Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

EFFECTS OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS ON HUMAN CHROMOSOMES.

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

Doctor of Philosophy

in

Genetics

at the

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

by

MOHAMMED ABDUL WAHAB

2005

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

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

In the field of EMF research, perhaps the most outstanding question that remains to be answered with certainty is how weak EMFs exert their effects at the molecular level. Various mechanisms are reviewed and evaluated in this thesis. From the results of the research performed in the current study which concentrated on testing and discovering genetic effects, a model is postulated that weak EMFs stimulate the production of free radicals which result in genetic damage. Further extensive research should be conducted to test this hypothesis. I wish to extend my sincere gratitude to my supervisors, Dr. R.E. Rowland, and Dr. J.V. Podd, without whom I would not have had the wonderful opportunity to tackle this piece of work. Their constant invaluable guidance, advice, encouragement and supervision kept me on track and from both of them I have learnt a lot.

I would like to express my sincere thanks to:

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

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

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

DEDICATED

TO

MY BELOVED WIFE

KAMRUNNAHAR BEGUM (Hira)

IN APPRECIATION OF HER CONTINUOUS HELP, SUPPORT AND LOVE.

Table of Contents.

Opening page	Ι
Title page	II
Abstract	III
Acknowledgments	IV
Dedication	V
Table of Contents	VI
List of Tables	XI
Lists of Figures	XII

Chapter One: Introduction.

1. INTRODUCTION	1
1.1 Sister Chromatid Exchange (SCE)	4
1.2 Micronucleus Assay (MN Assay)	5
1.3 The COMET Assay	5
1.4 Fluorescent In Situ Hybridization (FISH)	6
1.5 Aim, Objective and Prediction	7

Chapter Two: Review of Literature.

2. REVIEW OF LITERATURE	8
2.1 ELF EMFs and Living Organisms	8
2.1.1 Human Exposure to ELF EMFs	9
2.1.2 Electromagnetic Field Mechanism(s)	10
2.1.3 EMFs and Cancer	17
2.1.4 EMFs and Transcription	22
2.1.5 EMF and Enhancement of the Intracellular Ca ²⁺ Response	25

2.1.6	EMF and Plasma Membrane	26
2.1.7	Melatonin and ELF EMFs	27
2.1.8	Geomagnetic Field (GMF)	34
2.1.9	DNA Damage, Chromosomal Aberration and ELF EMFs	42
2.2 Sis	ter Chromatid Exchange (SCE)	46
2.2.1	Introduction	46
2.2.2	Genetic Basis of SCE	51
2.2.3	SCE Models	55
2.2.4	SCE Induction	77
2.2.5	SCE and Disease	80
2.2.6	Human Reproductive Hormones and SCE	86
2.2.7	Lymphocyte Concentration and SCE	87
2.2.8	Baseline SCE and the Influence of Genetic, Chemical,	
	and Environmental Agents	87
2.3 Mi	cronucleus Assay (MN Assay)	99
2.3.1	Introduction	99
2.3.2	Radiation Dose and Micronuclei	100
2.3.3	Radiosensitivity and Micronuclei	102
2.3.4	Genetic Studies Using the MN Assay	103
2.3.5	Micronucleus Frequency as a Biomarker of Cancer Risk	106
2.3.6	Effects of Age and Gender on Micronucleus Frequency	108
2.3.7	MN Assay and the Influence of Chemical and	
	Environmental Agents	112
2.3.8	Concentration of Cytochalasin B	129
2.4 Th	e COMET Assay	130
2.4.1	Introduction	130
2.4.2	Mechanism of Comet Tail Formation	132
2.4.3	The COMET Assay in Genotoxicity Testing	133
2.4.4	EMF Exposure and COMET Assay	135
2.4.5	Evaluation and Interpretation of Results	135
2.4.6	DNA Damage and Use of COMET Assay	136
2.5 Flu	orescent In Situ Hybridization (FISH)	138
2.5.1	Introduction	138
2.5.2	Advantage, and Limitations of FISH Analysis	139

Chapter Three: Materials and Methods.

3. MA	rerials and Methods	144
3.1 Sist	ter Chromatid Exchange (SCE)	144
3.1.1	Collection of blood samples	144
3.1.2	SCE protocol	145
3.2 Mi	cronuclei Assay (MN)	156
3.2.1	Collection of blood samples	156
3.2.2	MN assay protocol	156
3.3 CO	MET Assay	161
3.3.1	Materials	161
3.3.2	Generation of EMFs and Field Exposure	166
3.3.3	Collection of Blood Samples	166
3.3.4	Lymphocyte Cultures	166
3.3.5	Lymphocyte Preparation	166
3.3.6	Lymphocyte Concentration and Cell Viability	167
3.3.7	Pre-treatment Method	169
3.3.8	The COMET Assay Methodology	171
3.3.9	Slide Analysis (Quantitative)	173
3.4 Flu	orescent in situ Hybridization (FISH)	176
3.4.1	Collection of Blood Samples	177
3.4.2	FISH Protocol	177

Chapter Four: Results.

4. Results	181
4.1 Sister Chromatid Exchange (SCE)	181
4.1.1 Analysis of Round-1 (SCE)	182
4.1.2 Analysis of Round-2 (SCE)	189
4.1.3 Complex MF	193

140

4.2 Micronucleus Assay (MN Assay)	
4.2.1 Complex MF	205
4.3 COMET Assay	205
4.4 Fluorescent in situ Hybridization (FISH)	209
4.5 Chromosomal Anomalies	209

Chapter Five: Discussion.

5. DISCUSSION	211
5.1 SCE Analysis	211
5.2 MN Assay	224
5.3 COMET Assay	230
5.4 FISH	233
5.5 FUTURE RESEARCH	234

Chapter Six: Summary and Limitations.

6. SUMMARY AN	ID LIMITATIONS	237
6.1 Summary		237
6.2 Limitations	of Present Study	239
References.		242
Appendix One (a)	: A simplified comparison between electrical pressure	
	and water pressure.	296
Appendix One (b): A simplified comparison between electrical fields		
	and magnetic fields.	297
Appendix One (c)	: Magnetic field decreases with increasing distance	
	from the source and measured in Tesla (T).	298
Appendix Two	: Personal Questionnaire	299
Appendix Three	: Consent Form Appendix Two: Consent Form	311
Appendix Four	: Showing 50 (1-50) random microscope coordinates	
	at (X and Y positions) for each of the 2 gels per slide	312
Appendix Five	: Figure 1a & 1b	314

Appendix Five	: Figure 2a & 2b	315
Appendix Five	: Figure 3a & 3b	316
Appendix Five	: Figure 4a & 4b	317
Appendix Six	: Sinusoidal wave & Square wave	318
Appendix Seven	: Table 1a - Recorded Temperatures	
	(Control condition: coil off & no MF)	319
	: Table 1b- Recorded Temperatures	
	(Coil on @ 1mT MF)	320
	: Figure 1a & 1b - Graph of recorded temperatures	
	(Control condition: coil off & no MF)	321-322
	: Figure 1c & 1d - Graph of recorded temperatures	
	(Coil on @ 1mT MF)	323-324

List of Tables.

Table 4.1.	Overall mean SCE and standard deviations of the control group	
	and that of the entire set of experimental groups for R1.	182
Table 4.2.	Mean SCE and standard deviations of all experiments in R1 based	
	on all three donors.	185
Table 4.3.	Overall ANOVA for R1.	186
Table 4.4.	Overall mean SCE and standard deviations of the control group	
	and that of the entire set of experimental groups for R2.	189
Table 4.5.	Mean SCE and standard deviations of all experiments in R2 based	
	on all three donors.	191
Table 4.6.	Overall ANOVA for R2.	192
Table 4.7.	Overall SCE mean of control and complex experiments in R1.	193
Table 4.8.	Overall SCE mean of control and complex experiments in R2.	194
Table 4.9.	M _a 's and standard deviations of control group and that of	
	the entire set of experimental groups.	195
Table 4.10	M_a 's and standard deviations of all experiments based on all	
	three donors.	196
Table 4.11	. Overall ANOVA for M _a .	197
Table 4.12	M_b 's and standard deviations of control groups and that of the	
	entire set of experimental groups.	200
Table 4.13	M_b 's and standard deviations of all experiments based on all three	
	donors.	201
Table 4.14	. Overall ANOVA for M_b .	202
Table 4.15	. Overall M_a of control and complex experiments of MN assay.	205
Table 4.16	. Overall M_b of control and complex experiments of MN.	205
Table 4.17	. Summary results of three parameters (Tail length, Tail moment	
	and Olive tail moment) of the COMET assay experiments.	207
Table 4.18	. <i>t</i> -tests for all three parameters (Tail length, Tail moment and Olive	
	tail moment) between the three COMET assay experiments.	208
Table 4.19	. Summary results of control and one FISH experiment.	209

List of Figures.

Figure 1.1.	Showing the flow of current and magnetic field (Giancoli, 2000).	1
Figure 2.0.	ure 2.0. Pathways of reactive oxygen species (ROS) involvement in cellular	
	reactions subjected to short- and long-term EMF-exposure.	14b
Figure 2.1.	Biological signal transduction pathways involving first- and	
	second-messenger systems.	16
Figure 2.2.	Showing geographic and geomagnetic poles.	35
Figure 2.3.	A c-metaphase chromosome spread of a dividing peripheral blood	
	Lymphocyte showing 10 SCEs.	46
Figure 2.4.	Showing how SCE staining method produces differentially stained	
	chromatids by the incorporation of BrdU.	48
Figure 2.5.	Showing "single" and "twin" sister chromatid exchanges.	50
Figure 2.6.	Showing the single stranded (unineme) nature of chromosomes.	51
Figure 2.7.	SCE model of Kato.	57
Figure 2.8.	Multiple lesion pathways leading to a small number of	
	SCE-inducing lesion states.	59
Figure 2.9.	Replication crosslink bypass model of SCE.	60
Figure 2.10.	Alternate rejoining processes for the replication bypass of SCE.	62
Figure 2.11.	Model for possible effects of DNA-damaging agents on cluster	
	replication and segregation.	64
Figure 2.12.	Double-strand recombination at the junction between replicon	
	clusters.	65
Figure 2.13.	Showing the Cleaver's SCE model.	67
Figure 2.14.	Topoisomerase II subunit exchange model of SCE.	69
Figure 2.15.	Replication detour SCE model of Ishii and Bender.	70
Figure 2.16.	Strand switching by homologous displacement at blocked	
	replication forks.	72
Figure 2.17.	Strand switching in the model of Shafer.	75
Figure 2.18.	SCE between replicated strands by two topoisomerase.	
	II-mediated strand switches.	76
Figure 3.1.	A. Incubator apparatus.	146
Figure 3.2.	Helmholtz coils .	148

Figure 3.3.	Lollipop-plot of the resultant vectors in a longitudinal section	
	through the 'Z-axis' of a Helmholtz coil.	150
Figure 3.4.	Magnitude of resultant vectors in 'mT' for the pair of	
	Helmholtz coils.	150
Figure 3.5.	Magnitude of resultant vectors in ' μ T' for the pair of	
	Helmholtz coils.	151
Figure 3.6.	Angles in Cartesian co-ordinates of resultant magnetic field vectors	
	relating to the pair of Helmholtz coils.	151
Figure 3.7.	A Giemsa-stained c-metaphase chromosome complement.	
	(2n = 46.)	153
Figure 3.8.	Diagrammatic representation of various differential staining patterns	
	observed after incorporation of BrdU into replicating DNA.	155
Figure 3.9.	Cytokinesis-blocked (CB) binucleated cells (a-h) with varying	
	numbers (0-7) of micronuclei.	159
Figure 3.10.	Binucleate cells which can be scored for micronuclei.	160
Figure 3.11.	Typical appearance and relative size of micronuclei in	
	binucleated cells that meet the scoring criteria.	160
Figure 3.12.	Cellular structures which resemble micronuclei but were	
	not scored.	161
Figure 3.13.	Illustrative diagram of what was observed after centrifugation of	
	whole blood during isolation using Ficol, concentration gradient.	167
Figure 3.14.	Diagram representing the dimensions of a haemocytometer	
	counting chamber.	168
Figure 3.15.	Illustrative image showing how the gel layers were formed	
	using the pre-treatment method. Image not to scale.	170
Figure 3.16.	Image indicating the placement of microscope slides in	
	electrophoresis tank.	172
Figure 3.17.	Image capturing hardware used in the current study.	173
Figure 3.18.	Orientation of cell (directed left to right) for analysis by	
	the CASP software.	174
Figure 3.19.	Image outlining 7 of the 13 different variables that are measured	
	by the CASP [™] software.	176

XIII

Figure 4.1.	Graph of mean SCE of control groups and EMF exposed groups	
	of three donors in R1.	183
Figure 4.2.	A c-metaphase chromosome spread from a sham exposed	
	control showing 11 SCEs.	184
Figure 4.3.	A c-metaphase chromosome spread from an EMF exposed	
	experiment showing 20 SCEs.	184
Figure 4.4.	A c-metaphase chromosome spread of a dividing peripheral blood	
	lymphocyte that has undergone three rounds of DNA replication.	185
Figure 4.5.	Graph of mean SCE of different Wave and Form in R1.	187
Figure 4.6.	Graph of mean SCE of different Wave and Strength in R1.	187
Figure 4.7.	Graph of mean SCE of different Form and Strength in R1.	187
Figure 4.8.	Graph of mean SCE of different Wave and three Donors.	188
Figure 4.9.	Graph of mean SCE of different Form and three Donors.	188
Figure 4.10.	Graph of mean SCE of different Strength and three Donors.	188
Figure 4.11.	Graph of mean SCE of control groups and EMF exposed groups	
	of three donors in R2.	190
Figure 4.12.	Graph of mean SCE of different Wave and Form in R2.	192
Figure 4.13.	Graph of mean SCE of different Form and Strength in R2.	193
Figure 4.14.	Graph of M_a of control groups and EMF-exposed groups for three	
	donors.	195
Figure 4.15.	Graph of M _a of different Wave and Strength.	198
Figure 4.16.	Graph of M _a of different Form and Strength.	198
Figure 4.17.	Graph of M_a of different Form and Wave.	198
Figure 4.18.	Graph of M _a of different Wave and three Donors.	199
Figure 4.19.	Graph of M_a of different Form and three Donors.	199
Figure 4.20.	Graph of M_a of different Strength and three Donors.	199
Figure 4.21.	Graph of M_b of control groups and EMF-exposed groups for	
	three donors.	200
Figure 4.22.	Graph of M_b of different Wave and Strength.	203
Figure 4.23.	Graph of M_b of different Form and Strength.	203
Figure 4.24.	Graph of M_b of different Form and Wave.	203
Figure 4.25.	Graph of M_b of different Wave and three Donors.	204
Figure 4.26.	Graph of M_b of different Form and three Donors.	204
Figure 4.27.	Graph of M_b of different Strength and three Donors.	204

Figure 4.28.	Photographs showing single-strand DNA migration pattern	
	of individual peripheral blood lymphocytes.	206
Figure 4.29.	Mean values for Tail length, Tail moment, Olive tail moment for	
	control, Square continuous 1 mT and Square pulsed 1 mT.	208
Figure 4.30.	Photographs showing a human peripheral blood lymphocyte cell	
	with six whole chromosome (2, 3 and 5) labelled with FITC (green).	210