assumed an effect at cellular level, where cells respond to EMF by reacting as they would to an unspecific stressor (Simko & Mattsson, 2004). However, the proposed hypothesis by Cuppen *et al.* (2007) needed to be further investigated, specifically in mammals. Research will also need to focus on ELF-EMF signal variables with respect to the specific EMF intensity, waveform and frequency. Further investigation on the biological mechanism of action could also contribute toward outcomes in this regard.

1.2 Problem Statement

If there is evidence that short-term exposure to the ELF-EMF Immunent Activator signal can stimulate the immune system of fish and chickens (Cuppen et al., 2006), the question arises whether it would be possible to reproduce the findings in mammals, specifically mice (*Mus. musculus*) and, by so doing, validate the findings of previous research in lower animals. It is therefore important to test the effect of the ELF-EMF Immunent Activator signal on the immune system of mammals.

1.3 Aim of the study

The aim of this study was to investigate whether short-term exposure to ELF-EMF from the specific Immunent Activator signal influenced the immune system in mice (*Mus. musculus*) and to determine if the duration of exposure to the signal had any significant effect on the results.

1.4 Objectives

The following were the objectives of the study:

- Obtain a sample of one hundred experimental mice.
- Set up a housing facility in which mice can live comfortably while being exposed to the Immunent Activator signal.
- Divide mice into four groups and expose them to the Immunent Activator signal as follows: Present in same sequence as in *Figure 1.1*.
    - Continuous exposure (24 hours)
    - Exposure of four hours per day
    - Exposure of one hour per day
    - Control group which received no (sham) exposure
- Following exposure for one week, perform the following biological analyses:
    - Full blood count
    - Immunophenotyping, using CD3, CD4, CD8 and CD19 markers (to determine total T-lymphocyte, T-helper, T-suppressor and B-lymphocyte counts)
- Data analyses:
    - Compare data from exposure groups with the control group
    - Compare data among the three exposure groups.

Figure 1.1 presents a summary of the objectives and the design of the study.

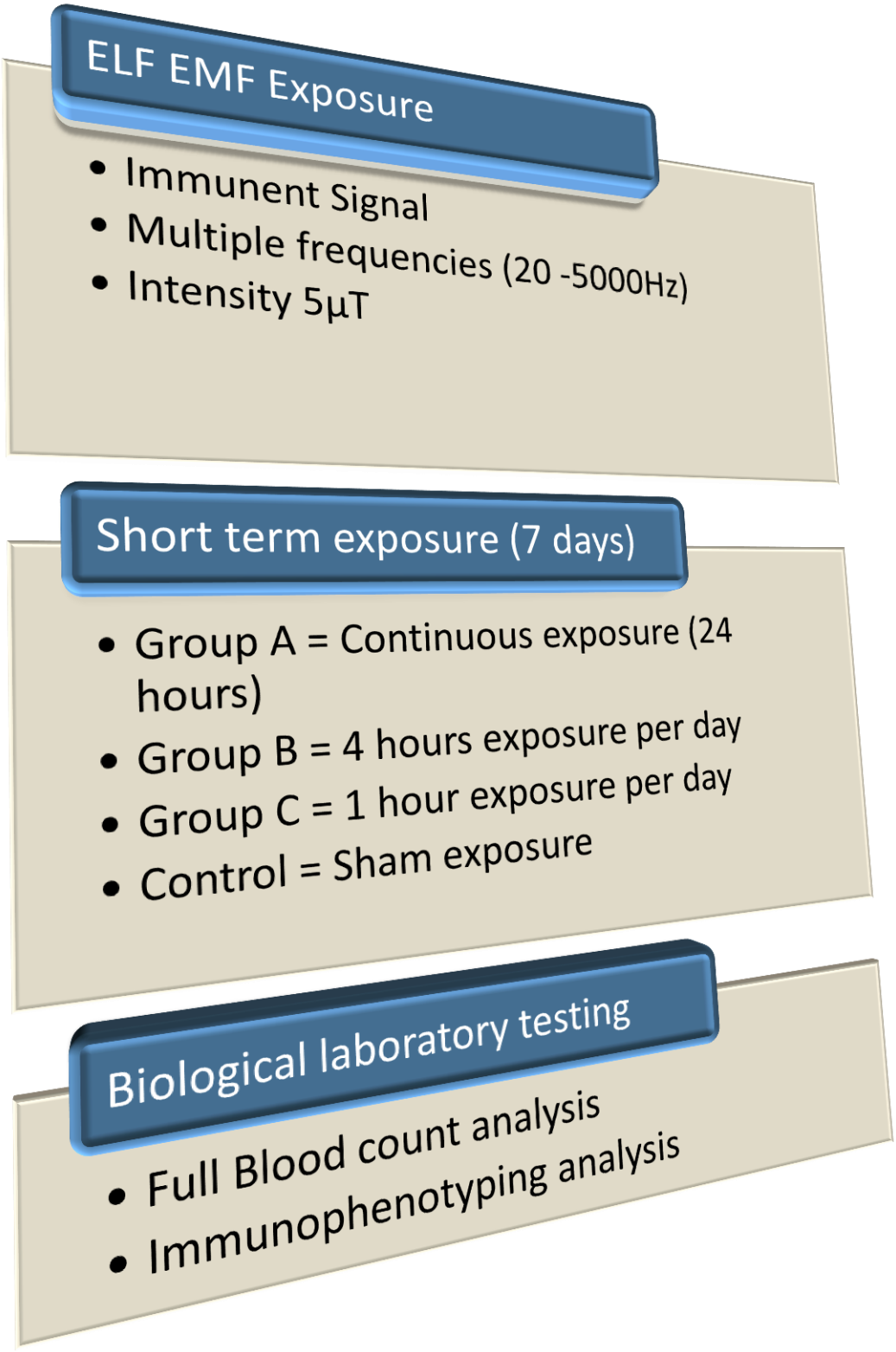

Figure 1.1: Schematic layout of study protocol

1.5 Summary

As several studies have shown that the immune system in fowl and fish can be activated by exposure to ELF-EMFs, it left the scope for supportive research to be conducted in order to determine whether the same results could be achieved in mammals. This research would therefore extend previous studies to include higher animals and it would test additional immune parameters to those tested in earlier studies. In addition to this, the length of exposure would be investigated to determine if the desired effects could be influenced by the duration of exposure.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Considering the nature and origin of electromagnetic fields (EMFs), it is inevitable that questions will arise as to whether EMFs have any biological effect on the human body and, moreover, by which biological mechanisms this effect takes place. Life itself is an "electromagnetic event" (Markov *et al.*, 2006), and it is well known that exposure to HF-EMFs (from the ionizing segment of the spectrum) can cause serious tissue damage, such as burns (Blank, 1995; Ahlbom & Feychting, 2003). For this reason strong regulations have been established internationally to prevent exposure to these fields. Evidence has been provided that exposure to low frequency (LF-EMFs) in individuals living close to power stations (Wertheimer & Leeper, 1979) and long-term exposure to lower field strengths (de Bruyn & de Jager, 1994) could be detrimental to human health. There is, however, no evidence that short-term exposure to extremely low frequency EMFs is detrimental to human health (Boorman *et al.,* 1999; Repacholi & Greenbaum, 1999; Ahlbom & Feychting, 2003).

It is mostly household appliances that create ELF-EMFs, and the implications of exposure to these EMFs have been questioned, since the exposure of individuals to these fields occurs on a daily basis. Although literature has supplied a plethora of controversial evidence as to whether ELF-EMFs are harmful or not, there is currently insufficient research evidence to substantiate any of the findings. Where strong evidence has been provided that EMFs could cause adverse effects in humans (Wertheimer & Leeper, 1979), the mechanism of action is unknown (Ahlbom & Feychting, 2003).

The major component of research performed in this field has been directed toward the harmful effects of EMFs on the human biological system. Although magneto therapy with ELF-EMF has been used for almost five decades (Vallbona & Richard, 1999), research has begun to consider the possibility of additional

therapeutic benefits of some of these fields due to their ability to induce the synthesis of stress proteins (Blank & Goodman, 2000). However, it seems that this benefit is only obtained from short-term exposure, as de Bruyn & de Jager (1994) have indicated that long-term exposure can be detrimental due to chronic stress induction.

2.2 Electromagnetism

If a magnet is allowed to spin around in the vicinity of a coil, electricity will be generated (Boorman *et al.,* 1999). This forms the basic principle according to which electricity is generated in power stations. The area around the magnet forms the MF. MFs are lines of force that cannot be seen. The power of the magnetic field is strongest closest to the magnet. EFs and EMFs will be created wherever electricity is generated, transmitted or used (Boorman *et al.,* 1999).

EMFs consist of two components; electric and magnetic fields. EFs are created due to the presence of an electric charge. This is the power (extent) and direction of the force it exerts on a positive electric charge. A magnetic field is created by the movement of electric charges. This movement forms an electric current which gives the number of charges per second passing through the conductor (a conductor being a substance which allows electricity to flow through it). The majority of electricity used consists of alternating currents. The current is pulsed to-and-fro during a specific length of time. It is the number of times a current is pulsed to-and-fro during a specific time that is known as the frequency, which is measured in Hertz (Hz). The more alternating currents within a given time, the higher the frequency will be. It is then this frequency that creates the electric and magnetic field, of which the strength is proportional to the frequency (e.g., 50 Hz alternating current will create a 50 Hz electric and magnetic field). The magnitude of the magnetic field is proportional to the current flow in a conductor, regardless of the voltage present (Boorman *et al.,* 1999).

EMF signals vary considerably in their physical characteristics. The signal from one field differs from the other even if there is just one different variable (Markov & Hazlewood, 2009). When looking closely at the physics and engineering of EMF signals, there is always a basic frequency with a range of additional frequencies which creates the harmonics of the signal. Hence, when dealing with a specific signal, it is essential to understand and describe the harmonics of that signal.

2.2.1 Measurement of electromagnetic field strength

Electromagnetic fields are indicated as frequency or wavelength, where the wavelength is inversely proportional to the frequency (Ahlbom & Feychting, 2003). The shorter the wavelength, the higher the frequency will be. *Figure 2.1* demonstrates the relationship between wavelength and frequency and illustrates some sources of these fields. The lower electromagnetic fields refer to the frequency and the higher electromagnetic fields refer to wavelength. Voltage determines the extent of the electric fields in a location, the strength of which is measured in volt/meter (V/m) or kilovolt/meter (KV/m) (Boorman *et al.,* 1999). Current refers to the amount of the MFs in a location (strength = magnetic flux density or magnetic strength, where magnetic flux density and magnetic strength are proportional to the amount of current). Magnetic flux density is measured in gauss (G), or tesla (T), where 1mG is equivalent to 0,1μT. Magnetic strength is measured in standard international (SI) units of amperes/meter (A/m).

2.2.2 The electromagnetic spectrum

The electromagnetic spectrum ranges from ELF to HF wavelengths. Included in this spectrum are various sources and strengths of EMFs, such as x-ray radiation, ultraviolet (UV) radiation, radio frequency (RF) fields, static fields and visible light (Ahlbom & Feychting, 2003). The characteristics of EMFs depend on their frequency or wavelength (Ahlbom & Feychting, 2003), which will determine the energy they create. The ionizing segment of the electromagnetic spectrum is associated with heat production and can break chemical bonds (Ahlbom & Feychting, 2003). Exposure to these fields of radiation will cause cell damage (Blank, 1995). These fields include the HF fields with a short wavelength (Ahlbom & Feychting, 2003). They begin with the UV-band and extend to gamma rays. The non-ionizing segment, on the other hand, is not associated with heat production and cannot break chemical bonds. This segment includes LF fields with a longer wavelength. The fields associated with the use of mobile telecommunication which fall within the RF band include frequencies between 450 - 2500M Hz. However, it is obvious that microwaves that fall within 10^9 - 10^{11} Hz) that are used for cooking will cause cell damage (Blank, 1995) as they are somewhat higher than RF. At the lowest end of the LF band are ELF fields which are associated with general production of electricity, including frequencies of

approximately 50 - 60 Hz (Ahlbom & Feychting, 2003). In most countries, electrical power is used at 50 - 60 Hz (Hashish *et al.*, 2008). LF fields are found between the latter two fields and have frequencies of up to 300G Hz. The distinction between the ionizing and non-ionizing spectrum is at approximately the upper end of the UV band.

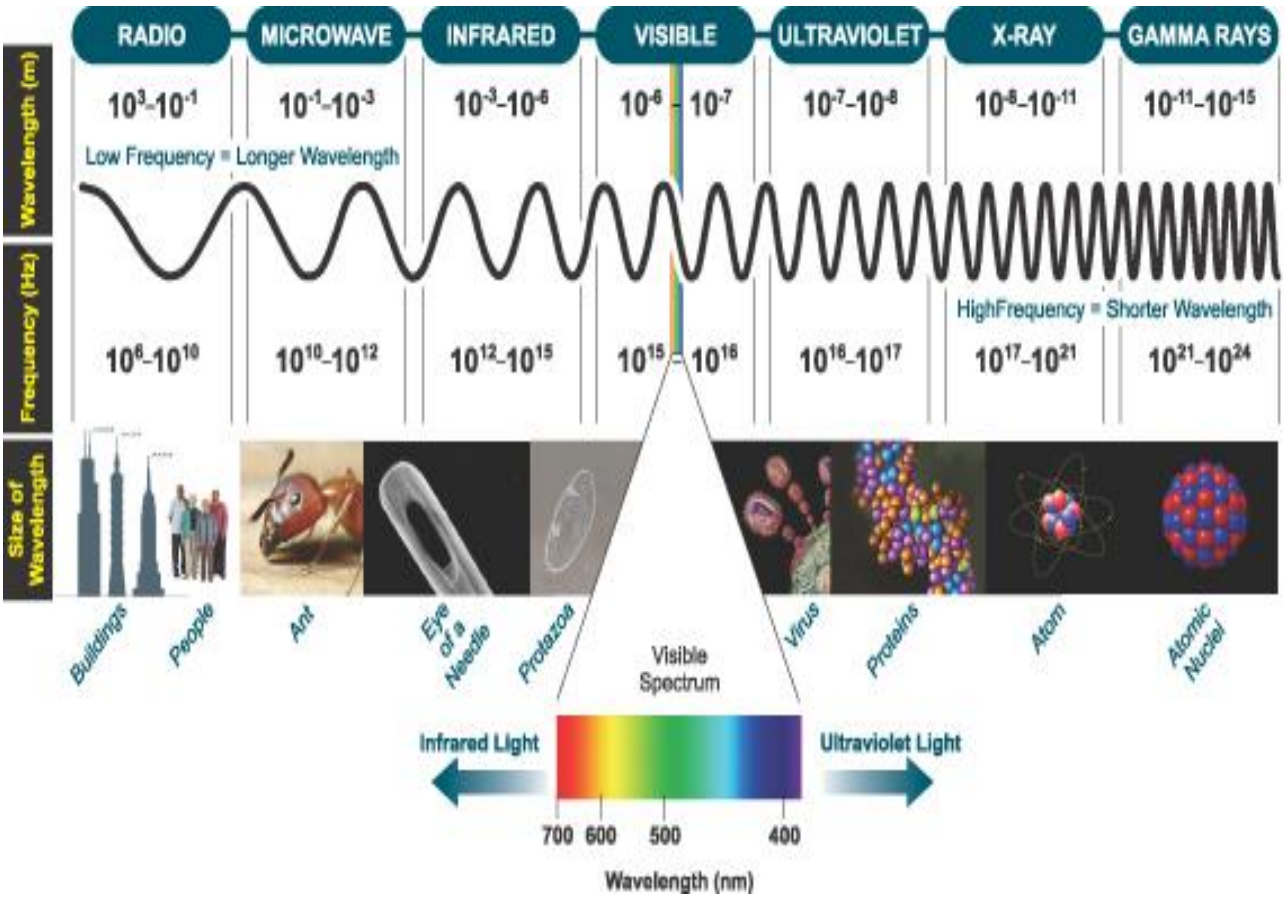

Figure 2.1: The electromagnetic spectrum, its frequencies, wavelengths and the sizes of the wavelengths (University of Virginia online (image from Andor Technology online))

2.2.3 Sources of electromagnetic fields

The electromagnetic spectrum covers many different types of electromagnetic fields, such as x-ray radiation, UV radiation, radio frequency fields, static fields and visible light (Ahlbom & Feychting, 2003). Electric fields can occur naturally in the atmosphere (approximately 120 - 150 V/m), but in thunderstorms theImmunent Activatory can reach up to 20 KV/m (Repacholi & Greenebaum, 1999). Naturally occurring environmental levels are also found, but it is not certain at which field parameters these can be determined (Repacholi & Greenebaum, 1999). High electrostatic fields (20 KV/m) can also be found near equipment using high voltages, including household appliances such as television

sets. Most public exposure to electromagnetic fields comes from electrical appliances, household wiring and alternating current (AC) transmission and distribution lines.

Examples of exposure levels include the following ELF-EMF strengths (Repacholi & Greenebaum, 1999):

- static geomagnetic field and naturally occurring - 0.01 - 0.1 mT (millitesla)
- direct current (DC) lines - 22 μT
- workers using DC equipment – 50mT
- occupational exposure at magnetic resonance imaging (MRI) departments (MRI operators – 5 mT and patients undergoing MRI – 2.5T)
- household appliances – 10-15 μT

A study was performed by Farag *et al.* (1998) to determine the most common exposure that people experience in a domestic environment. They tested residential sources such as electrical appliances in the home, grounding systems of residence lights and overhead power distribution lines. The largest EMFs were created by appliances in the homes, and the largest fields were experienced closest to the appliances while in use. The fields decreased exponentially as the distance from the appliance increased. The authors described two types of residential EMF sources. One is known as “area sources”. In these cases the fields extend for some distance beyond the source (e.g., currents on pipes, power and distribution lines). The other is known as “local sources”. In the latter case the fields are confined to the vicinity of the source (e.g., televisions, toasters, washing machines, hair dryers, to name a few).

2.2.4 Dosimetry

Dosimetry is also known as the “dose”. This is the amount of exposure which can cause a biological effect as a result of the exposure (Repacholi & Greenebaum, 1999). Dosimetry is measured at or near the point of interaction of the field with the biological system. *Macrodosimetry* involves exposure of the entire body, tissues or an organ, whereas *microdosimetry* involves exposure at cellular or sub-cellular level. In the clinical and scientific communities, the concept of dosimetry can often be misinterpreted. According to Markov & Hazlewood (2009),

dosimetry can be viewed from a physical and a biophysical point of view. *Physical dosimetry* relates to the characteristics of the signal from an engineering point of view. *Biophysical dosimetry* is the amount of EMF received by target tissues. The latter is, in fact, the "dose". In addition to the fact that EMFs vary considerably in their physical characteristics, there are also other variables (e.g., distance) that will determine the actual "dose" of exposure. The time or duration of exposure and the strength of the fields will determine the dosimetry. Some authors refer to the duration of exposure as the "working time" (Goraca *et al.*, 2010).

2.2.5 Biological effects of extremely low frequency electromagnetic fields

Since there is no conclusive scientific evidence that LF-EMFs and ELF-EMFs can cause health effects, investigations in this field are on-going. The strongest evidence found was that of a substantially increased risk of childhood cancer in subjects living in close proximity to power lines (Wertheimer & Leeper, 1979). Considering the lack of substantial evidence, research has hypothesized the possibility of adverse health effects due to long-term exposure to these field strengths of EMFs and it was found that there was evidence of a possible cause, but it was concluded that the mechanism was unknown (Ahlbom & Feychting, 2003). De Bruyn and de Jager (1994), however, suggested that long-term exposure to ELF-EMF resulted in adverse health effects due to the induction of chronic stress, hence implicating the chronic stress factor as a possible mechanism for adverse health effects. These findings confirmed similar findings by Mevissen *et al.* (1998). Feychting and Ahlbom (1993) demonstrated that the effect of exposure to LF-EMF depended on the dose of exposure. They postulated that the amount of EMF that an individual was exposed to (dosimetry) contributed to the increased risk of adverse health effects.

The duration of exposure plays a very important role in the outcome of effects that ELF-EMF has on biological systems. Goraca *et al.* (2010) confirmed that the duration of exposure or working time had an effect on the results. With exposure of rats to a 40Hz (7 mT) field for thirty minutes per day for two weeks, there was no alteration in reactive oxygen species (ROS) generation of heart tissue, but when exposing them to the same signal for sixty minutes per day for two weeks,

there was an increased ROS production and decreased antioxidant production. Therefore, they concluded that the effect was dependent on working time (i.e., duration of exposure).

Havas (2004) is convinced that scientists are very close to unravelling the effects of ELF-EMF on biological systems and the biological mechanisms involved in this process, arguing that the outcomes will be beneficial. This strongly warrants the necessity for research to achieve a specific outcome.

2.2.5.1 Public concern

The consequences of exposure to LF-EMFs and ELF-EMFs are of great public concern, since these are the fields the public are exposed to on a daily basis. Havas (2000) refers to these fields as "techno fields" since they are the fields created by technology. For this reason investigations have been ordered by various governments to determine the possible health effects due to exposure to these EMFs (Havas, 2000). In 1992 the United States (US) congress advised the National Institute of Environmental Health Sciences (NIEHS), the National Institute of Health (NIH) and the US Department of Energy (DOE) to initiate research and to collect evidence on the potential health risks from being exposed to ELF-EMF (Boorman *et al.,* 1999). This was an in-depth study to clarify whether there are any health risks being exposed to these fields. The report concluded that there was very little evidence showing that exposure to these fields posed a health risk, but it could also not be considered safe either. The reason for this conclusion was that there was a lack of consistency in the findings in animals and mechanistic studies.

Despite the research done in this area over the past decade, it is still unclear as to whether exposure to ELF-EMF is harmful to human biological systems or not. This area of research therefore still needs a great deal of epidemiological studies (Hashish *et al.*, 2008).

2.2.5.2 Therapeutic benefits and applications

In spite of the proliferation of evidence pointing to the detrimental health effects of long-term exposure to LF-EMFs, there is also evidence of beneficial effects from short-term exposure to ELF-EMFs (Blank & Goodman, 2000; Simko & Mattson,

2004; Markov *et al.*, 2006; Goraca *et al.*, 2010; Waite *et al.*, 2011). This evidence excludes the fact that EMFs have been used therapeutically for centuries. The use of pulsating electromagnetic therapy has been used for more than 50 years (Vallbona & Richard, 1999) and the interest in this form of therapy has increased immensely in the last few decades (Markov *et al.*, 2006). The reason for this is that "electroceutics" (a term introduced by Markov *et al.* in 2006) is cheaper and less invasive than drugs or surgery. Moreover, it has fewer side effects than those associated with drug treatment (i.e., the administration of pharmaceutics). The application of this technology is commonly used in many conditions varying from bone fracture and wound healing to treatment of sleep disorders. In fact, EMF devices have been used therapeutically since just after World War II, but very little is known about the applications at that time (Markov & Hazlewood, 2009). It is specifically the time varying (pulsed) EMFs that are applied therapeutically. The general aim of therapeutic signals is to create a signal that mimics the body's natural EMFs or, alternatively, alter certain biochemical processes in the body (Waite *et al.*, 2011).

The physics and engineering of EMF signals for therapeutic application are designed in a specific fashion to target a specific tissue, or part of a tissue, ensuring that the flux density is received by the target. A specific signal might be beneficial for application in one biological area, but not in another (Markov & Hazlewood, 2009).

The indication of a stress response which has repeatedly been implicated (Blank & Goodman, 2000; Simko & Mattsson, 2004; Cuppen *et al.*, 2007; Hashish *et al.*, 2008; de Kleijn *et al.*, 2011) has shown the possibility of therapeutic benefits and has opened a new area of interest in these fields. Studies have shown the possibility of therapeutic benefits from short-term exposure to ELF-EMFs (Goraca *et al.*, 2010; Marcov *et al.*, 2006; Simko & Mattson, 2004; Waite *et al.*, 2011; Blank & Goodman, 2000). A field commonly applied in magneto therapy is a 40 Hz, 7 mT exposure for thirty minutes per day, for two weeks (Goraca *et al.*, 2010). The question then, once again, arises as to what the mechanism of action is. The same stress induction mentioned by de Bruyn & de Jager (1994) is proposed to be the possible mechanism of action by Blank and Goodman (2000). However,

Blank and Soo (1996) suggest that overexposure to these EMFs could compromise the beneficial effect, which supports the findings of de Bruyn & de Jager (1994).

In a mini review article by Goodman and Blank (1998), the therapeutic application of EMFs is reiterated. Reference was made to Basset (1995), who stated that the application of magnetic fields for assistance in healing of bone fractures was approved by the Food and Drug Administration (FDA) in 1972. However, the biggest question or void in this area is the availability of research findings supporting the biological mechanisms.

Varani *et al.* (2002) indicate that exposure to LF-EMF results in an increase of A_{2A} adenosine receptor density. Adenosine interacts with receptors on the neutrophil surface as an anti-inflammatory agent. With this finding in mind, it has been proposed that these fields can well be used therapeutically for wound healing due to their anti-inflammatory effects.

Goats (1989) also discuss the various therapeutic applications of pulsed electromagnetic energy. The application can be used for pain, soft tissue injury, wound healing and nerve repair.

2.2.5.3 Biological mechanisms

Cuppen *et al.* (2007) propose that the mechanism of action is on a cellular level. This group of researchers proposes that EMFs act as the foreign stimulus. An immune response is launched against foreign substances or cells by means of promoters such as heat shock proteins and cytokines. When cells are stressed for any reason, there is immediate release of danger signals which then, in turn, alert the immune system to launch an action.

Blank (1995) devoted a great deal of time to investigate the possible molecular mechanisms of action that EMFs have on the human biological system. Blank and Soo had also studied the mechanism of action of EMFs on the cell membrane enzymes, cytochrome oxidase and Na, K-ATPase (Blank and Soo, 1992; Blank and Soo, 1996). They found that EMFs interact with charge movements during enzyme function and cause accelerated charge movements in

enzymes as well as within the deoxyribonucleic acid (DNA) of the cell, thus opening up the possibility that EMFs directly interact with cellular DNA.

Blank and Goodman (2009) state that there is a general acceptance that LF-EMFs activate DNA to synthesize proteins, this being the mechanism of increasing production of stress proteins by cells. This stress response is a natural mechanism of the body to protect itself against potentially harmful external influences. They also point out that the effect that these EMF fields have on the body is not only at cellular level, but that they also have physiological effects such as changes in heart and breathing rate or muscle activity. The cellular effects and stress proteins will be discussed in more detail later in this chapter (i.e., sections 2.5.3 and 2.5.4).

There is evidence that all cells (not only electrically excitable and nerve cells) produce EMFs in the visible light spectrum (Cifra *et al.*, 2011). This spontaneous emission of light by living cells, initially referred to as “mitogenic radiation”, was well established in the mid-1900s. Later it became known as ultra-weak photon emission (UPE) or, alternatively, biological luminescence or bio photons. Based on this phenomenon, it was proposed by Cifra *et* al. (2011) that inter-cellular interactions can take place through EMFs and, hence, that EMFs form an integral part of biological activity in the body. Cifra *et al.* (2011) propose that when cells are stimulated and undergo mitosis, there is a fluctuation of electrical charges in the cell accompanied by a fluctuation in EMF generation. The mitochondria are thought to be a source of EMF generation since they are an essential source of cellular ROS production, although any other cell structure is also considered a possible source of EMF generation. In this same article Cifra *et al.* (2011) discuss the findings of other authors that DNA is one of the main sources of UPE. The general consensus is that the main source of UPE is through ROS, which are excited molecules (Cifra *et al*., 2011). Apart from their review on the theories of EMF sources in living cells, Cifra *et al.* (2011) also investigated the effect of EMF on biological systems. They state that LF-EMFs do have an effect on biological systems, since they can penetrate deeper into the tissue. They can act on a level which affects cell interaction; i.e., through DNA, ribonucleic acid (RNA) and protein synthesis; hormone production and antioxidant enzyme activity.

A significant finding is that of increased free radical production in reaction to exposure to EMFs (Simko *et al.*, 2001; Hashish *et al*., 2007; Goraca *et al*., 2010; Cifra *et al*., 2011; Mattson & Simko, 2012). Free radicals are a very important mediator in cell metabolism.

Cifra *et al.* (2011) also found that it is hard to explain a mechanism of action other than thermal effects. One of the possibilities mentioned is that EMFs interact with cellular magnetic nanoparticles. This indeed shows a possible mechanism at a molecular and biophysical level, which will explain the cellular response to EMFs. When considering which units of the cell would most probably interact with the EMFs, the most probable would be the cellular sodium (Na) ion pump, membrane-bound enzymes, membrane macromolecules or cellular calcium (Ca^2).

To summarize the findings by Cifra *et al.* (2011) on the possible mechanism of action, it is clear that cells generate and interact with EMFs. EMFs interact with biological systems at the level of nanoparticles. This leads to response by cell DNA, RNA and macromolecules. Other than thermal reactions, processes that are affected in the cells are, among others, protein synthesis, mitochondrial activity, RNA and DNA synthesis, ROS production, free radical production, and hormone production.

It is evident that the biological mechanism of action of EMFs can be explained at various levels. For the purpose of this study, it would be best to look at this effect on cellular level, specifically with respect to immune cells.

2.3 Immunent

As mentioned before, signals differ from each other in as much as just one parameter may differ from another. Immunent BV, a company in the Netherlands, patented the Immunent Activator which is used to generate extremely low frequency electromagnetic fields (retrieved from www.immunent.com). The activator generates electromagnetic fields of extremely low frequency – i.e., below all safety guidelines developed by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) between 5 - 50 µT. The Immunent (IM) Activator signal is indeed different from all other commercial therapeutic signals in that the signal consists of multiple waveforms, creating complex, continuously changing EMFs with steep rise times and exponential decays, giving it a unique oscillation pattern. The unique characteristics of the Immunent signal are that it does not have a repeated waveform or pattern and it actually appears to have a characteristic of random electronic noise (Waite *et al.*, 2011). It is this "noise" feature that Waite *et al.* (2011) consider as a likely mechanism of action. They also speculate that the beat periodicity (rhythmic variations) can be a possible mechanism of action.

Extensive experiments were conducted by Immunent (Cuppen *et al*., 2006; Cuppen *et al*., 2007; Cuppen *et al.*, 2008), in which lower animals (fish and chickens) were exposed to extremely low frequency electromagnetic fields. In their first series of *in vitro* experiments, they found up to 40% increased oxidative burst activity in carp phagocytes after exposure to electromagnetic field strengths of 1.15 - 50 µT. Their second series of *in vivo* experiments involved exposure of commercial goldfish to electromagnetic fields similar to those of the first experiment. They found a decreased mortality rate of up to 50% after 18 days of treatment. Their third series of experiments were also *in vivo* experiments done on chicken broilers exposed to coccidiosis. Up to 40% of the animals showed reduced intestinal lesions after exposure to 6.5 µT field strengths.

2.4 The Immune System

Our environment hosts a large number of infectious agents which use the human body as a host to thrive and multiply (Delves *et al.*, 2011:3), resulting in disease. Fortunately, the body possesses an immune system specifically devised to eliminate any external factors which may interfere with the body's normal biological functions. The immune system has the ability to discriminate between "self" and "non-self", and in this way eliminates any "non-self" organism or entity from the body in an attempt to protect the body (Weir & Steward, 1993:3). The immune system is a complicated, multifaceted system which involves the interaction of cells, soluble biologically active substances and complement. The immune system also operates on different levels, depending on the biochemical properties of the foreign intruder. Sometimes the reaction is fast, simple and non-specific, but there are times when it may be necessary to react in a specific, systematic fashion.

2.4.1 Innate and acquired immunity

The immune system operates by means of two integral systems; first, the innate immune response and later a more specific response called acquired immune response. These two systems are interdependent of each other (Coico & Sunshine, 2009:2).

2.4.1.1 Innate immunity

Innate immunity is the immunity we are born with and is readily available at any given time to destroy any invading pathogen. It reacts in a non-specific fashion and constitutes of factors such as the skin, pH, enzymes, complement and specific cells. This form of immunity is innate in the sense that it is not affected by previous contact with any specific organism (Coico & Sunshine, 2009:2). Many pathogens can successfully evade our innate immune system in which case a more complicated system must be initiated, namely acquired immunity.

2.4.1.2 Acquired immunity

Acquired immunity is a process whereby immune cells are stimulated to secrete antibodies and biologically active substances, such as cytokines, to kill specific pathogens or antigens. Acquired immunity involves a cellular component and a

humoral component (Coico & Sunshine, 2009:18). Cellular immunity is the interaction between immune cells and the direct killing of invading pathogens through action of enzymes or cytokines. Acquired immunity involves mainly three types of cells which interact amongst each other. These cell types are T-lymphocytes (T-cells), B-lymphocytes (B-cells) and antigen-presenting cells (APCs). APCs are cells that have the ability to engulf foreign particles, process them and then present them to the other immune cells (e.g., macrophages). Humoral immunity is the activation of specific B-cells to produce specific antibodies which will neutralize the foreign antigen (Coico & Sunshine, 2009:2-3).

Innate and acquired immunity interrelate with one another through the cells and biologically active substances in a specialized manner to eliminate foreign organisms which gain access to the body. In *Figure 2.2,* the intricate interrelationship between these two segments of the immune system is illustrated.

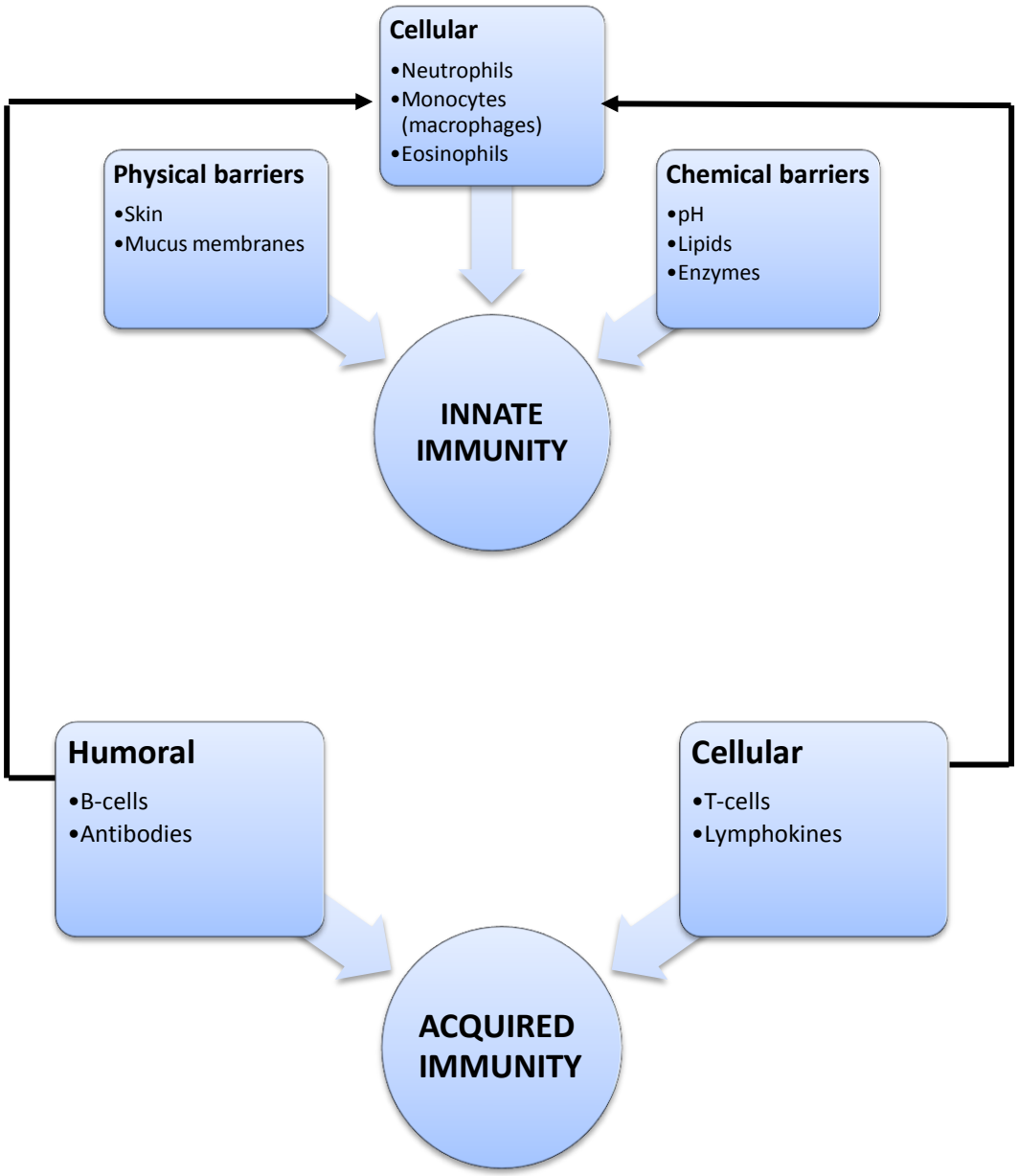

Figure 2.2: The interrelationship between the innate and acquired immune system

(Original figure retrieved from Immune Therapy Research Laboratory)

2.4.2 Cells of the immune response

Cells, specifically leukocytes, play a major role in the immune response. Leukocytes are also known as white blood cells (WBC). The cells involved in the immune response are haematopoietic cells and develop and differentiate from a pluripotent stem cell (Coico & Sunshine, 2009:11-25). These cells have specific antigens on their surfaces by which they are phenotyped. This is known as a cluster of differentiation (CD). Cluster of differentiation is a phenotypic

nomenclature used in leukocytes by identifying the specific antigens on the cell surface which determine the characteristic nature of that specific leukocyte. At the pluripotent stem cell stage, the cells express CD34 on their surface and are therefore indicated as $CD34^+$. If a cell expresses a specific gene of interest, it is referred to as "+" and if it does not express the gene, it is referred to as "-" (Coico, Sunshine & Benjamini, 2003:103). As they mature into specific cell types (unipotent) under the influence of soluble biologically active substances, they lose the expression of CD34 on their surface (Coico, Sunshine & Benjamini, 2003:18-19). Blood cells follow two pathways of differentiation from these pluripotent stem cells. One develops into red blood cells (erythrocytes), platelets (thrombocytes) and granulocytic leukocytes (e.g., neutrophils and monocytes), also known as granulocytes. The other pathway gives rise to development of lymphocytes.

2.4.2.1 Lymphocytes

Lymphocytes are central to the specific (acquired) immune response and consist of T-cells (CD3) and B-cells (CD19) (Coico, Sunshine & Benjamini, 2003:18). B-cells mature and differentiate in the bone marrow and are responsible for the production of antibodies which neutralize or kill antigens outside the cells of the host. Often the antigens can penetrate the host cells and it is in this case that T-cells are needed for the immune response, since they have specific antigen receptors on their surfaces. T-cells mature and differentiate in the thymus and are responsible for the launching (activation) and regulation of the immune response. All T-cells express CD3 (i.e., $CD3^+$) on their surface (Coico, Sunshine & Benjamini, 2003:103). T-cells are subdivided, once again, into two subsets. The one subset is known as helper T-cells (T_H cells) which are $CD4^+$ and the other subset is known as cytotoxic T-cells (T_C cells) which are $CD8^+$. Both these subsets of T-cells play a role in the antigen-specific immune response, depending on the nature or class of the antigen. These cells need to be stimulated to respond through soluble biologically active substances. Specialized T-cells, known as cytotoxic T-cells and natural killer (NK) cells, kill virus-infected cells and abnormal cells (Mayer & Nyland, 2010). NK cells can kill virus infected or abnormal cells without any stimulation, which is why they are involved in the early stages of these infections (Coico, Sunshine & Benjamini, 2003:15). $CD4^+$ T-cells are referred to as helper-T-cells because they work in cooperation with B-

lymphocytes, resulting in the production of antibodies by B-cells (Coico, Sunshine & Benjamini, 2003:137). They also cooperate with other T-cells (T_C cells) in the immune response. Other functions of T_H cells are inflammatory effects, regulatory effects and cytokine effects. T_H ($CD4^+$) cells also consist of subsets and are defined by the cytokines that they produce. They can be divided into three subsets: T_H0, T_H1 and T_H2. In response to certain cytokines, T_H0 will differentiate into T_H1 and T_H2 (Coico, Sunshine & Benjamini, 2003:137). Each of these subsets synthesizes different cytokines.

2.4.2.2 Monocytes

Monocytes migrate from the bone marrow to body tissues and lymphoid organs where they are then known as macrophages. Macrophages play a very important role in presenting foreign material to the T-cells, as well as in phagocytosing (ingesting) and killing foreign material through the release of oxidative enzymes in their cytoplasmic granules (Mayer & Nyland, 2010).

2.4.2.3 Neutrophils

Neutrophils are primarily involved in the nonspecific defences of innate immunity. They can phagocytose and subsequently kill infectious organisms with enzymes released from their granules. This is known as oxidative burst activity (Coico, Sunshine & Benjamini, 2003:14).

2.4.2.4 Eosinophils

Eosinophils are part of the group of granulocytes (they contain granules in their cytoplasm) in the leukocyte family (Coico, Sunshine & Benjamini, 2003:14).They play an important role in the protection against parasitic infections and are also involved in combating allergic conditions (Mayer & Nyland, 2010).

2.4.2.5 Basophils

Similar to the function of eosinophils, basophils and mast cells (which resemble basophils) release substances from their granules that promote inflammation and allergic reactions (Mayer and Nyland, 2010). They constitute a very small portion of the granulocytic leukocyte population. The granulocytic leukocyte population is collectively known as granulocytes. These include eosinophils, basophils and neutrophils.

2.4.3 Biologically active substances

These are substances that are harmful to microorganisms. Examples of these are degradative enzymes, toxic free radicals, inhibitors of growth, acute-phase proteins, complement, and cytokines (Coico, Sunshine & Benjamini, 2003:17). Most of these substances also play a crucial role in the inflammatory process, which comprises an essential component of the immune response.

2.4.3.1 Cytokines

Cytokines are substances secreted by immune cells. They play a major role in inducing an innate and adaptive immune response and, ultimately, the effector phases of the immune response (Weir & Stewart, 1993:12). Cytokines are released by cells in response to a stimulus such as an infective agent, and then they control the migration of various cells to areas of infection in the body (Coico, Sunshine & Benjamini, 2003:149). The release of specific cytokines results in the activation of other cells. Cytokines are synthesized by cells and then secreted, or they are expressed as proteins on the cell surface. $CD4^+$ T-cells are a major source of cytokine production. Cytokines can work in a cascade fashion, where the one cytokine stimulates the release of the next, or they can have inhibitory effects on one another. The basic functional categories of cytokines include immunoregulation, facilitation of immune response (including activation of the inflammatory response), and they affect the leukocyte movement and stimulation of haematopoiesis (Coico, Sunshine & Benjamini, 2003:152-156). *Table 2.1*, below, summarizes the most common cytokines involved in the immune response and some of their functions (Coico, Sunshine & Benjamini, 2003:153).

Table 2.1: Major Cytokines, their source and their major functions in the immune system

Cytokine	Source of production	Major functions
IL-1	Monocytes; some other cells	Involved in the facilitation of innate iummune response and activation of inflammatory response Fever production, stimulates synthesis of acute-phase proteins; promotes proliferation of some T_H cells
IL-2	T_H 0 and T_H1 cells	T-cell growth factor
IL-3	T_H cells; NK cells; mast-cells	Growth factor for Haematopoietic cells
IL-4	T_H2 $CD4^+$ T-cells; mast-cells	Growth factor for B-cells and T_H2 $CD4^+$ cells; inhibits T_H1 $CD4^+$ cells
IL-5	T_H2 cells; mast-cells	Growth factor for B-cells and eosinophils
IL-6	T-cells; other cells	Involved in the facilitation of innate iummune response and activation of inflammatory response Synthesis of acute-phase proteins; activation of T-cells and IL-2 production; stimulates antibody production by B-cells
IL-7	Bone marrow; thymic stromal cells; some T-cells	Growth factor for pre-T and pre-T-cells
IL-9	T-cells	Mast-cell activation
IL-10	T_H2 cells; macrophages	Inhibits production of T_H1 cells and macrophage function
IL-11	Fibroblasts	Stimulates platelet precursor growth
IL-12	B-cells; macrophages	Activates NK cells and promotes the generation of T_H1 $CD4^+$ cells
IL-13	T-cells	Growth factor for B-cells; antibody production
IL-14	T-cells	Development of memory B-cells
IL-15	T-cells; epithelial cells	T-cell growth factor
IL-16	T-cells; eosinpohils; mast cells	Chemotactic factor for T-cells; proinflammatory
IL-17	T-cells	Induction of proinflammatory cytokine secretion and hematopoietic progenitor cell differentiation
IL-18	Macrophages; monocytes and other cells	Induces *IFNγ* production; enhances NK cell lytic activity
IFNα		Involved in the facilitation of innate iummune response and activation of inflammatory

		response
IFNγ	T_H1 cells	Activates NK cells and macrophages; inhibits T_H2 $CD4^+$ cells
TGFβ	Lymphocytes; macrophages; platelets; mast-cells	Inhibits activation of monocyte and T-cell subsets; active fibroblast growth and wound healing
TNFα	Macrophages; mast-cells	Involved in the facilitation of innate iummune response and activation of inflammatory response Inflammatory response involvement; activates cells of the immune system and some epithelial cells; induces fever and septic shock
TNFβ	T-cells	Inflammatory response involvement; involves $CD8^+$ cell activity
GM-CSF	T-cells; monocytes	Promotes growth of granulocytes and macrophages
M-CSF	T-cells; monocytes	Promotes macrophage growth
G-CSF	T-cells; monocytes	Promotes macrophage growth

G-CSF = granulocyte colony-stimulating factor; BM-CSF = granulocyte-macrophage colony-stimulating factor; IFN = interferon; IL = interleukin; M-CSF = macrophage colony-stimulating factor; NK = natural killer; TGF = transforming growth factor; T_H = helper T-cell; TNF = tumor necrosis factor

(Source: Coico, Sunshine & Benjamini, 2003:152-156)

Cytokines can be pro-inflammatory (IL-1β, IL-6, IL-16 or TNFα), anti-inflammatory (IL-10) or chemotactic (IL-8) (de Kleijn *et al.*, 2011). Chemotactic means their secretion attracts other cells of the immune response. Hence, cytokines can have activation as well as a regulatory effect on the immune response.

2.4.3.2 Complement

Complement is a group of approximately 30 serum and membrane-bound proteins which can lyse antibody-coated red blood cells and destroy invading pathogens. It forms part of the innate and acquired immune response. Components of the complement system are activated in a cascade of events to perform their function (Coico, Sunshine & Benjamini, 2003:205). This activation takes place in response to antigen stimulation.

2.4.3.3 Toxic free radicals and ROS

During the energy metabolism and production in cell mitochondria, metabolic by-products of O_2 are formed which are highly reactive (Thannickal & Fanburg, 2000). These free radicals are toxic and can cause severe damage to cell structures. They have been implicated in a myriad of diseases of the human body. As a protective mechanism, cells produce antioxidant enzymes to neutralize or counteract these ROS. Once the rate of ROS production exceeds the antioxidant production, oxidants will accumulate resulting in a condition of "oxidative stress". Although ROS are potentially pathologic, they also have many physiological functions. One of the most important physiological benefits of these oxidative species is the killing of pathogenic organisms by phagocytic cells through superoxide and hydrogen peroxide production (Sorg, 2004). Ideally, the concentration of ROSs and free radicals should be kept to the minimum for good health, but environmental factors are always a problem which contributes to oxidative stress.

2.4.3.4 Acute-phase proteins

These are a group of powerfully active substances which are released by hepatocytes in response to injury and inflammation of body tissues. This acute phase response is initiated by the effect of released cytokines such as IL-1, IL-6 and INFα. After their release, these proteins then stimulate other areas of the innate immune response (Coico & Sunshine, 2009:16, 168). As an example, C-reactive protein (CRP) is one of the acute-phase proteins that are involved in the activation of the complement system.

In summary, the immune system is mediated and controlled by cells, cytokines and complement. All of these elements and cells form a fine balance to protect the body against harm and disease. They all work in a symphonic fashion with one another to maintain an "immunologic equilibrium" in the body. Various factors influence the immune system, hence causing an imbalance to this critical "Yin Yang" of the body (Lafaille & Mathis, 2002). Some factors include temperature changes, hormonal influences, stress, genetics, age and diet (Weir &

Stewart, 1993:17-19). Moreover, there is also evidence that ELF-EMF can influence the immune system (de Seze *et al.*, 1993; Cuppen et al., 2006).

2.5 *Effects of extremely low frequency electromagnetic fields on the immune system*

An area of specific concern with respect to the biological effect of exposure to ELF-EMF is the immune system. This is most likely due to strong evidence that biological effects of ELF-EMF are demonstrated at cellular level. This is important as a large component of the immune system works at cellular level. Several authors have speculated or hypothesized that short-term exposure to ELF-EMF can stimulate the immune response, specifically at cellular level, leading to the production of cytokines (Blank *et al.*, 1992; Simko & Mattson, 2004; Cuppen *et.al.*, 2007; Elmusharaf *et al.*, 2007; de Kleijn *et al.*, 2011). Markov *et al.* (2006) indicate an interaction between ELF-EMF and the immune system, although the signal used for their study was a signal with specific characteristics. This same signal that was discussed in section 2.3 was utilized in this study and will be discussed in more detail later in the literature review (see section 2.5.5).

The question arises as to which area of the immune system will be affected by these ELF-EMFs. Going back to the discussion on the possible mechanism of action of EMFs, it is obvious that the effect already begins at molecular level. It is logical that for the immune system it will occur at cellular level and exert its effects through cell interaction. ELF-EMF can penetrate tissue and hence cells due to their long wavelength (Goodman & Blank, 1998). It appears that the interaction begins at the cell membrane, which then activates signal transduction pathways.

The development of scientific evidence of a mechanism of interaction of EMF on the immune system is intense and slow, yet steady (Markov *et al.*, 2006). Some common features have been noticed and clarified, but the many contradictory findings still render specific knowledge of this mechanism unclear. Hence, continued research in this field is required to elucidate more evidence in order to illuminate this phenomenon.

2.5.1 Effect on cytokine production

In an *in vitro* study, Aldinucci & Pessina (1998) found that peripheral blood mononuclear cells that were exposed to 50 Hz (flux density 3 mT) fields secreted

increased amounts of INFγ and IL-6 after 24 hours of exposure. This effect was only seen once cells had been stimulated with the mitogen, phytohaemagglutinin (PHA). Cells that had not been challenged with PHA showed no increased release of these cytokines. This was not demonstrated by control (sham exposed) samples. They suggest a kind of "priming" event that results from exposure to the EMFs. These findings support the hypothesis presented by Cuppen *et al.*, 2006, that ELF-EMF could boost or stimulate the immune system by "putting it into a state of alert due to cytokine production".

In a study conducted in humans, young healthy men were exposed to 50/60 Hz (10 μT) fields for nine hours continuously and intermittently (one hour on, one hour off) (Selmaoui *et al.*, 2011). This study was performed in a twenty-four-hour period for the continuous and intermittent group. This study revealed an increased IL-6 in the subjects who were exposed to the intermittent field.

2.5.2 Effect on leukocyte quantity

Gobba *et al.* (2009) conducted a study in which humans were occupationally exposed to various levels of ELF-EMF. The NK-cell activity was determined and it was found that the subjects with the highest exposure had lower NK-cell activity compared to subjects in the lower exposure groups. The subjects in the lower exposure groups were those that were exposed to levels of 2 μT and lower, whereas the higher exposure groups were exposed to levels exceeding 2 μT. These findings are significant, since NK-cells play an important role in host defence against cancer. However, further investigation in this field is needed. Exposure to ELF-EMF in the industrial environment is contentious, since the sources and duration of exposure vary considerably. It is also difficult to identify the ideal control sample, since it is virtually impossible to identify a sample that has close to zero exposure in such environments.

Hashish *et al.* (2008) found that mice exposed to 50 Hz (1.4 mT) ELF-EMF for thirty days had a decreased total leukocyte count, platelets, monocytes, peripheral lymphocytes, splenic lymphocytes, T-cells and B-cells. There was also an increase in granulocyte concentration. In addition to these findings, evidence of increased oxidative stress was also found. The researchers conclude that the

decrease in leukocytes and platelets and the increase in granulocytes can be related to the increased free radical production and oxidative stress.

2.5.3 Effect on cell activity

It has been found that short-term exposure to fields in the ELF range affects the blood cell levels and results in lymphocyte proliferation (de Kleijn *et al.*, 2011). Several research studies have indicated an increased phagocytic activity in macrophages, hence implicating an effect on the innate immune response due to increased free radical production (Rollwitz *et al.*, 2004; Simko & Mattson, 2004; Hashish *et al.*, 2008; Goraca *et al.,* 2010; de Kleijn *et al.*, 2011). However, it was found that the effect of long-term exposure to ELF-EMF on the immune system had just the opposite effect, where it resulted in decreased blood cell levels (Bonhomme-Faivre *et al.*, 2003).

Markov *et al.* (2006) found striking evidence of EMFs interacting with lymphocytes. They felt, however, that this interaction was strongly dependent on the cells' metabolic state. If cells were metabolically stable, they were not affected by EMFs. However, EMFs would place metabolically unstable cells under more stress, which lead to further instability and disequilibrium. This then increased the production of free radicals, leading to oxidative stress.

Khadir *et al.* (1999) investigated the effect of 22 mT fields on granulocyte activity and reported an increased superoxide anion (O_2 / free radical) production in the cells that were pre-stimulated with phorbol 12-myristate 13-acetate (PMA). They contributed this effect to altered intracellular signaling. These findings agree well with those of Markov *et al.* (2006), who suggest that homeostatically unstable cells, but not metabolically stable cells, are affected by ELF-EMF. This could possibly explain the therapeutic benefit of these fields.

There is strong evidence that ELFs-EMFs have cell-activating capabilities. This was shown by numerous authors who described an increased production of free radicals, which will be discussed in the following section.

2.5.4 Effect on release of other biologically active substances

ELF-EMF can induce the expression of heat shock proteins which, in turn, provide cellular protection (Goodman & Blank, 1998). These heat shock proteins are stress proteins which are released when the body experiences any stress influence and then reacts in a protective manner. They are named with a prefix „hsp' followed by a number which indicates their molecular weight in kilodaltons (kD). These proteins are part of a family of approximately 20 with molecular weights ranging from just a few kD to over 100 kD. The major stress protein is hsp70 (Blank & Goodman, 2009). Blank and Goodman (2000) explain in their article how the synthesis of proteins begins with the activation of DNA. Any external stress factors (e.g., temperature, oxygen deprivation, dietary deficiencies) on the human body will result in the increased synthesis of certain stress proteins. They also indicate the increased synthesis of the hsp70 protein (also refer to Goodman and Blank, 1998) in reaction to exposure to 60 Hz EMFs with field strengths lower than 0.1 mT. The increased activation of stress proteins results in a cellular response which is one of the body's natural defence mechanisms. It is a cytoprotective response and when experienced for a short while, it will be beneficial. Blank and Goodman (2009) refer to an experiment where fertilized eggs from *Sciara coprophila* were divided into two groups. One group was pre-exposed to EMFs, after which both groups were heat shocked at 37^{o}C. They found that only 10% of the unexposed eggs survived, whereas 95% of the eggs exposed to the EMF survived this heat shock. They referred to this as "forced induction of stress proteins for stress conditioning".

One of the mechanisms through which ELFs-EMFs can affect biological systems, is through the production of free radicals (Goraca *et al.*, 2010). In this study it was found that the duration of exposure (working time) had an influence on the results in that longer exposure (sixty minutes *vs*. thirty minutes per day) resulted in a marked increase of ROS generation and a decrease in total antioxidant capacity. An *in vitro* study conducted by Rollwitz *et al*., (2004) explains a "cell-activating capacity of ELF-EMF" on murine bone marrow-derived promonocytes and macrophages. They found that ROS production was one of the key processes in this phenomenon. De Kleijn *et al*., (2011) cite Rollwitz (2004) in their publication and suggest that Rollwitz's study clearly demonstrated that the

innate immune system is affected by exposure to ELFs-EMFs through increased phagocytic activity and increased free radical production.

Findings of increased free radical production and accompanied oxidative stress have been published by numerous authors, but not all used the same field strength in their studies (Simko *et al.*, 2001 [0.5-1.5 mT]; Rollwitz *et al.*, 2004 [1 mT]; Simko & Mattson, 2004 [not specific – review]; Hashish *et al.*, 2008 [1.4 mT]; Goraca *et al.*, 2010 [7 mT]; Mattsson & Simko, 2012 [not specific]). In their study, de Bruyn and de Jager (1994) observed stress induction using lower magnetic flux density (5-10 μT), but this effect was due to long-term exposure.

2.5.5 *Summarizing the effect of the Immunent signal and other ELF-EMF signals on the immune system*

Numerous studies have been performed using the Immunent signal. Most of these studies have shown that this signal has a stimulatory effect on the immune system.

2.5.5.1 The findings of Cuppen et al. (2007)

Cuppen *et al.* (2007) proposed a hypothesis as to how ELFs-EMFs from the Immunent system (field strengths between 0.15μT - 1.5 mT and frequencies between 250 - 5000 Hz) can stimulate an immune response. This response is stimulated when an EMF induces stress in cells which, in turn, produce cytokines. The production of cytokines needs some form of stimulant. In this case, the stimulant is the stress production by means of EMFs through the release of "danger signals". This event alerts the immune cells to a possible action. Cuppen *et al.* (2007) suggested their hypothesis that EMFs result in the release of similar danger signals that are released in response to pathogens. *Figure 2.1* illustrates their hypothesis:

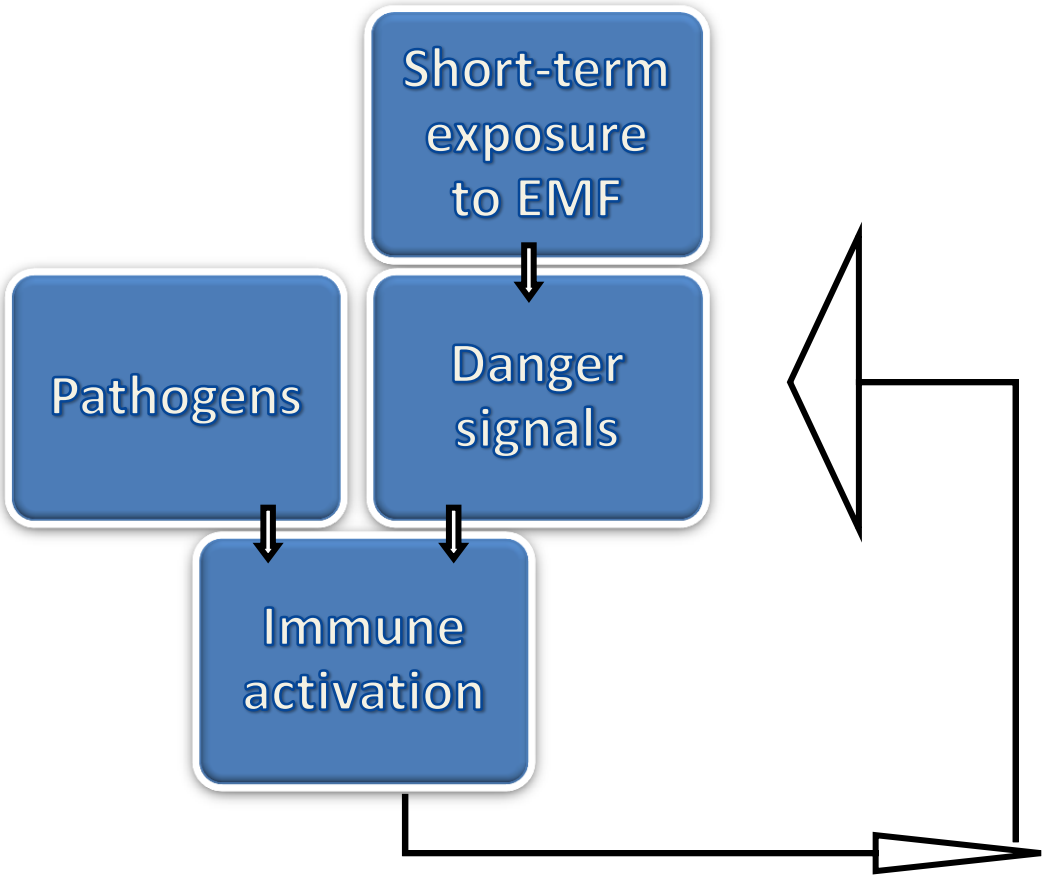

Figure 2.3: *The hypothesis by Cuppen et al. for immune activation by the EMF signal (After Cuppen et al., 2007)*

In two experiments on fish, this research group found a significant increase in ROS production of macrophages and neutrophils. In an *in vitro* experiment on broiler chickens exposed to *Coccidiodis eimeria*, they demonstrated a 40% decrease in intestinal lesions and an 8% increase in feed conversion. Findings from experiments on broiler chickens were also published by Elmusharaf *et al.* (2007).

These studies clearly demonstrated that this signal from Immunent stimulates the immune response. Their suggestion is that these EMFs have advantageous, practical applications in the farming industry and also hold economic benefits. Their study also opened the field to an area of research on the bioeffects of ELFs-EMFs.

2.5.5.2 The findings of Elmusharaf et al. (2007)

The findings published by Elmusharaf *et al.* (2007) were based on similar experiments as those of Cuppen *et al.* (2007). The published work by the former authors, however, only referred to the exposure of broiler chickens infected with coccidiosis. Similar to the findings by Cuppen *et al.* (2007), there was a 40% decrease in intestinal lesions following thirty minute exposure per twenty four hours for twenty one days. Of interest is the hypothesis proposed by Elmusharaf *et al.* (2007). They confirmed their hypothesis that EMF inhibits an inflammatory reaction which, in turn, enhances an immune response. This might sound contradictory, since inflammation is part of the innate immune response. Perhaps the authors are implying that if the inflammatory response (which results in tissue damage) can be kept to a minimum and the cellular immune response can be enhanced, the response can be directed toward the specific pathogen and not the surrounding tissues.

2.5.5.3 The findings of Keirs et al. (2005)

Keirs *et al.* (2005) are cited in an article by Elmusharaf *et al.* (2007). Keirs *et al.* (2005) found that exposure of commercial egg-layer hens to a specific EMF (in this case, not Immunent) resulted in increased egg production. Once again, we see the beneficial economic implications of EMF exposure.

2.5.5.4 The findings of de Kleijn et al. (2011)

In an *in vitro* study, the above authors investigated whether ELF-EMF exposure with the Immunent signal had an effect on inflammatory signaling pathways. They found that ELF-EMF exposure had no effect on IL-6 release, early cytokine production or transcriptional and translational processes when stimulating cells with live and attenuated pathogens.

2.5.5.5 The findings of Havas (2000)

This author reviewed reports by the NIEHS and the US Nuclear Regulatory Commission (NRC). The author reports on many other aspects of ELF-EMF exposure. Concerning the immune system specifically, the author reports a lack of evidence that ELF-EMF affects the immune system in experimental animals.

2.5.5.6 The findings of Johansson (2009)

Johansson feels very strongly that EMFs disturb the immune system by exerting an inflammatory response. He/She argues that this overreaction of the immune system results in tissue damage. However, this results when the duration of exposure is assumed to be long-term, i.e., chronic exposure. The public's attention is therefore alerted to this being a possible cause of increased incidences of allergies and asthma. He/she also postulates that this disturbance of the immune system could possibly predispose the individual to cancer.

2.5.5.7 The findings of Markov et al. (2006)

In this article the authors review and summarize findings of EMF studies on immune cells in an attempt to apply these EMFs therapeutically. After reviewing thousands of international papers and abstracts, they were astounded by the diversity of the findings with respect to beneficial or hazardous effects. They report a large body of evidence that short-term exposure to ELFs-EMFs enhances the immune system at cellular level. For example, one of the findings they present is that EMFs augment the intrinsic behaviour of lymphocytes. The authors suggest that EMFs do not affect metabolically stable cells, but will induce stress in cells that are homeostatically unstable. They consider the possibility that EMFs "target cells that are homeostatically unstable as a consequence of disease". If correct, this would have advantageous therapeutic implications.

One of the major obstacles they highlight is the lack of specification of the physical characteristics of the signals used in the reviewed studies. Their recommendation is that studies should use specific signals.

2.6 Summary

We cannot escape the fact that EMFs constantly surround us. However, it is important that regulations be set so that exposure levels be kept within safety limits. Let us not forget that EMFs have been used as a therapeutic tool for many centuries, but exposure requires a specific signal, dosimetry and working time. To put it simply, a person cannot stand in front of the television or microwave and expect therapeutic results.

It is evident that long-term exposure to ELFs-EMFs results in mild oxidative stress, hence promoting the inflammatory process (Mattsson & Simko, 2012). This can be detrimental to human health; but according to Mattsson & Simko (2012), these findings need to be supported by more realistic long-term exposure experiments (i.e., life-long experiments).

Although some research studies have indicated that the immune system can be activated by short-term exposure to ELFs-EMFs, it leaves scope for additional research to be conducted in this field in order to duplicate, and thus verify or refute, results from previous research. In this study it meant extending the research to higher animals and testing additional immune parameters to those tested in earlier studies. In addition to this, it was deemed beneficial to determine the optimal duration of exposure to obtain the desired effects. Mattsson and Simko (2012) suggest that further investigations should be performed to determine cytokine release and threshold identification. They also suggest investigating a comparison between activated cells and non-activated cells in an attempt to identify the mechanism of action between EMFs and cellular responses.

Electricity and its generation of electromagnetic fields is part of our daily existence. One can almost say that we live in an electromagnetic environment. This forms part of our daily existence. Moreover, human health is largely dependent on the effective functioning of the body's immune system which is influenced by various factors. If there is evidence that electromagnetic fields influence the immune system positively or negatively, it certainly warrants further investigation.

Lastly, following a review of numerous publications on the effect that the Immunent Activator signal has on the immune system, it became evident that there are growing indications that this signal has a stimulatory effect on the immune system. Hence, the therapeutic application and commercial value of this signal holds many advantages and should be investigated.

CHAPTER 3

METHODOLOGY

3.1 Introduction

The study involved causal-comparative (*ex post facto*) research where experimental mice were treated with the Immunent signal and control mice received no (sham) exposure. The findings among the exposed (experimental) mice were then compared, and the results were also compared with those of the control mice.

Three groups of mice were exposed to an Immunent BV ELF-EMF signal with an intensity of 5 μT for seven days. Each group was exposed to this EMF signal for a different length of time per day. Simultaneously, a control group of mice was kept in a separate room under identical conditions, with the exception of EMF exposure (sham exposure). After seven days, following completion of the exposure period, all the mice from the three groups were anaesthetized, whole blood was collected and the mice were sacrificed. The whole blood was used for full blood count (FBC) and immunophenotyping analysis. Results for all the parameters measured were captured on an excel spreadsheet and statistically analyzed.

The results of the exposure groups were compared with those of the control group to determine whether there was a statistically significant difference between the control and the exposure groups. In addition to this, the three exposure groups were compared to one another to determine whether the duration of exposure had any influence on the results. *Figure 3.1* provides a schematic layout of the exposure protocol and laboratory analysis.

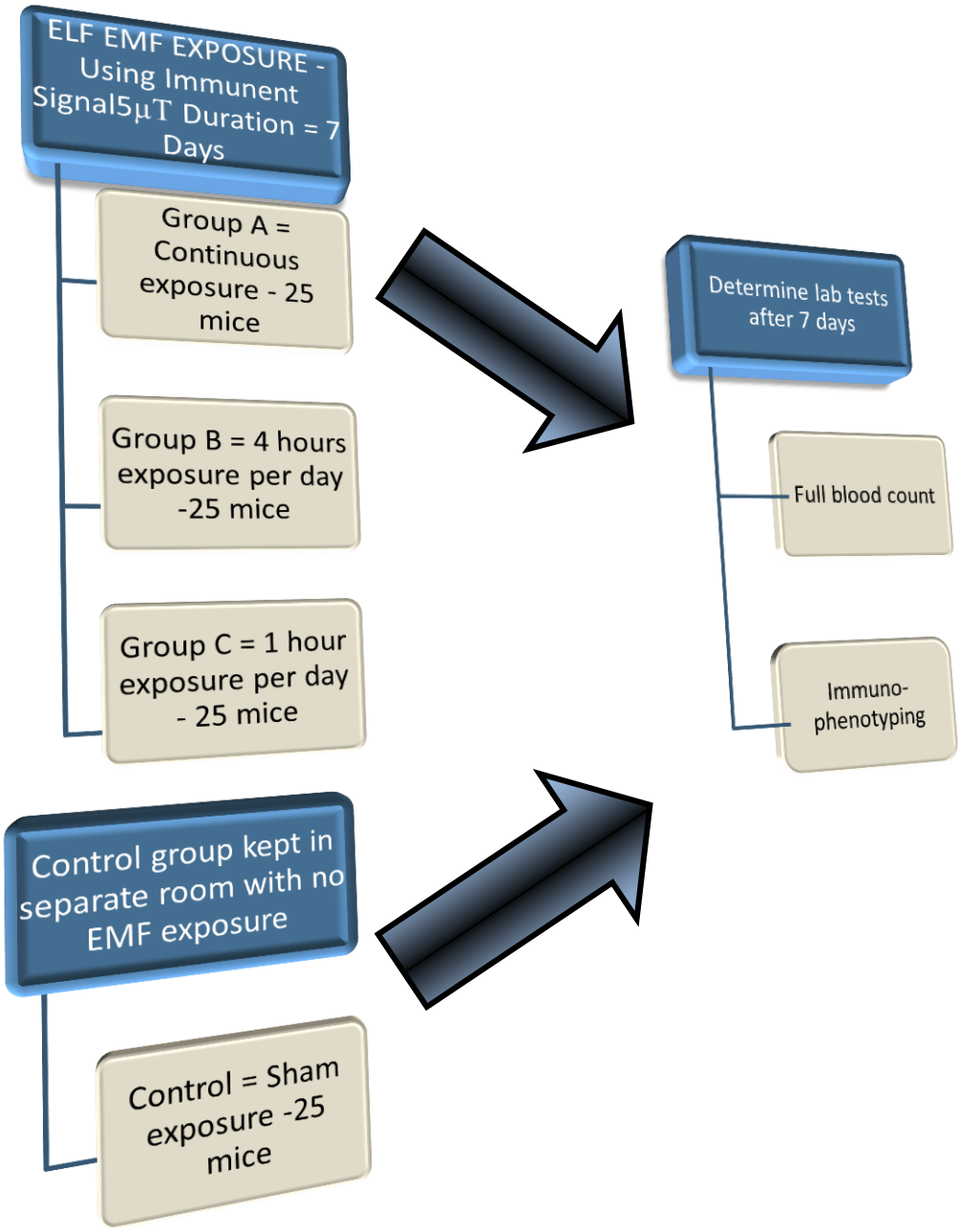

Figure 3.1: Schematic summary of research methodology

3.2 Materials and apparatus

While the exposure of the animals took place at an experimental animal facility, the biological analyses were performed in a laboratory. The materials and apparatus used in the experiment are described and discussed in this section. These include the experimental animals, their housing, the exposure system and the biological laboratory analyses.

3.2.1 Exposure system

The exposure system consisted of the Immunent Activator signal, cylinders and housing, each of which are described below. The generator created the specific Immunent Activator signal which was wired to the cylinders. The cylinders were designed to hold the housing cages with the mice for the exposure period.

3.2.1.1 Exposure system signal

Figure 3.2 below depicts the control box containing the generator for the exposure system used in the study. The generator was patented and supplied by Immunent BV (Werfberg 12, Veldhoven, The Netherlands).

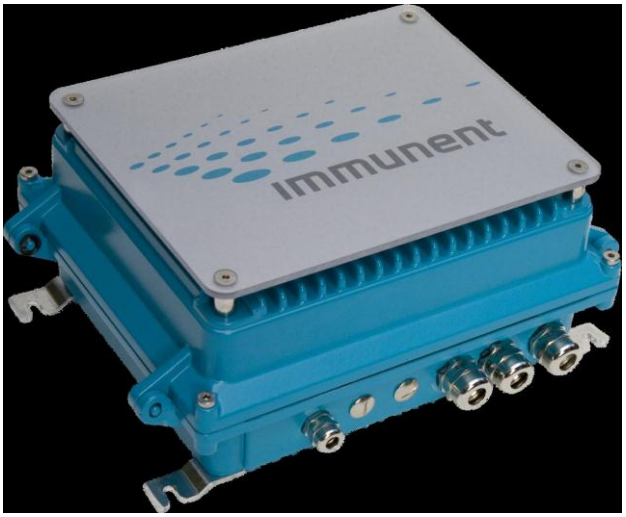

Figure 3.2: Immunent (IM) signal source

The signal generated by Immunent (IM) BV is a specific signal consisting of multiple waveforms creating complex, continuously changing electromagnetic fields (EMFs) with steep rise times and exponential decays as illustrated in *Figure 3.3*. The Immunent exposure system uniformly exposed the experimental group of mice to EMFs (multiple frequencies between 20 - 5000 Hz) with an intensity of

5 µT. At these low exposure settings, there was no chance of heat or sound generation. A computerized time switch was installed to regulate the various exposure durations.

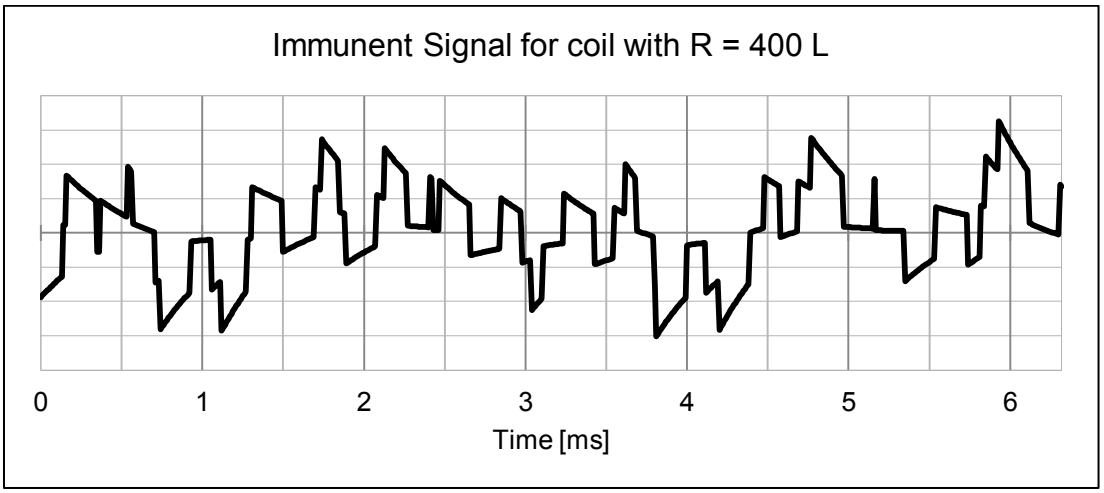

Figure 3.3: Illustration of signal

3.2.1.2 Exposure cylinders

Custom-made plastic cylinders were constructed to house the cages containing the mice while they were exposed to the signal. The cylinders were fixed onto wooden tables inside the experimental room (*Figure 3.4*). Copper coils were wound around each cylinder to create the electromagnetic field controlled from the Immunent exposure control system. The dimensions of the cylinders were 20 cm in diameter with a length of 120 cm. These dimensions allowed up to three levels of cages within the cylinders. Holes were drilled into the cylinders to permit good ventilation and light inside the cylinders. *Figure 3.4* illustrates the cylinders that had been constructed.

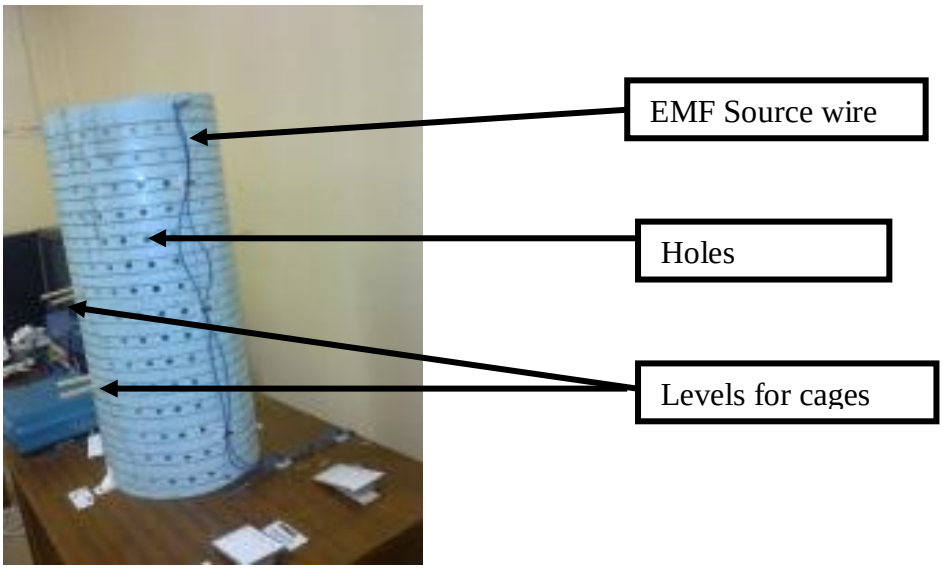

Figure 3.4: Exposure cylinders

3.2.2 *Experimental animals*

Mice (*Mus. musculus*) were used for the study. The animals were supplied by the University of the Free State (UFS) Animal Unit. This specific strain of mice is bred at the animal unit under controlled conditions for experimental use. The sample of experimental animals consisted of one hundred healthy young adult (approximately six weeks old) male mice of the BALB/c N1H strain. The mean weight of the mice was 23+/-1.5 grams. The weight was kept within this range for all the mice to avoid dominant behaviour by larger mice.

Figure 3.5: Mice (Mus. Musculus) BALB/c N1H strain

3.2.3 Housing and facility

The experiment was conducted at the UFS Animal Unit facility. Both the experimental and the control room were approximately three meters by seven meters in size. The experimental room was equipped with twelve tables containing fixed exposure cylinders (*Figure 3.6*) and the control room was equipped with four tables containing fixed cylinders. For the housing of the animals, specifically designed cages were custom made with depth x breadth x

length (DxBxL) dimensions of 15x15x30 cm. These cages could comfortably accommodate three to four mice each. The cages were made of hard plastic with a layer of absorbent saw dust on the floor of the cage. The top of the cage was covered with a perspex lid containing sufficient ventilation holes. The lid was designed in such a way that the water bottle nozzle could fit into it, facing towards the inside. Glass tubes supplied slits large enough for food to be reached and nibbled by the mice, but also small enough that the mice were not able to escape. The use of steel in the cages was avoided to eliminate the possibility of interactions between steel and the EMFs.

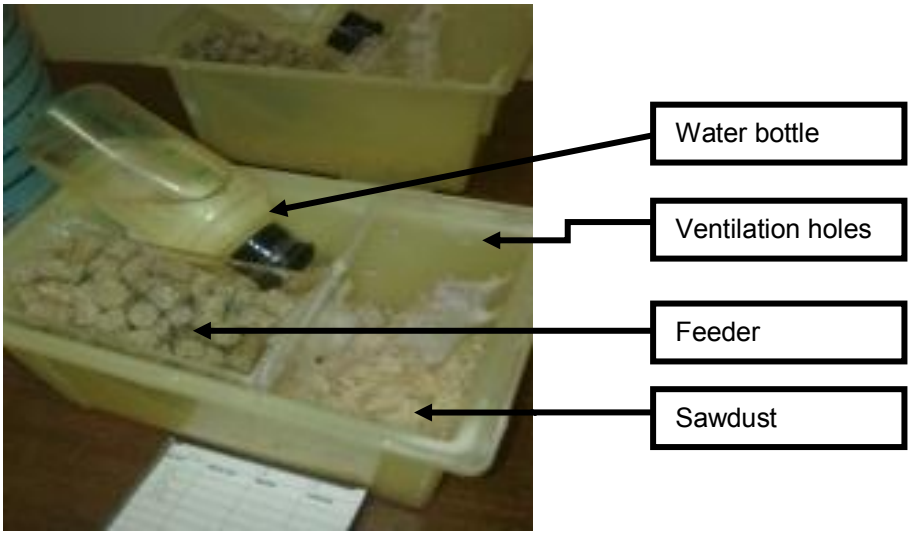

Figure 3.6: Housing cage with feeder

3.2.4 Experiment setup

The experiment was set up to comprise of four groups of mice. Each group consisted of four cylinders containing two cages each. Each cage housed three mice, with one of the cages in the fourth cylinder housing four mice, giving a total of twenty five mice per group. Each group received a different duration of exposure.

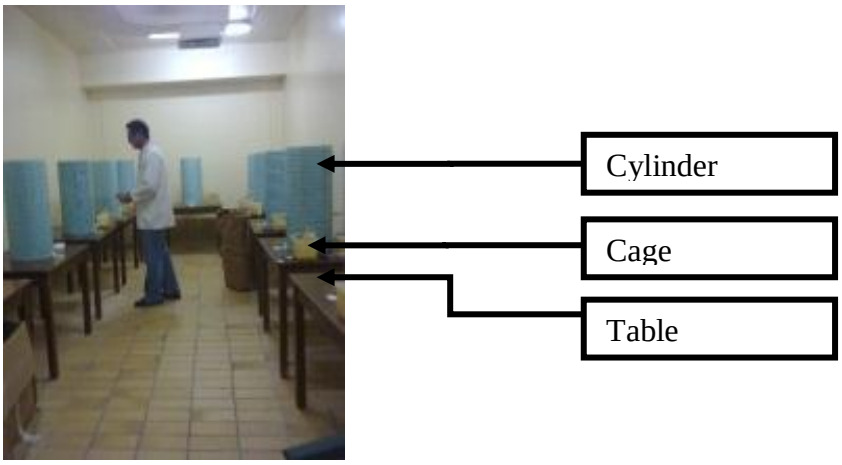

Figure 3.7: Experiment (exposure) room setup

The four cylinders for the control group were kept in a separate room where there was no exposure to EMFs. In all other aspects, the control group was treated in the same way as the experimental group.

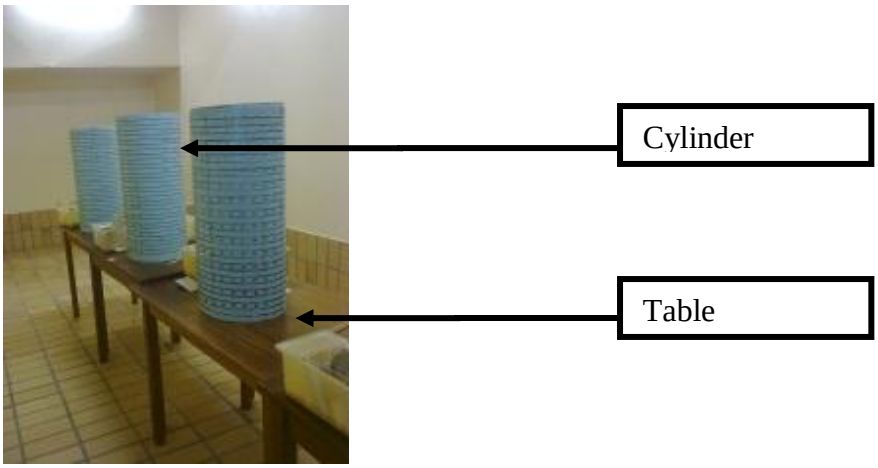

Figure 3.8: Control room setup

Equipment and materials used for the exposure protocol are included in *Table 3.1*. Some of the equipment used had to be custom made to fit into the room and to comply with the requirements of the study, but where possible, the animal unit supplied most of the equipment and material.

Table 3.1: Animals, housing equipment and material used for exposure procedure

EQUIPMENT / APPARATUS	SOURCE	DESCRIPTION
Mice (*Mus. musculus*)	Animal Unit, UFS	BALB/c N1H strain
Housing cages	Custom-made	Dimension (DxBxL): 15x15x30 cm
Signal source	Immunent (IM)	Werfberg 12, Veldhoven, The Netherlands
Exposure cylinders	Custom-made	20 cm diameter, 120 cm long
Exposure room	Animal Unit, UFS	300 cmx800 cm
Control room	Animal Unit, UFS	300 cmx800 cm
Food	Animal Unit, UFS	EPOL balanced mouse pellets

3.2.5 Biological Analysis

The biological parameters measured were performed on whole blood obtained from the mice. Approximately 1 ml whole blood was collected in blood tubes containing ethylenediaminetetraacetic acid (EDTA) powder. Haematological parameters measured included the full blood count and immunophenotyping of lymphocyte subsets.

3.2.5.1 Full blood count

An ABX Pentra 60C+ analyzer was used to measure the full blood count (FBC) parameters. The analyzer is able to determine the type and size of all the cells and haemoglobin concentration contained in blood. The data were transferred to a computer with compatible software and then printed. The reagents and controls used were compatible with the specific analyzer.

(*Horiba ABX Pentra C60+ Haematology Analyzer, online*)

Figure 3.9: Pentra 60C+ analyzer

Equipment and reagents used for the FBC are presented in *Table 3.2*. Suppliers and model / brand of the reagent and / or equipment are also included in the table.

Table 3.2: Equipment and reagents for full blood count

APPARATUS / REAGENTS	SUPPLIER	MODEL / CODE
Full blood count analyzer	Horiba, Japan	Pentra 60C+ Analyzer
LYSEBIO	Scientific Group	ABX/0906013
BASOLYSE 1L PENTRA 60	Scientific Group	ABX/0906003
CLEANER 1L PENTRA 60	Scientific Group	ABX/0903010
DILUENT 20L PENTA 60	Scientific Group	ABX/0901020
EOSINOFIX 1L PENTRA 60	Scientific Group	ABX/0206010
DIFFTROL NORMAL Control	Scientific Group	ABX/2062203
DIFFTROL LOW Control	Scientific Group	ABX/2062207
DIFFTROL HIGH Control	Scientific Group	ABX/2062208

3.2.5.2 Immunophenotyping

All reagents used were of research quality and obtained from a reputable firm. The antibody markers were specific for mice (rat-anti-mouse). A control was included for each specific antibody isotype and fluorescent marker. Analyses were performed on a Flourescence activated cell sorting (FACS) Calibur analyzer (Becton Dickinsin). The reagents, controls and instrumentation used for the immunophenotyping are presented in *Table 3.3.*

Table 3.3: Equipment and reagents for immunophenotyping

APPARATUS / REAGENTS	SUPPLIER	MODEL / CODE
Immunophenotyping analyzer	UFS Haematology (Becton Dickinsin)	FACS Calibur
CD3 FITC / IgG2a	Beckman Coulter	731992
CD4 PE / IgG2b	Beckman Coulter	733259
CD8 PE / IgG2a	Beckman Coulter	733264
CD19 FITC / IgG2a	Beckman Coulter	732058
Mouse IgG2a FITC Control	Beckman Coulter	A12690
Mouse IgG2a PE Control	Beckman Coulter	A09141
Mouse IgG2b PE (clone A-1) Control	Beckman Coulter	731601

3.3 Methods

The methods involved four processes:

- exposure of the animals to the EMF
- the bleeding and sacrificing of the animals
- laboratory testing
- data capturing / processing for statistical analyses.

The methods had been planned well in advance to ensure fluent and efficient flow of procedures. After the exposure had been completed, analyses were performed on the same day as bleeding / sampling. Good laboratory practice and procedures were adhered to, ensuring quality control and accuracy throughout all procedures. The rules and regulations of the South African National Standards (SANS) (SANS 10386:2008 The care and use of animals for scientific purposes and guidelines on ethics for medical research) were strictly adhered to.

3.3.1 *Ethical aspects*

Approval for the study was granted (No. 20/08) by the interfaculty animal ethics committee of the UFS (Appendix 1). Guidelines for the welfare of experimental animals were strictly adhered to according to the Experimental Animal Act (South African veterinary Foundation, online). These guidelines could be followed with the assistance of the personnel at the animal unit of the UFS, since the personnel at this unit had been trained and were qualified to work with experimental animals.

3.3.2 *Experimental animals*

Once the mice were received, they were checked for any defects and it was confirmed that all the mice were in good health. The sample of mice was divided into four groups of twenty five each. Three groups served as the experimental (exposure) groups, which were to be exposed to the Immunent signal. The fourth group served as a control and received sham (no) exposure.

3.3.3 *Exposure protocol*

The exposure protocol consisted of three groups with four cylinders per group (*Figure 3.10*). Group A was exposed to the Immunent signal continuously (24 hours), group B was exposed for four hours per day and group C was exposed for one hour per day. Each cylinder had two levels (one cage per level) with three mice per cage, with the last cage of each group housing four mice. These numbers mounted to a total of 25 mice per group. The control group was kept in a separate room under identical conditions as the experimental exposure group, with the exception of EMF exposure. Exposure was administered for seven days. Daily visits were made to the facility to check on the mice with respect to feed, water, room temperature and the exposure system. During daily feeding times, the cages were rotated on the levels to ensure equal amount of signal, light exposure and oxygen for all the mice.

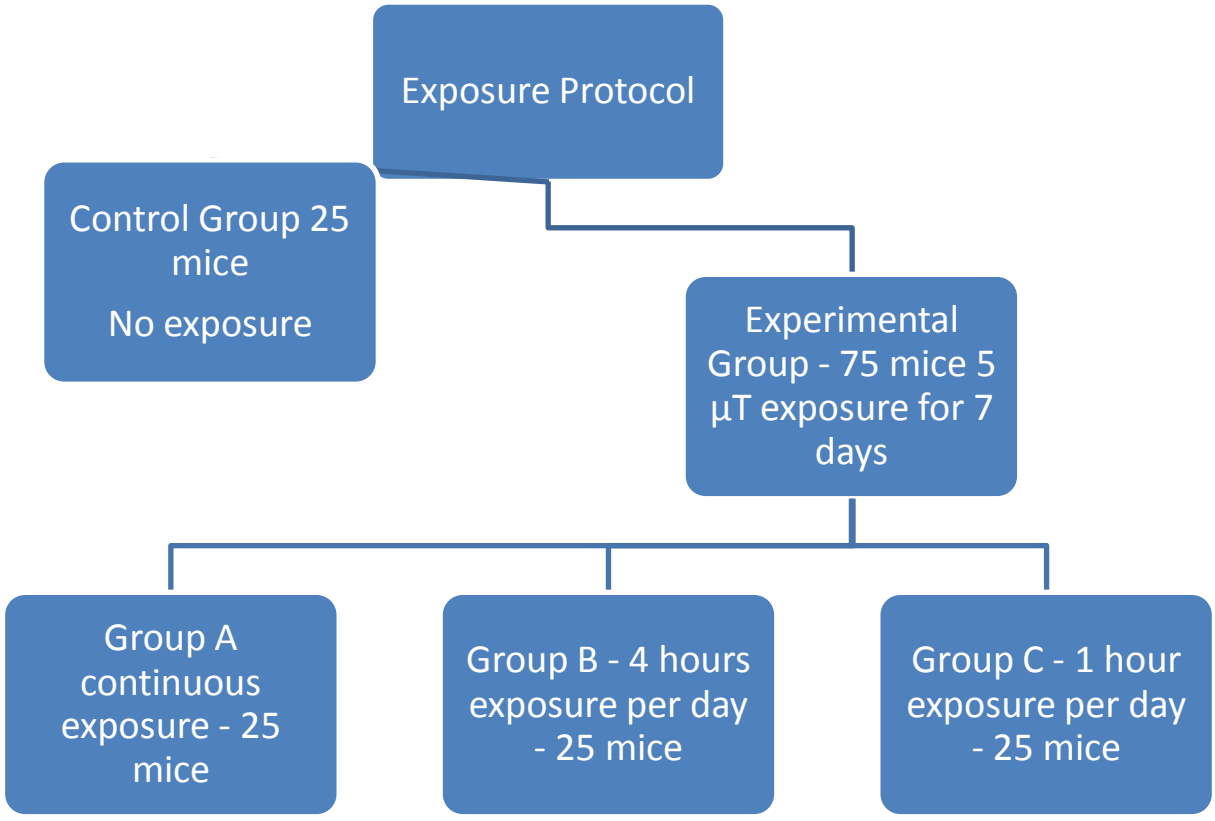

Figure 3.10: Schematic representation of exposure protocol

3.3.4 *Housing and exposure*

The exposure system supplied the EMF signal to each cylinder which housed two cages per cylinder. Three to four mice were housed in each cage. The cages were lined with a two centimeter layer of sawdust for bedding and absorbance of urinary excretions. The mice were provided with constant access to food and water. The mouse diet consisted of *Epol* balanced mouse pellets, supplied by the UFS animal unit. Visits were made daily to the facility to check on food and water. During these visits the cages in each cylinder were alternated to ensure equal exposure of light and EMFs. Cages were identified with a card indicating the cage number and initial weight of each mouse. Controlled environmental conditions were adhered to, with constant room temperature of 21^{o}C and light switches controlling a twelve-hour day and a twelve-hour night routine. Human handling of the mice was kept to a minimum to prevent induction of stress in the animals.

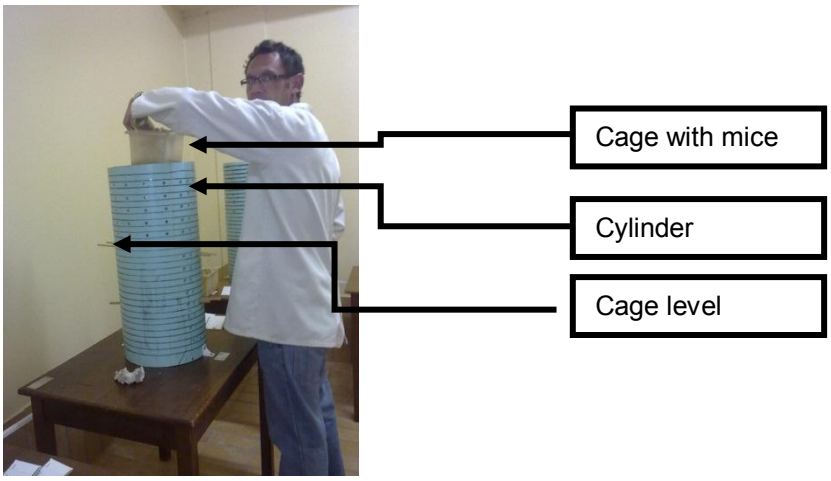

Figure 3.11: Cages were rotated on a daily basis

3.3.5 Sample collection and preparation

The sample collection and preparation were carried out over three days, since it was not possible to attend to the entire sample in one day. On the first day, 30 samples were prepared and analyzed and 35 were completed on each of the second and third days.

After completion of seven days of exposure, the mice were transferred to a theatre room at the animal unit which is specially equipped for the bleeding procedures that were to be performed. Taking one cage from each group at a

time, the mice were transferred from their cages to an anaesthetic chamber containing Allantoin gas. Once fully anaesthetized, approximately 1 ml whole blood was collected from each mouse by means of orbital bleeding. The bleeding, anaesthetics and sacrificing were performed by trained personnel at the animal unit. Whole blood was collected into marked / numbered 5 ml blood tubes containing EDTA (powder) anticoagulant for full blood count and immunophenotyping. Whole blood was immediately re-suspended in the anticoagulant to prevent clotting of blood. While still anesthetized, the mice were sacrificed by cervical dislocation.

Once the blood was properly mixed, equal amounts of blood were transferred to two separate eppendorf tubes. A 500 µl aliquot was used for the full blood count and a 500 µl aliquot was used for the immunophenotyping.

3.3.6 Full Blood Count

The 500 µl aliquot whole blood samples were transported at room temperature to the Haematology laboratory. Upon arrival at the laboratory, the blood tubes were placed on a roller to keep the blood mixed until the analyses were performed. Full blood analyses were performed within one hour of reaching the laboratory using a Pentra 60 C+ (from ABX Diagnostics) analyzer and the results were printed. The analyses were done within several hours of blood collection. A minimum of 200 µl whole blood (EDTA) was required for these analyses.

The parameters measured included white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (Hb), haematocrit (Hct), mean corpuscular volume (MCV) and platelet count (Plt). Lymphocyte, monocyte, neutrophil, eosinophil and basophil differential counts were also determined.

Controls included high, low and normal controls, as well as interlaboratory control run in collaboration with the National Health Laboratory Services (NHLS) Haematology laboratory as an additional control.

3.3.7 Immunophenotyping

The other 500 µl aliquots of whole blood were transported at room temperature to the Haematology laboratory at UFS where the immunophenotyping was performed that same day. Immunophenotyping involves identification of specific

antigens on lymphocyte surface, known as cluster of differentiation (CD), in order to define the specific lymphocyte subtype. Aliquots of 100 µl whole blood with EDTA anticoagulant were placed into marked tubes for CD3, CD4, CD8 and CD19. 10 µl Specific fluorescent antibody markers (rat anti-mouse) were added to whole blood and incubated to facilitate binding of antibodies to specific antigens. Red blood cells were lysed with a FACS lysing solution and a stabilizing solution was added to the marked cells. Results were analyzed on a FACS Calibur analyzer (Becton Dickinsin) to sort and count the specific lymphocyte populations. Isotype-specific controls were run for each marker. Result printouts were filed as hard copies.

3.3.8 Quality Control

Good laboratory practice (GLP) standards were conformed to, with quality control procedures and the necessary controls implemented where necessary. Prior to the study, a validation process of the entire study was run to ensure effective flow of the work and that all procedures were feasible. Fluency was critical, since the number of procedures and samples was extremely time-consuming and had to be precisely planned.

3.4 Data Collection and Statistical Analyses

Printed copies of the results for FBC and immunophenotyping analysis were collected and, once all data had been captured, the hard copies were filed for future reference. This section will explain how the raw data were captured and it will also illuminate the statistical analyses that were performed.

3.4.1 Data collection

Raw data were compiled onto an excel worksheet and included (Appendix 2). The raw data included all the parameters measured in the FBC as well as the immunophenotyping values. Parameters were expressed in their specific units of measurement.

3.4.2 Statistical analyses

The raw data were analyzed by a statistics consultant (Maryn Viljoen, Statistics Consulting Services) for analyses. Descriptive statistics namely means and standard deviations (or medians and percentiles) were calculated for each of the three exposure groups and the control group.

The mean or median values of each of the exposure groups were compared individually to the mean or median values of the control group using either the t-test or Kruskal-Wallis Test for independent samples. Where the results did not give a well distributed curve, the median values were used, but where a well distributed curve was obtained, the mean values were used. A significance level of 0.05 was used. The *ANOVA* test or Kruskal-Wallis Test was used to determine statistically significant mean or median differences ($p<0.05$) of the exposure groups (A = continuous exposure, B = four hours, C = one hour) amongst themselves. The Kruskal Wallis test was used for median values. Any outliers and clotted samples were excluded from the statistical analyses.

3.5 Conclusion

In summary, the methodology involved exposing mice to a specific Immunent ELF-EMF signal for a short period of time (seven days). The experimental group consisted of three groups of mice which were exposed to the EMF signal for different lengths of time. The control group was kept away from any source of EMF, but in all other aspects it was treated in the same way as the experimental group. Interference with the social behaviour of the mice was kept to a minimum in an attempt to prevent stress in the mice.

Ethical aspects with respect to the treatment of the experimental animals were strictly adhered to (SANS 10386:2008). This was made possible with the help of qualified personnel at the UFS animal unit.

After seven days, the mice were anaesthetized and bled. The blood was sent for biological analyses. The results were captured on an excel worksheet and sent for statistical analyses. A complete layout of the research protocol and a discussion of the results follow in Chapter 4.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

For this comparative study, the results of the EMF-exposed groups were compared with the sham-exposed (control) group. The results of the exposure groups were compared with those of one another as well as with those of the control group to determine if any statistically significant differences existed. In addition to this, the results of the various exposure groups were compared with each other for statistically significant differences. As stated in section 3.4.2, where the results did not give a well distributed curve, the median values were used, but where a well distributed curve was obtained, the mean values were used. Outlier values were excluded from the statistical analyses.

For the presentation of the results, the exposure and control groups are referred to as follows:

- Continuous exposure group (24 hours) - Group A
- Four-hour exposure group - Group B
- One-hour exposure group - Group C
- Control group - Group K

Figure 4.1 presents a diagrammatic summary of the experiment and the parameters according to which the results are discussed in this chapter.

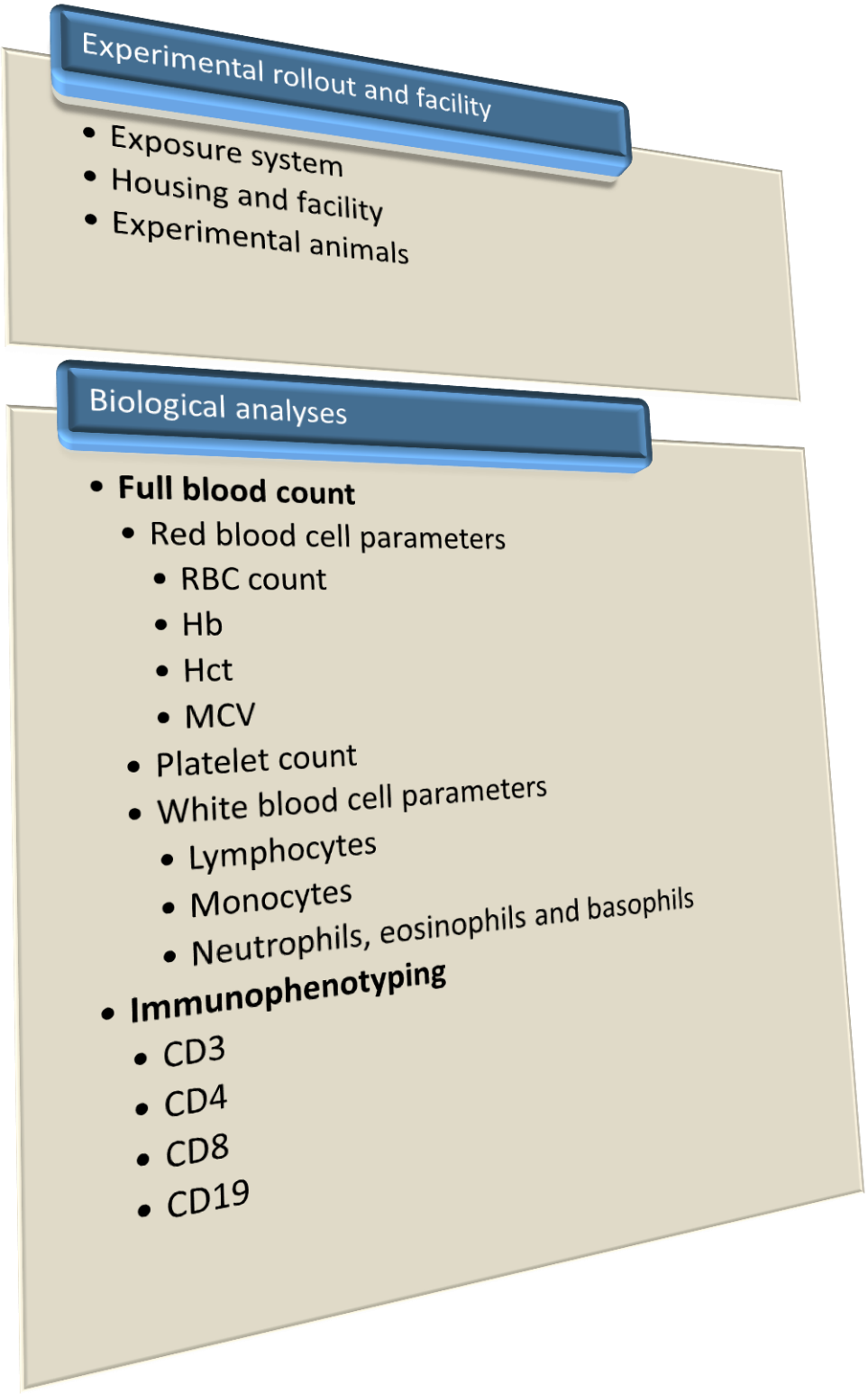

Figure 4.1: Schematic summary of presentation of results

4.2 Experimental rollout and facility

During the daily visits to the facility a close inspection of the exposure system, room temperature, housing, feeding, behaviour and condition of the mice was done. An exposure worksheet was created to record the significant findings.

4.2.1 Exposure system

The exposure system functioned without interruption. A computer program connected to the exposure system to monitor the exposure times indicated no evidence of error during the exposure period. No power failures were reported by the personnel of the experimental animal facility. Thus, it could be deduced that the exposure protocol was completed successfully.

4.2.2 Housing and facility

All aspects of the housing were found to be in good order. The room temperature measured constantly between 21-22^{o}C. With the doors permanently closed, unnecessary noise was also filtered from the room. The only people that entered the rooms were the researchers involved with the study. This also lessened the likelihood of the mice being exposed to foreign scent.

4.2.3 Experimental animals

The BALB/c N1H strain mouse specie was chosen due to their small size. This made it possible for a large enough number of mice (one hundred) to be housed comfortably in the space available at the Animal Unit facility. From previous experiments, a sample of one hundred mice was found to be sufficient. The maximum number of mice that could be completed in one day from bleeding to completion of all tests was thirty five.

At the age of six weeks, the mice had reached sexual maturity and were considered young, but fully grown. Male mice were selected for the study to exclude sexual hormonal interference as would have been the case in female mice.

There were no significant behavioural changes in the mice during the exposure period. The mice fed well and, evidently, appeared to experience minimal stress throughout the experiment. The animals appeared to be comfortable with the

housing, feed and bedding provided. Likewise, the control mice also appeared to be comfortable and fed well during the trial period. No differences were noted between the behaviour of the experimental and the control animals.

The animals were handled with extreme care during the anaesthesia period. The practice of limiting the handling and feeding of the animals to one (at most two) persons would have contributed minimizing stress in the animals.

The entire sample of one hundred mice were bled and sacrificed for the study. However, a small number of the collected blood samples could not be used for analysis due to clotting of blood when collection time was prolonged. The bleeding process proved to be challenging in the sense that the blood often began to clot before the bleeding process was complete. Administering heparin to the animals prior to the bleeding could moderately decrease this problem, but then there was the possibility of placing the animals under stress due to handling, since heparin needs to be administered by injection. For this reason it was decided against heparin administration prior to bleeding of the animals.

4.3 Biological analyses

The biological analyses included the full blood count (FBC) and immunophenotyping on whole blood collected in EDTA. Each group of biological parameters was recorded in a table, after which each parameter was represented graphically. This was done for the exposure groups *vs*. the control as well as for the intergroup comparisons. The results for each parameter were depicted in a table where the data included the mean / median, standard deviation (SD) as well as the p-value for the t-test / Kruskall-Wallis test of each exposure group compared to that of the control group. Following each table presenting the data, are figures with histograms presenting a diagrammatic representation of each set of data. SD error bars and trend lines were included.

4.3.1 Full blood count

The presentation of the results for the FBC was grouped into erythrocyte parameters, platelet counts and leukocyte parameters. The parameters of most significance for this study were those of the leukocytes, since these cells are involved in the immune response and are also known as immune cells (Delves, Martin, Burton & Roitt, 2011:35). Hence, the specific differential leukocyte counts were determined and either shown to have statistical significant relevance with respect to the immune response, or shown to present no relevance at all.

4.3.1.1 Red blood cell (erythrocyte) parameters

The descriptive statistics and statistical significance for differences between the experimental groups (A, B and C) and the control group for the erythrocyte parameters are depicted in *Table 4.1*. The erythrocyte parameters, and their units of measurement, included the following:

- Erythrocyte count, expressed as 10^{12} erythrocytes per litre (10^{12}/L) whole blood.
- Haemoglobin (Hb), expressed as grams per decilitre (g/dl) whole blood.
- Haematocrit (Hct), expressed as percentage (%) whole blood.
- Mean corpuscular volume (MCV), expressed as femtolitre (fl) whole blood.

Table 4.1: Descriptive statistics and p-values for mean / median erythrocyte parameters

Parameter		A	B	C	K
Erythrocyte count	Mean (10^{12}/L)	9.32	9.26	9.21	9.23
	SD	0.28	0.53	0.52	0.49
	p-value t-test	0.4748	0.8340	0.8833	
Hb	Mean (g/dl)	14.76	14.76	14.66	14.75
	SD	0.51	0.71	0.79	0.64
	p-value t-test	0.9327	0.9419	0.6795	
Hct	Mean (%)	44.12	43.88	43.86	43.72
	SD	1.37	2.6	2.45	2.33
	p-value t-test	0.4713	0.8271	0.8441	
MCV	Median (fl)	47.00	47.00	47.00	47.00
	Inter-quartile range	47.00-48.00	47.00-48.00	47.00-48.00	47.00-48.00
	p-value Kruskal-Wallis Test	0.2422	0.8513	0.0688	

($p<0.05$ indicates statistically significant difference)

Figures 4.2 (a – d) depict the comparative mean / median values for the parameters in *Table 4.1*. Histograms were used to depict the values for the three different exposure groups and the control group.

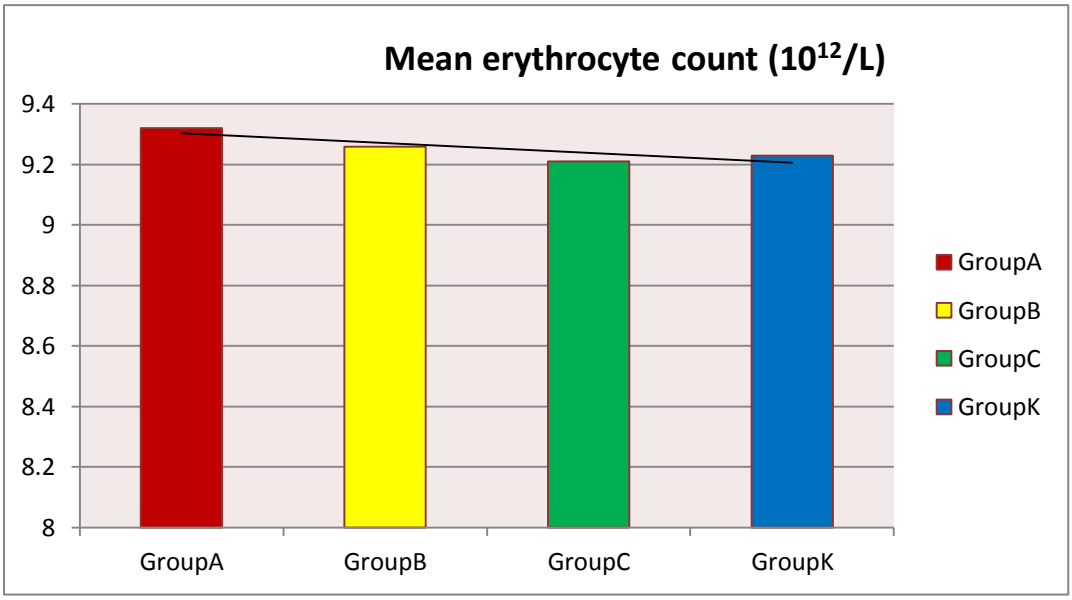

Figure 4.2 (a): Mean / median values for erythrocyte count of all the groups

The trend line showed a slight decrease in erythrocyte values from group A to group C. In spite of this slight trend, there were no statistically significant differences between the EMF exposure groups and the control group (*Figure 4.2a*).

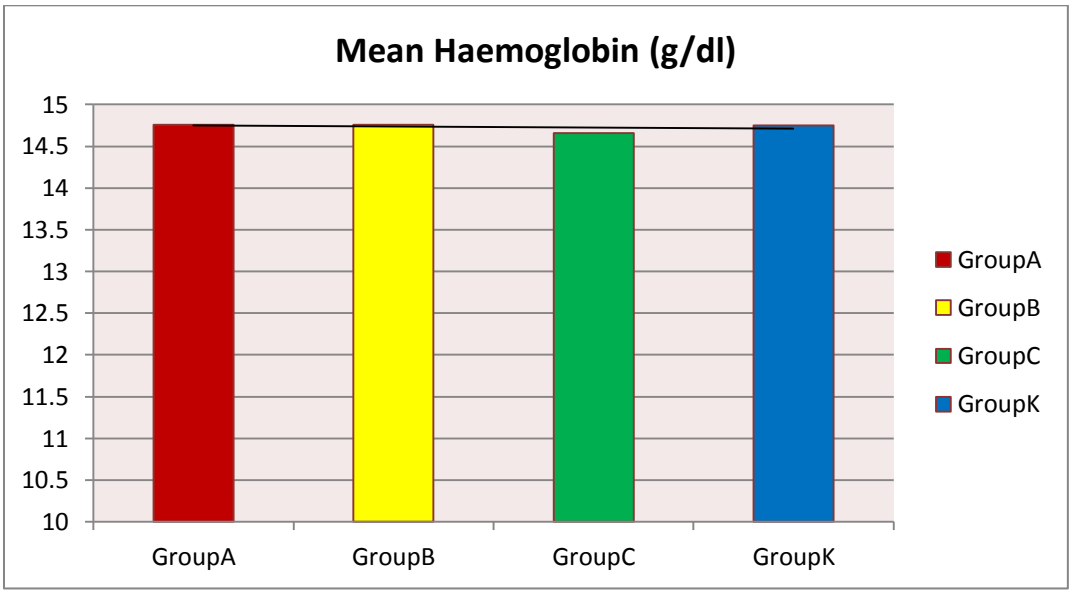

Figure 4.2 (b): Mean values for Hb concentration of all the groups

There were no statistically significant differences for Hb levels between the exposed groups and the control group. There was also absence of a trend in this regard (*Figure 4.2b*).

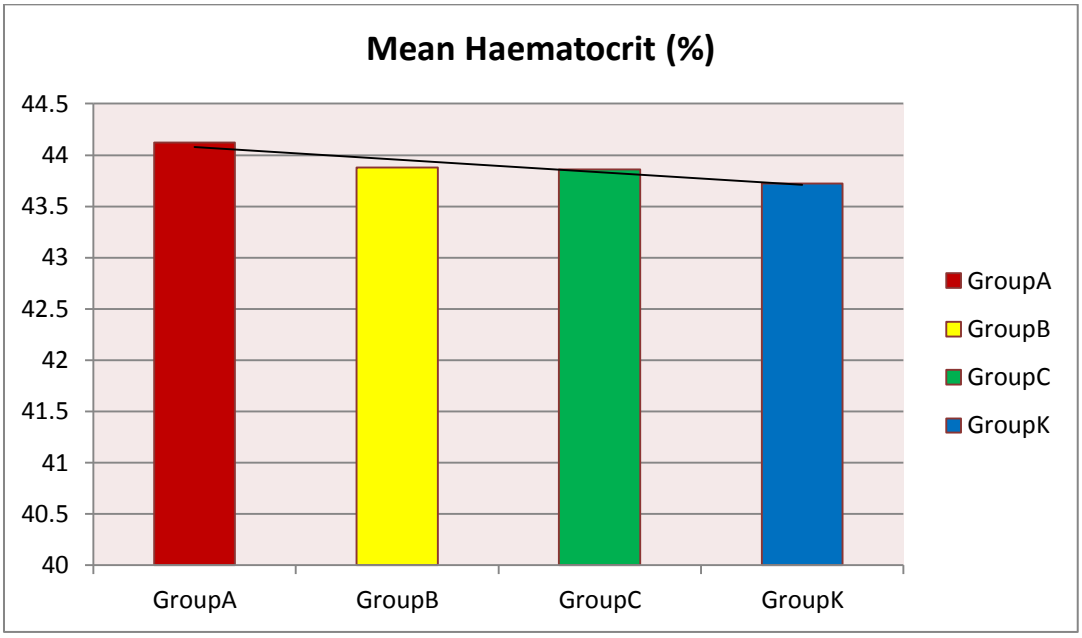

Figure 4.2 (c): Mean values for Hct of all the groups

Once again, there was a slight trend indicating increasing Hct values as the exposure duration increased. However, the differences were not statistically significant (*Figure 4.2c*).

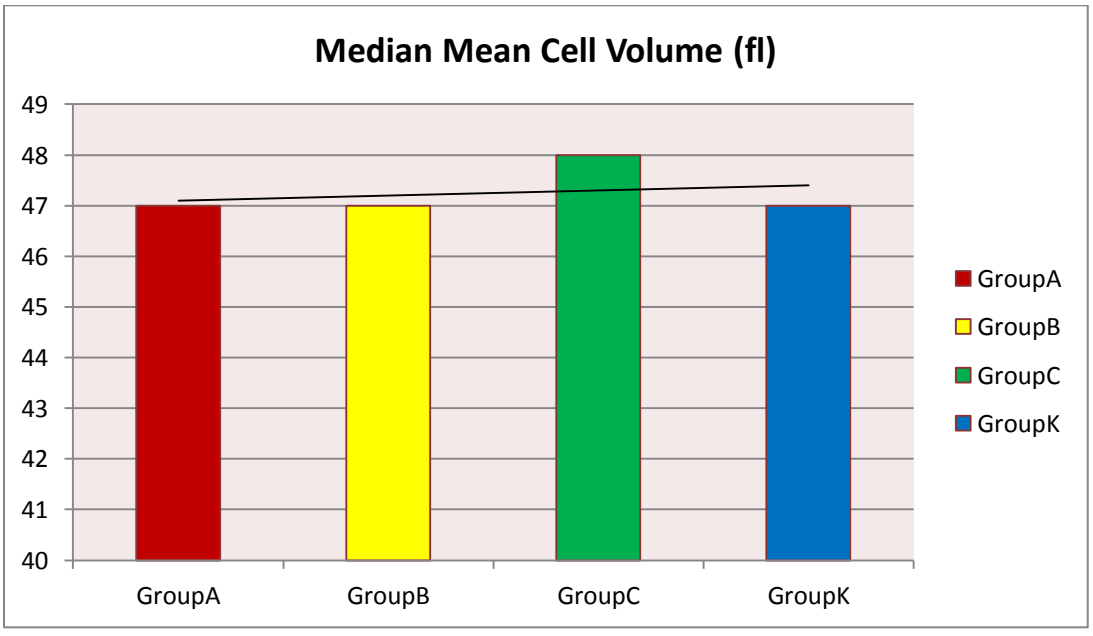

Figure 4.2 (d): Median values for MCV of all the groups

There were no statistically significant differences between the exposed groups and the control group. The spike of increased values for group C was, therefore, not statistically significant (*Figure 4.2d*).

4.3.1.1.1 Summary of comparative mean / median values for the erythrocyte parameters

To summarize, there were no statistically significant differences between the exposure groups and the control group for any of the erythrocyte parameters. The p-values for the t-test and the Kruskal-Wallis Test were found to be above the statistically significance indicator of 0.05 ($p>0.05$).

Table 4.2 below represents the p-values of mean / median differences for erythrocyte parameters between all the groups (ANOVA test or Kruskal-Wallis Test) and between the individual groups (intergroup comparison).

Table 4.2: Intergroup comparison for mean / median erythrocyte parameters

Parameter	A vs. B vs. C (ANOVA t-test / Kruskal-Wallis Test) p-value	A vs. B (t-test / Kruskal-Wallis Test) p-value	A vs. C (t-test / Kruskal-Wallis Test) p-value	B vs. C (t-test / Kruskal-Wallis Test) p-value
Erythrocyte	0.7267	0.6672	0.3868	0.7281
Hb	0.8355	1.0000	0.5988	0.6392
Hct	0.8957	0.6810	0.6416	0.9778
MCV	0.2256	0.3244	0.4146	0.0954

($p<0.05$ indicates statistically significant difference)

Figures 4.3 (a - d) depict the graphics for the mean / median intergroup comparison of the erythrocyte parameters. The histograms depict the comparison of each group with the other.

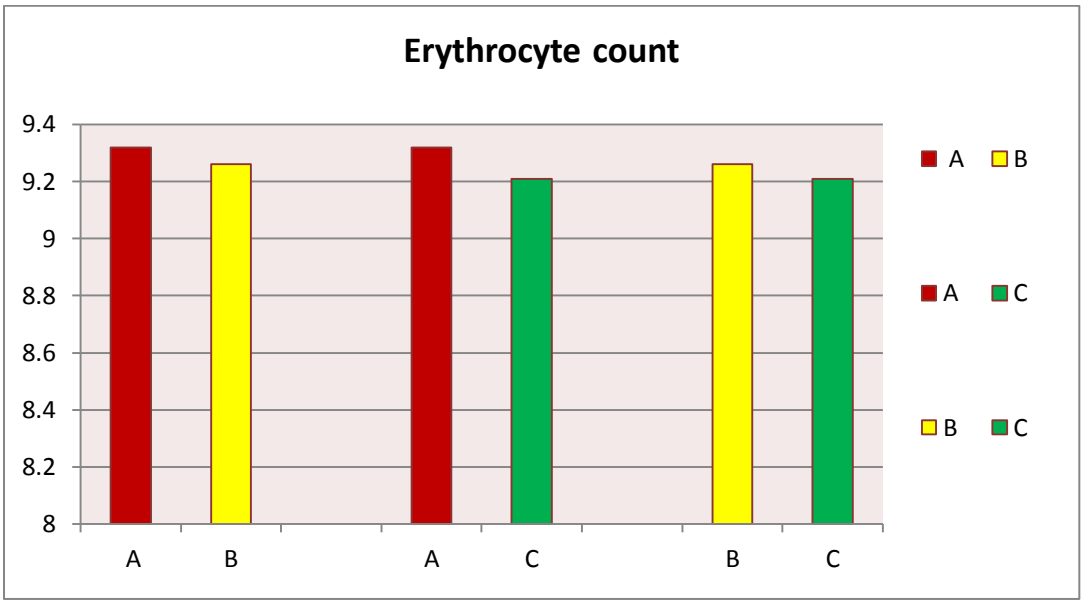

Figure 4.3 (a): Intergroup comparison of mean erythrocyte count

Although the largest difference found was between groups A and C for the erythrocyte count, the difference was not statistically significant. There were no statistically significant differences between any of the exposure groups for the erythrocyte counts (*Figure 4.3a*)

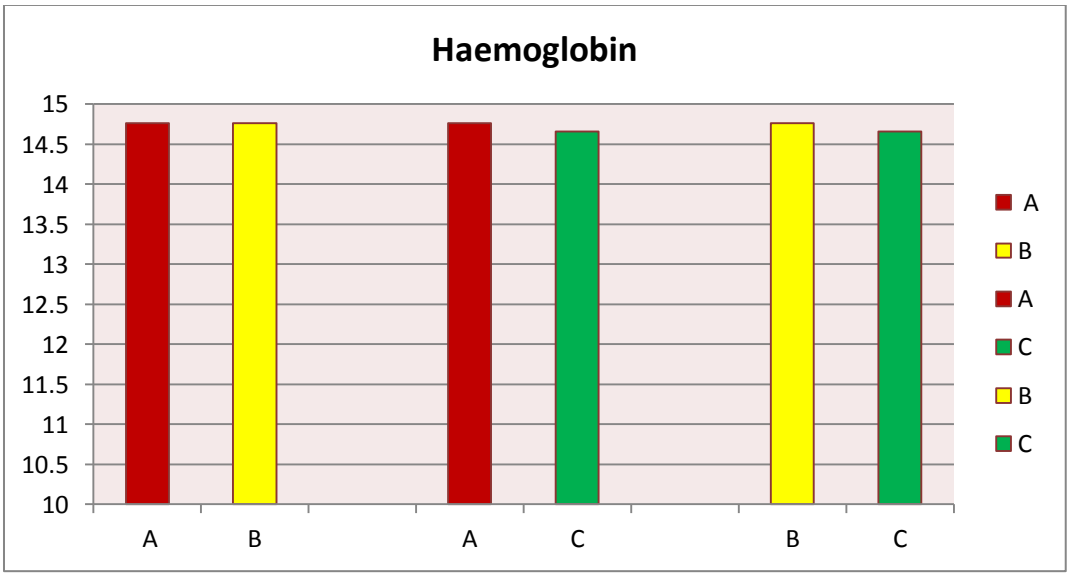

Figure 4.3 (b): Intergroup comparison of mean Hb concentration

As with the erythrocytes, there were no statistically significant differences between any of the exposure groups for the Hb concentration. All the values were very similar (*Figure 4.3b*).

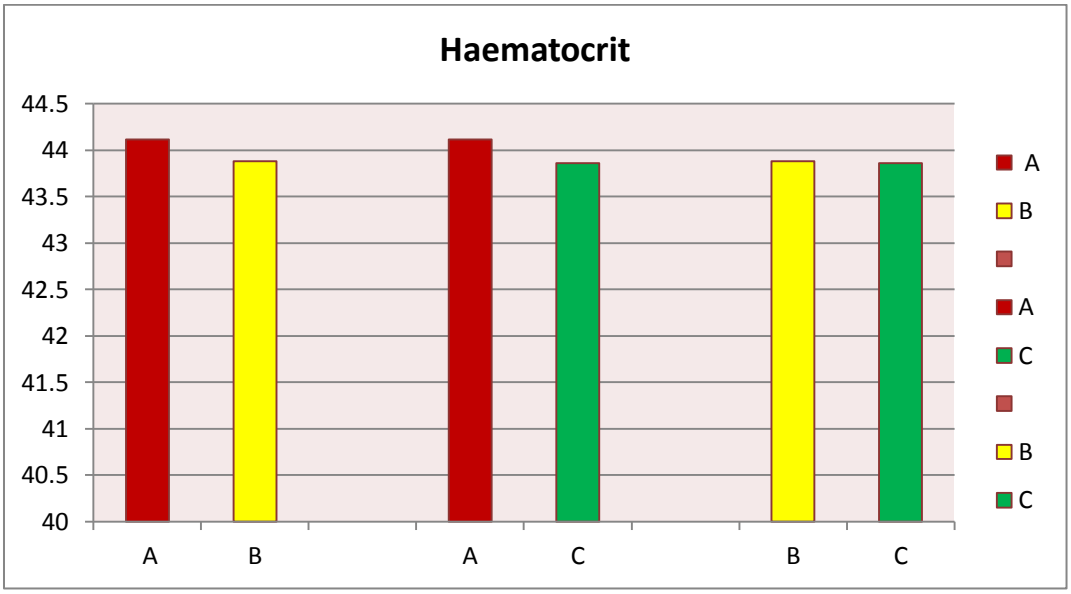

Figure 4.3 (c): Intergroup comparison of mean Hct percentage

There was a slight variation in Hct concentration between groups A and B as well as between groups B and C, but it was not statistically significant. No statistically significant differences for the intergroup comparisons were found between any of the exposure groups (*Figure 4.3c*).

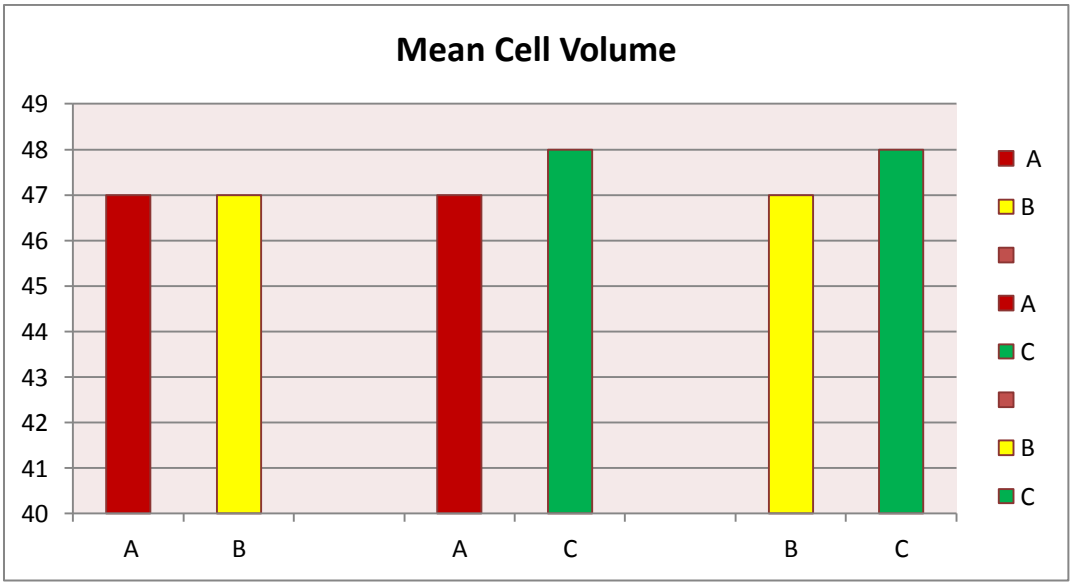

Figure 4.3 (d): Intergroup comparison of median MCV concentration

The intergroup comparison (data in *Table 4.2* and *Figures 4.3 (a - d)* indicated no statistically significant mean or median differences between the exposure groups for any of the erythrocyte parameters ($p<0.05$). The ANOVA test / Kruskal-Wallis Test (A vs. B vs. C) showed no statistically significant mean or median differences ($p>0.05$) for any of the erythrocyte parameters.

4.3.1.1.2 Summary of intergroup comparisons for mean / median erythrocyte parameters

To conclude this group of analyses, the erythrocyte parameters in FBC indicated no statistically significant differences between any of the groups (A, B, and C) and the control group. In addition, there was no statistically significant difference in erythrocyte parameters for the intergroup comparison. In spite of the lack of statistically significant differences, there was a general trend for higher erythrocyte values in group A, and to a lesser extent, also group B. It can therefore be deduced that exposure to the ELF-EMF had no effect on the erythrocyte parameters of the mice. The duration of exposure (twenty four, four and one hours) also had no effect on the results of the erythrocyte parameters.

4.3.1.1.3 Discussion of findings for erythrocyte parameters

Evidently, in the current study there was no anticipation that the ELF-EMF would have any effect on the erythrocytes, since the immune system involves predominantly the leukocytes (Coico & Sunshine, 2009:11-25).

In a study where rabbits were exposed to radiofrequency EMFs, their erythrocyte parameters did not differ from those of the control group (Sarookhani *et al.*, 2012). However, the fields in the study by Sarookhani *et al*. (2012) were stronger (950M Hz) than those in the current study. In another study where the EMFs were similar to those used in the current study, it was also found that there were no statistically significant differences in erythrocyte parameters between the exposed groups and the control group (Cakir *et al.*, 2009). This study differed from the current study in the sense that exposure took place over a longer period (fifty and one hundred days).

El-Bialy *et al.* (2012) conducted a study in which rats were exposed to ELF-EMFs for an extended period (thirty days). They found that erythrocyte, Hb and Hct

levels were decreased in the exposed group. However, no significant difference was found in this study.

4.3.1.2 Platelet count

The descriptive statistics and significance for median differences between the experimental groups (A, B and C) and the control group for the platelet count are depicted in the *Table 4.3*. The platelet count was expressed as $10^3/mm^3$ whole blood.

Table 4.3: Descriptive statistics and p-values for median platelet count

Parameter		A	B	C	K
Platelets	Median($10^3/mm^3$)	854.00	879.00	890.00	828.00
	Inter-quartile range	785.00-932.00	783.00-945.00	775.00-1029.00	761.00-1028
	p-value Kruskal-Wallis Test	0.9045	0.9362	0.6672	

(p<0.05 indicates statistically significant difference)

The histogram below (*Figure 4.4*) facilitates a graphic view and representation of the median values in *Table 4.3*.

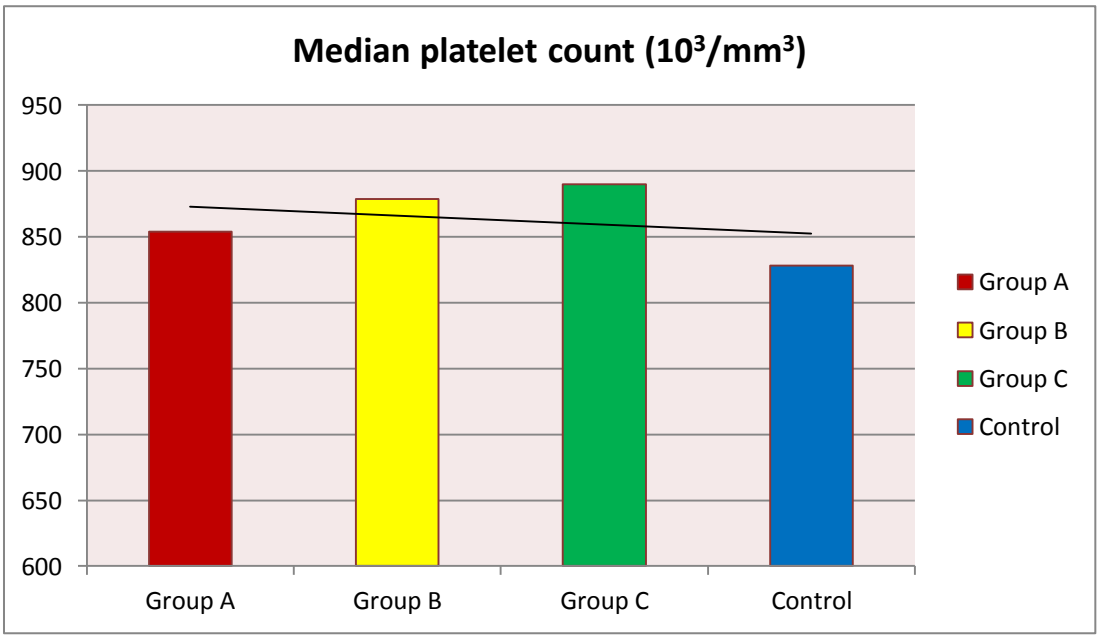

Figure 4.4: Median platelet count of all the groups

From *Figure 4.4* it is evident that the platelet count increased as the exposure period decreased. The control value was lower than the values of the three ELF-

EMF-exposed groups. However, none of the differences were statistically significant.

4.3.1.2.1 Summary of comparative median values for the platelet parameters

There were no statistically significant differences ($p>0.05$) between any of the exposure groups and the control group for the platelet count. This was found with reference to the descriptive statistics and significance for median differences between the experimental groups (A, B and C) and the control group as depicted in *Table 4.*3.

Table 4.4 below indicates the p-values for median differences in platelet counts between all the groups (using the Kruskal-WallisTest) and between the individual groups (intergroup comparison).

Table 4.4: Intergroup comparison for median platelet count

Parameter	A vs. B vs. C (Kruskal-Wallis Test) p-value	A vs. B (Kruskal-Wallis Test) p-value	A vs. C (Kruskal-Wallis Test) p-value	B vs. C (Kruskal-Wallis Test) p-value
Platelets	0.6334	0.7859	0.3986	0.4434

(p<0.05 indicates statistically significant difference)

A graphic view and representation of the median intergroup comparison can be seen in the histogram below (*Figure 4.5*):

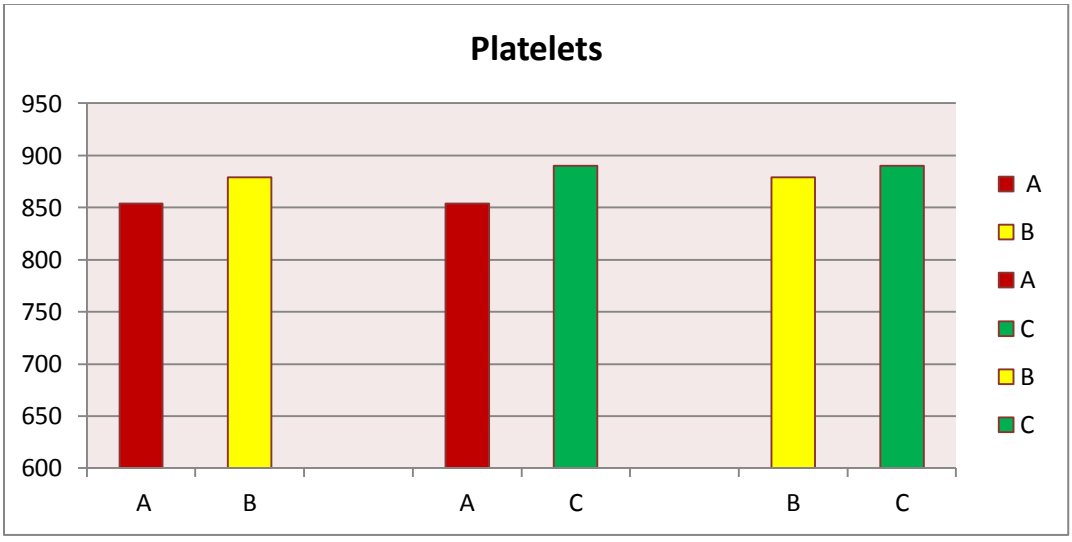

Figure 4.5: Intergroup comparison of median platelet counts

Although there appeared to be a marked difference between groups A and B, it was not statistically significant. The difference between groups A and C was even more prominent, yet it was not statistically significant either.

4.3.1.2.2 Summary of intergroup comparison for median platelet parameters

For the intergroup comparison, there were no statistically significant median differences ($p>0.05$) between any of the exposure groups themselves (A, B or C) for the platelet counts. The Kruskal-Wallis Test (A *vs*. B *vs*. C) also indicated no statistically significant differences ($p>0.05$) between the groups.

4.3.1.2.3 Discussion of findings for platelet parameters

It was clear that ELF-EMF exposure did not have any effect on the peripheral platelet counts of the experimental mice. The duration of exposure also had no effect on the platelet count.

The study by Sarookhani *et al.* (2012) produced different results. They exposed rabbits to mobile phone radiation with a frequency of 950 Mhz (3 and 6 Watts). In the 3 Watt group, the platelet counts were decreased, but the counts were increased in the 6 Watt group. However, these results have no direct bearing on the current study since the frequency of the fields was much higher than in the current study (50-5000 Hz). The results of the study by Cakir *et al.* (2009) on platelets concur with those of the current study, whereas the platelet counts in a study by El-Bialy *et al.* (2012) were found to be statistically significantly increased in the exposed group. However, the exposure period in the latter study, was thirty days compared to the seven days of the current study.

4.3.1.3 Leukocyte parameters

The descriptive statistics and statistical significance for mean or median differences between the experimental groups and the control group for the leukocyte parameters are depicted in *Table 4.5*. Where statistically significant differences were found, the value is highlighted in the table. An asterix (*) is assigned to the column of the graph in *Figures 4.6 (a - f)* where a statistically significant difference was found. The leukocyte parameters and their units of measurement were the following:

- The total leukocyte count, expressed as 10^9 leukocyte per litre (10^9 /L) whole blood. This count included all the leukocyte counts below.
- The lymphocyte count, expressed as a percentage of the total leukocytes.
- The monocyte count, expressed as a percentage of the total leukocytes.
- The neutrophil count, expressed as a percentage of the total leukocytes.
- The eosinophil count, expressed as a percentage of the total leukocytes.
- The basophil count, expressed as a percentage of the total leukocytes.

Table 4.5: Descriptive statistics and p-values for mean / median leukocyte parameters

Parameter		A	B	C	K
Total Leukocytes	Mean (10^9/L)	7.26	7.06	6.66	6.33
	SD	1.66	1.44	1.42	1.39
	p-value t-test	0.0391	0.0773	0.4032	
Lymphocytes	Median (%)	89.25	87.30	87.10	87.10
	Inter-quartile range	87.3-92.55	85.60-90.90	85.50-89.20	84.25-89.05
	p-value Kruskal-Wallis Test	0.0149	0.4715	0.5555	
Monocytes	Median (%)	2.60	3.00	3.10	3.25
	Inter-quartile range	1.70-3.60	2.30-3.80	2.20-4.10	2.50-4.90
	p-value Kruskal-Wallis Test	0.0833	0.5549	0.6309	
Neutrophils	Median (%)	6.50	8.60	8.70	9.00
	Inter-quartile range	5.60-8.70	6.90-10.10	7.20-10.20	7.80-9.85
	p-value Kruskal-Wallis Test	0.0014	0.2712	0.6169	
Eosinophils	Median (%)	0.10	0.20	0.20	0.20
	Inter-quartile range	0.10-0.20	0.10-0.30	0.10-0.40	0.10-0.40
	p-value Kruskal-Wallis Test	0.1360	0.9030	0.8469	
Basophils	Median (%)	0.40	0.40	0.40	0.40
	Inter-quartile range	0.30-0.50	0.30-0.50	0.30-0.40	0.30-0.50
	p-value Kruskal-Wallis Test	0.6656	0.8356	0.2562	

(p<0.05 indicates a statistically significant difference)

The histograms below (*Figures 4.6 a – f*) represent the data in *Table 4.5.* A separate graph was created for each specific parameter, in spite of the fact that several units of measurement were the same. This was to simplify visualization of the group results compared to those of the control.

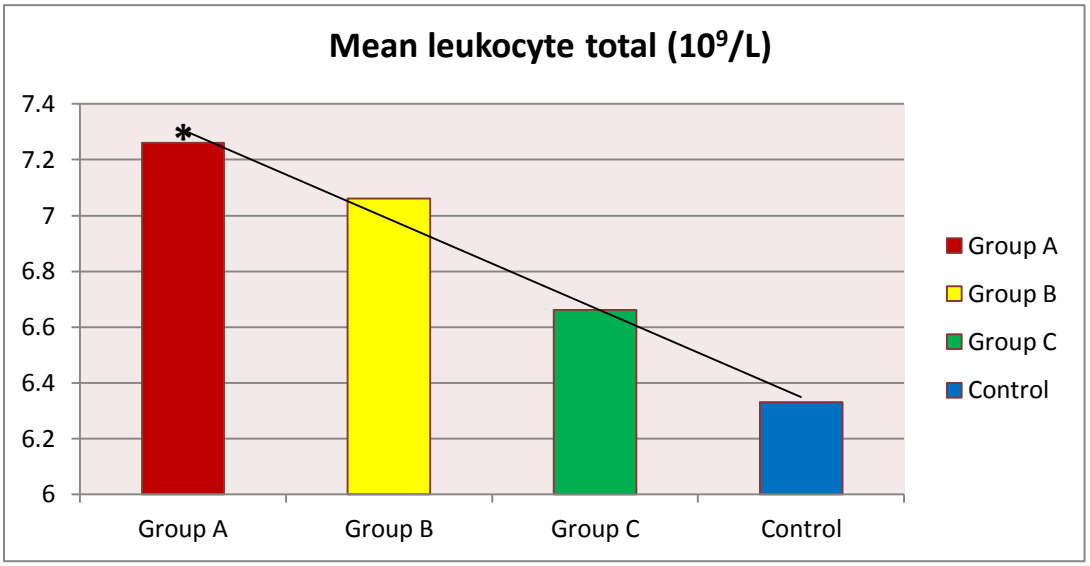

Figure 4.6 (a): Mean values for the total leukocyte count of all the groups

** = Statistically significant difference (p<0.05)*

Figure 4.6a shows a statistically significant difference in leukocyte count between group A and the control group. There was no statistically significant difference between groups B, C and the control group. The trend line showed a decrease in leukocyte count as the exposure duration decreased, being statistically significant only in group A.

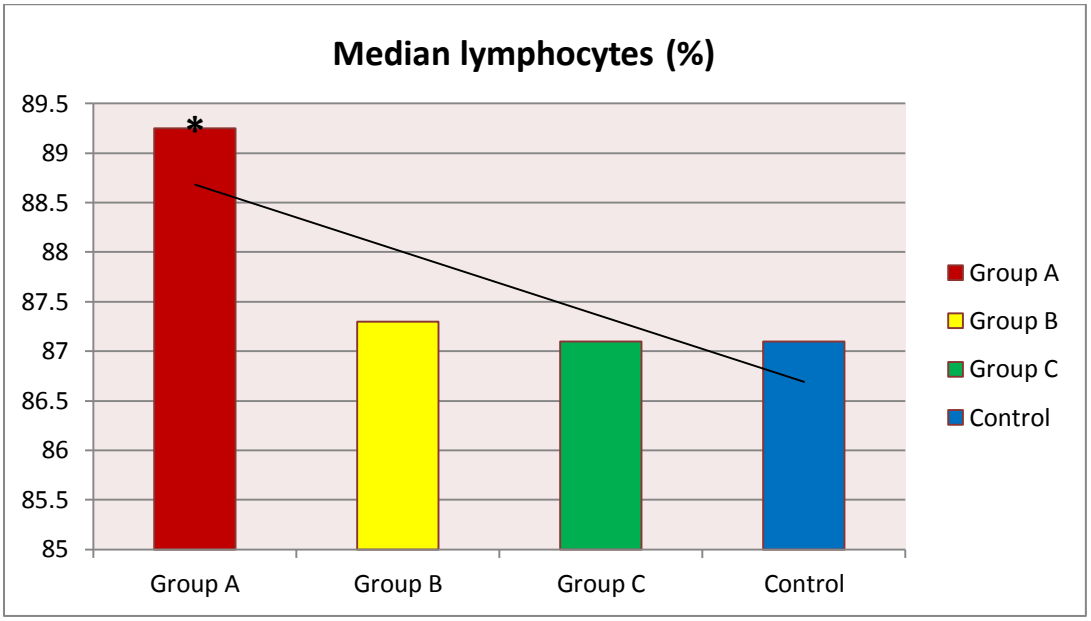

Figure 4.6 (b): Mean values for the total lymphocyte percentage of all the groups

** = Statistically significant difference (p<0.05)*

From *Figure 4.6b* it is evident that there was a statistically significant difference between group A and the control group for the lymphocyte count. There was no evidence of any differences between groups B, C and the control group.

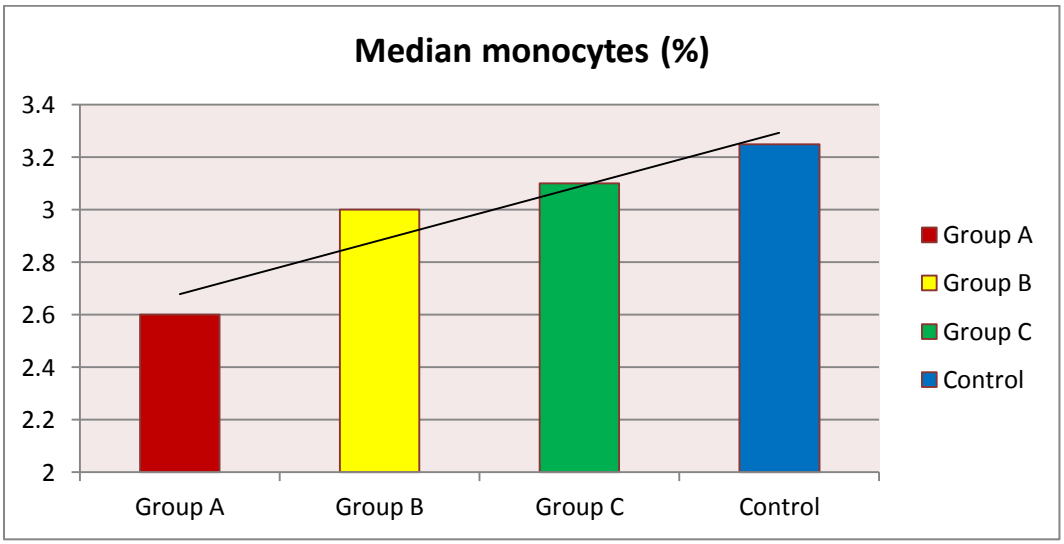

Figure 4.6 (c): Median values for the monocyte percentage of all the groups

The trend line in *Figure 4.6c* indicated a gradual increase in monocyte percentage as the duration of exposure decreased. Exposure group A (24-hour exposure) had the lowest monocyte percentage compared to the control group. Despite this observation, the differences were not statistically significant.

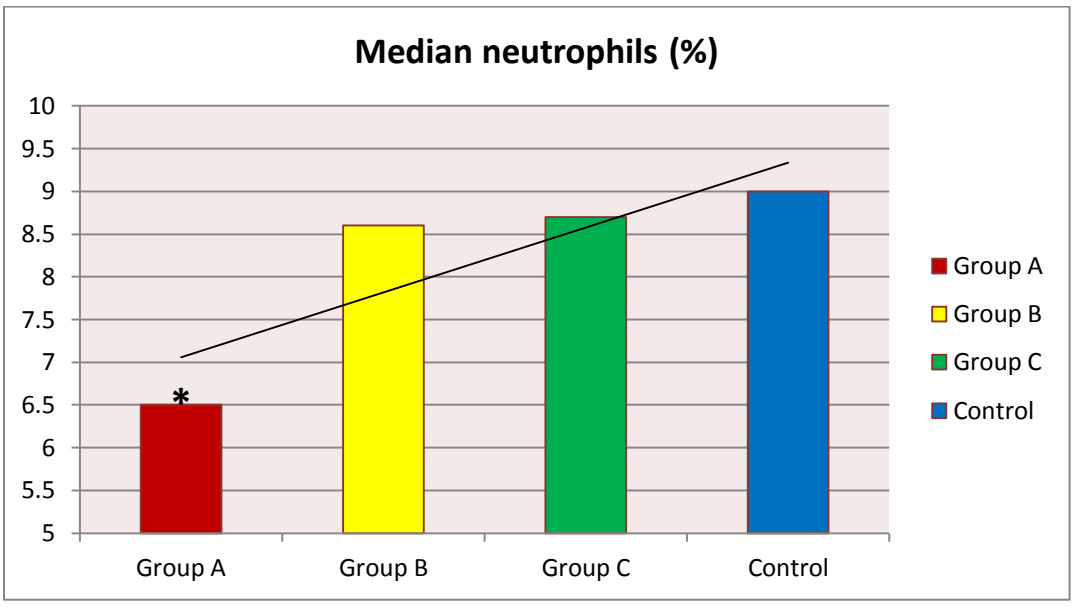

Figure 4.6 (d): Median values for the neutrophil percentage of all the groups

* = *Statistically significant difference ($p<0.05$)*

Figure 4.6d indicates that the neutrophil percentage for group A was statistically significantly lower than that of the control group. The percentages of group B and C were also lower than that of the control group, but the differences were not statistically significant.

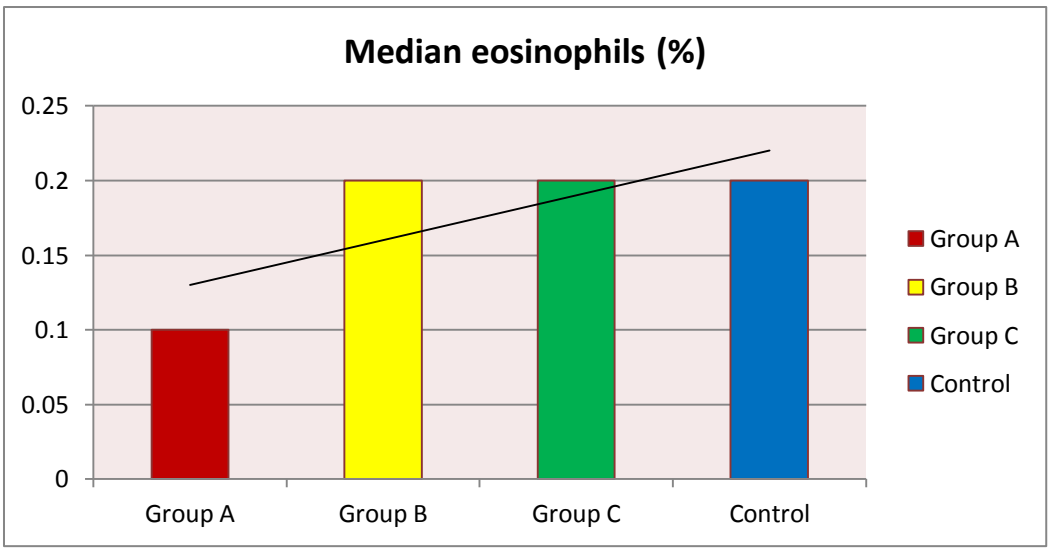

Figure 4.6 (e): Median values for the eosinophil percentage of all the groups

Although the eosinophil percentage for group A was 50% lower than the percentages for groups B, C and the control group, the differences were not statistically significant (*Figure 4.6e*).

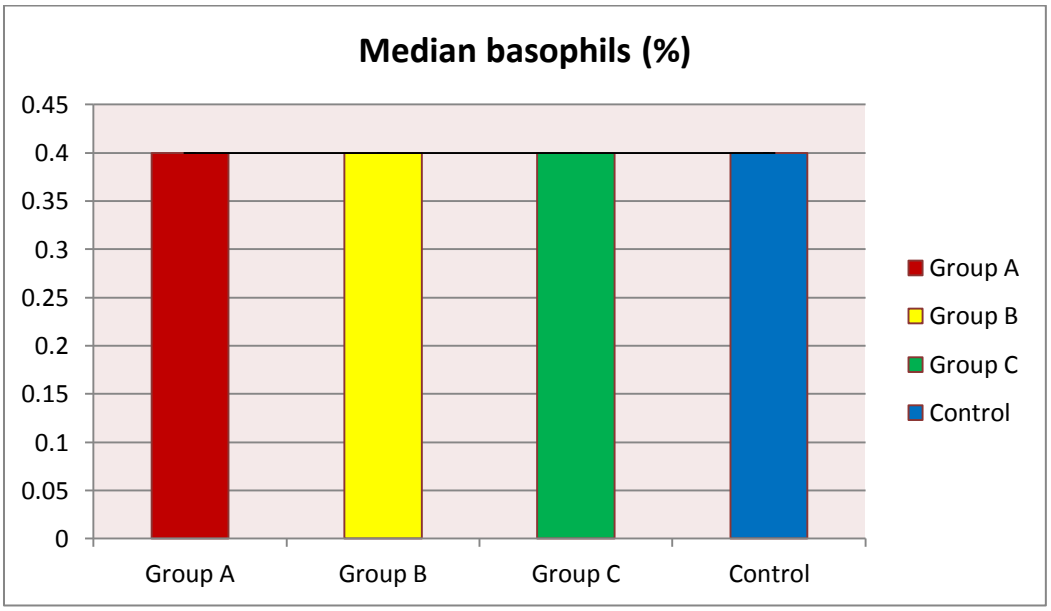

Figure 4.6 (f): Median values for the basophil percentage of all the groups

From *Figure 4.*6f it is evident that there were no statistically significant differences in basophil percentages between any of the exposure groups and between the exposure groups and the control group.

4.3.1.3.1 Summary of comparative mean / median values for the leukocyte parameters

The data revealed a statistically significant mean or median difference in total leukocyte (p=0.0391), lymphocyte (p=0.0149) and neutrophil (p=0.0014) values between group A (24-hour exposure) and the control group. For the monocytes, eosinophils and basophils, there were no statistically significant median differences between the experimental groups and the control group (p>0.05).

There was an increased mean total leukocyte count and the median lymphocyte count for the mice in the 24-hour exposure group (group A). *Figure 4.6 (b)* illustrates that the lymphocyte counts increased exponentially, with the lowest count found in group C and the highest count in group A. Although group B and group C indicated no statistically significant difference from the control group, it was noted that there seemed to be a trend where the lymphocyte values increased with increased duration of exposure.

In contrast to the increased lymphocyte count, the neutrophil count for group A was statistically significantly lower (p=0.0014) than that of the control group. As with the analysis of the former group, *Figure 4.6 (d)* reveals that there was an exponential decrease, but that the difference between group B and group C was not statistically significant.

Figures 4.6 (c) and *4.6 (e)* reveal that there were lower monocyte (p=0.0833) and eosinophil (p=0.1360) values for group A compared to the control group, but these were not statistically significant. For the monocytes, there was an apparent trend to decrease with increased exposure time. In spite of the differences not being statistically significant, the monocytes showed a clear exponential increase from group A (24-hour group) to group C (1-hour group).

It was observed that the statistically significant difference between the exposure groups and the control group seemed to increase as the duration of exposure

increased. Moreover, in spite of the differences not all being statistically significant, many parameters became statistically significant in group A (24-hour exposure), which was the maximum exposure duration.

Table 4.6 depicts the differences for leukocyte parameters between all the groups using the ANOVA and Kruskal-Wallis test and between the individual groups (intergroup comparison) using the t-test or Kruskal-Wallis test. Keep in mind that the Kruskal Wallis test was used for data with median values.

Table 4.6: Intergroup comparison for leukocyte parameters

Parameter	A vs. B vs. C (ANOVA/ Kruskal-Wallis Test) p-value	A vs. B (t-test/ Kruskal-Wallis Test) p-value	A vs. C (t-test/ Kruskal-Wallis Test) p-value	B vs. C (t-test/ Kruskal-Wallis Test) p-value
Total Leukocytes	0.3772	0.6520	0.1830	0.3385
Lymphocytes	0.0338	0.0423	0.0151	0.7195
Monocytes	0.1563	0.1272	0.0771	0.7709
Neutrophils	0.0190	0.0435	0.0072	0.4786
Eosinophils	0.2636	0.2379	0.1219	0.6228
Basophils	0.1475	0.7251	0.0945	0.0879

($p<0.05$ indicates statistically significant difference)

The histograms in *Figures 4.7 (a-f)* depict the data for the intergroup comparison in *Table 4.6*.

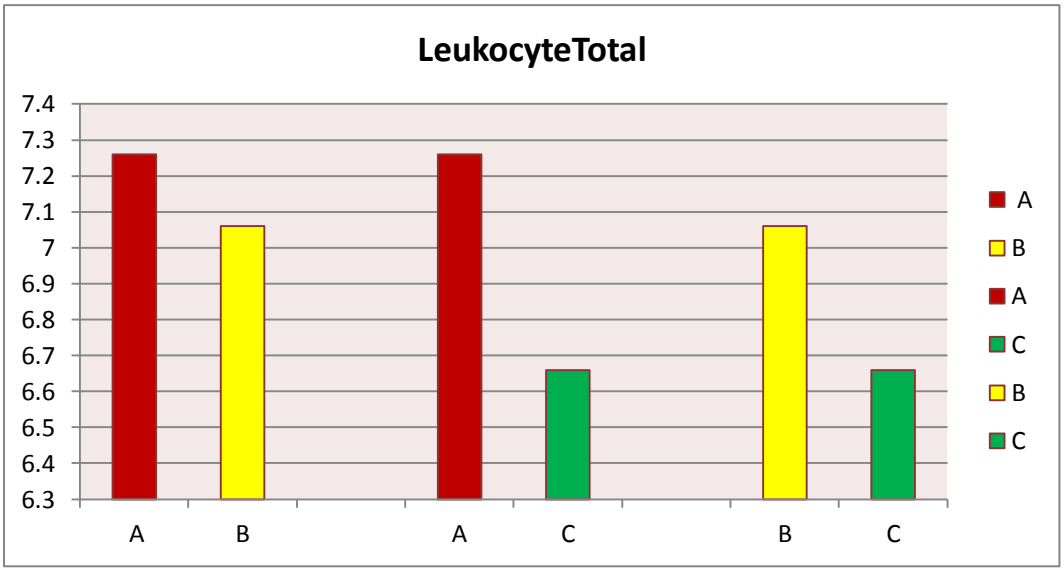

Figure 4.7 (a): Intergroup comparison of mean total leukocyte counts

Figure 4.7a shows that no statistically significant differences were found for the intergroup comparison between the exposure groups for the total leukocyte counts. The largest difference was observed between group A and group C.

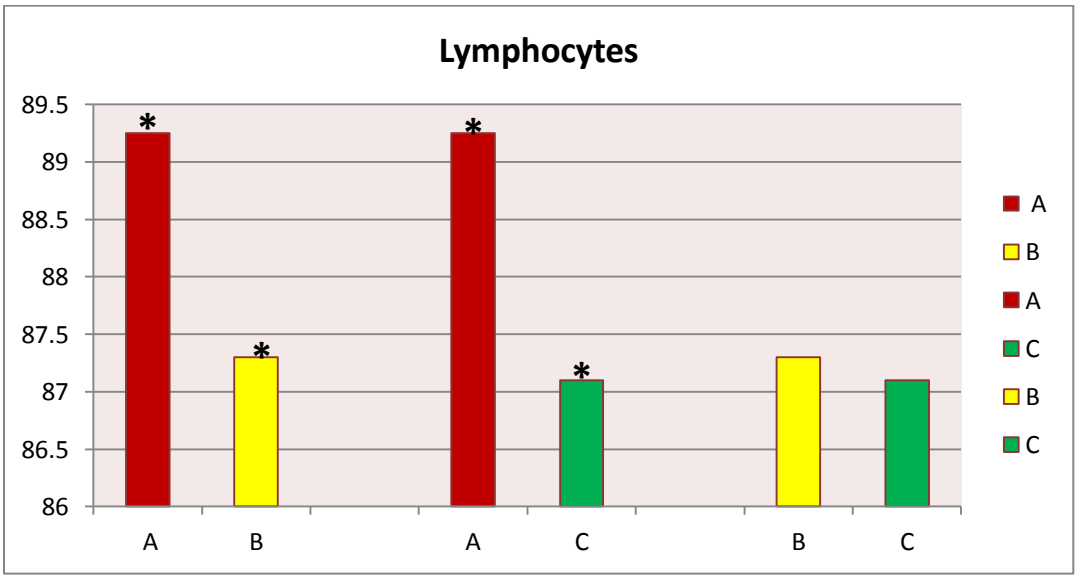

Figure 4.7 (b): Intergroup comparison of median lymphocyte percentages

* = *Statistically significant difference ($p<0.05$)*

From *Figure 4.7b* it is evident that there was a statistically significant difference between groups A and B as well as between groups A and C when conducting the intergroup comparison. The result of the intergroup comparison between groups B and C showed no statistically significant difference.

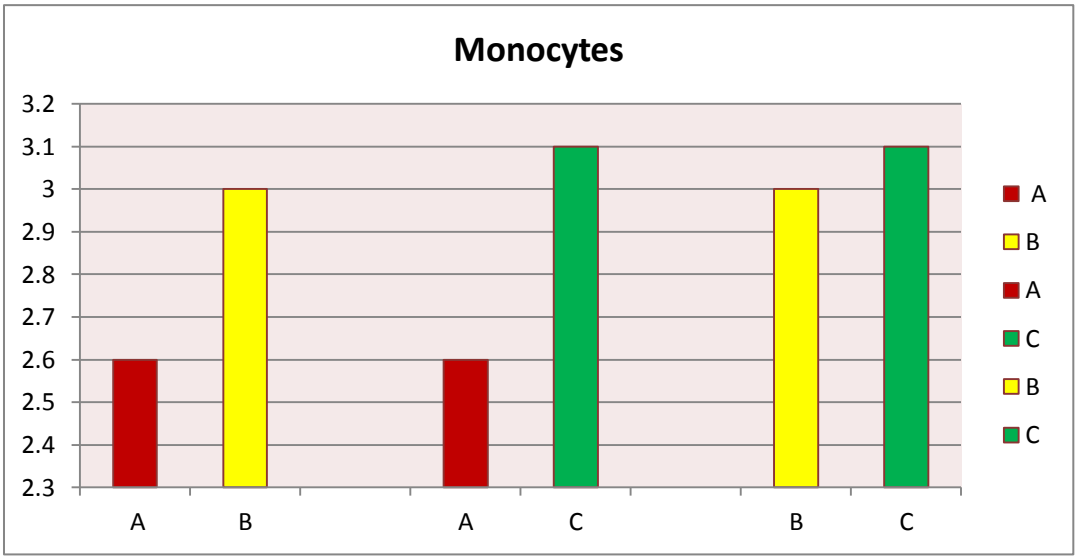

Figure 4.7 (c): Intergroup comparison of median monocyte percentages

The intergroup comparisons for the monocyte counts were not statistically significant for any of the groups as seen in *Figure 4.7c*. However, despite the fact that it was not statistically significant, a difference was noted between groups A and B as well as between groups B and C.

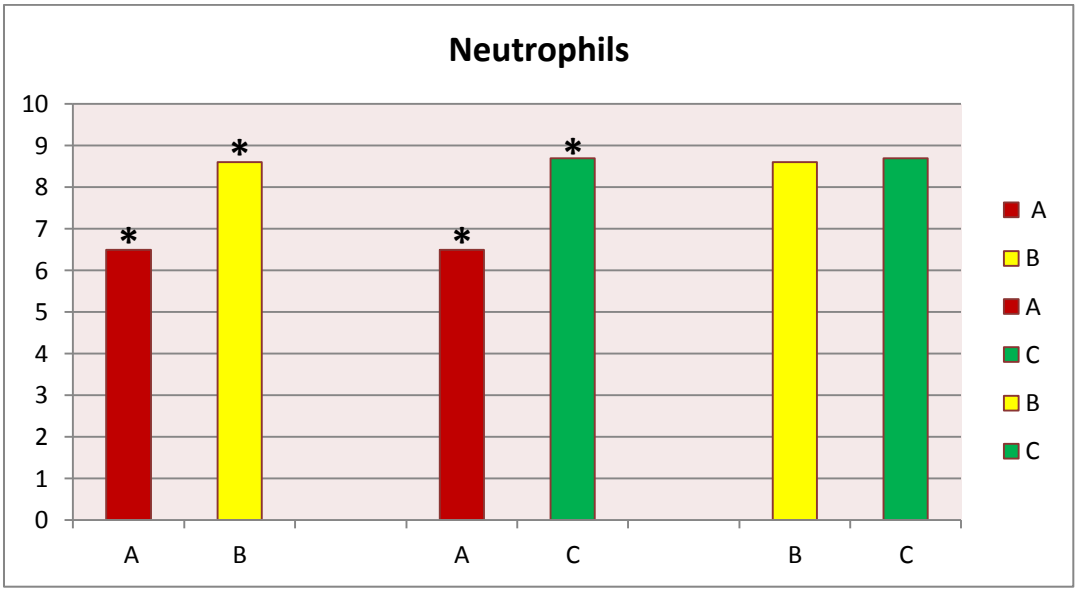

Figure 4.7 (d): Intergroup comparison of median neutrophil percentages

* *= Statistically significant difference ($p<0.05$)*

Figure 4.7d reveals that there was a statistically significant difference for the intergroup comparison of the neutrophil counts between groups A and B as well as between groups A and C. There was no significant difference between groups B and C.

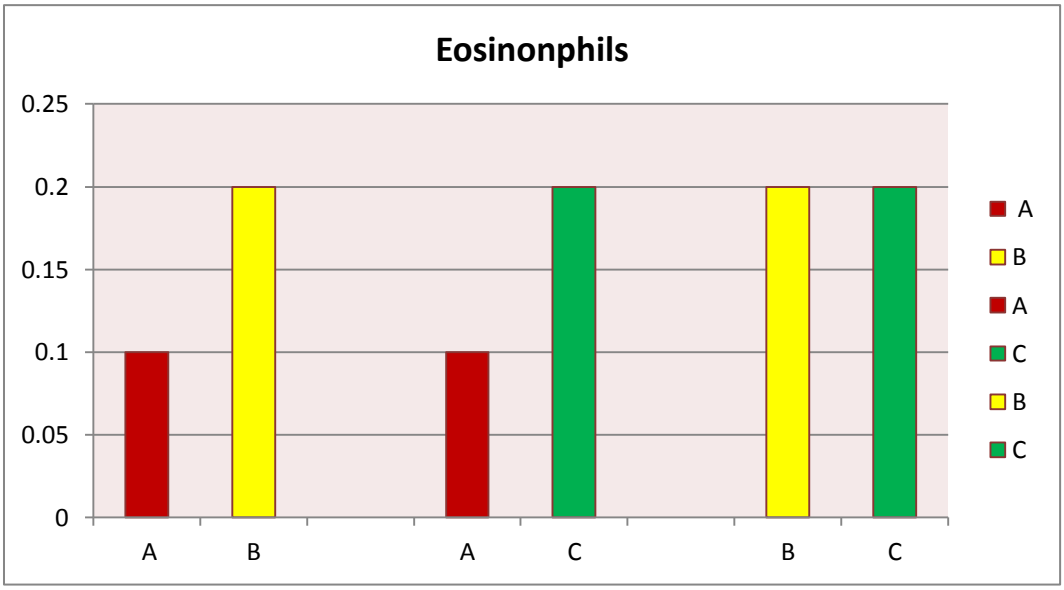

Figure 4.7 (e): Intergroup comparison of median eosinophil percentages

From *Figure 4.7e* it can be seen that although there were 50 % intergroup differences between groups A and B as well as between groups A and C for the eosinophil counts, these differences were not statistically significant.

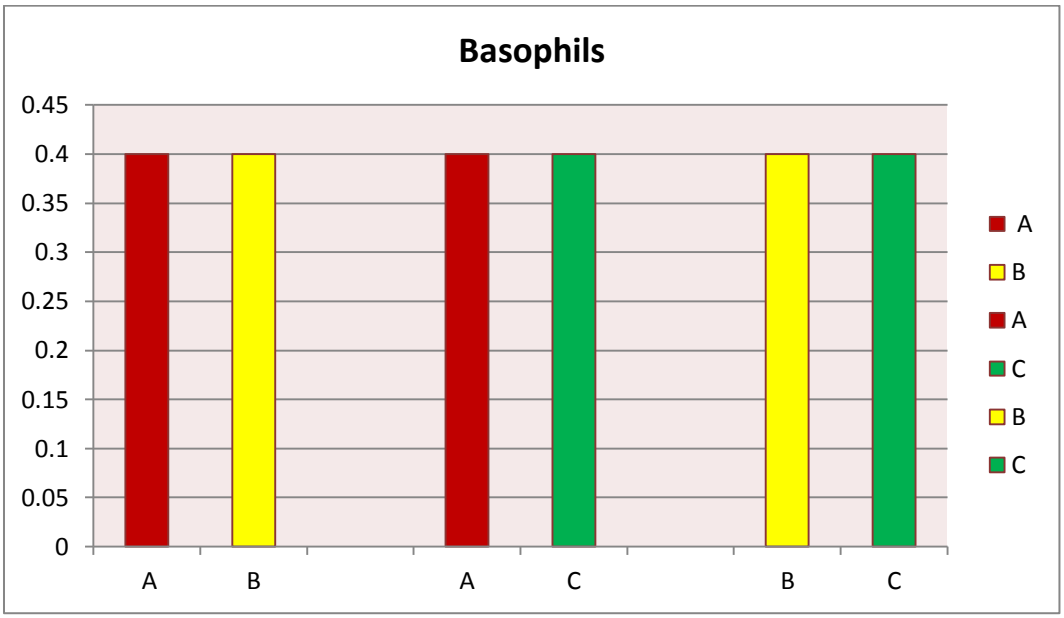

Figure 4.7 (f): Intergroup comparison of median basophil percentages

The basophils were the last of the leukocyte differential counts. In *Figure 4.7f* it is clear that there was no intergroup variation between the exposure groups for the basophil counts.

4.3.1.3.2 Summary of intergroup comparison for mean / median leukocyte parameters

The results presented in *Table 4.6* and *Figures 4.7 (a-f)* indicate no statistically significant intergroup variation for the total leukocyte, monocyte, eosinophil and basophil ($p>0.05$) counts. However, using the ANOVA test there was a statistically significant variation between the three groups for the lymphocyte ($p=0.0338$) and neutrophil ($p=0.0190$) counts.

The results for lymphocyte counts indicated a statistically significant median difference between groups A and B ($p=0.0423$) as well as groups A and C ($p=0.0151$), but none was found between groups B and C ($p>0.05$). For the neutrophils there was a statistically significant median difference between groups A and B ($p=0.0435$) as well as between groups A and C ($p=0.0072$). However, as with the lymphocytes, no significant difference was found between groups B and C ($p>0.05$). For both the parameters the most statistically significant median differences were found between group A and group C. From these results it is evident that longer exposure duration had an effect on the results.

4.3.1.3.3 Discussion of findings for leukocyte parameters

From the results as reported above it was evident that exposure to the ELF-EMF signal influenced various leukocyte counts. There were statistically significant differences in the lymphocyte and the neutrophil counts when comparing group A to the control group. The total leukocyte count for group A which was statistically significantly higher than that of the control group was most likely due to the increased lymphocyte count. The lymphocyte count was increased, whereas the neutrophil count was decreased. There was also an apparent variation between the groups where the inclination increased with the duration of exposure. The biggest difference was found in group A, which was the experimental group that received the maximum daily exposure (24-hour exposure). Hence, the longer the exposure, the more statistically significant the differences were. This confirmed the hypothesis that the duration of exposure plays an important role or has a significant effect on the immune cell counts. This effect due to duration of exposure was also reported by Goraca *et al.* (2010). These authors explained

that the oxidative stress parameters achieved in their study depended on the "working time" (i.e., duration of exposure) of the ELF-EMF.

The increased leukocyte count in the current study could possibly indicate an immune stimulation. In the human body, immune stimulation is characterized by an increased lymphocyte count since the lymphocytes become activated when stimulated by a "foreign agent or stimulus" (Coico & Sunshine, 2009:2). Markov *et al.,* (2006) found that ELF-EMFs do indeed interact with lymphocytes. Selmaoui *et al*., (2011) found an increased IL-6 in healthy young men exposed to a 9h intermittent field (10 μT). An increased leukocyte count can, therefore, possibly be due to increased cytokine production (Aldinucci & Pessina, 1998; Cuppen et al., 2006; Selmaoui *et al.*, 2011). This could possibly also explain the association with the increased lymphocyte concentration in this study. The reason for this is that activation of the innate immune system is accompanied by cytokine production (de Kleijn *et al.*, 2011). These cytokines play an important role in T-lymphocyte differentiation in the immune response. In *Table 2.1* it is indicated that IL-6 is responsible for activation of T-cells.

These findings are contradictory to the findings of Hashish *et al.* (2008). In their study they found a decrease in leukocyte and platelet numbers and an increase in granulocyte numbers. However, the exposure period was longer than in this study and the signal strength differed from the Immunent signal in the current study.

Markov (2006) found that ELF-EMFs affected homeostatically unstable cells. From this finding, one can possibly deduce that the immune system becomes homeostatically unstable when exposed to these EMFs. This will concur with the hypothesis of Cuppen *et al.* (2006) that ELF-EMFs can "put the immune system into a state of alert".

The decreased neutrophil count in the current study does not support this statement, but the findings by Elmusharaf *et al*. (2007) could provide a possible explanation for the decreased neutrophil count. In their study conducted on broiler chickens with intestinal coccidiosis lesions caused by the *Eimeria* species, it was found that there was a reduction in intestinal lesions after exposure to ELF-

EMFs (Elmusharaf *et al.*, 2007). This finding suggested an antagonistic effect on coccidiosis by the ELF-EMFs. The authors suggest that this could possibly be due to an increased blood flow, similar to an inflammatory reaction, as well as the relocalization or colonization of phagocytic cells to damaged tissues. This then resulted in an increased phagocyte infiltration to damaged tissues to aid in the response to the pathogen. The researcher is of the opinion that this suggestion by Elmusharaf *et al.,* (2007) could possibly explain the reduced neutrophil count in the current study. In other words, the neutrophil count was decreased in the peripheral blood due to relocation of these cells to the other tissues in the body when being stimulated by the applied ELF-EMF.

de Kleijn *et al.,* (2011) mention that inflammatory cytokines play a role in regulation of other immune cells such as neutrophils. Coico, Sunshine and Benjamini (2003:151) explain that neutrophil migration (also known as transendothelial migration) occurs under the influence of cytokines. This migration is illustrated in *Figure 4.8*. One of the cytokines involved in this process is IL-8.

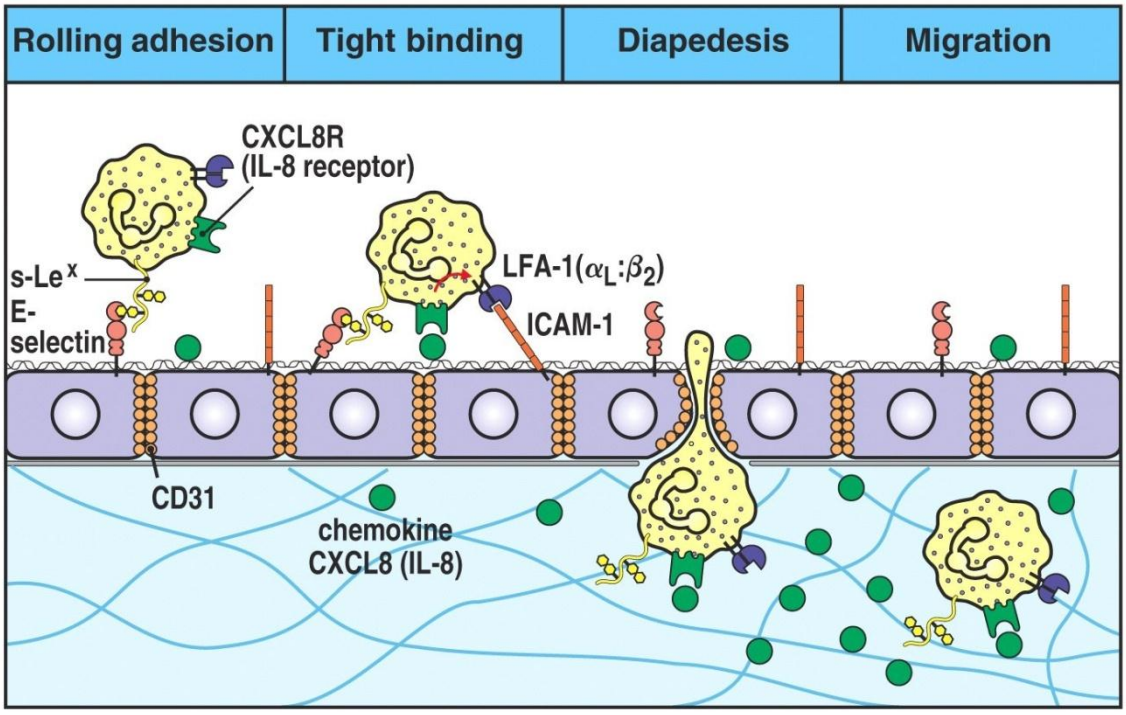

Figure 2-44 part 3 of 3 Immunobiology, 6/e. (© Garland Science 2005)

http://www.bio.davidson.edu/courses/immunology/students/spring2006/, accessed 18 September 2012

Figure 4.8: The process of transendothelial migration by neutrophils

The finding of increased phagocytic activity following short-term exposure to ELF-EMFs has been described by several authors (Rollwitz *et al.*, 2004; Simko & Mattson, 2004; Hashish *et al.*, 2008; Goraca *et al.,* 2010; de Kleijn *et al.*, 2011). Increased granulocyte activity is associated with increased superoxide anion (O_2/ free radical) production (Khadir *et al.*, 1999). Consider the findings of Varani *et al.,* (2002) as presented in section 2.2.5.2, who found that ELF-EMF exposure resulted in an increase of $A2_A$ adenosine receptor density. They mention that adenosine receptors interact on the neutrophil surface as an anti-inflammatory agent. Once again, we have another possible biological mechanism to consider in explaining the involvement of neutrophils in the immune response following exposure to ELF-EMFs.

A great deal of literature has been published indicating the effect that ELF-EMF exposure has on the release of biologically active substances such as stress proteins (Goodman & Blank, 1998; Blank & Goodman, 2000; Blank & Goodman, 2009) and the production of free radicals (Simko *et al.*, 2001; Rollwitz *et al.*, 2004; Simko & Mattsson, 2004; Hashish *et al.*, 2008; Goraca *et al.*, 2010; Cifra *et al.*, 2011; de Kleijn *et al.*, 2011; Mattsson & Simko, 2012). Reactive oxygen species (ROS) are produced by phagocytes once they have been stimulated. Hence, ROS production seems to be a key process in the stimulatory effect of ELF-EMFs. Once again, as indicated by de Bruyn and de Jager (1994), this process results in stress when exposed long term. The findings of Hashish *et al.* (2008) concur with this. In their study they found that mice exposed to a 50 Hz (1.4 mT) ELF-EMF for thirty days had a decreased total leukocyte, platelet, monocyte, peripheral lymphocyte, splenic lymphocyte, T-cell and B-cell counts. This finding is contradictory to the findings of the current study, but it must be kept in mind that in their study the exposure duration was longer and the field strength was stronger (1.4 mT).

Hence, short-term exposure to ELF-EMFs seems to be a key factor to achieving the effects of immune stimulation. The increased lymphocytes provide evidence of immune stimulation. In addition the theory of transendothelial migration also provides evidence of immune stimulation.

4.3.2 Immunophenotyping

The descriptive statistics and statistical significance for mean differences between the experimental groups and the control group for the immunophenotyping parameters are summarized in *Table 4.7*. The immunophenotyping parameters and their units of measurement included the following:

- CD3 (Total T-lymphocytes), expressed as a percentage of the total lymphocyte count.
- CD4 (Suppressor T-Lymphocytes), expressed as a percentage of the total lymphocyte count.
- CD8 (Cytotoxic T-lymphocytes), expressed as a percentage of the total lymphocyte count.
- CD19 (B-lymphocytes), expressed as a percentage of the total lymphocyte count.

Table 4.7: Descriptive statistics and p-values for mean immunophenotyping parameters

Parameter		A	B	C	K
CD3	Mean (%)	34.13	34.17	35.77	25.70
	SD	8.86	11.64	11.01	13.73
	p-value t-test	0.0218	0.0372	0.0137	
CD4	Mean (%)	26.52	25.72	26.87	19.20
	SD	7.01	8.65	8.81	11.11
	p-value t-test	0.0138	0.0400	0.0193	
CD8	Mean (%)	8.37	8.43	9.94	6.7
	SD	2.96	3.35	2.85	3.19
	p-value t-test	0.0762	0.0934	0.0015	
CD19	Mean (%)	23.08	23.22	17.32	17.88
	SD	12.10	12.03	12.78	13.77
	p-value t-test	0.1849	0.1885	0.8933	

(p<0.05 indicates statistically significant difference)

Figures 4.9 (a - d) present a graphic view and representation of the data in *Table 4.7*. Separate histograms were created for each specific parameter, even though the units of measurement were the same. This was to simplify visualization of the exposure group results compared to those of the control group.

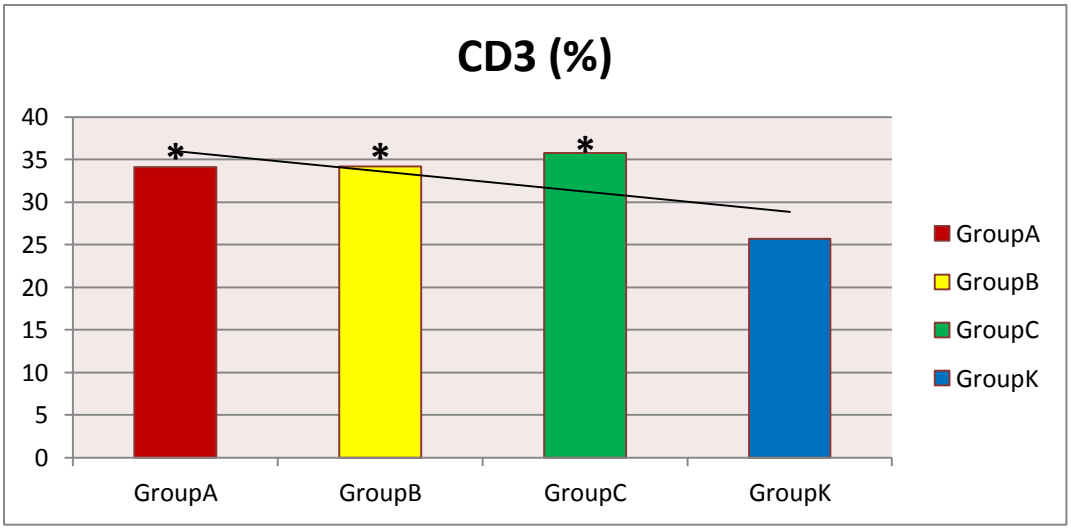

Figure 4.9 (a): Mean values for CD3 percentages of all the groups

** = Statistically significant difference ($p<0.05$)*

The CD3 percentages for all three exposure groups were statistically significantly higher than the percentage for the control group as can be seen in *Figure 4.9a*. There was a slight increase in values as the exposure duration decreased.

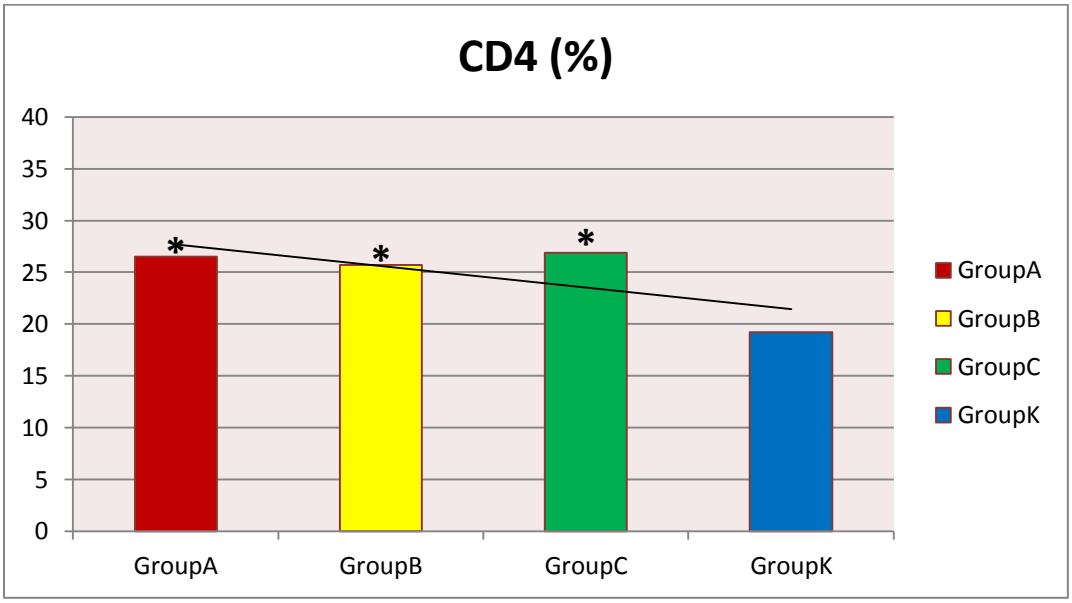

Figure 4.9 (b): Mean values for CD4 percentages of all the groups

** = Statistically significant difference ($p<0.05$)*

In *Figure 4.9b* it was found, once again, the CD4 percentages of all three the exposure groups were found to be statistically significantly higher than the the percentage for the control group. In this case there was no trend regarding the duration of exposure.

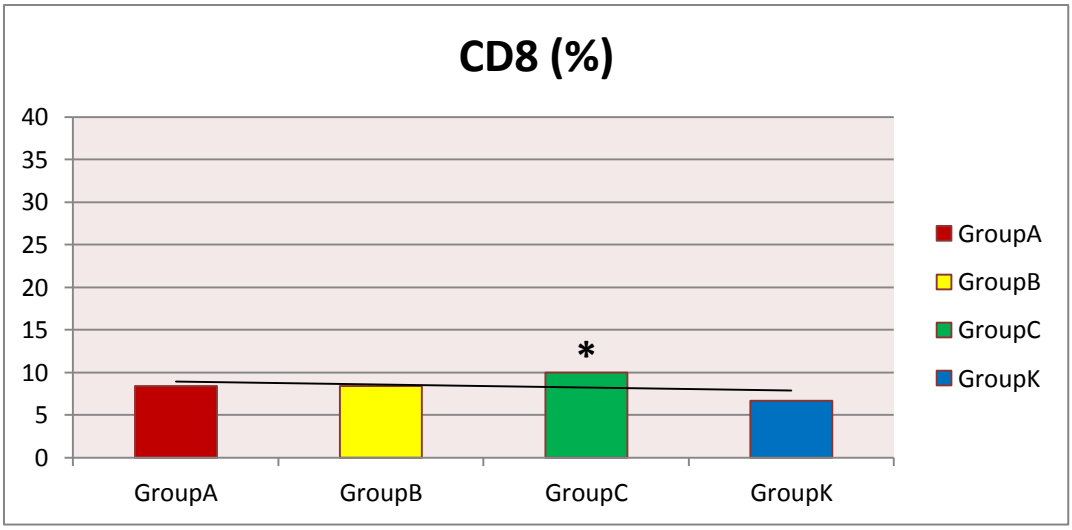

Figure 4.9 (c): Mean values for CD8 percentages of all the groups

* *= Statistically significant difference ($p<0.05$)*

For the CD8 analysis, *Figure 4.9c* shows that only group C was found to be statistically significantly higher than the control group. The differences for groups A and B were not statistically significant.

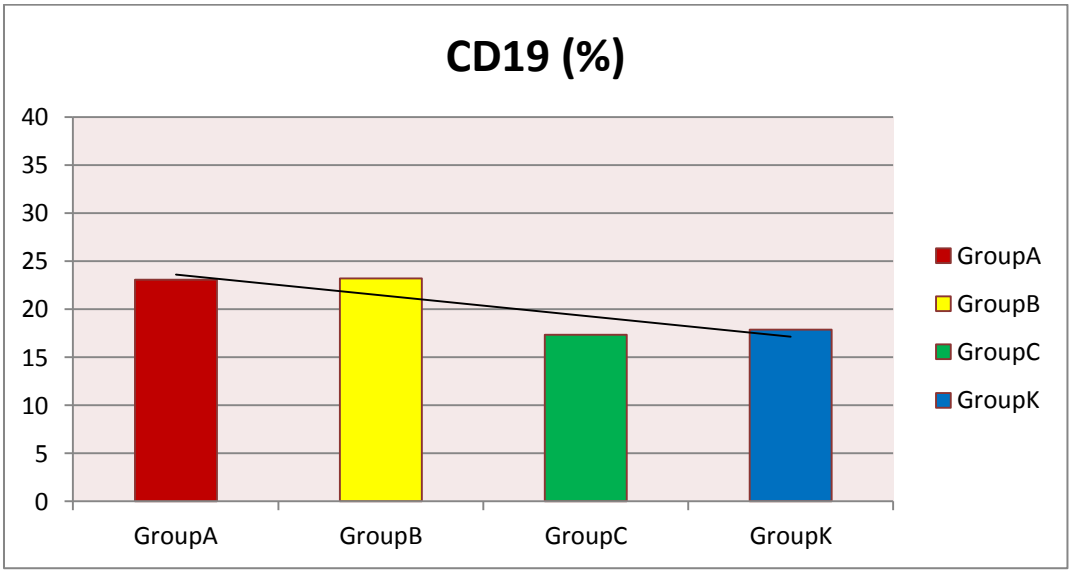

Figure 4.9 (d): Mean values for CD19 percentages of all the groups

Upon determination of the CD19 markers, there were no statistically significant differences between the exposure groups and the control group. *Figure 4.9d* shows that group A and group B seemed to be somewhat higher than the control, in spite of the lack of statistical significance.

Figure 4.10 represents a collective summary of histograms for all the CD markers. This summary includes the total T-lymphocyte (CD3), T-helper lymphocyte (CD4), cytotoxic T-lymphocyte (CD8) and the B-lymphocyte markers.

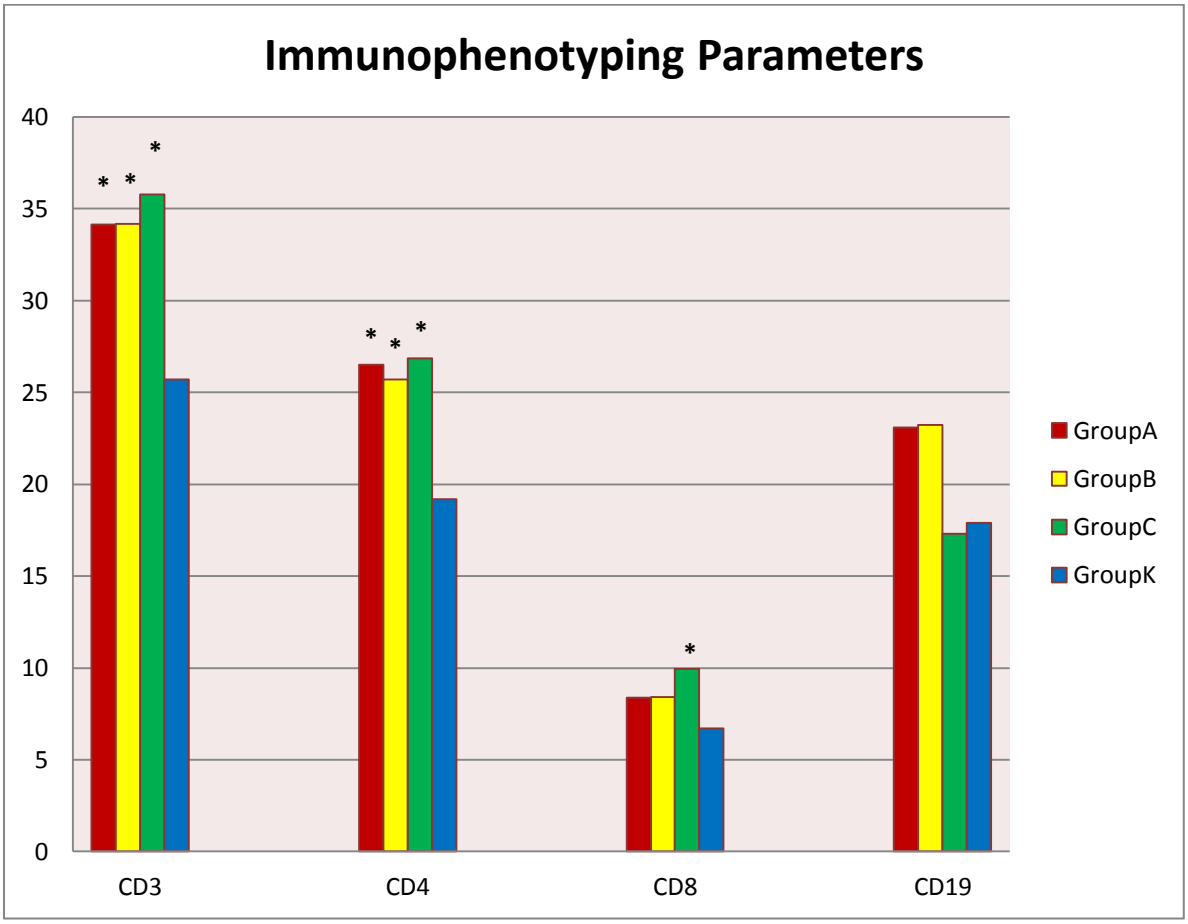

Figure 4.10: *Combined histogram of all immunophenotyping parameters showing a comparison between the exposure and the control groups*

* = *Statistically significant difference ($p<0.05$)*

4.3.2.1 Summary of comparative mean values for the immunophenotyping parameters

There was a statistically significant mean difference in the CD3 counts between groups A (p=0.0218), B (p=0.0372), C (0.0137) and the control group. In addition, a statistically significant mean difference in CD4 counts was found between groups A (p=0.0138), B (p=0.0400), C (p=0.0193) and the control group was

found. For the CD8 counts, only group C showed a statistically significant mean difference (p=0.0015) compared to that of the control group. No statistically significant mean differences were found between any of the exposure groups and the control group for CD19 counts.

Table 4.8, below, indicates the differences between all of the groups using the ANOVA test and between the individual groups (intergroup comparison).

Table 4.8: Intergroup comparison for immunophenotyping parameters

Parameter	A vs. B vs. C (ANOVA) p-value	A vs. B (t-test) p-value	A vs. C (t-test) p-value	B vs. C (t-test) p-value
CD3	0.8480	0.9892	0.5869	0.6545
CD4	0.8974	0.7330	0.8859	0.6767
CD8	0.1800	0.9438	0.0815	0.1295
CD19	0.2194	0.9683	0.1331	0.1360

(p<0.05 indicates statistically significant difference)

The graphic representations of the intergroup comparisons are presented in *Figures 4.11 (a - d)*.

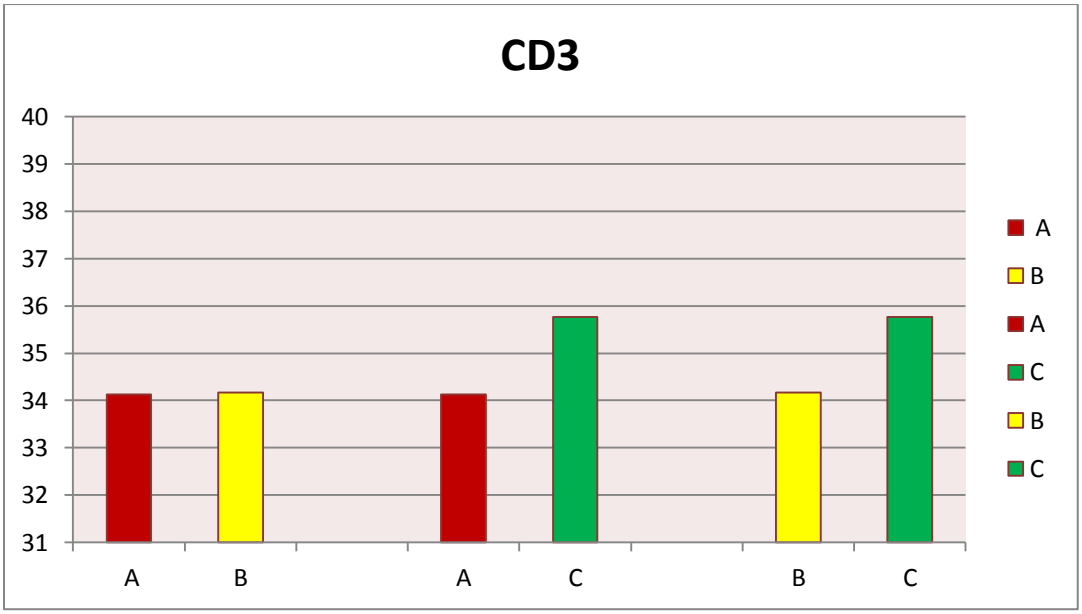

Figure 4.11 (a): Intergroup comparison of mean CD3 percentages

From the histogram in *Figure 4.11a* it is clear that there were no statistically significant differences for the intergroup comparisons. It did appear as though

the value for group C was higher than the values for groups A and B, but the differences were not statistically significant.

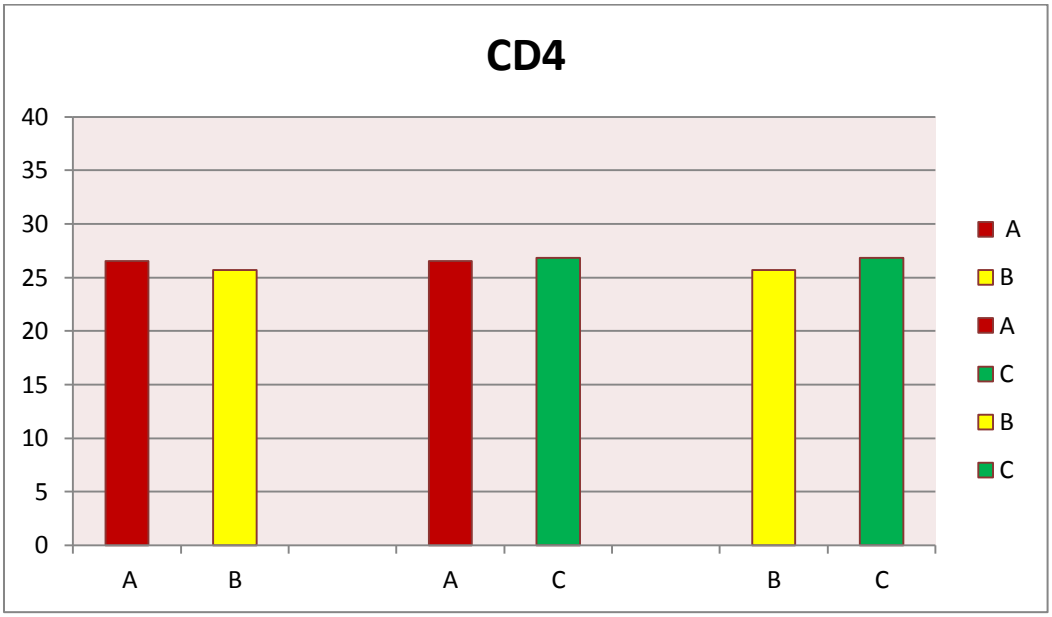

Figure 4.11 (b): Intergroup comparison of mean CD4 percentages

It is apparent in *Figure 4.11b* that there were no intergroup variations for the CD4 analysis. This was also evident due to the lack of statistical significance. There was a slight variation between groups A and B as well as between groups B and C.

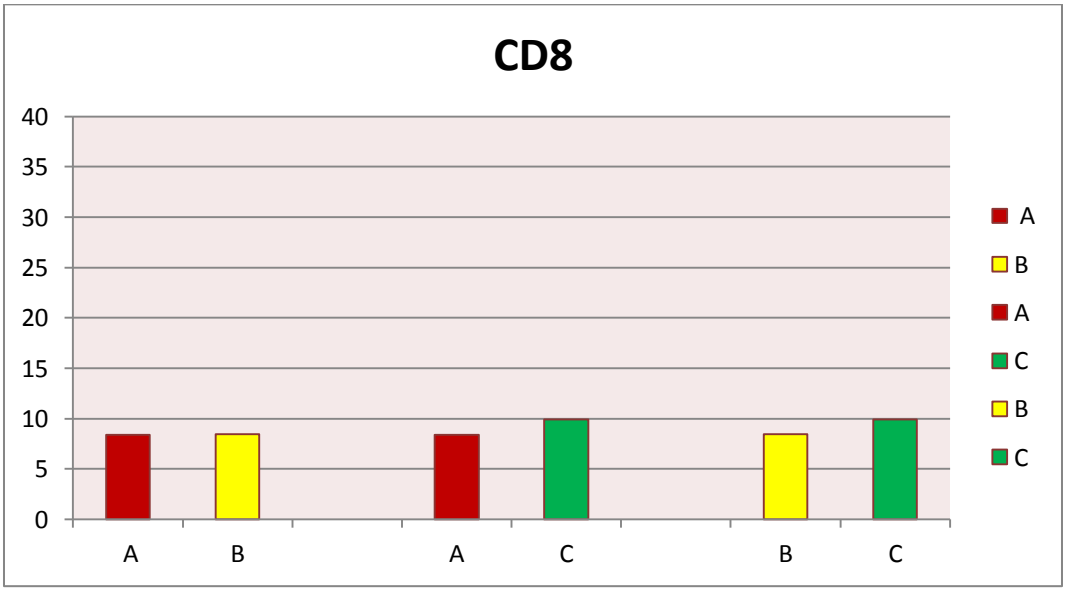

Figure 4.11 (c): Intergroup comparison of mean CD8 percentages

There was no evidence of intergroup variation for the CD8 analysis. As seen in *Figure 4.11c* the value for group C seemed to be slightly higher than the values for groups A and B, but the differences lacked statistical significance.

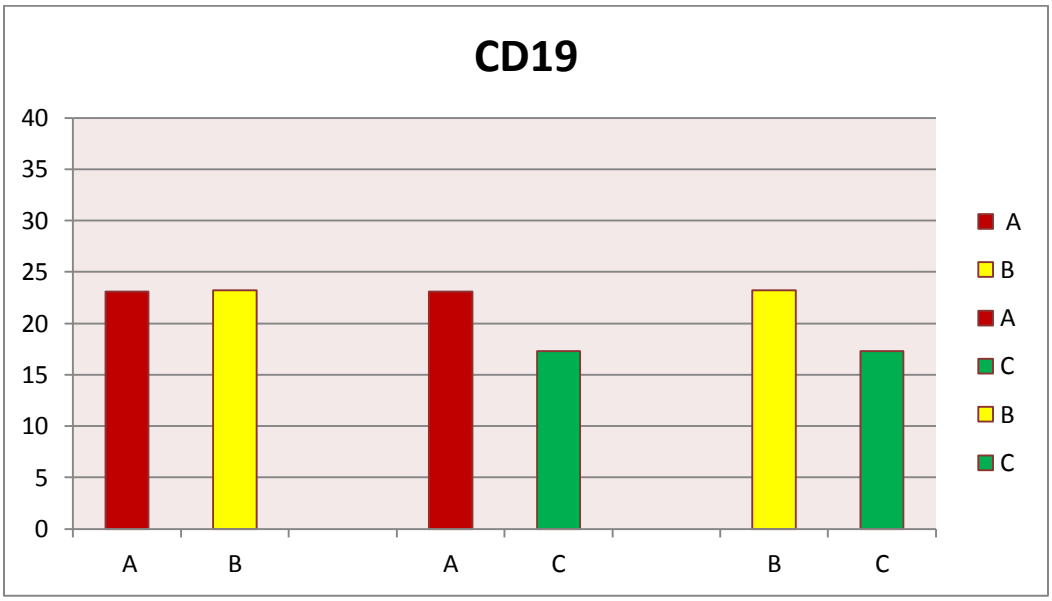

Figure 4.11 (d): Intergroup comparison of mean CD19 percentages

When looking at *Figure 4.11*d, it seemed as though there was a significant intergroup variation between groups A and C as well as between groups B and C for the CD 19 analysis. However, these variations were not statistically significant.

4.3.2.2 Summary of intergroup comparison for mean immunophenotyping parameters

There were no statistically significant differences ($p>0.05$) between any of the exposure groups themselves (24-hour, 4-hour and 1-hour exposure) for the immunophenotyping parameters. The ANOVA test (A vs. B vs. C) indicated no statistically significant differences ($p>0.05$) between the exposure groups for the immunophenotyping parameters. Thus, the duration of exposure did not seem to have any effect on the immunophenotyping results.

4.3.2.3 Discussion of findings for immunophenotyping parameters

Taking a critical view at the results obtained for the immunophenotyping, it was found that short-term exposure to the selected ELF-EMF resulted in increased CD3 and CD4 lymphocyte counts for all the exposure groups (24-hour, 4-hour and 1-hour exposure). The most significant increase in values was seen in group

C (one hour exposure). There was also a significant increase in CD8 counts for group C. There were no statistically significant differences for CD19 counts.

It merits mentioning that the increased total leukocyte counts for the leukocyte parameters were probably due to the increased lymphocyte counts. It can be concluded that T-lymphocytes (CD3) represented the specific group of lymphocytes that were increased.

Therefore, short-term exposure of the specific Immunent signal resulted in increased T-cell counts, but had no effect on B-cell counts. Since there were no statistically significant differences found in the intergroup comparison, it can be deduced that the duration of exposure did not affect the results of the immunophenotyping.

A possible explanation for the increased $CD3^+$ and $CD4^+$ lymphocyte counts for all the exposure groups (24-hour, 4-hour and 1-hour exposure) could be that the T-lymphocytes were signalled by the increased IL-6 levels. This confirms the findings of Selmaoui *et al.* (2011), who reported raised IL-6 levels in young men when exposed to 50 Hz EMFs. The increase, and possible activation, of $CD4^+$ cells can then result in activation and hence an increased number of $CD8^+$ cells (Coico, Sunshine and Benjamini, 2003:139).

In evaluating the findings, it seems important to keep the findings of Aldinucci & Pessina (1998) in mind, as they found an increased INFγ and IL-6 with 50 Hz exposure. It is known that IL-6 activates T-cells and IL-2 production (Coico, Sunshine and Benjamini, 2003:153). CD4 cells also produce IL-2 which, in turn, induces proliferation and differentiation of $CD8^+$ cells (Coico, Sunshine and Benjamini, 2003:139). This could possibly be the underlying biological process resulting in the increased CD4 counts and also the increased CD8 count in group C. These results agree well with the results of the leukocyte parameters reported in section 4.3.1.3. Hence, it was clear that the lymphocyte counts were limited to the T-lymphocytes.

4.4 Summary of discussion

4.4.1 Literature summary

Wertheimer and Leeper (1979) found the strongest evidence of adverse effects resulting from exposure to EMFs. They found that there was a substantial increased risk of childhood cancer in families living close to power lines. In this case their argument carries a great deal of weight, but the EMFs they investigated do not correspond with the fields investigated in this study.

Research in this field later indicated toward evidence of adverse health effects due to long-term exposure to ELF-EMFs (Ahlbom & Feychting, 2003; de Bruyn & de Jager, 1994; Mevissen *et al.*, 1998). Goraca *et al.* (2010) also found that the effect was dose-dependent. Johansson (2009) felt that the effect of chronic exposure to EMFs is underestimated. This author suggested increased safety limits for public exposure.

Gobba *et al.* (2009) reported that occupational exposure of subjects to higher field strength ELF-EMFs had lower NK cell counts than subjects exposed to the lower field strengths (less than 2 μT). Limited publications could be found that reported on leukocyte counts or counts of other immune cells to support these findings.

In spite of the concern for the possibility of adverse health effects, research has also indicated that exposure to ELF-EMFs can be beneficial to human health and that they have therefore been used therapeutically for various conditions (Vallbona & Richards, 1999; Markov *et al.*, 2006; Waite *et al.*, 2011). However, most of these findings lacked evidence of the biological mechanism.

One very common feature emanating from a large number of scientific investigations with regard to biological mechanism is the fact that ELF-EMFs increase ROS production (Simko *et al.*, 2001; Rollwitz *et al.*, 2004; Simko & Mattson, 2004; Hashish *et al.*, 2008; Goraca *et al.*, 2010; Mattsson & Simko, 2012). This provides strong evidence that the innate immune system is triggered.

Then there were also the findings of increased cytokine production which may indicate towards stimulation of the immune response (Aldinucci & Pessina, 1998; Selmaoui *et al*., 2011; de Kleijn *et.al.*, 2011). However, it is evident that any beneficial effects are limited to short-term exposure to ELF-EMFs. Several investigations have shown that there is an interaction between EMFs and immune cells, but there still seems to be some basic information lacking in this field (Markov *et al.*, 2006). There is a possibility that the link to this understanding perhaps lies in the specificity of the signal and the dosimetry.

Since it has been strongly suggested that exposure to ELF-EMFs results in increased ROS production, thereby eliciting an anti-inflammatory response, it is evident that this effect takes place at cellular level. Markov *et al.* (2006) concentrated on the effects of these fields at immune cell level. They hypothesized that beneficial health effects, specifically on the immune system, could be obtained by regulation of the inflammatory response. Based on their findings, they argued strongly that further research should concentrate on fields with specific physical characteristics.

A factor to consider in the therapeutic application of EMFs is that the physics and engineering of EMF signals are specific (Markov & Hazlewood, 2009). A specific signal might be beneficial for application in one biological area, but not in another. With regard to this specificity, the Immunent Activator signal seems most appropriate, since this signal is specific and unique.

Supportive research on the benefits obtained by exposure to the Immunent Activator signal may have significant financial implications. Benefits in the farming industry have been suggested (Cuppen *et al.,* 2007). A platform of intense investigation with a wide field of research opportunities has therefore been created. However, it has become apparent that this is an area of research which, to a great extent, needs supportive research. In this regard the Immunent Activator signal was tested by, among others, Waite *et al*., (2011), who suggested the possibility of therapeutic use due to its specific characteristics.

4.4.2 Summary of findings from the present study

The most significant statistical differences were found in the leukocyte parameters, and such differences were also evident in and confirmed by the immunophenotyping results. For the full blood count this significant difference was not observed in all of the exposure groups, but for CD3 and CD4 counts a statistically significant difference was evident in all of the exposure groups. For the intergroup analysis there was a statistically significant difference between some of the exposure groups. Most of the results indicated toward group A as the group with the most common and most statistically significant differences.

For the leukocyte parameters, the lymphocyte counts in group A were increased and the neutrophil counts were decreased. In spite of the lower neutrophil counts, the total leukocyte count in group A was still higher than the count of the control group.

The lower neutrophil counts could be associated with transendothelial migration. If this were to be true, the findings of many authors indicating increased ROS production will corroborate this biological process.

From the immunophenotyping results it is evident that short-term exposure to the Immunent Activator signal resulted in stimulation of specifically the T-lymphocytes. This could be deduced from the fact that a significant increase was observed in the CD3 (total T-lymphocyte population), CD4 (helper T-lymphocytes) and CD8 (cytotoxic T-lymphocytes). No statistically significant differences were found for the CD19 (B-lymphocyte population).

Cuppen *et al.* (2007) hypothesized that the immune system is put into a "state of alert" when exposed to ELF-EMFs. This is as a result of stress induction on the cells. There was evidence from this study that the T-lymphocytes and the neutrophils were activated. This was possibly due to stress induction on the cells by the EMF.

A prominent observation in the current study was the increased leukocyte count. This was most likely due to the increased lymphocyte count, which related specifically to T-lymphocytes (CD3).

From the current study, there is strong evidence of immune stimulation. This was evident from the total leukocyte count that was statistically significantly higher in the exposed groups compared to the unexposed group. Nihal *et al.* (2012) found similar results in their study.

Upon investigation of the specificity of the increased leukocytes it was apparent that this increased value was specific for the lymphocytes. This finding was largely limited to the 24-hour exposure group. It was also evident that the duration of exposure had an effect on the lymphocyte count. The significance indicated a tendency to increase as the duration of exposure increased. An increase in lymphocyte count signifies immune stimulation. A mechanism of action to be proposed is one of increased T-lymphocyte count due to stimulation by increased interleukin production.

The immunophenotyping results showed that the increased lymphocyte count was specific for T-lymphocytes, since there was a statistically significant increase in CD3 and CD4 cells for all the exposure groups, with the highest significance levels in the 24-hour and the 1-hour exposure groups. However, for CD8 cells this was only observed in the one hour exposure groups.

The decreased neutrophil count for the 24-hour exposure group was the parameter which was found most challenging to explain. In view of the hypothesis suggested by Elmusharaf *et al*. (2007), the low neutrophil count could possibly indicate toward relocalization or colonization of phagocytic cells to the tissues. If the finding of increased ROS production holds true, the neutrophil counts from this study would support the theory of Elmusharaf *et al*. (2007). The possible increased production of interleukins could account for increased ROS production and relocation of phagocytic cells.

Since research has indicated that long-term exposure to ELF-EMFs results in oxidative stress due to prolonged ROS production, the researcher is of the opinion that short-term exposure could alert the immune system and be used therapeutically as was hypothesized by Simko & Mattsson (2004). It is, therefore, possible that the Immunent Activator signal can be used to therapeutically

manipulate the immune response by serving as an antigenic signal. This conclusion is based on the evidence of increased T-lymphocytes.

EMF exposure tends to elicit an inflammatory response (Johansson, 2009; Mattson & Simko, 2012). If this inflammatory response is elicited for only a short period of time, it could very well put the immune system into a state of alert. The inflammatory response is beneficial if there is an appropriate feedback mechanism and the response is limited to a short period. If the inflammatory response continues for an extended period there is a possibility of tissue damage. This has been indicated by de Bruyn and de Jager (1994). This study strongly agrees with the suggestions made by Markov *et al.* (2006) that EMF therapy can be used for treatment of inflammatory diseases and pain, since T-lymphocytes are one of the key modulators of inflammation. Hence, these EMFs can, in effect, be used as regulators of the inflammatory process.

Selmaoui *et al*. (2011) found an increased IL-6 in healthy young men exposed to a nine hour intermittent field (10 μT). This could possibly also explain the association with the increased lymphocyte concentration in this study. The reason for this is that activation of the innate immune system is accompanied by cytokine production (de Kleijn *et al.*, 2011). These cytokines play an important role in T-lymphocyte differentiation in the immune response. Considering that IL-6 is secreted by T-cells and is also responsible for the activation of T-cells and IL-2 production, it would fit in with the findings of increased lymphocyte (specifically T-cells) counts in the current study and the findings by Selmaoui *et al.,* (2011) of increased IL-6. These findings are supported by those of Aldinucci & Pessina (1998), whose study revealed enhanced release of IL-6 and INFγ after exposure to 50 Hz ELF-EMF, which resulted in improved biological responses. INFγ is also secreted by T_H1 cells and is responsible for activation of NK cells and macrophages as well as the inhibition of T_H2 cells (Coico, Sunshine and Benjamini, 2003:154).

The cells which indicated the most obvious interaction with ELF-EMFs in the current study were the T-lymphocytes and neutrophils. These leukocytes are key regulators of the immune response. The researcher suggests that T-lymphocytes proliferate in response to ELF-EMF exposure in an attempt to launch an immune

response to a “stimulus”. In this case the immune response is alerted in response to a pathogen introduced to the body. Evidently this also has a beneficial effect on the healing process of the body. As far as the neutrophils were concerned, the researcher believes this finding coincides well with the finding by Elmusharaf *et al*. (2007), where the neutrophils migrated to the tissue in response to EMF exposure, hence resulting in decreased peripheral blood counts.

CHAPTER 5

CONCLUSIONS AND REFLECTIONS

5.1 Introduction

Despite constant concern regarding the effects that daily exposure to ELF-EMFs has on human health, there is limited evidence that these EMFs can adversely affect human health. These "*technofields"* as described by Havas (2000), surround us daily and in some instances, all day. One can argue that the human race has adapted to this exposure as technology has advanced throughout the centuries, but the argument would hold no weight in the absence of supportive scientific evidence. In spite of extensive investigations conducted by the National Institute of Environmental Health Sciences and the Department of Energy in this field, conclusive evidence that exposure to these fields poses a health risk remains absent or, at best, nebulous. Yet there is equally insufficient evidence to show that exposure to these fields could be considered safe or beneficial. This study did, however, indicate that short-term exposure to the Immunent Activator signal which creates ELF-EMFs can stimulate the immune response. These findings can be helpful in identifying a possible therapeutic application for ELF-EMFs.

5.2 Conclusions

The following are the most significant conclusions of the study:

- The comparative data revealed that there was a significant difference in lymphocyte and neutrophil counts between the exposure groups and the control group, hence indicating that short-term exposure to the Immunent Activator signal did in fact stimulate an immune response.
- Intergroup comparisons revealed that there was a statistically significant variation between the different groups for the lymphocyte and neutrophil counts, hence confirming that the duration of exposure had an effect on the cell counts.
- The immunophenotyping analysis for specific lymphocyte sub-populations revealed that only T-lymphocytes presented any significant differences. These included the CD3, CD4 and CD8 lymphocyte populations. No statistically significant difference was found for the B-lymphocytes (CD19).

5.3 Limitations of the study

Several challenges were faced during the course of the study which included the following:

- Since research has indicated a correlation between short-term exposure to ELF-EMFs and an increased ROS production in phagocytes such as neutrophils and macrophages, it would have been ideal to include the oxidative burst test which determines ROS production. This investigation was not included in the present study for the following reasons:
    - Failure to validate a method before initializing the study.
    - The volume of blood obtained from mice is limited and it would have been impossible to do all the biological analyses on the small amount of blood collected.
    - Financial constraints.
- In some instances the blood tended to clot during the bleeding procedure, therefore clotted samples could not be included in the biological analyses.
- Initially, it was intended to include CD56 (natural killer cells) counts in the immunophenotyping. This marker is costly and financial constraints made it impossible to include this marker for analysis.

5.4 Recommendation for future investigations

This study provided supporting evidence that confirmatory studies should be performed on the Immunent Activator signal. There was a strong indication that interleukins played one of the key roles in the findings from this study. Hence, the following future investigations are advised:

- Further studies on interleukin production are required to investigate the mechanism behind the increased T-lymphocyte counts. The specific interleukins for investigation should be IL-2, IL-6 and INFγ. Investigation on pro-inflammatory cytokines is also advised. These would include IL-8 and IL-16.
- Future investigations on IL-8 to investigate the suggestion of neutrophil migration should be conducted, since this cytokine stimulates the migration of phagocytes.
- A successive study to confirm the ROS production of phagocytes using the Immunent Activator signal is recommended.
- It is recommended that the investigation be repeated to compare results of healthy mice with disease-challenged mice. Hence, a follow-up study is advised where mice are challenged with a "disease" or "organism". Such a study would coincide with a recommendation by Markov *et al.* (2006) who suggested further studies on cells which are "out of equilibrium". To determine the effect of the Immunent Activator signal on challenged animals could corroborate the findings of this study and other studies related to this one.

5.5 Concluding comments

Numerous studies have been performed to investigate possible health effects of ELF-EMF exposure. It has become clear that long-term exposure to these fields has detrimental health effects, whereas short-term exposure could hold beneficial health effects. However, many of the studies were not clear on the biological mechanism of action and a few hypotheses have been offered suggesting an inflammatory response due to cytokine release. A review of findings reported in the literature repeatedly indicated towards the involvement of cytokines. When integrating the results from this study with those reported in the literature, there is a substantiation of cytokine involvement. Hence, the role of cytokines as a mediator for immune stimulation is strongly suggested.

The researcher is of the opinion that the critical issues in this study were dosimetry and the physical characteristics of the ELF-EMF signal. It is for these reasons that it is agreed that future investigations should be from a mechanistic point of view. Hence the Immunent Activator exposure system for further investigations is advised.

The findings of the study lead to the conclusion that short-term (one week) exposure to the Immunent Activator signal can stimulate the immune response. Hence, the effects and potential therapeutic application of this signal could be beneficial to human health.

REFERENCES

Andor Technology. *What is light?* [Online]. Available from: http://www.andor.com/library/light/ [Accessed 28 August 2012]

Ahlbom A and Feychting M. 2003. Electromagnetic Radiation. *British Medical Bulletin*, 68(1): pp 157-165

Aldinucci C and Pessina GP. 1998. Electromagnetic fields enhance the release of both interferon γ and interleukin-6 by peripheral blood mononuclear cells after phytohaemagglutinin stimulation. *Bioelectrochemistry and Bioenergetics*, 44 (2): pp 243-249

Blank M and Goodman R. 2000. Stimulation of the stress response by low-frequency electromagnetic fields: possibility of direct interaction with DNA. *Plasma Science*, 28(1): pp 168-172

Blank M and Goodman R. 2009. Electromagnetic fields stress living cells. *Pathophysiology*, 16(2): pp 71-78

Blank M and Soo L. 1992. The threshold for alternating current inhibition of the Na,K-ATPase. *Bioelectromagnetics*, 13(4): pp 329-333

Blank M and Soo L. 1996. The threshold for Na,K-ATPase stimulation by electromagnetic fields. *Bioelectrochemistry and Bioenergetics*, 40: pp 63-65

Blank M. 1995. Biological effects of environmental electromagnetic fields: molecular mechanisms. *Biosystems*, 35: pp 175-178

Bonhomme-Faivre L, Marion S, Forestier F, Santini R and Auclair H. 2003. Effects of Electromagnetic Fields on the Immune Systems of Occupationally

exposed Humans and Mice. *Archives of Environmental Health*, 58(11): pp 712-717

Boorman GA, Bernheim NJ, Galvin MJ, Newton SA, Parham FM, Portier CJ and Wolfe MS. 1999. *NIEHS Working Group Report on Health Effects from Exposure to Power-Line Frequency Electric and Magnetic Fields* [Online]. USA. Available from: http://www.niehs.nih.gov/health/materials/niehs [Accessed 17 September 2012]

Cakir DU, Yokus B, Akdag MZ, Sert C and Mete N. 2009. Alterations of haematological variations in rats exposed to extremely low frequency magnetic fields (50 Hz). *Archives of Medical Research*, 40(5): pp 352-356

Cifra M, Fields JZ and Farhadi A. 2011. Electromagnetic cellular interactions. *Progress in Biophysics and Molecular Biology*, 105: pp 223-246

Coico R, Sunshine G and Benjamini E. 2003. *Immunology Short Course*. 5th ed. New Jersey: John Whiley & Sons

Coico R and Sunshine G. 2009. *Immunology Short Course*. 6th ed. New Jersey: John Whiley & Sons

Cuppen JJM, Wiegertjies GF, Molenaar M, Geervliet J and Smink W. 2006. Immune Stimulation Effects in Fish and Fish Immune Cells Through Weak Low Frequency Electromagnetic Fields With Variable Field Strength. *In: 4th International workshop on Biological Effects of Electromagnetic Fields*, Crete 16-20 October 2006

Cuppen JJM, Wiegertjies GF, Lobee HWJ, Savelkoul HFJ, Elmusharaf MA, Beynen AC, Grooten HNA and Smink W. 2007. Immune stimulation in fish and chicken through weak low frequency electromagnetic fields. *Environmentalist 27(4)*: pp 577-583

Cuppen JJM, Parmentier HK and Savelkoul HFJ. 2008. Immune Stimulation in Fish and Farm Animals Through Weak Low Frequency Electromagnetic Fields: A. Hypothesis. *In: 30th Bioelectromagnetics Society Annual Meeting,* San Diego 8-12 June 2008. Curran Associates Inc.: pp 57-60

De Bruyn L and de Jager L. 1994. Electric Field Exposure and Evidence of Stress in Mice. *Environmental Research*, 65: pp 149-160

de Kleijn S, Bouwens M, Verburg-van Kemenade BML, Cuppen JJM, Ferwerda G, Hermans PWM. 2011. Extremely low frequency electromagnetic field exposure does not modulate toll-like receptor signaling in human peripheral blood mononuclear cells. *Cytokine*, 54: pp 43-50

Delves PJ, Martin SJ, Burton DR and Roitt IM. 2011. *Essential Immunology*. 12th ed. Chichester: Wiley-Blackwell

de Seze R, Bouthet C, Tuffet S, Deschaux P, Caristan A, Moreau JM and Veyret B. 1993. Effects of Time-Varying Uniform Magnetic Fields on Natural Killer Cell Activity and Antibody Response in Mice. *Bioelectromagnetics*, 14(5): pp 405-412

El-Bialy NS, El-Gebaly RH and Rageh MM. 2012. Studies on Haematological Parameters and DNA Structure in Newborn Rats Exposed to Extremeley Low Frequency Magnetic Fields. *Life Science Journal*, 9(1): pp 489-495

Elmusharaf MA, Cuppen JJ, Grooten HNA, Beynen AC. 2007. Antagonistic Effect of Electromagnetic Field Exposure on Coccidiosis Infection in Broiler Chickens. *Poultry Science*, 86: pp 2139-2143

Farag AS, Dawoud MM, Selim SZ, Cheng TC, Marcus AM and Penn D. 1998. Electromagnetic fields in the home. *Electric Power Systems Research*, 45: pp 73-89

Feychting M and Ahlbom A. 1993. Magnetic fields and cancer in children residing near Swedish high voltage power lines. *American Journal of Epidemiology*, 138: pp 467-481

Goats GC. 1989. Pulsed electromagnetic (short-wave) energy therapy. *British Journal of Sports Medicine*, 23: pp 213-216

Gobba F, Bargellini A, Scaringi M, Bravo G and Borella P. 2009. Extremely low frequency magnetic fields occupational exposure and natural killer activity in peripheral blood lymphocytes. *Science of the Total Environment*, 407: pp 1218-1223

Goodman R and Blank M. 1998. Magnetic field stress induces expression of hsp70. *Cell Stress and Chaperones*, 3(2): pp 79-88

Goraca A, Ciejka E and Piechota A. 2010. Effects of Extremely Low Frequency Magnetic Field on The Paramaters of Oxidative Stress in Heart. *Journal of Physiology and Pharmacology*, 61(3): pp 333-338

Hashish AH, El-Missiry MA, Abdelkader HI and Abou-Saleh RH. 2008. Assessment of biological changes of continuous whole body exposure to static magnetic field and extremely low frequency electromagnetic fields in mice. *Ecotoxicology and Environmental Safety*, 71: pp 895-902

Havas M. 2000. Biological effects of non-ionizing electromagnetic energy: A critical review of the reports by the US National Research Council and the US National Institute of Environmental Health Sciences as they relate to the broad realm of EMF bioeffects. *Environmental Reviews*, 8(3): pp 173-253

Havas M. 2004. Biological Effects of Low Frequency Electromagnetic Fields. *In*: Clements-Croome D, ed. *Electromagnetic Environments and health in Buildings*. London: Spon Press, pp 207-232

HORIBA ABX Pentra C60+ Haematology Analyzer [Online]. From: http://www.fishersci.com/ecomm/servlet/fsproductdetail [Accessed 30 May 2013]

Immune Therapy Research Laboratory. *The Immune System* [Online]. Available from: http://www.oramune.com/custom.aspx?id=6 [Accessed 17 September 2012]

Immunent Werfberg 12 – Veldhoven – The Netherlands [online]. *Immune stimulation in fish and farm animals through weak low frequency electromagnetic fields*. Available from: http://www.immunent.com [Accessed online 14 May 2013]

Johansson O. 2009. Disturbance of the immune system by electromagnetic fields - A potentially underlying cause for cellular damage and tissue repair reduction which could lead to disease and impairment. *Pathophysiology*, 16(3): pp 157-177

Keirs RW, Peebles ED, Sarjeant WJ, Gerard PD and Terner D. 2005. Assessment of the effects of electromagnetic field modification on egg-laying hens in commercial flocks as indicated by production measures. *American Journal of Veterinary Reasearch*, 66(8): pp 1425-1429

Khadir R, Morgan JL, Murray JJ. 1999. Effects of 60 Hz magnetic field exposure on polymorphonuclear leukocyte activation. *Biochemica et Biophysica Acta*, 1472(2): pp 359-367

Lafaille JJ and Mathis D. 2002. Immunological Yin-Yang. *Current Opinion in Immunology*, 14(6): pp 741-743

Markov M, Nindl G, Hazelwood C and Cuppen J. 2006. Interactions between Electromagnetic Fields and Immune System: Possible Mechanism for Pain Control. *Bioelectromagnetics*, 27(3): pp 213-225

Markov MS and Hazlewood CF. 2009. Electromagnetic field dosimetry for clinical application. *The Environmentalist*, 29(2): pp 161-168

Mattsson MO and Simkó. 2012. Is there a relation between extremely low frequency magnetic field exposure, inflammation and neurodegenerative diseases? A review of in vivo and in vitro experimental evidence. *Toxicology*, 301(1): pp 1-12

Mayer G and Nyland J. Cells involved in Immune Responses and Antigen Recognition [Online]. University of South Carolina: Available from: http://pathmicro.med.sc.edu/bowers/immune.htm [Accessed online 11/03/2013]

Mevissen M, Haussler M, Szamel M, Emmendorffer A, Thun-Battersby S and Loscher W. 1998. Complex Effects of Long-term 50 Hz Magnetic Field Exposure in Vivo on Immune Functions in Female Sprague-Dawley Rats Depend on Duration of Exposure. *Bioelectromagnetics*, 19(4): pp 259-270

National Institute of Environmental Health Sciences. Electric & Magnetic Fields [Online]. Available from: http://www.niehs.nih.gov/health/topics/agents/emf/ [Acessed 05 September 2012]

Repacholi MH and Greenebaum B. 1999. Interaction of Static and Extremely Low Frequency Electric and Magnetic Fields With Living Systems: Health Effects and Research Needs. *Bioelectromagnetics*, 20(3): 133-160

Rollwitz J, Lupke M and Simko M. 2004. Fifty-hertz Magnetic Fields Induce Free Radical Formation in Mouse Bone Marrow-derived Promonocytes and Macrophages. *Biochemica et Biophysica Acta*, 1674: 231-238

Sarookhani MR, Safari A, Zahedpanah M, Asiabanha Rezaei M and Zaroushani V. 2012 Effects of 950 MHz Mobile Phone Electromagnetic Fields on the Peripheral Blood Cells of Male Rabbits. *African Journal of Pharmacy and Pharmacology*, 6(5): 300-304

Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). 2009. *Health Effects of Exposure to EMF* [Online]. Available from

http://ec.europa.eu/health/scientific_committees/emerging/opinions/scenihr_opinions. [Acessed on 18 December 2011]

Selmaoui B, Lambrozo J, Sackett-Lundeen L, Haus E and Touitou Y. 2011. Acute Exposure to 50 Hz Magnetic Fields Increases Interleukin-6 in Young Healthy Men. *Journal of Clinical Immunology*, 31(6): pp 1105-1111

Simko M, Droste S, Kriehuber R and Weiss DG. 2001. Stimulation of phagocytosis and free radical production in murine macrophages by 50 Hz electromagnetic fields. *European Journal of Cell Biology*, 80(8): pp 562-566

Simkó M and Mattsson MO. 2004. Extremely Low Frequency Electromagnetic Fields as Effectors of Cellular Responses in Vitro: Possible Immune Cell Activation. *Journal of Cellular Biochemistry*, 93(1): 83-92

South African Veterinary Foundation [Online]. Animal Protection Act (Act 71 of 1962). Animal Care legislation Review. South Africa. Available from http://www.savf.org.za/WN [Accessed 18 September 2012]

Sorg O. 2004. Oxidative stress: a theoretical model or a biological reality? *Comptes Rendus Biologies,* 327(7): pp 649-662

Thannickal VJ and Fanburg BL. 2000. Reactive oxygen species in cell signaling. *American Journal of Physiology Lung Cellular and Molecular Physiology*, 279(6): pp L1005-L1028

The Electromagnetic spectrum [Online]. Available from: http://csep10.phys.utk.edu/astr162/lect/light/spectrum.html [accessed 28 August 2012]

University of Virginia. Department of Astronomy [Online]. Available from: http://www.astro.virginia.edu/~rsl4v/PSC/light.html [accessed 28 August 2012]

Vallbona C and Richard T. 1999. Evolution of magnetic therapy from alternative to traditional medicine. *Physical Medicine and Rehabilitation Clinics of North America*, 10(3): pp 729-754

Varani K, Gessi S, Merighi S, Iannotta V, Cattabriga E, Spisani S, Cadossi R and Borea PA. 2002. Effect of low frequency electromagnetic fields on A2A adenosine receptors in human neutrophils. *British Journal of Pharmacology*, 136(1): pp 57-66

Waite GN, Egot-Lemaire SJP and Balcavage WX. 2011. A Novel View of Biologically Active Electromagnetic Fields. The *Environmentalist*, 31(2): pp 107-113

Weir DM and Stewart J. 1993. *Immunology*. 7th edition. Edinburgh: Churchill Livingstone

Wertheimer N and Leeper E. 1979. Electrical Wiring Configurations and Childhood Cancer. *American Journal of Epidemiology*, 109(3): 273-284

http://www.bio.davidson.edu/courses/immunology/students/spring2006/, accessed 18 September 2012

UNIVERSITEIT VAN DIE VRYSTAAT
UNIVERSITY OF THE FREE STATE
YUNIVESITHI YA FREISTATA

Direkteur: Fakultêre Administrasie / Director: Faculty Administration
Fakulteit Gesondheidswetenskappe / Faculty of Health Sciences
Internal Post Box / Interne Posbus G40 E-mail address StraussHS@ufs.ac.za
Tele: / Tel: (051) 4444256

Me / Ms H Strauss 2011 10 31

MS MK WIESE
BIOMEDICAL TECHNOLOGY
SCHOOL OF HEALTH TECHNOLOGY
FACULTY OF HEALTH AND ENVIRONMENTAL SCIENCES
CENTRAL UNIVERSITY OF TECHNOLOGY, FREE STATE
BLOEMFONTEIN
9300

Dear Ms Wiese

ANIMAL EXPERIMENT NR 20/08
PROJECT TITLE: "IMMUNE STIMULATION IN MICE (MUS. MUSCULUS) WITH EXPOSURE TO EXTREMELY LOW FREQUENCY (ELF) ELECTROMAGNETIC FIELDS (EMF)"

You are hereby kindly informed that the Interfaculty Animal Ethics Committee approved the following amendments to the above study at the meeting held on 27 October 2011

a) ***Change specie name in project title to Mus. musculus***
b) ***Extension of approval to 2011***
c) ***Change number of animals to 520***

Kindly take note of the following:

1. ***Fully completed and signed applications have to be submitted electronically to StraussHS@ufs.ac.za and a hard copy has to be submitted too.***
2. ***A signed progress report with regard to the above study has to be submitted electronically to StraussHS@ufs.ac.za while a hard copy has to be submitted to Ms H Strauss, Room D115, Francois Retief building, Faculty of Health Sciences. A report has to be submitted when animals are physically involved and after completion of the study. Guidelines with regard to progress reports are available from the secretary and on the network i:\algemeen\navorsing-research\etiekkomittees.doc.***
3. ***Researchers that plan to make use of the Animal Experimentation Unit must request a quotation from the Head, Mr Seb Lamprecht***
4. ***Contract research: Fifty (50%) of the quoted amount is payable when you receive the letter of approval.***

Regards

.....
CHAIR:
INTERFACULTY ANIMAL ETHICS COMMITTEE

Group	Weight (g)	WBC (103/mm3)	RBC (106/mm3)	HGB (g/dl)	HCT (%)	MCV (µm3)	PLT (103/mm3)	Lymph (%)	Mono (%)	Neutr (%)	Eosin (%)	Baso (%)	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)
A1	26.1	4.3	9.2	14.6	43	47	854	91.3	1.7	6.9	0	0.1	33.1	26.4	10.6	17.2
A2	29	6.3	9.4	15.1	44.3	47	784	91.6	1.9	6	0.1	0.4	17.1	13.5	1.7	1.1
A3	29.9	7.2	9.3	15.2	44.6	48	16.4	93.1	0.8	5.6	0.1	0.4	27.1	21.3	3.6	6.5
A4	25	5.9	9.7	15.5	46.5	48	822	92,2	1.4	6.1	0	0.3	40.1	29.5	13.9	16.3
A5	31.2	8.7	9.6	14.8	45	47	932	93.4	1.7	4.6	0	0.3	55.5	44.8	13.9	30.1
A6	32.3	6	9.8	15.5	46.5	47	847	92	1.4	6.3	0	0.3	39.9	29.6	8.7	14.5
A7	29.7	7.6	9.9	15.9	46.9	48	861	94.4	1.1	3.8	0.1	0.6	33.3	25.7	9.4	35.7
A8	32.6	7.2	8.9	13.9	41.8	47	974	94.1	1.4	3.7	0.3	0.5	28.4	22.5	9.1	15
A9	37.3	6.9	9	14.1	43.7	48	829	90.5	2.8	6.1	0.1	0.5	36.7	29.5	10.2	9.6
A10	27	5.9	9.5	15.2	45.5	48	960	88.2	2.6	8.7	0.1	0.4	30.3	23.7	7.1	29.2
A11	30.1	7	9.2	14.6	43.8	48	776	88.3	2.6	8.7	0.1	0.3	32.3	25.1	9.9	10.4
A12	23.9	8.3	9	14.2	42.4	47	871	86.7	3.1	9.4	0.5	0.3	40.3	31.4	10.3	33.7
A13	24.9	6.8	9.6	15.5	45.7	48	785	84.4	3.2	11	1	0.4	27.8	20.2	5.8	17.3
A14	26.8	3.8	9.2	14.4	43.1	47	844	87.9	2.3	8.5	0.7	0.6	30	23.7	6	21
A15	28.2	6.6	9.6	15	45.5	48	781	86.4	3.6	9.2	0.3	0.5	33.5	26	8.2	39.9
A16	30.8	8.6	8.9	14.4	42.5	48	1023	94.6	1.6	2.7	0.2	0.9	43.2	34	9.6	43.8
A17	26.7	11.6	9.4	14.6	43.9	47	1004	79.4	13.6	6.5	0	0.5	42.3	32.3	9.6	30.9
A18	28.6	8.1	9.4	14.9	44.2	47	457	94.7	1.7	3	0.1	0.5	38	28.6	10.1	12.1
A19	29.9	6.6	9.4	15	44.3	47	944	86.8	4.1	8	0.5	0.6	4.8	3.3	1.3	2.7
A20	24.3	9.5	9.3	14.4	43.3	47	1006	87.8	4.1	7.4	0.1	0.6	26.6	21	6.7	31.6
A21	27.2	8.3	9.2	14.2	43.1	47	725	88.3	2.6	8.7	0.1	0.3	29.8	22.4	7.3	21.1
A22	27.3	7.7	9.3	14.4	44.2	48	888	87.9	3.7	7.9	0.1	0.4	34.3	26.6	8.5	37.1
A23	25.3	7	9.2	14.4	43.4	47	879	91.8	1.8	6	0.1	0.3	44.9	35.3	10.1	36.4
A24	28.3	9.6	8.9	14.3	43	48	854	90.2	3.6	5.5	0.1	0.6	40.2	31.9	7.6	33.9
A25	26.6	5.9	9	14.9	42.8	48	853	83.8	3.6	12	0.2	0.4	14.3	11.5	2.9	9.4
Average	**26.35**	**5.1**	**9.1**	**14.75**	**42.9**	**47.5**	**853.5**	**87.55**	**2.65**	**9.45**	**0.1**	**0.25**	**23.7**	**18.95**	**6.75**	**13.3**

Group	Weight (g)	WBC (103/mm3	RBC (106/mm3	HGB (g/dl)	HCT (%)	MCV (µm3)	PLT (103/mm3)	Lymph (%)	Mono (%)	Neutr (%)	Eosin (%)	Baso (%)	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)
B1	26.7	9.1	10.2	16.1	48.3	47	773	92.3	2.3	5.1	0	0.3	25.2	19.8	8.5	12.8
B2	26.4	9.4	9.5	15	45.1	48	915	91.2	1.3	7.1	0	0.4	49.2	35.4	10.8	30.6
B3	25.9	6.9	9.5	15.1	45.2	48	895	91.3	1.4	6.9	0	0.4	43.6	33.7	12.5	15.5
B4	26.8	6.9	8.8	14.6	41.8	48	954	82.5	3.2	12.7	1.1	0.5	9.2	6.8	2.9	0.4
B5	27.1	5	9.9	15.7	47	48	808	88.3	2.6	8.7	0.1	0.3	16.4	11.7	3	6.5
B6	25.1	7.3	8.5	14	40.3	47	442	87.6	3.6	8.2	0.1	0.5	5.4	2.7	2.8	0.7
B7	27.9	6.4	8.9	14.5	42	47	1020	85.4	2.2	11.4	0.7	0.3	28.8	20	7.9	32
B8	25.6	6.5	9.8	15.4	46.6	48	922	87.1	2	10.1	0.3	0.5	42.1	31	10.4	30.4
B9	26.8	4.8	9.4	14.6	44.4	47	661	85.6	3.5	10.1	0.3	0.5	38	28	8.9	9.5
B10	26.2	5.3	8.8	13.9	41.4	47	827	85.1	3.5	10.2	0.7	0.5	42.2	31.9	13.4	15.5
B11	25.8	7.8	9.3	14.8	44.1	47	991	86.3	2.9	10	0.4	0.4	23.4	19	5.4	15.3
B12	28.1	8.2	10	15.5	47.1	47	539	85.7	4.9	8.9	0	0.5	59.6	43.9	15	9.4
B13	23.3	6.3	9.1	14.5	43.9	48	1012	87.3	4.4	7.5	0.3	0.5	36	28.4	9.5	26.7
B14	27.1	7.4	9	14.5	43.2	48	945	86.6	3.9	8.6	0.4	0.5	34.4	25.3	9.2	28.7
B15	28.6	5.3	9.4	14.9	44.4	47	961	84.7	15	0	0	0.3	38	27.9	10.5	30.7
B16	25.3	9	9.9	15.5	46.8	47	902	88.8	3	7.7	0.2	0.3	37.3	29.9	7.7	32.1
B17	24.7	8.9	8.5	14.1	40.2	47	1119	83.2	4.3	11.5	0.6	0.4	14.2	10.6	2.4	13.2
B18	26.9	9.5	9.4	15	44.6	47	930	91.4	1.7	6.3	0.1	0.5	42	32.9	8.9	44.2
B19	27.1	7.3	9.9	15.6	46.6	47	428	87.9	2.9	8.7	0.1	0.4	14.8	11.6	2.7	3.2
B20	23.1	7.5	9.4	15.2	45.5	48	783	90.9	2.3	6.4	0.1	0.3	34.5	26.4	8.8	29.4
B21	24.4	7.6	9.5	14.8	44.5	47	845	92.7	1.4	5.5	0.1	0.3	39.2	29.7	8.6	36.5
B22	24.6	7.1	8.9	14.2	41.7	47	817	86	3.8	9.5	0.3	0.4	5.9	4.3	1.3	1.8
B23	27.7	6	8.8	14.1	41.5	47	872	88.6	3.5	7.3	0.2	0.4	21.2	15.7	4.6	21.5
B24	29.2	4.6	8.1	12.8	37.5	46	688	84.1	4.8	10.2	0.3	0.6	3.4	2.4	1	1.9
B25	25.7	6.3	9.1	14.6	43.2	48	879	91.5	2.4	5.3	0.3	0.5	37.4	27.3	8.4	43.9
Average	**26.2**	**7.7**	**9.65**	**15.35**	**45.75**	**47.5**	**826**	**91.9**	**2.35**	**5.2**	**0.15**	**0.4**	**31.3**	**23.55**	**8.45**	**28.35**

Group	Weight (g)	WBC (103/mm3	RBC (106/mm3	HGB (g/dl)	HCT (%)	MCV (µm3)	PLT (103/mm3)	Lymph (%)	Mono (%)	Neutr (%)	Eosin (%)	Baso (%)	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)
C1	31.2	6.9	9.7	15.6	46.7	48	659	89.8	2.4	7.4	0.1	0.3	15.2	11.2	7	1.8
C2	28	8.1	9.9	15.6	47.2	48	685	87.8	3.8	8	0	0.4	26.8	15.7	5	3.8
C3	28.7	5.7	8.8	14.4	41.9	48	924	87.1	2.9	9.6	0.1	0.3	6.8	4.9	1.9	3
C4	29	6.9	9.5	15.1	45.4	48	884	91.2	1.7	6.8	0	0.3	50.6	39.4	15.7	3.9
C5	30.4	7.1	9.6	14.8	45	47	775	89.2	3.3	7.2	0	0.3	7.9	5.4	4.1	4.9
C6	24.3	7.9	9.5	14.8	44.8	47	812	93.2	1.5	5	0	0.3	40.7	31.8	11.7	30.4
C7	30	6.6	8.9	13.8	41.8	47	1032	87.3	3.6	8.7	0.1	0.3	36.2	28.1	8.9	16.2
C8	26	5.8	8.6	13.6	41.2	48	990	89.1	2.4	7.7	0.4	0.4	39.9	31.2	11.2	20.4
C9	29.3	6.3	9.6	15.4	46.2	48	694	85.1	2.5	11.7	0.3	0.4	21.3	15.5	8.6	4.5
C10	27.4	4.1	9.5	14.9	44.9	47	947	85.1	2.2	11.9	0.5	0.3	45.7	33.1	13.2	8.7
C11	28	4.5	9.1	14.7	43.4	48	818	86.1	1.4	11.9	0.3	0.3	47.7	34.7	14	22.4
C12	25.5	5.7	9	14.2	42.9	47	1063	89.7	1.8	8	0.2	0.3	45.7	34.6	12.1	26.3
C13	24.6	5.7	9.6	15.5	45.8	48	890	80.1	4.1	14.5	0.8	0.5	35.8	27.7	9.7	7.9
C14	24.6	6.3	8.4	13.4	39.6	47	1076	89.2	3.7	6.1	0.5	0.5	38	29.6	10.9	30.7
C15	27.2	5.1	7.7	12.4	37.9	49	1049	85.5	7	6.8	0.2	0.5	40.1	30.6	10.5	4.6
C16	28.1	7	9.1	14.3	41.7	46	1029	81.6	4.9	12.3	0.8	0.4	3.7	2.9	1	4.4
C17	28.1	6.4	9.6	15.5	46.3	48	828	88.3	2.1	9.1	0.2	0.3	50.2	39.2	12.1	23.1
C18	21	7.1	8.7	14	41.4	48	654	85.7	4.9	9	0.1	0.3	7	5.3	2.3	2.3
C19	24.8	9.3	9.4	14.8	45.1	48	1085	92.7	1.6	5.2	0.1	0.4	37.9	29.6	9.1	45.5
C20	23.7	6.9	9.5	15.2	45.1	47	702	86.2	3.1	9.8	0.4	0.5	12.1	8.5	5.8	7.6
C21	23.1	10.9	10.1	15.7	48.1	48	500	86.2	4.6	8.1	0.6	0.5	35.3	26.5	8.6	11.4
C22	26.7	5.8	9	14.5	42.6	47	910	85	3.9	10.2	0.4	0.5	7.7	6.3	2.1	4.7
C23	24.7	7.6	9.3	15	44	47	1254	87.7	4.3	7.2	0.4	0.4	27	19.6	6.4	39.5
C24	26.6	6.8	9.3	14.8	44.4	48	1007	86.1	4.4	9.1	0.1	0.3	27	21	7	25.5
C25	31.2	6.1	8.9	14.5	43	48	811	85.2	2.5	11.5	0.4	0.4	42.1	29.7	11.3	12.2
Average	**31.2**	**6.5**	**9.3**	**15.05**	**44.85**	**48**	**735**	**87.5**	**2.45**	**9.45**	**0.25**	**0.35**	**28.65**	**20.45**	**9.15**	**7**

Group	Weight (g)	WBC (103/mm3	RBC (106/mm3	HGB (g/dl)	HCT (%)	MCV (µm3)	PLT (103/mm3)	Lymph (%)	Mono (%)	Eosin (%)	Baso (%)	Neutr (%)	CD3 (%)	CD4 (%)	CD8 (%)	CD19 (%)
K1	26.3	6.1	9.5	15.2	45.4	48	775	89.1	3.2	0	0.3	7.4	7.4	5.3	2.7	2.2
K2	27.9	3.2	9.2	14.6	43.3	47	858	84.1	5.2	0.2	0.6	9.9	7.4	5.1	2.6	2
K3	27.3	5.3	10.3	16.2	48	47	659	86.4	3.5	0.2	0.4	9.5	17.7	12.5	4.8	3.8
K4	26.2	6.1	9.2	14.7	43.7	47	809	92.6	1.2	0	0.3	5.9	42.3	33.9	9.4	29.6
K5	26.2	5.6	10.1	15.9	47.6	47	831	84.1	5	0.1	0.2	10.6	10.5	8.3	2.3	6.5
K6	28	7.1	9.3	14.7	44.1	47	818	90.5	1.6	0	0.3	7.6	41.3	31.6	7	26.1
K7	28.7	8	8.8	14	41.3	47	347	88.2	3.2	0.1	0.3	8.2	13.4	8.4	5.3	5.1
K8	24.2	7.2	9	14.8	42.6	47	926	92.6	0.9	0.2	0.4	5.9	13	8.5	4.6	13.2
K9	25	5.2	9.1	14.6	42.8	47	587	88.9	1.1	0.5	0.5	9	16.9	11.6	4.7	4.9
K10	24.4	6.1	8.8	14.2	41.7	47	642	82.9	5	0.4	0.5	11.2	13.1	9.3	3.8	4
K11	28	6.4	9	14.1	42.8	48	867	86.1	5.2	0.2	0.6	7.9	32.5	25.6	9.5	14.1
K12	25	No blood	No blood	No blood	No blood	No blood	No blood	No blood	No blood	No blood	No blood	No blood	1.8	1.5	1	1.4
K13	26.4	4.4	8.4	13.6	39.9	47	747	87.7	3.6	0.4	0.6	7.7	11.6	7.3	4.4	4.9
K14	24.4	7.4	9.2	14.7	43.4	47	792	84.9	3.7	1	0.6	9.8	16.1	10.5	8.5	24.7
K15	31.2	4.9	8.9	14	41.2	46	405	84.4	5.5	0.1	0.3	9.7	14.3	9.4	5.4	4.3
K16	28.5	7.9	9.5	14.7	44.9	47	1066	88	2.5	0.1	0.4	9	39.8	30.6	8.6	40
K17	26.3	7.8	8.6	14.4	41.1	48	1031	82	4.8	0.8	0.5	11.9	7.9	5.7	2	5.9
K18	26.2	9	9.8	15.3	46.5	47	1161	89.1	2.8	0.1	0.4	7.6	41.8	31.7	9.9	41.4
K19	25.2	5.1	9.1	14.7	43.7	48	825	84.8	3.3	0.2	0.4	11.3	26.2	19.6	8.3	8
K20	28.2	5.8	9.6	15.2	45.7	48	1025	89.3	1.9	0.2	0.5	8.1	56.2	42.6	14.4	29.9
K21	25.7	7.8	9.7	15.2	45.8	47	807	88.1	2.5	0.1	0.4	8.9	31.8	22.4	8.8	19.3
K22	25.1	5.5	8.4	13.9	39.3	47	1205	82.8	5.8	0.9	0.7	9.8	11.4	8.6	1.3	5.4
K23	24	5.1	9.1	14.8	43.7	48	1185	83.4	3.1	0.3	0.3	12.9	19.3	14.9	3.1	15.6
K24	28.6	7.1	9.8	15.8	46.9	48	1093	89	2.5	0.1	0.3	8.1	41.9	33.8	10.4	44.1
K25	25.3	7.7	9.2	14.6	43.9	48	1026	86.5	3.3	0.6	0.3	9.3	28.6	22	6.2	30.6
Average	**25.8**	**6.9**	**9.35**	**14.9**	**44.65**	**48**	**900.5**	**87.8**	**3.25**	**0.3**	**0.3**	**8.35**	**18**	**13.65**	**4.45**	**16.4**