The Clastogenic Effects of Cyclophosphamide and a Sixty Hertz Electronmagentic Field on Bone Marrow Cells of CD-I Mice

Kevin K. Block

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THE CLASTOGENIC EFFECTS OF CYCLOPHOSPHAMIDE AND
A SIXTY Hertz ELECTROMAGNETIC FIELD ON BONE
Marrow CELLS OF CD-1 MICE

by

Kevin K. Block

A Dissertation
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Doctor of Philosophy
Department of Science Studies

Western Michigan University
Kalamazoo, Michigan
August 1997
THE CLASTOGENIC EFFECTS OF CYCLOPHOSPHAMIDE AND A SIXTY HERTZ ELECTROMAGNETIC FIELD ON BONE MARROW CELLS OF CD-1 MICE

Kevin K. Block, Ph.D.
Western Michigan University, 1997

Extremely low frequency (ELF) electromagnetic fields (EMF) have been correlated with the induction of cancer in a number of epidemiological studies (Feychting and Ahlbom, 1993; Lin and Lee, 1994; London et al. 1991; Lovely et al., 1994; Tomenius, 1986; Wertheimer and Leeper, 1979, 1982). Two hypothesis were tested in the present study. The first hypothesis tested was that exposure to a 7.3 G magnetic field for 24 hours would increase the frequency of clastogenic and/or mutagenic events in bone marrow cells of mice. The second hypothesis tested was that exposure to a 7.3 G magnetic field for 24 hours would increase the frequency of clastogenic and/or mutagenic events in bone marrow cells of mice also treated with cyclophosphamide, and hence may enhance the potential for cancer initiation by a known clastogen, mutagen, and carcinogen (Povirk and Shuker, 1994).

Eight groups of mice, 12 animals per group (6♀ and 6♂), were administered a single i.p. injection of cyclophosphamide at a dose of 0, 5, 25, or 50 mg/kg b.w. Each group of mice was separated by sex into two plastic
cages and the two cages placed side-by-side within the center region of an energized or nonenergized Helmholtz coil. The 60 Hertz alternating current was regulated to flow through the coil for 19 seconds, followed by a 19 second interval of no current flow, ad infinitum. As measured, the energized coil produced a magnetic field intensity of 7.3 gauss and an electric field intensity of 17.0 V/m at the center region of the coil.

After 20 hours of ELF EMF or sham exposure, the mice were injected with a single i.p. injection of colchicine at a dose of 4 mg/kg b.w., and returned to their cage within the coil for 4 more hours of ELF EMF or sham exposure. After 24 hours of ELF EMF or sham exposure, the mice were sacrificed by cervical dislocation, their femurs removed, and bone marrow metaphase spreads prepared on glass slides.

The scoring of the slides revealed that a 24 hour exposure to a magnetic field intensity of 7.3 G did not significantly increase the frequency of clastogenic and/or mutagenic events in mice that had (or had not) been treated with cyclophosphamide. The results did not support either of the two research hypothesis tested in the present study.
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Kevin K. Block
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CHAPTER I

INTRODUCTION

Historical Background to Problem

Ubiquity of Electromagnetic Fields

In a technological society, such as the United States, the widespread use of electrical energy is a common fact of life. During the year 1983, the average household used about 9000 kilowatt-hours (kWh) of electricity, and this was forecasted to increase to about 10,671 kWh of electricity during the year 2000. In addition, during the year 1984, both residential and commercial use of electricity was estimated at 780 billion kWh, and this was forecasted to increase to 1320 billion kWh during the year 2000 (Aldrich and Easterly, 1987).

Coupled with the ever increasing use of electricity, is the increased potential for humans to be exposed to electromagnetic fields (EMFs) created by 50 or 60 Hertz (Hz) electricity flowing within conductors. We cannot see, hear, or feel the low energy EMFs created by the extremely low frequency (ELF) alternating electric currents, but the fact remains, most people in a modern society are immersed in ELF EMFs of varying intensity and for
varying durations during each day of their life. During the course of an average day, a person in the United States may be in close proximity to several items that are powered by electricity. Such items include electric powered toothbrushes, radios, hairdryers, lights, razors, small and large appliances, televisions, stereos, computers, blankets, heaters, waterbeds, clocks, spas, hand held power tools, machinery, and the list goes on and on. People are also exposed to EMFs generated from electricity flowing through the electrical wiring in their homes and at their work locations, and to EMFs generated from electricity flowing through overhead (or buried) electric power transmission lines. In addition, people are also exposed to EMFs generated by certain types of medical diagnostic tests and medical therapy.

**Components of Electromagnetic Fields**

There are two physical components of EMFs: (1) an electric field (EF), and (2) a magnetic field (MF). Both EFs and MFs occur as a result of electric charges.

**Electric Fields**

Electric fields exert attractive or repulsive forces on electric charges. There are positive electric charges (protons) and negative electric charges (electrons) found throughout nature. Each positive or negative electric charge is surrounded by an electric field (EF) which exerts a force on other
positive and negative electric charges. The EF around a positive electric
circle, a positive electric charge repels other positive electric charges and attracts negative electric
charges. Conversely, an EF around a negative electric charge repels other
negative electric charges and attracts positive electric charges. The intensity
of an EF is measured in units of volts per meter (V/m).

Electric fields are found throughout nature. Static electricity is one
example of an EF that occurs in nature. All of us are familiar with the build
up of static electric charges from walking across a carpeted room and the
associated discharge of those charges when we touch a doorknob or another
person. Lightning is another example of static electric charges that have
accumulated and are then discharged with each lightning bolt. Other
examples of EFs that occur in nature include the EFs that hold the atoms
and molecules of both living and non-living matter together, and the EFs
found to exist across the plasma membranes of living cells.

**Magnetic Fields**

An electric current is the movement or flow of electric charges in the
same direction. The greater the current, the greater the strength of the
associated MF that is generated. The strength of an electric current is
measured in units of amperes (A).

When electric charges are in motion in a conductor such as with
alternating electric current moving in transmission lines, the moving electric
charges create a field that exerts a force on other moving electric charges. The field around the moving electric charges in alternating electric current is called a magnetic field (MF). The intensity of a MF is measured in units of gauss (G) or tesla (T), with the equivalence between the two units being: 1T = 10,000G.

Similar to EFs, MFs are also found throughout nature. Examples include the small magnetic fields originating from electric currents created by the electric depolarization of neuron membranes and the depolarization of cardiac muscle. Other examples include the earth's MF created by electric charges flowing within the earth's molten center and the MF created by a bar magnet as a result of a certain alignment of electron spins within the iron atoms of the magnet. MFs are generated around electric powered items and around any conductor that has an electric current flowing through it.

Properties of Magnetic and Electric Fields

Magnetic Field

Magnetic fields are force fields generated by moving electric charges which exert a force upon other moving electric charges. The electric charges must be in motion in order to generate and maintain a MF. When 60 Hz electricity is flowing through a conductor, a MF is generated around the conductor. However, when the electricity is shut off, the electric current
stops flowing in the conductor and the MF ceases to exist.

Magnetic fields, as they propagate through space, will easily pass through most objects (including human beings), which is the reason that overhead or buried residential power lines will contribute to the MFs within our houses and the buildings we are in while at work. The strongest MFs are always found closest to the conductor and decrease rapidly in intensity with increasing distance from the conductor.

Electric Field

Electricity must be flowing in a conductor in order for a MF to be generated. However, an EF may be generated near an electric powered item merely by having the item connected to a voltage source even when the item is not in operation.

Unlike the propagation of MFs through space, the propagation of EFs through space are stopped by most objects around us, such as the walls and roofs of buildings and houses, or even by the trees so often found in residential neighborhoods. Like MFs, the strongest electric fields are found closest to the conductor and decrease rapidly in intensity with increasing distance from the conductor.

Properties of 60 Hertz Alternating Electricity

The electricity that is generated in the United States is 60 Hz
alternating current (AC), which means the electric current alternates back and forth in direction, sixty times in every second. This alternation of electric current affects both the MFs and EFs generated by the alternating current. Unlike the earth's MF which has a fixed direction, the MFs generated from 60 Hz AC, vary periodically with a period of 1/60 second. In each period, the MF builds up from zero to a maximum value, then decreases to zero, and then builds up to a maximum value in the opposite direction, and once again returns to a zero value.

The wavelengths, frequencies, and energies of the EMFs generated by 60 Hz AC electricity are part of a spectrum known as the electromagnetic spectrum (see Figure 1). The velocity, in air, of the EMFs generated from 60 Hz electricity is the same as it is for the velocity of all other types of electromagnetic energy in air, namely the speed of light ($3 \times 10^8 \text{ m/s}$). The intensity of EMFs coming from a point source, such as the EMFs coming from a hand held electric drill, vary inversely with the square of the distance from the point source. The intensity of EMFs coming from a line source, such as the EMFs coming from electric transmission lines, vary inversely with the distance from the line source.

**Public Concern Over EMFs**

Since the early 1980's, largely due to epidemiological studies that have at times been sensationalized by the press, there has been increasing public
<table>
<thead>
<tr>
<th>Wavelength (m x 2.9979)</th>
<th>Energy (eV x 4.1354)</th>
<th>Frequency (Hertz)</th>
<th>Type of Electromagnetic Radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^8$</td>
<td>$10^{-15}$</td>
<td>1</td>
<td>ELF 60 Hz AC Electricity</td>
</tr>
<tr>
<td>$10^6$</td>
<td>$10^{-13}$</td>
<td>$10^2$</td>
<td>AM Radio</td>
</tr>
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<td>$10^4$</td>
<td>$10^{-11}$</td>
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<td>Television/radar</td>
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<td>$10^2$</td>
<td>$10^{-9}$</td>
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<td>Microwaves</td>
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<td>1</td>
<td>$10^{-7}$</td>
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<td>Visible Light</td>
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<td>$10^{-8}$</td>
<td>10</td>
<td>$10^{16}$</td>
<td>Gamma Rays</td>
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<td>$10^7$</td>
<td>$10^{22}$</td>
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<tr>
<td>$10^{-16}$</td>
<td>$10^9$</td>
<td>$10^{24}$</td>
<td></td>
</tr>
</tbody>
</table>

Wavelength ($\lambda$) = $c/f = 2.9979 \times 10^8$ m s$^{-1}$ / frequency (Hz)

Energy ($E$) = $hf = 4.1354 \times 10^{-15}$ eV s$^{-1}$ x frequency (Hz)

Figure 1. Approximate Wavelengths, Energies, and Frequencies of Various Types of Electromagnetic Radiations.
concern over the possibility that exposure to ELF EMFs may increase the incidence of certain types of cancer.

One type of epidemiological study that has reported statistically significant correlations between cancer incidence and exposure to ELF EMFs is concerned with the population of workers whose occupations have required them to receive sustained exposures to ELF EMFs at their work locations (Lin, Dischinger, Conde, and Farrell, 1985; Savitz and Calle, 1987; Pearce, Reif, and Fraser, 1989; Bastuji-Garin, Richardson, and Zittoun, 1990; Garland, et al., 1990; Loomis and Savitz, 1990; Demers et al., 1991; Tornqvist, Knave, Ahlbom, and Persson, 1991; Tynes, Anderson, and Langmark, 1992; Richardson et al., 1992; Floderus et al. 1993; Matanoski, Elliot, Breysse, and Lynberg, 1993; Theriault et al. 1994; Floderus, Tornqvist, and Stenlund, 1994; Savitz and Loomis, 1995).

A second type of epidemiological study that has reported finding statistically significant correlations between cancer incidence and exposure to ELF EMF is concerned with the populations of children and adults who received sustained exposures to ELF EMFs within their own homes (Wertheimer and Leeper, 1979; Wertheimer and Leeper, 1982; Tomenius, 1986; Savitz, John, and Kleckner, 1990; London et al., 1991; Feychtting and Ahlbom, 1993; Lovely et al., 1994; Lin and Lee, 1994).

In a review of 53 epidemiological studies that reported finding statistically significant correlations between the incidence of certain types
of cancer and exposure to ELF EMFs (Bates, 1991), 46 of the studies also reported the risk odds ratio for acquiring the cancers. A risk odds ratio equal to 1 means the probability of acquiring a certain type of cancer is the same as the national incident rate for that type of cancer; a risk odds ratio equal to 2 means the probability of acquiring a certain type of cancer is 2 times that of the national incidence rate for that type of cancer, and so on. Of the 46 studies reporting risk odds ratios, 42 studies reported risk odds ratios between 1 (same as the national incident rate) and 4 (4 times the national incident rate), 3 studies reported risk odds ratios between 4 to 10, and 1 study reported a risk odds ratio greater than 10. The public is cautioned to put these reported risk odds ratios in perspective since risk odds ratios in the 1 to 4 range do not demonstrate a strong relationship between a variable (i.e. ELF EMFs) and cancer incidence. Risk odds ratios of 10 and above show a much stronger relationship between a variable and cancer incidence such as in the epidemiological studies which reported statistically significant correlations between smoking and lung cancer. Such studies reported risk odds ratios of between 20 to 30 (Hendee and Boteler, 1994).

Another thing that has not always been made clear to the public about these epidemiological studies, is that a statistical correlation does not mean the same thing as cause and effect. As Morgan (1992) explains, statistically significant correlations between two variables such as ELF EMFs and cancer incidence do not prove that exposure to ELF EMFs caused
the higher incidence rate of a cancer. These studies do not (and cannot) look at all of the possible variables or agents that in fact could actually account for higher incidence rates of cancer. In real life, we have different lifestyles and diets, exposing us to a number of different carcinogenic agents. This makes it very difficult to propose a cause and effect relationship between ELF EMFs and higher incidence rates of cancers.

One important finding of the epidemiological studies is that MFs, generated by 60 Hz electricity, show a higher correlation figure with cancer incidence than the correlation figure of EFs with cancer incidence. One possible explanation for this finding may be that MFs are known to penetrate most materials easily (including the human body), whereas EFs do not penetrate most materials very easily (including the human body). Because the energy of the electric field is dissipated very quickly upon entering the human body, it does not penetrate very deeply into the tissues of the body. However the energy of a magnetic field is not dissipated quickly upon entering the human body and as a result, the magnetic field penetrates deeply into the tissues of the human body. It is for this reason that magnetic fields may be principally responsible for any biological effects induced by ELF EMFs.

Biological Effects of EMF

Laboratory studies on the biological effects of ELF EMFs have created
controversies for a number of reasons. First, a number of the studies have reported finding biological effects while other studies have reported finding no biological effects. Second, attempts to reproduce certain positive studies that reported finding biological effects, have not always been successful. Third, there is no generally accepted mechanism for how ELF EMFs can produce biological effects in organisms. Fourth, certain studies have reported finding no linear dose response between biological effects and the increase of dose rates or total dosages of ELF EMFs. In addition, some studies have reported thresholds of ELF EMF frequency and intensity whereby biological effects are seen at a certain frequency and/or intensity and then disappear at higher or lower frequencies and/or intensities (Goodman et al., 1995).

The two best understood mechanisms for producing biological damage in living tissue by EMFs, are those of ionization and thermal effects. At the frequency of 60 Hz electricity, each particle (photon) of the EMF has a very small energy, \(2.5 \times 10^{-13} \text{ eV}\) (see Figure 1). Unlike the energies of gamma rays, X-rays, or even the higher energy ultraviolet electromagnetic radiations, the ELF EMFs generated by 60 Hz electricity do not have sufficient energy to ionize atoms, break molecular bonds in biological molecules, or ionize water molecules in cells resulting in the production of highly reactive free radicals. All of these processes would require photons with energies greater than \(1 \times 10^{-2} \text{ eV}\).
It is generally accepted (Morgan, 1992) that the EMFs generated from 60 Hz electricity can move ions within the tissues of our bodies, thereby producing electric currents in these tissues. However, the electric currents produced in tissues from ELF EMFs are several times weaker than the electric currents that naturally occur in the body. The electric currents produced in the tissues surrounding the heart (as a result of the depolarization of cardiac tissue) are 100 to 1000 times greater than the electric currents produced in the body by 60 Hz ELF EMFs. (Pool, 1990).

One result of the weak electric currents produced in tissues by 60 Hz ELF EMFs, is that some heat is produced within the tissues. However, EFs in the air would have to be between a magnitude $10^7$ and $10^8$ V/m, or the MFs would have to be between a magnitude of $10^4$ and $10^5$ G, in order to produce electric currents sufficient in magnitude to produce heating effects comparable to those that exist naturally within the human body. However, EFs greater than $10^6$ V/m cannot be maintained naturally in air due to spark discharge of the field, and MFs between $10^4$ and $10^5$ G are not found to occur naturally (Aldrich and Easterly, 1987).

Although the ELF EMF energies created by 60 Hz AC electricity are insufficient to ionize atoms, break molecular bonds, or produce thermal effects of any significance within tissues, several laboratory studies have shown that ELF EMFs are somehow interacting with organisms and are affecting the biological processes within them. Exactly how ELF EMFs may
produce the biological effects is still not known.

While several of the laboratory studies have reported finding biological effects associated with 60 Hz EMFs, there have also been a number of studies that have reported finding no biological effects. Some of the reported biological affects include the following: (Morgan, 1992)

1. Changes in the amount of ion flow across cell membranes, especially for the Ca$^{++}$ ions, but also for $K^+$, $Li^+$, and $Mg^{++}$.

2. Changes in the production of melatonin, a hormone important in regulating the body's daily biological cycle.

3. Changes in the duration of the cell-cycle, whereby the growth rate of cells and division of the cells is affected.

4. Changes in the rate that DNA molecules are copied and the rates and amounts of ribonucleic acid (RNA) copied from the DNA.

5. Changes in the immune response by certain immune cells.

6. Changes in the activities of certain enzymes.

7. Changes in the response of cells to mitogens.

8. Enhancement in the ability of broken bone to reunite.

9. Enhanced mutagenic effects have been reported in a few studies, however the majority of studies have reported no such effects.

Carcinogenesis

Moolgavkar and Knudson (1981) have described a two stage model for
carcinogenesis based on two independent events: (1) tumor initiation; and (2) tumor promotion. Under the two stage model, two separate DNA mutations must eventually occur before a cell can be transformed into a malignant cell capable of transformed clonal expansion. The first DNA mutation occurs with initiation, whereby the DNA of a cell is changed either by spontaneous mutation or by the affects of a chemical or physical mutagenic agent. The initiated cell is now called an intermediate cell. The intermediate cell may now divide resulting in an intermediate lesion in the DNA that may or may not be repaired in the cell. The process that increases the proliferation of the intermediate cells is called promotion, and the agents (usually not mutagenic themselves) that facilitate the division of intermediate cells, are called promoters. An intermediate cell, with a mutation, may now undergo a second mutation resulting in a transformed cell that is malignant and whose clone of cells will result in a malignant tumor. In essence, a promoter increases the pool of intermediate cells having the required first mutation, thereby increasing the overall possibility of a second mutation to occur to one of these cells and for a malignant transformation of the cell to occur (Severson and Davis, 1991).

In addition to promoting factors, there are also co-promoting factors. Co-promoters are not direct tumor promoters themselves, but assist in the carcinogenic process by enhancing the tumor promoting affects of promoters (Loscher and Mevissen, 1994). For many types of tumors in humans, the
latent period between the initiating event in a cell and the development of the malignant tumor is 15 to 45 years depending on the activities of the tumor promoters and co-promoters (Koifman, 1993).

**ELF EMF as a Cancer Promoter/Co-promoter**

There have been a small number of in vitro and in vivo laboratory studies that have reported finding mutagenic effects in cells as a result of exposures to ELF EMFs. On the other hand, there have been a far greater number of laboratory studies that have reported finding no mutagenic effects due to exposures to ELF EMFs. Taken in total, the preponderance of negative findings have caused many scientists to conclude that DNA is not significantly damaged by exposure to ELF EMFs. As a result, many scientists believe that ELF EMFs may act as cancer promoters or co-promoters rather than as a cancer initiator (Goodman et al., 1995).

Mechanisms proposed to explain how EMFs may act as promoter/co-promoters include the following:

1. Disruption of intercellular communication: EMFs may act synergistically with promoters at the cell membrane whereby distorted signals are then sent inward to the nucleus and other organelles. This may result in a disruption of the normal cell-to-cell communication through gap junctions, leading to autonomous cell growth (Adey, 1988; 1990).

2. Disruption of intracellular calcium homeostasis: EMFs disruption
of a cell's calcium homeostasis may lead to increased production of free radicals within the cell which in turn may affect the cell's ability to protect itself from oxidative attack by toxic chemicals or ionizing radiation (Stevens, 1993).

3. Reduced levels of melatonin: Melatonin is the principal hormone released by the pineal gland in the brain. Melatonin acts as the neuroendocrine controller of the biological clock by synchronizing the release of hormones from other endocrine glands. Melatonin's action on other endocrine glands is generally inhibitory. Since ELF EMFs have been shown to depress melatonin secretion from the pineal gland, reduced circulating levels of melatonin could result in increased circulating levels of testosterone and estrogen. Increased circulating levels of these two steroids could enhance the carcinogenic process for those types of cancers with positive receptors for these steroids. Such cancers would include breast and prostate cancer (Stevens, 1993).

4. Reduced immune functions: There is some evidence that ELF EMFs can suppress the cytotoxicity of T-lymphocytes (Lyle et al., 1983). The reduced immune functions could result in reduced elimination of initiated cells as well as the progression of transformed cells via reduced T-cell activity (Severson and Davis, 1991).

5. Altered gene expression: Disruption of a cell's calcium homeostasis by ELF EMFs could affect the activation of certain genes associated with
growth processes of the cell, which in turn could be involved in the 
carcinogenic process (Goodman, 1990).

**Leukemia**

As explained by Kumar, Cotran, and Robbins (1997), "The leukemias are 
malignant neoplasms of the hematopoietic stem cells characterized by 
diffuse replacement of the bone marrow (cells) by neoplastic cells" (p 373).

The two major classifications of leukemias are (1) acute leukemias, and (2) 
chronic leukemias. Both the acute leukemias and the chronic leukemias 
may be classified further into (1) lymphocytic, and (2) myelocytic. Thus a 
particular leukemia may be placed into one of four different classifications, 
(1) acute lymphocytic leukemia (ALL), (2) chronic lymphocytic leukemias 
(PLL), (3) Acute myelocytic leukemia (AML), or (4) chronic myelocytic 
leukemia (CML) (Kumar et al., 1997).

Chromosome abnormalities have been found in people who have been 
exposed to various physical agents (i.e. gamma radiation or X-rays) or to 
various chemical agents (i.e. cyclophosphamide, nitrosourea, benzo(a)pyrene, 
7,12-dimethylbenzanthracene). Chromosome abnormalities have also been 
found in people suffering from certain diseases. Cytogenetic examination 
of bone marrow cells taken from people suffering from leukemia have found 
both numerical and morphological chromosome abnormalities in a 
percentage of the leukemia patients, although a percentage of the patients
have also shown no chromosome abnormalities. The numerical abnormalities found in leukemia patients include both aneuploidy and euploidy chromosome abnormalities. The morphological abnormalities include deletions, breaks, gaps, fragments, marker chromosomes, and dicentrics (Woodliff, 1971).

Certain types of chromosome abnormalities are associated with certain types of leukemia. As explained by Kumar et al (1997),

Approximately 90% of patients with ALL have nonrandom karyotypic abnormalities. Most common is hyperdiploidy (>50 chromosomes/cell) in early B-cell ALL. The Philadelphia chromosome is present in 2% to 5% of children with ALL and in 25% to 30% of adults with ALL. Several cytogenetic changes have been noted in AML...Of particular interest is the t(15:17) translocation...In approximately 90% of patients with CML, the Ph (Philadelphia) chromosome, usually representing a reciprocal translocation from the long arm of chromosome 22 to the another chromosome (usually the long arm of chromosome 9) can be identified...Approximately 50% of (CLL) patients have karyotypic abnormalities, the most common of which is trisomy 12.

(Kumar et al, 1997, pp374-378)

Although chromosome abnormalities are found in only a percentage of untreated leukemia patients, chromosome abnormalities are more commonly found in leukemia patients who have undergone therapy with physical and/or chemotherapeutic agents. The increase in the number and types of chromosome abnormalities in patients after therapeutic treatments attest to the ability of the physical and chemical agents to induce chromosome abnormalities (Weatherall and Walker, 1965).

Cancer therapy has in many cases been shown to be a two edged
sword. The very agent(s) used to put a cancer into remission may later be responsible for inducing a secondary cancer. Ionizing radiation (gamma radiation or X-rays) can cause several different types of chromosomal aberrations and can also lead to acute leukemia (Woodliff, 1971). Several chemicals used as a cancer chemotherapeutic can cause chromosome damage (i.e. cyclophosphamide) and must be considered potentially leukemogenic (Woodliff, 1971)

Statement of Research Problem

Extremely low frequency (ELF) electromagnetic fields (EMF) have been correlated with the induction of cancer in a number of epidemiological studies (Feychting and Ahlbom, 1993; Lin and Lee, 1994; London et al., 1991; Lovely et al., 1994; Tomenius, 1986; Wertheimer and Leeper, 1979, 1982).

Since damage to chromosomes can initiate the carcinogenic process, it is important to know if ELF EMFs are mutagenic to chromosomes. The first hypothesis tested in the present study was that exposure of mice to a 7.3 G magnetic field, for 24 hours, would increase the frequency of clastogenic and/or mutagenic events in the bone marrow cells of the mice exposed to the magnetic field.

It is also important to know if ELF EMFs can enhance the mutagenicity of known cancer initiators. Cyclophosphamide is a known
clastogen, mutagen, and carcinogen (Povirk and Shuker, 1994). The second hypothesis tested in the present study was that exposure of mice to a 7.3 G magnetic field for 24 hours would increase the clastogenic and/or mutagenic effects of cyclophosphamide in the bone marrow cells of mice. In the present study, mice were treated with a single intraperitoneal (i.p.) injection of cyclophosphamide at a dose of either 0, 5, 25, or 50 mg/kg body weight, and then exposed to a 7.3 G magnetic field intensity for a period of 24 hours.

A significant increase in the frequency of clastogenic and/or mutagenic events in the bone marrow cells of mice exposed to both cyclophosphamide and the 7.3 G magnetic field would suggest that ELF EMFs can enhance the cancer initiation process by cyclophosphamide.
CHAPTER II

LITERATURE REVIEW

Cyclophosphamide

Introduction

The nitrogen mustard, cyclophosphamide, is a bifunctional alkylating agent that is used extensively as an anticancer chemotherapeutic in the treatment of several different types of neoplastic diseases. In addition, due to cyclophosphamide's immunosuppressive properties, the drug is also administered as an immunosuppressive agent prior to organ transplants and in the treatment of such nonneoplastic diseases as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, psoriatic arthritis, chronic hepatitis, scleroderma, glomerulonephritis, and chronic interstitial pneumonia (Anderson, Bishop, Garner, Ostrosky-Wegman, and Selby, 1995).

In the 1950s, anticancer chemotherapy relied extensively on the use alkylating agents, including nitrogen mustards. It was not long after the regular use of alkylating agents for anticancer therapy, that case reports started appearing in the literature about patients developing secondary cancers after having received prior treatment with alkylating agents (Povirk
and Shuker, 1994). Of all the alkylating agents used as anticancer chemotherapeutics, the nitrogen mustards are among the most carcinogenic, (Kaldor, Day, and Hemminki, 1988). After reviewing the available information, the International Agency for Research on Cancer (IARC) designated cyclophosphamide as being carcinogenic to humans (IARC, 1981) (IARC, 1982) (IARC, 1987).

A number of studies have reported a link between the use of cyclophosphamide as a chemotherapy agent and the later onset of secondary cancers. The onset of secondary bladder cancer in patients who previously received cyclophosphamide therapy, has been reported in a number of studies (Fairchild, Spence, Solomon, and Gangai, 1979; Kinlen, 1985; Pederson-Bjergaard, Ersboll, and Hansen, 1988; Travis, Curtis, Boice, and Fraumeni, 1989; Thrasher, Miller, and Wettlaufer, 1990; Sigal, Tomaszewski, Brooks, Wein, and LiVasi, 1991; Cannon, Linke, and Cos, 1991; Alivizitos et al., 1991; Ortiz, Gonzales-Parra, Alveraz-Costa, and Egido, 1992). In addition, there have been a number of studies that have reported on the onset of leukemia following cyclophosphamide therapy (Greene, Boice, and Greer, 1982; Pederson-Bjergaard et al., 1985; Baglin, Galvin, and Pollock, 1987; Geller et al., 1989; Gibbons and Westerman, 1988; Escalante, Kaufman, and Beardmore, 1989; Henry-Amar and Dietrich, 1993).
Metabolism of Cyclophosphamide

The parent cyclophosphamide molecule must first undergo transformation by hepatic cytochrome P450 enzymes in order to become an active alkylating agent (see Figure 2). Cyclophosphamide first undergoes an oxidation in the hepatocytes from the parent molecule to 4-hydroxycyclophosphamide. The 4-hydroxycyclophosphamide molecule may then either nonenzymatically equilibrate with the aldophosphamide structure, or be metabolized further in the hepatocytes to the biologically inactive 4-ketocyclophosphamide molecule. The aldophosphamide molecule may then be metabolized in the liver to the biologically inactive carboxyphosphamide molecule or may enter the circulatory system. The carboxyphosphamide molecule, while itself not being toxic, may then be metabolized to the nornitrogen molecule which is a very strong alkylating agent. Inside the cells of the body, the aldophosphamide molecule may then undergo nonenzymatic cleavage into an acrolein molecule and a phosphoramidemustard molecule. The phosphoramidemustard molecule is thought to be the major alkylating agent giving rise to a series of mono- and crosslinked adducts resembling the nornitrogen molecule. (Anderson et al., 1995; Stanton and Legendre, 1986; Povirk and Shuker, 1994).
Figure 2. Metabolites of Cyclophosphamide.
**Cyclophosphamide: Mechanism of Action**

Alkylating agents act by forming covalent bonds between an alkyl group(s) contributed by the alkylating agent and biological molecules in the body. As explained by Stanton and Legendre (1986),

Alkylating agents act by forming covalent bonds with organic compounds, particularly nucleic acids, thereby replacing a hydrogen bond (on the nucleic acid or protein) with an alkyl group (from the alkylating agent). This results in the cross-linking of DNA strands, the breakage of DNA strands, the formation of linkages between different locations on the same DNA strand, and the inhibition of DNA synthesis. Cyclophosphamide's main cytotoxic effects result from alkylating reactions with nuclear DNA, cytoplasmic RNA, and various other cytoplasmic and membrane proteins. These reactions result in the prevention of mitosis because of interference with DNA replication, transcription, and RNA translation. Cyclophosphamide, similar to all chemotherapeutic drugs, is most effective on cell populations undergoing rapid division.

(Stanton and Legendre, 1986, pp 1319-1320)

In regards to Cyclophosphamide's carcinogenic properties, the drug's carcinogenic mechanism(s) are still not clearly understood but are thought to arise from the contribution of one or more mechanisms that include chromosome point mutations, intragenic deletions, large scale chromosome aberrations, disturbances in genetic regulation, defects in immunological surveillance, and/or activation of latent viruses (Povirk and Shuker, 1994; Stanton and Legendre, 1986).

**Cyclophosphamide: Mutagenicity In Vitro**

There are a number of in vitro studies that have reported on the
mutagenicity of cyclophosphamide. Nasr, Goldman, Klein, and Dacre (1988) reported finding a significant increase in the frequency of sister-chromatid exchanges (SCEs) in cultured Chinese hamster ovary (CHO) cells activated with rat liver microsomes (S9) and treated with cyclophosphamide at a dose concentration of $10^{-4}$ M. Arnsdorf-Roubicek and Targa (1990) reported on an in vivo exposure/in vitro assay to assess the mutagenicity of cyclophosphamide's metabolites in the blood of rats. Rats were treated with cyclophosphamide at doses of 2.5, 5.0, 10.0, or 20.0 mg/kg body weight (b.w.) and then blood plasma was removed from the treated rats. The authors reported finding a statistically significant increase in the frequency of SCEs in cultured human or rat lymphocytes treated with the blood plasma taken from the cyclophosphamide treated rats.

Sharma, Sobti, Chaudhry, and Dhar (1987) reported finding a statistically significant increase in the frequency of chromosome aberrations (CAs) in the polytene chromosomes from the salivary gland cells of Anopheles stephensi mosquito larvae treated with cyclophosphamide. The mosquito larvae were introduced to 20.7 $\mu$g/ml of cyclophosphamide in distilled water as a rearing medium. Twenty-four hours post treatment, the larvae were transferred to ordinary water and allowed to grow. Healthy larvae were then dissected to obtain the salivary gland cells for chromosome analysis. Miller (1991) reported finding a statistically significant increase in the frequency of CAs in mitogen-stimulated human B- and T-lymphocyte
populations activated with S9 mix and treated with cyclophosphamide at concentrations of 10, 15, 20, or 30 µg/ml. Monteith and Vanstone (1995) reported finding increased frequencies of CAs in cultured V79 Chinese hamster cells exposed to cyclophosphamide at doses of 4 or 8 µg/ml.

Oshiro, Piper, Balwierz, and Soelter (1991) reported at least a two-fold increase in the frequency of micronuclei (MN) found in cultured Chinese hamster ovary cells exposed to cyclophosphamide at concentrations of 12, 20, 80, or 160 µg/ml.

Table 1 presents a summary of the in vitro studies reviewed in this paper on the mutagenicity of cyclophosphamide.

Cyclophosphamide: Mutagenicity In Vivo

There are a number of in vivo studies that report on the mutagenicity of cyclophosphamide. Rabello-Gay, Carvalho, Otto, and Targa (1985) reported a significant increase in the frequency of CAs in bone marrow cells taken from mice treated with cyclophosphamide at a dose of 50 mg/kg b.w.. McFee and Tice (1990) reported dose related increases in the frequency of CAs in first division metaphase bone marrow cells taken from mice treated with cyclophosphamide at doses of 18.75, 37.5, or 75.0 mg/kg b.w.. Nersessian, Zilfian, and Koum Koumadjian (1992) reported finding a significant increase in the frequency of CAs in bone marrow cells taken from Armenian hamsters treated with a single intraperitoneal injection (i.p.) of
<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism/Cell Type</th>
<th>Dose (CP)</th>
<th>End-point</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharma et al. (1987)</td>
<td>Anopheles mosquito larvae/polytene chromosomes</td>
<td>20.7 μg/ml in larvae rearing medium</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>Nasr et al. (1988)</td>
<td>CHO</td>
<td>10⁻⁴ M</td>
<td>SCE</td>
<td>++</td>
</tr>
<tr>
<td>Rosselli et al. (1990)</td>
<td>rat/lymphocytes</td>
<td>10 mg/kg to rat.</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>Arnsdorf-Roubicek et al. (1990)</td>
<td>rat/lymphocytes human/lymphocytes</td>
<td>plasma dose not given</td>
<td>SCE</td>
<td>++</td>
</tr>
<tr>
<td>Miller (1991)</td>
<td>human B- and T-lymphocytes</td>
<td>10-30 μg/ml</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>Oshiro et al. (1991)</td>
<td>CHO</td>
<td>10/160 μg/ml</td>
<td>HGPRT</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>CHO</td>
<td>5/10/20 μg/ml</td>
<td>MN</td>
<td>&gt;2x controls</td>
</tr>
<tr>
<td>Monteith et al. (1995)</td>
<td>Chinese hamster/V79</td>
<td>4/8 μg/ml</td>
<td>CA</td>
<td>+</td>
</tr>
</tbody>
</table>

++ = positive results and statistically significant
+ = positive results but not statistically significant
CA = chromosome aberration
SCE = sister chromatid exchange
MN = micronucleus
HGPRT = a specific mutation locus
CP = cyclophosphamide
CHO = Chinese hamster ovary cells

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cyclophosphamide at doses of 25, 50, 100, 200, or 300 mg/kg b.w.. Rosselli et al. (1990) reported finding a significant increase in the frequency of CAs in the bone marrow cells of rats treated with cyclophosphamide at a dose of 10 mg/kg b.w.. The authors also reported significant increases in the frequency of CAs in cultured lymphocytes prepared from rats treated with a single dose of cyclophosphamide at a dose of 10 mg/kg b.w..

Reimer and Singh (1982) reported finding a significant increase in the frequency of SCEs in the bone marrow cells of five different strains of mice treated with a single i.p. injection of cyclophosphamide at a dose of 45 mg/kg b.w.. Huang, Tan, Sirianni, Pacholec, and Chapman (1990) reported a dose related increase in the frequency of SCEs in bone marrow cells taken from three different strains of mice treated with cyclophosphamide at doses of 5 or 10 mg/kg b.w..

Jenderny, Walk, Hackenberg, and Rohrman (1988) reported finding a significant increase in the frequency of SCEs and CAs in bone marrow cells of mice after the mice inhaled cyclophosphamide at doses of 8, 23, or 57 mg/kg b.w. for females, and 21, 49, or 114 mg/kg b.w. for males. The authors also reported finding a significant increase in the frequency of SCEs and CAs in bone marrow cells of Chinese hamsters after the animals inhaled cyclophosphamide at doses of 30 or 80 mg/kg b.w. by females, and 25 or 40 mg/kg b.w. by males. The authors also reported significant increases in the frequency of SCEs and CAs in bone marrow cells of mice and Chinese
hamsters that were administered a single i.p. injection of cyclophosphamide at a dose of 29 or 56 mg/kg b.w. to male mice, 15 or 32 mg/kg b.w. to male hamsters, 22 or 42 mg/kg b.w. to female mice, and 15 or 32 mg/kg b.w. to female hamsters.

Krishna, Kropko, Ciaravino, and Theiss (1991) reported finding a statistically significant increase of micronucleated polychromated erythrocytes and CAs in bone marrow cells taken from mice treated with cyclophosphamide at doses of 20 or 40 mg/kg b.w.. Monteith and Vanstone (1995) reported an increase in the frequency of MN in bone marrow cells of mice treated with cyclophosphamide at a dose of 40 mg/kg b.w.. Krishna, Petrere, Anderson, and Theiss (1995) reported finding a statistically significant increase in micronucleated polychromated erythrocytes in bone marrow cells of mice that received cyclophosphamide at doses of 30 or 40 mg/kg b.w..

Krishna et al. (1995b) reported on a dominant lethal (DL) assay in which male mice were administered cyclophosphamide at a dose of 40 mg/kg b.w. and then mated with eight different groups of female mice (2 females per group) over an eight week breeding period. The authors reported that the total number of mouse embryo implants had been significantly reduced for matings taking place in weeks 1, 2, 3, 6, and 7 of the eight week breeding period. The authors also reported a significant increase in the number of mouse embryo implants (dead and resorbed) for breeding weeks 1, 2, and 3.
Synaptonemal complex (SC) analysis has been used to detect structural chromosome damage in the germ cells of rodents treated with cyclophosphamide. Allen, DeWeese, Gibson, Poormand, and Moses, (1987) reported finding increased frequencies of synaptonemal complex (SC) damage in the spermatocytes of mice treated with cyclophosphamide at doses of 30 or 300 mg/kg b.w.. Allen et al. (1988) reported finding a statistically significant increase in the frequency of SC damage in the spermatocytes of mice treated with cyclophosphamide with a dose as low as 10 mg/kg b.w..

Table 2 presents a summary of the in vivo studies reviewed in this paper on the mutagenicity of cyclophosphamide.

Extremely Low Frequency Electromagnetic Fields (ELF EMFs)

ELF EMFs: Mutagenicity In Vitro

Nordenson, Mild, Nordstrom, Sweins, and Birke (1984) reported finding no significant increases in the frequency of CAs in cultured human lymphocytes exposed to an electric field current density of 1 mA/cm². The authors also reported finding no significant increases in the frequency of CAs in cultured lymphocytes exposed to a peak electric field intensity of 2.5 or 3.0 kV/cm created by ten spark discharges, with each spark discharge lasting 3μs in duration. However a significant increase in CAs was found in cultured lymphocytes exposed to a peak electric field intensity of 3.5 kV/cm
## Table 2

**Summary of In Vivo Studies on Cyclophosphamide Mutagenicity**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism/Cell Type</th>
<th>Dose (CP)</th>
<th>End-point</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reimer et al. (1982)</td>
<td>5 strains of mice/bone marrow cells</td>
<td>45 mg/kg</td>
<td>SCE</td>
<td>++</td>
</tr>
<tr>
<td>Rabello-Gay et al. (1985)</td>
<td>mice/bone marrow cells</td>
<td>50 mg/kg</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>Allen et al. (1987)</td>
<td>mice/spermatocytes</td>
<td>30/300 mg/kg</td>
<td>SC</td>
<td>+</td>
</tr>
<tr>
<td>Allen et al. (1988)</td>
<td>mice/spermatocytes</td>
<td>10 mg/kg</td>
<td>SC</td>
<td>++</td>
</tr>
<tr>
<td>Jenderny et al. (1988)</td>
<td>mice/bone marrow cells</td>
<td>8-114 mg/kg</td>
<td>SCE</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Chinese hamster/bone marrow cells</td>
<td>15-80 mg/kg</td>
<td>SCE</td>
<td>++</td>
</tr>
<tr>
<td>Huang et al. (1990)</td>
<td>3 strains of mice/bone marrow cells</td>
<td>5/10 mg/kg</td>
<td>SCE</td>
<td>++</td>
</tr>
<tr>
<td>Rosselli et al. (1990)</td>
<td>rats/bone marrow cells</td>
<td>10 mg/kg</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>Mcfee et al. (1990)</td>
<td>mice/bone marrow cells</td>
<td>18.75/37.5/75 mg/kg</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>Krishna et al. (1991)</td>
<td>mice/bone marrow cells</td>
<td>20/40 mg/kg</td>
<td>MN</td>
<td>++</td>
</tr>
<tr>
<td>Nersessian et al. (1992)</td>
<td>Armenian hamsters/bone marrow cells</td>
<td>25/50/100/200/300 mg/kg</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>Monteith et al. (1995)</td>
<td>mice/bone marrow cells</td>
<td>40 mg/kg</td>
<td>MN</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 2—Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism/Cell Type</th>
<th>Dose(CP)</th>
<th>End-point</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krishna et al. (1995b)</td>
<td>mice/bone marrow cells</td>
<td>30/40 mg/kg</td>
<td>MN</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>mice/embryo implants</td>
<td>40 mg/kg to males</td>
<td>DL</td>
<td>++</td>
</tr>
</tbody>
</table>

++ = positive results and statistically significant  
+  = positive results but not statistically significant  
SC = synaptonemal complex  
DL = dominant lethal  
CA = chromosome aberration  
SCE = sister chromatid exchange  
MN = micronucleus  
CP = cyclophosphamide

created by ten spark discharges, with each spark discharge lasting 3\(\mu\)s in duration. d'Ambrosio et al. (1985) reported finding a significant increase in the frequency of CAs in cultured bovine lymphocytes that were exposed to a 50 Hz electric field with a current density of 2.4 \(\mu\) A/cm\(^2\), for a period of 48 or 72 hours. However, the authors reported finding no significant increase in the frequency of SCEs in cultured lymphocytes exposed to electric fields of the same parameters and for the same period of time.

Rosenthal and Gunter (1989) reported finding no significant increase in the frequency of CAs or SCEs in cultured human lymphocytes exposed to a continuous 60-Hz magnetic field intensity of 50 G, for periods of 48 or 72
hours. However, the same authors reported finding a significant increase in the frequency of SCEs in cultured human lymphocytes that were pretreated with methylnitrosourea (NMU) or trenimon (TRN) and exposed to a magnetic field intensity of 75 G, as compared to lymphocytes pretreated with NMU or TRN and not exposed to the magnetic field. Khalil and Qassem (1991) reported finding a statistically significant increase in the frequency of CAs in cultured human lymphocytes exposed to a 50-Hz pulsed EMF (PEMF) with a magnetic field intensity of 10.5 G, for a period of 24, 48 or 72 hours. However, the same authors reported that cultured human lymphocytes under the same exposure conditions, showed no significant increase in SCEs after 24 or 48 hours of the PEMF exposure, but did show a significant increase in SCEs after 72 hours of exposure to the PEMF.

Cohen, Kunska, Astemborski, and McCulloch (1986a) reported on a study that looked at the frequency of CAs in cultured peripheral lymphocytes taken from 10 normal human adults (5 male and 5 females). The ELF EMF used in the study had an electric field current density of 30 $\mu$A/cm$^2$ with either a 1 or 2 G magnetic field intensity. The ELF EMF exposure period for the cultured lymphocytes was 69 hours. The authors reported finding no significant difference in the number of CAs found in the exposed cells as compared to the nonexposed cells. Garcia-Sagredo, Parada, and Monteagudo (1990) reported finding a significant increase in the frequency of CAs in cultured human lymphocytes exposed to a PEMF with
a magnetic field intensity of 10, 20, or 40 G, for a period of 24 hours, as compared to the cultured human lymphocytes not exposed to PEMF.

Cohen, Kunska, Astemborski, McCulloch, and Paskewitz (1986b) reported on a study that looked at the frequency of SCEs and CAs in cultured human peripheral lymphocytes taken from 10 normal adults (5 male and 5 female). The cultures were either exposed, or not exposed, to an ELF EMF with an electric field current density of 30 μA/cm² and a magnetic field intensity of either 1 or 2 G, for a period of 69 hours. The authors reported finding no significant increase in the frequency of SCEs or CAs in the exposed cells as compared to the nonexposed cells. The same authors reported finding no significant increase in CAs in cultured lymphoblastoid cells taken from patients having conditions with deleterious genetic predispositions such as ataxia telangiectasia, Bloom syndrome, Fanconi anemia, or xeroderma pigmentosum. The cultured lymphoblastoid cells were exposed to an ELF EMF with an electric field current density of 30 uA/cm² and a magnetic field intensity of either 1 or 2 G.

Takahashi, Kaneko, Date, and Fukada (1987) reported the results of two experiments concerned with exposing Chinese hamster V79 cells to PEMF consisting of 25μ second pulses at a magnetic field intensity of 1.8, 4.4, 6.6, 12, 17, 21, or 25 G. The first experiment exposed V79 cell cultures to varying magnetic field intensities (1.8-25.0 G) for 24 hours with addition of 5-bromodeoxy uridine (BrdU) after the PEMF exposure. The second
experiment exposed V79 cell cultures to the PEMF under the same exposure conditions except the BrdU was added to the cultures prior to the PEMF exposures. The authors reported finding no significant increase in the frequency of SCEs of the exposed cells as compared to nonexposed cells in either of the two experiments. Garcia-Sagredo et al (1990) reported finding no significant increase in the frequency of SCEs in cultured human lymphocytes that were exposed to a PEMF with a magnetic field intensity of 10, 20, or 40 G, for a period of 48 hours, as compared to lymphocytes not exposed to the PEMF.

Livingston, Witt, Gandhi, Cahtterjee, and Roti Roti (1991) reported finding no significant increase in the frequency of SCEs or MN in cultured human lymphocytes or cultured CHO cells exposed to 60-Hz ELF EMF for 72 hours. The ELF EMF consisted of an electric field current density of 3, 30, 300, or 3,000 μA/cm², with a constant magnetic field intensity of 2.2 G. Zwingelberg et al. (1993) reported on an in vivo exposure/in vitro assay in which female Wistar rats were exposed to a continuous 50-Hz EMF with an electric field intensity of 50 kV/m and a magnetic field intensity of 300 G, for 24 hours a day over a period of either 7 or 28 days. Cultures were made of the peripheral lymphocytes taken from the rats both prior to ELF EMF exposure and then again 7 days after exposure to the ELF EMF. The authors reported finding no significant increase in the frequency of SCEs between the exposed cells and the pre-exposed control cells. Antonopoloulas,
Yang, Stamm, Heller, and Obe (1995) reported finding no significant increase in the number of SCEs in cultured human lymphocytes exposed to 50-Hz ELF EMF for periods of 48, 52, 56, 60, 64, or 68 hours as compared to human lymphocytes not exposed to the ELF EMF for the same periods. The intensity of the magnetic field component was 5 G and the electric field induced in the cultures by the magnetic field was determined to be 0.005 V/m.

Reese, Jostes, and Frazier (1988) reported finding no significant differences in the frequency of DNA single strand breaks (SSB) in cultured Chinese hamster ovary (CHO) cells that were either exposed or not exposed to 60-Hz magnetic field intensities of 1 or 20 G, or to electric field intensities of 1 or 38 V/m, or to a combined magnetic and electric fields of 20 G and 38 V/m respectively, for a period of 1 hour. Fiorani et al. (1992) reported finding no increase in the frequency of DNA SSB in cultured human tumor cell line #562 exposed to 50-Hz electric fields of varying intensities (0.2 to 20 KV/m) or to magnetic fields of varying intensities (0.002 to 2 G), or to a combination of electric and magnetic field of varying intensities, for a period of 1, 4, 6, 2 or 24 hours. Fairbairn and O'Neill (1994) reported finding no significant increase in the frequency of DNA SSB in cultured HL-60 cells, Raji cells, Hela cells, or human lymphocytes that were exposed to a pulsed (3 msec.) EMF with a magnetic field component of 50 G, for varying periods of time.
Fiorio, Morichetti, Vellosi, and Bronzetti (1993) reported finding no significant increase in the frequency of HGPRT mutants in Chinese hamster V79 cell cultures that were exposed to a continuous 50-Hz ELF EMF with a magnetic field component of 2 G, for periods of 2, 5, 7, or 10 days.

Tabrah, Mower, Batkin, and Greenwood (1994) reported finding no significant increase in the number of revertant colonies of strain TA100 cells (Salmonella typhimurium) that were exposed to ELF EMF for a period of 48 hours. The 60-Hz ELF EMF had an electric field intensity of 21 mV/cm, an electric current density of 0.21 μA/cm², and a magnetic field intensity of 2 G. However, the same authors did report a significant increase (14%) in the number of revertant colonies of TA100 cells that were exposed to the chemical azide coupled with ELF EMF exposure as compared to TA100 cells treated with azide but not exposed to the ELF EMF.

Table 3 presents a summary of the in vitro studies reviewed in this paper on the mutagenicity of ELF EMF.

**ELF EMFs: Mutagenicity In Vivo**

The number of in vivo studies that have investigated the mutagenicity of ELF EMF are relatively few in number compared to the number of in vitro studies. In regard to in vivo studies, Haider et al. (1994) reported finding a significant increase in the frequency of MN in mother pollen cells from the Tradescantia plant that were exposed to ELF EMF from radio broadcasting
### Table 3

**Summary of In Vitro Studies on ELF EMF Mutagenicity**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism/Cell Type</th>
<th>Exp. Time</th>
<th>ELF EMF</th>
<th>End-point</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nordenson et al.</td>
<td>human/lymphocytes</td>
<td>(10)3 µs sparks</td>
<td>(EC) 1 mA/cm² OR (EF) 2.5 kV/cm (EF) 3.0 kV/cm (EF) 3.5 kV/cm</td>
<td>CA</td>
<td>-</td>
</tr>
<tr>
<td>d'Ambrosio et al.</td>
<td>bovine/lymphocytes</td>
<td>48/72 h</td>
<td>(EC) 2.4 µA/cm²</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>Cohen et al.</td>
<td>human/lymphocytes</td>
<td>69 h</td>
<td>(EC) 30 µA/cm² (MF) 1 or 2 G</td>
<td>CA</td>
<td>-</td>
</tr>
<tr>
<td>Cohen et al.</td>
<td>human/lymphocytes</td>
<td>69 h</td>
<td>(EC) 30 µA/cm² (MF) 1 or 2 G</td>
<td>SCE</td>
<td>-</td>
</tr>
<tr>
<td>Takahashi et al.</td>
<td>Chinese hamster/V79</td>
<td>24 h</td>
<td>PEMF 25 µs pulses (MF) 1.8-25 G</td>
<td>SCE</td>
<td>-</td>
</tr>
<tr>
<td>Reese et al.</td>
<td>CHO</td>
<td>1 h</td>
<td>(EF) 1 or 38 V/m (MF) 1 or 20 G</td>
<td>SSB</td>
<td>-</td>
</tr>
<tr>
<td>Rosenthal et al.</td>
<td>human/lymphocytes</td>
<td>48/72 h</td>
<td>(MF) 50 G OR (MF) 75 G &amp; MNU or TRN</td>
<td>CA</td>
<td>-</td>
</tr>
<tr>
<td>Garcia-Sagredo et al.</td>
<td>human/lymphocytes</td>
<td>48 h</td>
<td>PEMF 26 µs pulses (MF) 10/20/40 G</td>
<td>SCE</td>
<td>-</td>
</tr>
<tr>
<td>Garcia-Sagredo et al.</td>
<td>human/lymphocytes</td>
<td>24 h</td>
<td>PEMF 26 µs pulses (MF) 10/20/40 G</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>Reference</td>
<td>Organism/Cell Type</td>
<td>Exp. Time</td>
<td>ELF EMF</td>
<td>End-point</td>
<td>Effect</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------------</td>
<td>-----------</td>
<td>-----------------</td>
<td>-----------</td>
<td>--------</td>
</tr>
<tr>
<td>Khalil et al.</td>
<td>human/lymphocytes</td>
<td>24/48/or 72 h</td>
<td>PEMF-10 ms pulses (MF) 10.5 G</td>
<td>SCE</td>
<td></td>
</tr>
<tr>
<td>(1991)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Livingston et al.</td>
<td>human/lymphocytes</td>
<td>72 h</td>
<td>(MF) 2.2 G</td>
<td>SCE</td>
<td></td>
</tr>
<tr>
<td>(1991)</td>
<td>or CHO</td>
<td></td>
<td>(EC) 3/30/300/ or 3000 µA/cm²</td>
<td>MN</td>
<td></td>
</tr>
<tr>
<td>Fiorani et al.</td>
<td>human/tumor cell</td>
<td>1/4/6/or 24 h</td>
<td>(EF) 0.2-20 kV/m (MF) 0.002-2 G</td>
<td>SSB</td>
<td></td>
</tr>
<tr>
<td>(1992)</td>
<td>line #562</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fiorio et al.</td>
<td>Chinese hamster/ V79</td>
<td>2/5/7/or 10 days</td>
<td>(MF) 2 G</td>
<td>HGPRT</td>
<td></td>
</tr>
<tr>
<td>(1993)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fairbairn et al.</td>
<td>HL-60 cells, Raji cells, Hela cells, or human lymphocytes</td>
<td>24 h</td>
<td>(MF) 50 G</td>
<td>SSB</td>
<td></td>
</tr>
<tr>
<td>(1994)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tabrah et al.</td>
<td>Salmonella typhimurium/ TA 100 cells</td>
<td>48 h</td>
<td>(EF) 21 mV/cm revert.</td>
<td>(EC) 0.21 µA/cm² colonies</td>
<td></td>
</tr>
<tr>
<td>(1994)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

++ = positive results and statistically significant
- = negative results; no difference between treated and controls
CA = chromosome aberrations
SCE = sister chromatid exchange
MN = micronucleus
HGPRT = a specific mutation locus
SSB = single strand breaks
(EF) = electric field intensity
(EC) = electric current density
(MF) = magnetic field intensity
MNU = methylnitrosourea; TRN = trenimon

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antennae. Tradescantia plant cuttings with flower buds were placed at various distances away from five different radio broadcasting antennae and exposed to 10 to 21 MHz ELF EMF composed of varying electric field intensities of 1 to 170 V/m and varying magnetic field intensities of <0.01 to 0.11 A/m for a period of 30 hours. The authors reported finding a significant increase in the frequency of MN in the mother pollen cells of the Tradescantia plant at all exposure sites near the antennae. Ma, Chu, and Zu (1992) reported finding a 3-fold increase in the frequency of MN in mother pollen cells of the Tradescantia plant when plant cuttings with flower buds were placed inside a Helmholtz coil and exposed to a magnetic field intensity of 10 G for a period of 6 hours.

Nahas and Oraby (1989) reported finding a significant increase in the number of MN in polychromatic erythrocytes taken from male Swiss mice exposed to a 50-Hz EMF electric field intensity of 100, 170, 200 or 290 kV/m for a 24 hour period. The electric field intensity of 100 kV/m gave a slightly elevated but statistically insignificant increase in the number of MN over that of the controls. However, the electric field intensities of 170, 220, or 290 kV/m caused a significant increase in the frequency of MN in the polychromated erythrocytes.

Bauchinger, Hauf, Schmid, and Dresp (1981) reported on the frequency of CAs and SCEs in cultured human peripheral lymphocytes prepared from a group of 32 healthy male workers who had been
occupationally exposed to 50-Hz magnetic and electric fields. The control peripheral human lymphocytes were prepared from a second group of 22 healthy male workers who had not been occupationally exposed to EMFs. The authors reported finding no significant differences in the frequency of CAs or SCEs in the lymphocytes of the adult workers occupationally exposed to the EMFs as compared to the lymphocytes of the nonexposed workers.

Nordenson et al. (1984) reported finding no significant increase in the frequency of CAs in cultured human lymphocytes prepared from 20 men exposed to high levels of EMF while working in a 400-kV electric switchyard as compared to a group of 17 men who had not been exposed to high levels of EMF.

Diebolt (1978) reported finding no significant increase in the frequency of sex-linked recessive lethal mutations in the progeny resulting from the mating of ELF EMF exposed male fruit flies (Drosophila melanogaster) to nonexposed female fruit flies. The male fruit flies were exposed to an electric field intensity of 0.3 kV/cm or to a magnetic field intensity of 9,226 G for a 24 hour period, and then mated to a nonexposed female fly.

Table 4 presents a summary of the in vivo studies reviewed in this paper on the mutagenicity of ELF EMF.
## Table 4

### Summary of In Vivo Studies on ELF EMF Mutagenicity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism/Cell Type</th>
<th>Exp. Time</th>
<th>ELF EMF</th>
<th>End-point</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diebolt et al.</td>
<td>Fruit flies: Drosophila melanogaster</td>
<td>24 h</td>
<td>(MF) 9,226 G (EF) 0.3 kV/cm</td>
<td>recessive lethal</td>
<td>_</td>
</tr>
<tr>
<td>(1978)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bauchinger et al.</td>
<td>human/lymphocytes</td>
<td>&gt; 20 yrs.</td>
<td>MF and EF @ 380 kV switch-yard</td>
<td>SCE</td>
<td>_</td>
</tr>
<tr>
<td>(1981)</td>
<td></td>
<td></td>
<td></td>
<td>CA</td>
<td>_</td>
</tr>
<tr>
<td>Nordenson et al.</td>
<td>human/lymphocytes</td>
<td>1-8 wks</td>
<td>MF and EF @ 400 kV switch-yard</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>(1984)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nahas et al.</td>
<td>mice/polychromatic erythrocytes</td>
<td>24 h</td>
<td>(EF) 170/200/ or 290 kV/m</td>
<td>MN</td>
<td>++</td>
</tr>
<tr>
<td>(1989)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ma et al.</td>
<td>Tradescantia plant/pollen cells</td>
<td>6 h</td>
<td>(MF) 10 G</td>
<td>MN</td>
<td>3 fold increase</td>
</tr>
<tr>
<td>(1992)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haider et al.</td>
<td>Tradescantia plant/pollen cells</td>
<td>30 h</td>
<td>(EF) 1-170 V/m (MF) .01-.11 A/m.</td>
<td>MN</td>
<td>++</td>
</tr>
<tr>
<td>(1994)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

++ = positive results and statistically significant  
- = no effect seen; no difference between treated and controls  
(MF) = magnetic field intensity  
(EF) = electric field intensity  
SCE = sister chromatid exchange  
MN = micronucleus  
CA = chromosome aberration

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Replication of Cells

Effects of Cyclophosphamide on Cell Replication

It is now well understood that due to the drastic genotoxic effects that mutagenic and/or clastogenic agents can have on the chromosomes of cells, these agents frequently cause cell cycle delay and also frequently cause "cell death because a great number of mutant cells are apparently incapable of life" (Novotna et al., 1990, p342). In support of the above statement, Krishna et al. (1995b) reported that cyclophosphamide, at doses of 30 or 40 mg/kg b.w., were cytotoxic to bone marrow tissue of mice as evidenced by significantly reduced numbers of different bone marrow cell types found in the bone marrow of the treated mice. Jenderny et al. (1988) reported that the replication rate of mouse bone marrow cells was significantly reduced after male and female mice received a single i.p. injection of cyclophosphamide at a dose of 42 mg/kg b.w. and 56 mg/kg b.w., respectively. The same authors also reported that the replication rate of Chinese hamster bone marrow cells was significantly reduced in both sexes after male and female hamsters received a single i.p. injection of cyclophosphamide at a dose of 32 mg/kg b.w.. Krishna et al. (1991) reported that the percentage of rat bone marrow cells found to be in mitosis (mitotic index) was significantly reduced in male rats that received a single i.p. injection of cyclophosphamide at a dose of 40 mg/kg b.w., and also in female rats that received a 20 or 40
Novotna et al. (1990) reported that cyclophosphamide administered to chick embryos at a dose of 3 \( \mu g \) caused mitotic inhibition in the blood cells of the embryo, and at a dose of 6\( \mu g \), caused mitotic inhibition of cells in the face and limbs of the embryo. The authors concluded:

This (mitotic) inhibition is probably related, above all, to the mutagenic effect proper. It is assumed that the damage of DNA with S-dependent substances is largely repaired in the course of the G\(_2\) phase. This process consists of two steps. The first step is a mitotic delay, and the second one is removal and repair of DNA lesions. The delay of mitosis can be induced by an arbitrary clastogen. Generally, the greater the clastogenic potential of a substance, the longer the G\(_2\) phase and consequently the entire cell cycle.

(Novotna et al., 1990, p347)

Table 5 presents a summary of the effects of cyclophosphamide on the replication of cells.

**Effects of ELF EMF on Cell Replication**

In regards to the effects of ELF EMFs on the replication of cells, several research studies have reported finding no such effects. Miller et al. (1976) reported that exposure of Fava bean (Vicia faba) root tips (in medium) to an electric field intensity of 10 V/m and an electric current density of 0.1 A/m\(^2\), or to a magnetic field intensity of 17, 500, or 5000 G for periods of 1 hour, 1 day, or 2 days, caused no change in the mitotic index of the root tip cells. Inoue et al. (1985) reported that exposure of the Fava bean (Vicia faba) in medium, to an electric field intensity of 200, 290, or 360 V/m
Table 5

Summary of Effects of Cyclophosphamide on Cell Replication

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism/Cell Type</th>
<th>Dose(CP)</th>
<th>End-point</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jenderny et al.</td>
<td>mouse/bone marrow cells</td>
<td>42 or 56 mg/kg</td>
<td>cell replication rate</td>
<td>++</td>
</tr>
<tr>
<td>(1988)</td>
<td>Chinese hamster/bone marrow cells</td>
<td>32 mg/kg</td>
<td>cell replication rate</td>
<td>++</td>
</tr>
<tr>
<td>Novotna et al.</td>
<td>chick embryo/blood cells</td>
<td>3μ gram</td>
<td>cell replication rate</td>
<td>+</td>
</tr>
<tr>
<td>(1990)</td>
<td>chick embryo/face &amp; limb cells</td>
<td>6μ gram</td>
<td>cell replication rate</td>
<td>+</td>
</tr>
<tr>
<td>Krishna et al.</td>
<td>male rat/bone marrow cells</td>
<td>40 mg/kg</td>
<td>mitotic index</td>
<td>++</td>
</tr>
<tr>
<td>(1991)</td>
<td>female rat/bone marrow cells</td>
<td>20 or 40 mg/kg</td>
<td>mitotic index</td>
<td>++</td>
</tr>
<tr>
<td>Krishna et al.</td>
<td>mice/bone marrow cells</td>
<td>30 or 40 mg/kg</td>
<td>mitotic index</td>
<td>++</td>
</tr>
<tr>
<td>(1995)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

++ = statistically significant inhibition of cell replication rate/mitotic index  
+ = inhibition of cell replication rate but not statistically significant  

caused no change in the mitotic index of the root tip cells. Brulfert et al. (1985) reported that exposure of pea (Pisum sativum) root tips in medium to an electric field intensity of 430 V/m and an electric current density of 3 mA/cm², for a period of 24 or 48 hours, caused no change in the mitotic index of the root tip cells. Benz et al. (1986) reported that mice exposed to an
electric field intensity of 15 kV/m or 50 kV/m combined with a magnetic field intensity of 3 or 10 G respectively, for periods of 1 to 28 days, caused no significant effect on the average length of the cell cycle. Cohen et al. (1986a; 1986b) reported that exposure of cultured human peripheral lymphocytes (HPLs) to an electric current density of 30μA/cm² and a magnetic field intensity of either 1 or 2 G for 69 hours, had no effect on the replication rate of the exposed cells. Takahashi et al. (1987) reported that exposure of cultured CHO cells to PEMF with varying magnetic field intensities of 1.8 to 25 G for a 24 hour period, had no significant effect on the mitotic index of the cultured CHO cells. Livingston et al. (1991) reported that exposure of cultured HPLs to an electric current density of 3, 30, 300, or 3000 μA/cm², combined with a magnetic field intensity of 2.2 G for a period of 72 hours, had no effect on the mitotic index of the exposed cells. The same authors also reported that cultured CHO cells exposed to the same ELF EMF intensities as the HPLs, showed no significant change in the CHO cell's mitotic index. Zwingleberg et al. (1993) reported that exposure of rats to an electric field intensity of 50 kV/m and a magnetic field intensity of 300 G for 24 hours, did not result in a significant change in either the mitotic index or the proliferation index in the peripheral lymphocytes taken from the animals. Fiorani et al. (1992) reported that exposure of cells from the human tumor cell line #562 to varying electric field intensities of 0.2 to 20 kV/m, or to varying magnetic field intensities of 0.002 to 2.0 G, or to a combination of
these electric and magnetic intensities, caused no significant change in the exposed cell's replication rate.

In contrast to the aforementioned studies, there are also a few research studies that report finding ELF EMF effects on the mitotic index and length of the cell cycle of cells exposed to the ELF EMFs. Robertson et al. (1981) reported that exposure of pea (Pisum sativum) root tips in medium to electric field intensities of 140 V/m or 490 V/m resulted in an inhibition of the mitotic index of the root tip cells with the electric field intensity of 490 V/m. Conti et al. (1983) reported that varying magnetic field intensities from 23 to 65 G inhibited the blastogenesis of HPLs stimulated in vitro by the mitogens PHA and Con A. The authors speculated that since Ca^{++} are involved in the control of lymphocyte proliferation, the magnetic fields somehow alter Ca^{++} influxes across the membrane of the cells resulting in inhibition the blastogenesis of HPLs. Brulfert et al. (1985) reported that exposure of pea (Pisum sativum) root tips in medium to an electric field intensity of 430 V/m and an electric current density of 3 mA/cm², for 24 or 48 hours, caused the duration of the cell cycle to increase by 10%, which correlates with a reduction of the normal cell replication rate. Mooney et al. (1986) reported that the blastogenic response of the mitogen (PHA) stimulated peripheral blood mononuclear cells, was significantly inhibited by exposure to a magnetic field intensity of 45 G for a period of 72 hours, but not for an exposure period of 12 hours. Khalil et al. (1991) reported that
exposure of cultured HPLs to PEMF with a magnetic field intensity of 10.5 G for periods of 24, 48, or 72 hours, resulted in a significant suppression of the mitotic index of the cells. In addition, the cell cycle progression was significantly decreased as a result of the 72 hour exposure period. Conti et al. (1985) reported that the mitogens PHA and PMA caused an increase in the influx of Ca$^{++}$ into HPLs and the replication rate of the HPLs was increased. However when the mitogen stimulated HPLs were treated with verapamil, a well known Ca$^{++}$ blocker, the influx of Ca$^{++}$ was reduced and so was the replication rate of the HPLs. When the mitogen stimulated HPLs were treated with verapamil and also exposed to a magnetic field intensity of 60 G, the Ca$^{++}$ influx and the replication rate of the HPLs were reduced even further. The authors speculate that the magnetic fields may affect the movement of Ca$^{++}$ across the HPL plasma membrane resulting in inhibition of the HPLs replication rate.

Table 6 presents a summary of the effects of ELF EMF on the replication of cells.

**Summary**

It is apparent from the research studies reviewed in this paper that cyclophosphamide is highly mutagenic to several different types of cells as shown in the results of several in vitro and in vivo research studies that investigated the mutagenicity of the drug. It is also known with certainty
Table 6

Summary of Effects of ELF EMF on Cell Replication

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism/ Cell Type</th>
<th>ELF EMF</th>
<th>End-point</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller et al. (1976)</td>
<td>Fava bean/ root tip cells</td>
<td>(EC) 0.1 A/m² (EF) 10 V/m OR (MF) 17/500/500/or 5000 G</td>
<td>mitotic index</td>
<td>_</td>
</tr>
<tr>
<td>Inoue et al. (1985)</td>
<td>Fava bean/ root tip cells</td>
<td>(EF) 200/290 or 360 V/m</td>
<td>mitotic index</td>
<td>_</td>
</tr>
<tr>
<td>Brulfert et al. (1985)</td>
<td>pea/root tip cells</td>
<td>(EF) 430 V/m (EC) 3 mA/cm²</td>
<td>mitotic index</td>
<td>_</td>
</tr>
<tr>
<td>Benz et al. (Abstract) (1986)</td>
<td>mice/bone marrow cells</td>
<td>(EF) 15 kV/m (MF) 3 G or (EF) 50 kV/m (MF) 10 G</td>
<td>cell cycle time</td>
<td>_</td>
</tr>
<tr>
<td>Cohen et al. (1986a) (1986b)</td>
<td>human/ lymphocytes</td>
<td>(EC) 30 μA/cm² (MF) 1 or 2 G</td>
<td>cell replication</td>
<td>rate</td>
</tr>
<tr>
<td>Takahashi et al. (1987)</td>
<td>CHO</td>
<td>(MF) 1.8-25 G</td>
<td>mitotic index</td>
<td>_</td>
</tr>
<tr>
<td>Livingston et al. (1991)</td>
<td>human/ lymphocytes or CHO</td>
<td>(MF) 2.2 G (EC) 3/30/300/ or 3000 μA/cm²</td>
<td>mitotic index</td>
<td>_</td>
</tr>
<tr>
<td>Zwingleberg et al. (1993)</td>
<td>rat/ lymphocytes</td>
<td>(EF) 50 kV/m (MF) 300 G</td>
<td>mitotic index</td>
<td>_</td>
</tr>
<tr>
<td>Fiorani et al. (1992)</td>
<td>human/tumor cell line #562</td>
<td>(EF) 0.2-2 kV/m (MF) .002-2.0 G</td>
<td>cell replication</td>
<td>rate</td>
</tr>
<tr>
<td>Reference</td>
<td>Organism/Cell Type</td>
<td>ELF EMF</td>
<td>End-point</td>
<td>Effect</td>
</tr>
<tr>
<td>--------------------</td>
<td>------------------------</td>
<td>------------------------</td>
<td>------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Robertson et al. (1981)</td>
<td>pea/root tips</td>
<td>(EF) 140 or 490 V/m</td>
<td>mitotic index</td>
<td>+</td>
</tr>
<tr>
<td>Conti et al. (1983)</td>
<td>human/lymphocytes</td>
<td>(MF) 23-65 G</td>
<td>blastogenesis of lymphocytes with themitogens PHA or Con A</td>
<td>+</td>
</tr>
<tr>
<td>Brulfert et al. (1985)</td>
<td>pea/root tips</td>
<td>(EF) 430 kV/m (EC) 3 mA/cm²</td>
<td>cell cycle time</td>
<td>+</td>
</tr>
<tr>
<td>Conti et al. (1985)</td>
<td>human/lymphocytes</td>
<td>(MF) 60 G</td>
<td>blastogenesis of lymphocytes with the mitogens PHA or PMA</td>
<td>+</td>
</tr>
<tr>
<td>Mooney et al. (1986)</td>
<td>human/peripheral blood mononuclear cells</td>
<td>(MF) 45 G</td>
<td>blastogenesis of PBMC with the mitogen PHA</td>
<td>++</td>
</tr>
<tr>
<td>Khalil et al. (1991)</td>
<td>human/lymphocytes</td>
<td>(MF) 10.5 G</td>
<td>mitotic index cell cycle time</td>
<td>++</td>
</tr>
</tbody>
</table>

++ = statistically significant inhibition
+ = inhibition seen but not statistically significant
- = no effect seen
(MF) = magnetic field intensity
(EF) = electric field intensity
(EC) = electric current density

that cyclophosphamide is carcinogenic to humans and animals (IARC, 1981; 1982; 1987) which is in accord with the latest theories on chromosome
damage and the carcinogenic process. It is also known, with some degree of certainty, that the genotoxicity of cyclophosphamide contributes to an inhibition of the normal rate of cell replication in the cells exposed to cyclophosphamide.

In regards to ELF EMF, the vast majority of the in vitro research studies have reported ELF EMFs not to be mutagenic. However, there are a small number of in vivo research studies that reported ELF EMFs may be mutagenic under certain conditions. In addition, as of yet, there are no controlled research laboratory studies that provide clear, hard evidence that ELF EMFs are carcinogenic to humans or animals. In regards to the effects of ELF EMFs on the replication of cells, while there are a few studies that report that ELF EMFs are inhibitory to the replication of cells, the largest number of research studies report that ELF EMFs are not inhibitory to normal cell replication rates.
CHAPTER III

DESIGN OF STUDY

Introduction

The present study is concerned with learning more about the mutagenic and/or clastogenic potential of ELF EMFs. Since carcinogenesis involves damage to the integrity of chromosomes as a prerequisite to the formation of transformed cancer cells, it is important to know what mutagenic and/or clastogenic potential ELF EMFs may have on living cells. It is also important to know if ELF EMFs can enhance the mutagenic and/or clastogenic potential of known carcinogens such as cyclophosphamide, thereby enhancing the carcinogen's potential to initiate carcinogenesis.

Two hypothesis were tested in the present study. The first hypothesis tested was that mice exposed to a 7.3 G magnetic field would display a significantly increased frequency of mutagenic and/or clastogenic events in their bone marrow cells as compared to control animals. The second hypothesis tested was that mice treated with cyclophosphamide and exposed to a 7.3 G magnetic field would display a significantly greater number of mutagenic and/or clastogenic events in their bone marrow cells as compared to mice treated with cyclophosphamide only.
Experimental Animals

Male and female CD-1 strain mice were obtained from Charles River Laboratories, Wilmington, Massachusetts. Upon receipt of the mice, the animals were housed in plastic cages (11" x 7' x 5") with 6 mice of the same sex per cage. The animals were acclimated for a period of 16 days prior to the start of the study. During the acclimatization period, light periodicity was controlled at 12 hours of artificial light alternated with 12 hours of darkness. The temperature was thermostatically controlled at 72 degrees fahrenheit, and the animals were allowed food and water ad libitum.

During the 24 hour period of exposure to ELF EMF, or during the 24 hour period of sham exposures, six male mice were housed within a single plastic cage and six female mice were housed within a second plastic cage, and both plastic cages were positioned, side by side, at the internal center of the Helmholtz coil. Animals were allowed food and water ad libitum during either the ELF EMF or sham exposure periods.

The group of male mice used in the present study had a mean body mass of 36.0 g, with individual body mass ranging from 30.0 g to 46.0 g. The male mice used in the study were 8 weeks of age at the time of exposures. The group of female animals used in the study had a mean body mass of 27.5 g, with individual body mass ranging from 25.0 to 30.0 g. The female animals used in the study were 10 weeks of age at the time of
exposures. At the time the animals were ordered from Charles River Laboratories, the decision was made to use male animals that were two weeks younger than the female animals due to the fact that male CD-1 mice will grow more rapidly than the female animals.

**Chemicals Used**

Cyclophosphamide was selected for the present study because of its known mutagenic, clastogenic, and carcinogenic potential (Povirk and Shuker, 1994). The cyclophosphamide (No. 21870-7) was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. The drug was dissolved in isotonic saline (0.9% NaCl) and concentrations of cyclophosphamide solution were prepared which allowed for doses of the drug to be administered at 5, 25, or 50 mg/kg b.w..

Colchicine was used in the present study because of its ability to arrest cells at the metaphase stage of the cell's replication cycle (mitosis). The cells in the arrested metaphase stage can then be examined under the light microscope for chromosome damage. The colchicine was obtained from the Sigma Chemical Company, St. Louis, Missouri. The drug was dissolved in isotonic saline (0.9% NaCl) and a concentration of the colchicine solution was prepared which allowed for a dose of colchicine to be administered at 4 mg/kg b.w.
**ELF EMF Exposure Apparatus**

The Helmholtz coil used in this study consisted of two horizontal loops with an equidistant spacing of 13 inches between the two loops. Each loop measured 26 inches in internal diameter (I.D.) and was constructed from a 26 inch (I.D.) aluminum hoop with insulated 14 gauge copper wire wound circumferentially around the aluminum hoop, 63 times. Each of the two plastic animal cages that housed six male or six female mice during the ELF EMF or sham exposures, had physical dimensions of 11"x7"x5". The two plastic cages were positioned side-by-side within the internal center of the Helmholtz coil and were supported by a wooden stand in which aluminum nails were used in the construction (see Figure 3).

The electrical circuit that energized the two coil loops consisted of the following elements: 110 V. alternating current from an electrical outlet flowed into a 145 V. variable-adjust step-up transformer (Staco Variac, No. GJ901V), and then directly into a step-down transformer (General Electric-3KVA, No. 9T51B53). From the step-down transformer the current flowed through a solid state cycle repeat timer (Artisan Controls Company, Model No. 4610A-6-2-A) and then sequentially through the two coil loops and returned to the wall outlet. The solid state cycle repeat timer pulsed the electric current through the coil loops in 19 second pulses: Current flowed through the Helmholtz coil loops for a period of 19 seconds followed by 19
seconds where there was no flow of electrical current through the coil loops, with the cycle repeating ad infinitum. An electrical meter (Fluke-45) was connected in series with the circuit in order to monitor the circuit amperage, and an electrical meter (Fluke-45) was connected across the circuit to monitor the circuit voltage (see Appendix A).

The intensity of the magnetic or electric field produced by the energized Helmholtz coil was monitored with a meter (Polytek Manufacturing Incorporated, Scotia, N.Y., Model No. 100-7-8) that had the capacity to monitor either type of field. During the monitoring of magnetic field intensities, the meter required the use of a remote monitor probe.
attached to the body of the instrument by a cord. The dimensions of the
monitor probe were small enough so as to allow for magnetic field
monitoring at each of the four interior corners as well as the middle region
of each plastic cage. The magnetic field intensity readings at each of the 6
alternative locations within each plastic cage were essentially the same (see
Appendix B). The magnetic field intensities were also found to be essentially
the same inside of either plastic cage, at any single period of time. During
this study, the mice were exposed to a magnetic field intensity of 7.3 G when
the coil was electrically energized, or to an ambient magnetic field intensity
of 0.015 mG when the coil was not energized during sham exposures.

The electric field intensity produced by the energized Helmholtz coil
was monitored using the body of the instrument instead of the monitor
probe. Since the body of the instrument was too large to allow for it to be
placed inside the plastic animal cages, electric field intensity readings were
taken with the instrument resting on top of the plastic cages. For the
present study, when the Helmholtz coil was electrically energized, the meter
recorded an electric field intensity of 17.0 V/m. Through an oversight,
ambient electric field intensities were not recorded when the Helmholtz coil
was not energized. Although not quantified, logic would dictate that the
magnitude of the electric field would be negligible when the Helmholtz coil
is not energized as during sham exposures.
Experimental Design

Ninety-six mice were used in the present study. Mice were assigned to one of eight different groups and each mouse was assigned a number from 1 to 96. Each of the 8 groups of mice contained 6 male and 6 female animals. All animals had their body mass measured on a triple beam balance prior to receiving any intraperitoneal (i.p.) injections and being placed within an energized or nonenergized Helmholtz coil.

Two groups of mice received a single i.p. injection of vehicle only (0.9% saline solution without cyclophosphamide); two groups of mice received a single i.p. injection of cyclophosphamide at a dose of 5 mg/kg b.w.; two groups of mice received a single i.p. injection of cyclophosphamide at a dose of 25 mg/kg b.w.; and two groups of mice received a single i.p. injection of cyclophosphamide at a dose of 50 mg/kg b.w.

One group of mice that received the injection vehicle, one group that received a 5 mg/kg b.w. dose of cyclophosphamide, one group that received a 25 mg/kg b.w. dose of cyclophosphamide, and one group that received a 50 mg/kg b.w. dose of cyclophosphamide, were each divided equally by sex and placed into two 11"x7"x5" plastic cages, with 6 males in one cage and 6 females in the other cage. The two plastic cages containing the animals were then placed within the energized Helmholtz coil and exposed to a magnetic field intensity of 7.3 G for a period of 24 hours. This was done sequentially.
for each of the four groups of mice (see Table 7).

Each of the remaining four groups of mice that had received either a single i.p. injection of vehicle or a 5, 25, or 50 mg/kg b.w. dose of cyclophosphamide, were also divided by sex placing 6 male mice in one plastic cage and 6 female mice in the other cage. The two plastic cages containing the animals were then placed within the same nonenergized Helmholtz coil and the animals were sham exposed for a period of 24 hours. This was done sequentially for each of the four groups of mice (see Table 7).

At 20 hours of ELF EMF or sham exposure, each animal was briefly removed from it's plastic cage within the Helmholtz coil, and administered a single i.p. injection of colchicine at a dose of 4 mg/kg b.w.. The animal was then immediately placed back into it's respective plastic cage within the the Helmholtz coil and received four more hours of either EMF or sham exposure.

After spending a total period of 24 hours within either an electrically energized or nonenergized Helmholtz coil, each animal was removed from it's cage, body mass measurements taken on a triple beam balance, and then sacrificed by cervical dislocation. The mid-torso region of the dead animal was then wet down with a 50% methanol solution, and using scissors, an incision was made through the skin circumferentially around the mid-torso region of the animal. The skin of the lower torso was then pulled down the lower torso until the muscles covering the femur bones were fully exposed.
Table 7

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Number of Mice</th>
<th>Numbers Assigned to Animals</th>
<th>CP Dose</th>
<th>ELF EMF Exposures</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>#1 to #12</td>
<td>5 mg/kg b.w.</td>
<td>(MF) 7.3 G</td>
<td>6M/6F</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>#13 to #24</td>
<td>25 mg/kg b.w.</td>
<td>(MF) 7.3 G</td>
<td>6M/6F</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>#25 to #36</td>
<td>50 mg/kg b.w.</td>
<td>(MF) 7.3 G</td>
<td>6M/6F</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>#37 to #48</td>
<td>0 mg/kg b.w.</td>
<td>(MF) 7.3 G</td>
<td>6M/6F</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>#49 to #60</td>
<td>5 mg/kg b.w.</td>
<td>sham exposed</td>
<td>6M/6F</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>#61 to #72</td>
<td>25 mg/kg b.w.</td>
<td>sham exposed</td>
<td>6M/6F</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>#73 to #84</td>
<td>50 mg/kg b.w.</td>
<td>sham exposed</td>
<td>6M/6F</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>#85 to #96</td>
<td>0 mg/kg b.w.</td>
<td>sham exposed</td>
<td>6M/6F</td>
</tr>
</tbody>
</table>

(MF) = magnetic field intensity
M = male
F = female

With the femurs still attached to the animal, the muscle covering the two femurs was snipped away with scissors.

Both femurs were removed from the animal carcass by using small scissors to cut through the cartilage capsule at both articulating ends of the femur. After both femurs were removed from the animal, residual muscle fibers and residual cartilage was carefully removed from the femur, and the femur was rubbed clean using a paper tissue. The hard bone articulating...
processes at both ends of the femur were then carefully cut with scissors. Several, very thin sequential cuts were made through the hardbone processes until the red colored soft bone would first begin to appear through the cut end of the bone process.

Using a 26 gauge hypodermic needle attached to a 3 ml syringe filled with Hanks Buffered Saline Solution (HBSS), both trimmed ends of the femur were punctured with the needle. Starting at one end of the femur, pressure was applied to the syringe until its needle tip had passed through the denser region of the soft bone and entered the medulary canal of the femur. The needle was then retracted from the femur and the same procedure was performed at the second end of the femur. However, the needle was not retracted from second end of the femur until the bone marrow cells had been flushed out the opposite punctured end of the femur using 3 ml. of HBSS under the pressure of the syringe plunger.

**Slide Preparation**

The slides used for scoring chromosome/chromatid aberrations were prepared in the following manner:

1. Cells flushed from femurs with HBSS, are centrifuged at 1000 revolutions per minute (RPMs) for 10 minutes.

2. Pipette off excess HBSS leaving a small volume of HBSS over the pelletized cells.
3. Resuspend cells by vigorously tapping bottom of centrifuge tube.

4. Add 7 ml of 0.65% KCl, at 37° Celsius, to tube with cells.

5. Place tube with cells in 37° Celsius water bath for 15 minutes.

6. Centrifuge tube with cells at 1000 RPM for 10 minutes.

7. Pipette off supernatant leaving a small volume of 0.65% KCl covering the cell pellet. Try not to disturb the cell pellet when pipeting off the 0.65% KCl.

8. Resuspend cells by vigorously tapping bottom of tube.

9. Add 3 ml of fresh cell fixative (3 parts Absolute Methanol and 1 part Glacial Acetic Acid). Invert tube 3 times.

10. Centrifuge tube and cells at 1000 RPM for 10 minutes.

11. Pipette off supernatant leaving a small amount of fixative covering the cell pellet.

12. Resuspend cells by vigorously tapping bottom of centrifuge tube. Cell suspension should be visibly turbid, but not a milky white. If milky white, add a little more fixative.

13. Pull up turbid cell suspension into a pipette.

14. At arms length distance away from a slide previously chilled in the refrigerator, drop three drops of cell suspension along the length of the slide. **BE SURE TO WRITE ASSIGNED ANIMAL NUMBER ON SLIDE WITH PENCIL.**

15. Place slides under flame to burn off alcohol in fixative.
16. Dry slides for 48 hours.

17. Immerse slides in Giemsa staining solution for 5 minutes.

18. Rinse slides in tap water tray by 10 up and down movements of the slide tray.

19. Allow slides to dry and coverslip slides when dry.

Scoring Chromosome Aberrations

After preparation of the Giemsa stained metaphase chromosome spreads on glass slides, the animal number that had been assigned each animal and written on each slide, was covered by non-transparent tape, and each slide was then assigned a 5 digit random number to ensure a blind scoring of the slide. Records of assigned animal numbers and corresponding assigned random numbers were kept by an individual who was not involved in the scoring of the slides for chromosome damage. Before scoring any slides, all slides were placed in a slide box in increasing order of the random numbers to further randomize the selection of slides during the scoring process.

One hundred suitable metaphase chromosome spreads were scored per animal. In selecting chromosome spreads judged suitable for scoring, it was required that the chromosomes were sufficiently spread out so as not to be compacted together with chromosomes overlapping each other. Another criteria for selecting suitable chromosome spreads was that the chromosomes
could not be overly condensed making the chromosomes appear very thick and short thereby not allowing for definitive scoring of aberrations. Another criteria for selecting suitable chromosome spreads was that a spread could not have staining artifact or other types of debris obscuring the structure of any of the chromosomes. In addition, in a study such as this, many chromosome spreads will not display the normal diploid number of chromosomes (40 for a mouse), since some of the chromosomes will be lost from the spread when the cell bursts and the chromosomes are released to the surface of the slide. Recognizing this fact, in this study, a chromosome spread was scored as normal only when no chromosome aberrations were found in the spread, and there was a total of at least 37 chromosomes present in the spread (see Figure 4).

Metaphase chromosome spreads were scored for the following four different types of chromosome aberrations:

1. Chromatid breaks/chromatid fragments in which there is visible damage to only one chromatid resulting in a break, a gap, or a displaced section of chromatid (see Figure 5).

2. Isochromatid breaks /bi-chromatid fragments in which there is visible damage to both attached chromatids resulting in breaks, gaps, or displaced sections of both chromatids (see Figure 6).

3. Chromatid exchanges /rearrangements (see Figure 7).

4. Chromosomal rings (see Figure 7).
Figure 4. Normal Chromosome Spread.

Figure 5. Chromatid Break and Chromatid Fragment.
Figure 6. Isochromatid Breaks and Bichromatid Fragments.

Figure 7. Chromosome Exchanges and Chromosome Rings.
Statistical Analysis

Six different endpoints were looked at in the present study: (1) Number of chromatid breaks/chromatid fragments per 100 cells; (2) number of isochromatid breaks/bichromatid fragments per 100 cells; (3) number of exchanges/rearrangements per 100 cells; (4) number of rings per 100 cells; (5) total number of aberrant cells per 100 cells; and (6) the number of highly damaged (shattered) cells per 100 cells. In this study highly damaged cells were defined as cells displaying chromosome spreads with greater than 15 chromatid and/or isochromatid breaks/fragments (see Figure 8).

Figure 8. Highly Damaged Cells: > 15 Breaks/ Fragments.
Statistical analysis was performed in order to determine if the difference in the frequency of the chromosome aberration endpoints that occurred between selected groups of mice was significant. Among the types of statistical tests used to test for significance were the Chi-square test, Fisher's exact test, Student's t-test, and a Conditional Poisson test. In addition, other testing was done in the context of a logistic or Poisson regression model with appropriate allowances for overdispersion. In each instance, the statistical test was selected to be consistent with observed features of the data, such as very small number of counts or overdispersion. Significance was determined at probability values less than 5 % (P<0.05).
CHAPTER IV

RESULTS

Body Mass Measurements

Table 8 displays the mean body mass measurements of the groups of male and female mice used in this study. Body mass measurements were taken prior to each animal receiving an i.p. injection of vehicle or cyclophosphamide and again after 24 hours of ELF EMF or sham exposure.

Each group of female or male mice that received either a 5, 25, or 50 mg/kg dose of cyclophosphamide showed significant loss of body mass (P<0.05) as compared to groups of mice of the same sex that received the same ELF EMF or sham exposure, but had not received any cyclophosphamide. Of the two groups of female mice that received the injection vehicle only (0 mg/kg dose of cyclophosphamide) with one group exposed to ELF EMF and the other group sham exposed, the two groups showed no significant differences in body mass (P>0.05). Of the two groups of male mice that received the injection vehicle only (0 mg/kg dose of cyclophosphamide) with one group exposed to ELF EMF and the other group sham exposed, the two groups showed no significant differences in body mass (P>0.05) (see Table 8).
<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Sex</th>
<th>Mean Mass ±S.E. (g)</th>
<th>t value</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP EMF No.</td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 6 M</td>
<td>6</td>
<td>M</td>
<td>34.7±1.2</td>
<td>35.3±1.4</td>
<td>-2.00</td>
</tr>
<tr>
<td>0 - 6 F</td>
<td>6</td>
<td>F</td>
<td>26.0±0.7</td>
<td>26.2±0.7</td>
<td>-0.54</td>
</tr>
<tr>
<td>0 + 6 M</td>
<td>6</td>
<td>M</td>
<td>35.7±1.6</td>
<td>35.7±1.5</td>
<td>0.00</td>
</tr>
<tr>
<td>0 + 6 F</td>
<td>6</td>
<td>F</td>
<td>27.3±0.8</td>
<td>26.2±0.5</td>
<td>2.15</td>
</tr>
<tr>
<td>5 - 6 M</td>
<td>6</td>
<td>M</td>
<td>36.2±0.9</td>
<td>33.8±0.8</td>
<td>5.53</td>
</tr>
<tr>
<td>5 - 6 F</td>
<td>6</td>
<td>F</td>
<td>26.7±0.2</td>
<td>25.2±0.3</td>
<td>4.39</td>
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<tr>
<td>5 + 6 M</td>
<td>6</td>
<td>M</td>
<td>32.7±0.4</td>
<td>27.8±0.6</td>
<td>6.87</td>
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<tr>
<td>5 + 6 F</td>
<td>6</td>
<td>F</td>
<td>27.8±0.2</td>
<td>24.8±0.3</td>
<td>8.22</td>
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<tr>
<td>25 - 6 M</td>
<td>6</td>
<td>M</td>
<td>38.7±1.9</td>
<td>35.7±2.3</td>
<td>8.22</td>
</tr>
<tr>
<td>25 - 6 F</td>
<td>6</td>
<td>F</td>
<td>27.8±0.5</td>
<td>25.2±0.9</td>
<td>4.78</td>
</tr>
<tr>
<td>25 + 6 M</td>
<td>6</td>
<td>M</td>
<td>35.2±0.8</td>
<td>33.5±1.0</td>
<td>3.95</td>
</tr>
<tr>
<td>25 + 6 F</td>
<td>6</td>
<td>F</td>
<td>28.3±0.7</td>
<td>26.0±0.7</td>
<td>11.07</td>
</tr>
<tr>
<td>50 - 6 M</td>
<td>6</td>
<td>M</td>
<td>37.8±0.5</td>
<td>35.3±0.6</td>
<td>5.00</td>
</tr>
<tr>
<td>50 - 6 F</td>
<td>6</td>
<td>F</td>
<td>28.7±0.9</td>
<td>26.0±0.7</td>
<td>6.32</td>
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<tr>
<td>50 + 6 M</td>
<td>6</td>
<td>M</td>
<td>36.8±0.3</td>
<td>34.3±0.8</td>
<td>4.04</td>
</tr>
<tr>
<td>50 + 6 F</td>
<td>6</td>
<td>F</td>
<td>27.7±0.4</td>
<td>25.5±0.6</td>
<td>4.54</td>
</tr>
</tbody>
</table>

*Paired t-Test: Significant at P<0.05

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The results of the body mass measurements strongly suggest that EMF exposure at the field parameters used in this study was not a significant factor in the weight loss of the animals, and that animal weight loss was attributable to treatment of animals with cyclophosphamide.

Chromosome Aberrations

Table 9 / Figure 9, Table 10 / Figure 10, Table 11 / Figure 11, Table 12 / Figure 12, Table 13 / Figure 13, and Table 14 / Figure 14, respectively display the frequency of occurrence of the six endpoints looked at in this study: (1) Number of chromatid breaks (CB) and chromatid fragments (CF); (2) number of isochromatid breaks (IB) and bichromatid fragments (BF); (3) number of exchanges (E) and rearrangements (R); (4) number of rings; (5) number of highly damaged cells (HDC) whose metaphases displayed greater than 15 breaks and fragments; and (6) total number of aberrant metaphases (TAM). Within each table, the frequency of occurrence for the selected endpoint is displayed separately for the groups of male mice and for the groups of female mice for each of the eight different treatment scenarios used in this study.

Table 9 shows a nearly equal frequency of occurrence for CB/CF between the groups of male and female mice treated with injection vehicle only and then either exposed to ELF EMF or sham exposed. For the group of mice that received a 5 mg/kg b.w. dose of cyclophosphamide and then
sham exposed, the group of female mice showed a greater number of CB/CF with a larger fraction of animals displaying CB/CF than did the group of male mice. For the groups of mice that received a 5 mg/kg b.w. dose of cyclophosphamide and then exposed to ELF EMF, the group of male mice showed a slightly greater number of CB/CF with a slightly larger fraction of animals displaying CB/CF than did the group of female mice. For the groups of mice that received a 25 mg/kg b.w. dose of cyclophosphamide and then sham exposed, the group of male mice showed a greater number of CB/CF than did the group of female mice, although both the groups of female and male mice showed equivalent fractions of animals displaying CB/CF. For the groups of mice that received a 50 mg/kg b.w. dose of cyclophosphamide and then either sham exposed or exposed to ELF EMF, both groups of male mice showed a greater number of CB/CF than did the corresponding groups of female mice. However, using a conditional Poisson test for the groups of mice that received vehicle only, and a Poisson regression model (adjusting for overdispersion) for the groups of mice that received cyclophosphamide, there were no statistically significant differences found in the frequency of CB/CF between the male and female groups of animals for any of the eight possible treatment scenarios.

Figure 9 shows a graph of the combined frequencies of CB/CF for the combined male and female groups for each of the eight different possible treatment scenarios. Numerically, the combined frequencies of CB/CF for
Table 9

Number of Chromatid Breaks (CB) / Chromatid Fragments (CF)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CP</th>
<th>EMF</th>
<th>Sex</th>
<th>Number of Mice Analyzed (+)CB/CF</th>
<th>No. Cells Analyzed</th>
<th>No. of CB/CF</th>
<th>Mean No. per cell</th>
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<tr>
<td>0</td>
<td>-</td>
<td>M</td>
<td>6</td>
<td>0</td>
<td>600</td>
<td>3</td>
<td>.005</td>
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<tr>
<td>0</td>
<td>-</td>
<td>F</td>
<td>6</td>
<td>1</td>
<td>600</td>
<td>2</td>
<td>.003</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>M</td>
<td>6</td>
<td>2</td>
<td>600</td>
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<td>.003</td>
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<td>F</td>
<td>6</td>
<td>2</td>
<td>599*</td>
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<td>.005</td>
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<tr>
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<td>M</td>
<td>6</td>
<td>1</td>
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<td>1</td>
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<td>M</td>
<td>6</td>
<td>3</td>
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</tbody>
</table>

* Highly damaged cells were not included in cells analyzed for CB/CF

b Slides from one mouse were not scored due to poor readability of slides

c Slides from two mice were not scored due to poor readability of slides
Figure 9. Number of Chromatid Breaks/ Chromatid Fragments.
the combined groups of male/female mice that received either a 5, 25, or 50 mg/kg b.w. dose of cyclophosphamide and sham exposed, is greater than the combined frequencies of the corresponding male/female groups that received either a 5, 25, or 50 mg/kg b.w. dose of cyclophosphamide and exposed to ELF EMF.

Data were statistically analysed using a conditional Poisson test for the combined male/female groups of mice that received vehicle only, and a Poisson regression model (adjusting for overdispersion) for the combined male/female groups of mice that received either a 5, 25, or 50 mg/kg b.w. dose of cyclophosphamide. A statistically significant difference in the number of CB/CF was found only between the two combined male/female groups that received a 25 mg/kg b.w. dose of cyclophosphamide and then either sham exposed or exposed to ELF EMF (P=0.04).

Table 10 shows that for the groups of mice that received a 5 mg/kg b.w. dose of cyclophosphamide and then sham exposed, the group of female mice showed a greater number of IB/BF with a larger fraction of animals displaying IB/BF than did the group of male mice from the same treatment group. For the groups of mice that received a 25 mg/kg b.w. dose of cyclophosphamide and then sham exposed, the group of female mice showed a greater number of IB/BF with a larger fraction of animals displaying IB/BF than did the group of male mice from the same treatment group. For the groups of mice that received a 50 mg/kg b.w. dose of cyclophosphamide and
then either sham exposed or exposed to EMF, both groups of male mice showed a greater number of IB/BF than did the corresponding groups of female mice. However, using a conditional Poisson test for the groups of mice that received vehicle only, and a Poisson regression model (adjusting for overdispersion) for the groups of mice that received cyclophosphamide, there were no statistically significant differences found in the frequency of CB/CF between the male and female groups of animals for any of the eight possible treatment scenarios.

Figure 10 shows a graph of the combined frequencies of IB/BF for the combined male and female groups for each of the eight different possible treatment scenarios. Numerically, the combined frequencies of IB/BF for the male/female groups that received either vehicle, or a 5, 25, or 50 mg/kg b.w. dose of cyclophosphamide and sham exposed, is greater than the combined frequencies of the corresponding male/female groups that received either vehicle or a 5, 25, or 50 mg/kg b.w dose of cyclophosphamide and exposed to ELF EMF.

Data were statistically analysed using a conditional Poisson test for the combined male/female groups of mice that received vehicle only, and a Poisson regression model (adjusting for overdispersion) for the combined male/female groups of mice that received a 5, 25, or 50 mg/kg b.w. dose of cyclophosphamide. No statistically significant difference in the number of IB/BF was found between any two of the combined male/female groups that
Table 10

Number of Isochromatid Breaks (IB) / Bichromatid Fragments (BF)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CP</th>
<th>EMF</th>
<th>Sex</th>
<th>Number of Mice Analyzed (+) IB/BF</th>
<th>No. Cells Analyzed</th>
<th>No. of IB/BF</th>
<th>Mean No. per cell</th>
</tr>
</thead>
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<td>0</td>
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<td>2</td>
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<td>M</td>
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<td>1</td>
<td>.002</td>
</tr>
<tr>
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<td>F</td>
<td>6</td>
<td>0</td>
<td>599(^a)</td>
<td>0</td>
<td>.000</td>
</tr>
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<td>-</td>
<td>M</td>
<td>6</td>
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<td>600</td>
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<td>4</td>
<td>.007</td>
</tr>
<tr>
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<td>.003</td>
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<td>M</td>
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<td>427(^a)</td>
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<td>5</td>
<td>595(^a)</td>
<td>9</td>
<td>.015</td>
</tr>
</tbody>
</table>

\(^a\) Highly damaged cells were not included in cells scored for IB/BF
\(^b\) Slides from one mouse were not scored due to poor readability of slides
\(^c\) Slides from two mice were not scored due to poor readability of slides
Figure 10. Number of Isochromatid Breaks/ Bichromatid Fragments.
had similarly received either vehicle or a specified dose of cyclophosphamide and then either sham exposed or exposed to ELF EMF.

Table 11 shows that for the groups of mice that received a 25 mg/kg b.w. dose of cyclophosphamide and then sham exposed, the group of female mice showed a slightly greater number of E/R with a slightly larger fraction of mice displaying E/R than did the group of male mice. For the groups of mice that received a 50 mg/kg b.w. dose of cyclophosphamide and then either sham exposed or exposed to EMF, the male animals in both treatment groups showed a greater number of E/R than did the corresponding female groups. However, using a conditional Poisson test or a Poisson regression model (adjusting for overdispersion), no statistically significant differences were found in the frequency of E/R between the male and female groups of animals for any of the eight possible treatment scenarios.

Figure 11 shows a graph of the combined frequencies of E/R for the combined male/female groups for each of the eight different treatment scenarios used in the study. Numerically, the combined frequencies of E/R for the male/female groups that received either a 25, or 50 mg/kg b.w. dose of cyclophosphamide and sham exposed, is greater than the combined frequencies of the corresponding male/female groups that received either a 25, or 50 mg/kg b.w dose of cyclophosphamide and exposed to ELF EMF.

Data were statistically analysed using a conditional Poisson test or the Poisson regression model (adjusting for overdispersion) for the combined
### Table 11

**Number of Exchanges (E) / Rearrangements (R)**

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<thead>
<tr>
<th>Treatment CP</th>
<th>EMF</th>
<th>Sex</th>
<th>Number of Mice Analyzed (+)E/R</th>
<th>No. Cells Analyzed</th>
<th>No. of E/R</th>
<th>Mean No. per cell</th>
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</table>

<sup>a</sup> Slides from one mouse were not scored due to poor readability of slides

<sup>b</sup> Slides from two mice were not scored due to poor readability of slides

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Figure 11. Number of Exchanges/ Rearrangements.
male/female groups of mice that received either vehicle, or a 5, 25, or 50 mg/kg b.w. dose of cyclophosphamide. No statistically significant difference in the number of E/R was found between any two of the combined male/female groups that had similarly received either vehicle or a specified dose of cyclophosphamide and then either sham exposed or exposed to ELF EMF.

Table 12 shows that for the groups of mice that received a 50 mg/kg b.w. dose of cyclophosphamide and then either sham exposed or exposed to ELF EMF exposed, the groups of male mice in both treatment groups showed a greater number of chromosome rings than did the corresponding groups of female mice. The groups of male mice in both treatment groups also show a larger fraction of animals displaying the rings than do the groups of female mice in each of the respective treatment groups. However, using a conditional Poisson test or a Poisson regression model (adjusting for overdispersion), no statistically significant differences were found in the frequency of E/R between the male and female groups of animals for any of the eight possible treatment scenarios.

Figure 12 shows a graph of the combined frequencies of chromosome rings for the combined groups of male and female mice in each of the eight different treatment scenarios used in the study. Numerically, the combined frequencies of rings for the male/female groups that received either a, 25, or 50 mg/kg b.w. dose of cyclophosphamide and sham exposed, is greater than
Table 12

Number of Rings

<table>
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<th>Treatment</th>
<th>CP</th>
<th>EMF</th>
<th>Sex</th>
<th>Number of Mice Analyzed (+) Rings</th>
<th>No. Cells Analyzed</th>
<th>No. of Rings</th>
<th>Mean No. per cell</th>
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<td>.000</td>
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<td>F</td>
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<td>600</td>
<td>0</td>
<td>.000</td>
</tr>
<tr>
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<td>M</td>
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<td>.000</td>
</tr>
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<td>M</td>
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<td>.000</td>
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<td>.000</td>
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* Slides from one mouse were not scored due to poor readability of slides

b Slides from two mice were not scored due to poor readability of slides
Figure 12. Number of Rings.
the combined frequencies of the corresponding male/female groups that received either a 25 or 50 mg/kg b.w dose of cyclophosphamide and exposed to ELF EMF.

Data were statistically analysed using a conditional Poisson test or the Poisson regression model (adjusting for overdispersion) for the combined male/female groups of mice that received either vehicle or a 5, 25, or 50 mg/kg b.w. dose of cyclophosphamide. No statistically significant difference in the number of chromosome rings was found between any two of the combined male/female groups that had similarly received either vehicle or a specified dose of cyclophosphamide and then either sham exposed or exposed to ELF EMF.

Table 13 shows that for the groups of mice that received a 50 mg/kg b.w. dose of cyclophosphamide and then either exposed to EMF or sham exposed, the groups of male mice in both treatment groups show a greater number of HDC than do the corresponding groups of female mice in each treatment group. The groups of male mice in both treatment groups also show a larger fraction of animals displaying the HDC than do the groups of female mice in each of the respective treatment groups. Using a Fisher's exact test, no statistically significant differences were found in the frequency of HDC between the male and female groups of animals treated with vehicle, 5, or 25 mg/kg dose of cyclophosphamide. However, using a logistic regression test (adjusting for overdispersion), a significant difference in the
frequency of HDC was found between the male groups of mice that received a 50 mg/kg b.w. dose of cyclophosphamide and then either sham exposed or exposed to ELF EMF, and the corresponding groups of female mice.

Figure 13 shows a graph of the combined frequencies of HDC for the combined groups of male and female mice for each of the eight different treatment scenarios. Numerically, the combined frequencies of HDC for the male/female groups that received a 25 or 50 mg/kg b.w. dose of cyclophosphamide and sham exposed is greater than the combined frequencies of the corresponding male/female groups that received the same 25 or 50 mg/kg b.w. dose of cyclophosphamide but exposed to ELF EMF.

Statistical analysis was performed using a Fisher's exact test or a logistic regression model (adjusting for overdispersion), for the combined male/female groups of mice that received either vehicle or a 5, 25, or 50 mg/kg b.w. dose of cyclophosphamide. No statistically significant difference in the number of HDC was found between any two of the combined male/female groups that had similarly received either vehicle or a specified dose of cyclophosphamide and then either sham exposed or exposed to ELF EMF.

Table 14 shows a nearly equal frequency of occurrence for TAM between the groups of male mice and groups female mice treated with injection vehicle only and then either sham exposed or exposed to EMF. For the group of mice that received a 5 mg/kg b.w. dose of cyclophosphamide and
Table 13

Number of Highly Damaged Cells (HDC): >15 Breaks/Fragments

<table>
<thead>
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<th>Treatment CP</th>
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<th>Number of Mice Analyzed (+) HDC</th>
<th>Number of Cells Analyzed (+) HDC</th>
<th>% of Cells</th>
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<tr>
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<tr>
<td>50</td>
<td>+</td>
<td>M</td>
<td>6</td>
<td>600</td>
<td>42</td>
</tr>
<tr>
<td>50</td>
<td>+</td>
<td>F</td>
<td>6</td>
<td>600</td>
<td>5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Slides from one mouse were not scored due to poor readability of slides

<sup>b</sup> Slides from two mice were not scored due to poor readability of slides

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Figure 13. Number of Highly Damaged Cells.
sham exposed, the group of female mice showed a greater number of TAM and had a larger fraction of animals displaying TAM than did the group of male mice of the same treatment. For the group of mice that received a 5 mg/kg b.w. dose of cyclophosphamide and exposed to ELF EMF, the group of male animals showed a slightly greater number of TAM. For the group of mice that received a 25 mg/kg b.w. dose of cyclophosphamide and sham exposed, the group of male mice showed a greater number of TAM than did the group of female mice, although both the group of female mice and the group of male mice showed an equivalent fraction of animals displaying TAM. For the group of mice that received a 25 mg/kg b.w. dose of cyclophosphamide and exposed to EMF, the group of female mice showed a greater number of TAM than did the group of male mice. For groups of mice that received a 50 mg/kg b.w. dose of cyclophosphamide and then either sham exposed or exposed to EMF, the male animals in both treatment groups showed a greater number of TAM than did the groups of female mice in the respective treatment groups. However both the group of female mice and the group of male mice in each of the two treatment groups showed an equivalent fraction of animals displaying TAM and had a larger fraction of animals displaying TAM than did the group of male mice of the same treatment group. Using a standard Chi-square test, no statistically significant differences were found in the frequency of TAM between the male and female groups of animals treated with vehicle, or a 5 mg/kg dose of
cyclophosphamide. Using a logistic regression test (adjusting for overdispersion), no significant difference in the frequency of TAM was found between the male groups of mice that received a 25 or 50 mg/kg b.w. dose of cyclophosphamide.

Figure 14 shows a graph of the combined frequencies of TAM for the combined groups of male and female mice for each of the eight different treatment scenarios. Numerically, the combined frequencies of TAM for the male/female groups that received a 25 or 50 mg/kg b.w. dose of cyclophosphamide and sham exposed is greater than the corresponding male/female groups that received the same 50 mg/kg b.w.dose of cyclophosphamide but exposed to ELF EMF.

Statistical analysis was performed using a standard Chi-square test or logistic regression model (adjusting for overdispersion), for the combined male/female groups of mice that received either vehicle or a 5, 25, or 50 mg/kg b.w. dose of cyclophosphamide. No significant difference was found in the number of TAM between the male/female groups that had similarly received either vehicle or a 5 or 50 mg/kg b.w. dose of cyclophosphamide and then either sham exposed or exposed to ELF EMF. Using a logistic regression model (adjusting for overdispersion), a statistically significant difference in the number of TAM was found between the two combined male/female groups receiving a 25 mg/kg b.w. dose of cyclophosphamide and then either being sham exposed or exposed to ELF EMF (P=0.016).

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Table 14

Total Number of Aberrant Metaphases (TAM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CP</th>
<th>EMF</th>
<th>Sex</th>
<th>Number of Mice Analyzed</th>
<th>(+) TAM</th>
<th>Number of Cells Analyzed</th>
<th>(+) TAM</th>
<th>% of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - M</td>
<td>6</td>
<td>2</td>
<td>600</td>
<td>3</td>
<td>.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - F</td>
<td>6</td>
<td>2</td>
<td>600</td>
<td>4</td>
<td>.007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 + M</td>
<td>6</td>
<td>3</td>
<td>600</td>
<td>3</td>
<td>.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 + F</td>
<td>6</td>
<td>2</td>
<td>600</td>
<td>4</td>
<td>.007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - M</td>
<td>6</td>
<td>1</td>
<td>600</td>
<td>1</td>
<td>.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 - F</td>
<td>6</td>
<td>5</td>
<td>600</td>
<td>9</td>
<td>.015</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 + M</td>
<td>6</td>
<td>3</td>
<td>600</td>
<td>6</td>
<td>.010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 + F</td>
<td>6</td>
<td>3</td>
<td>600</td>
<td>3</td>
<td>.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 - M</td>
<td>6</td>
<td>6</td>
<td>600</td>
<td>50</td>
<td>.083</td>
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<tr>
<td>25 - F</td>
<td>5a</td>
<td>5</td>
<td>500</td>
<td>34</td>
<td>.068</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 + M</td>
<td>4b</td>
<td>3</td>
<td>400</td>
<td>11</td>
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</tr>
<tr>
<td>25 + F</td>
<td>5a</td>
<td>4</td>
<td>500</td>
<td>15</td>
<td>.030</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>50 - M</td>
<td>5a</td>
<td>5</td>
<td>500</td>
<td>185</td>
<td>.370</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 - F</td>
<td>6</td>
<td>6</td>
<td>600</td>
<td>130</td>
<td>.217</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 + M</td>
<td>6</td>
<td>6</td>
<td>600</td>
<td>190</td>
<td>.317</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 + F</td>
<td>6</td>
<td>6</td>
<td>600</td>
<td>62</td>
<td>.103</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

* Slides from one mouse were not scored due to poor readability of slides

b Slides from two mice were not scored due to poor readability of slides
Figure 14. Number of Cells With Aberrant Metaphases of Any Type.
Summary

The present study reports finding no significant increase in the frequency of CAs in bone marrow cells of male and female CD-1 mice that were administered vehicle only (0.9% NaCl solution) and exposed to a magnetic field intensity of 7.3 G for 24 hours, as compared to male and female CD-1 mice administered vehicle only, but sham exposed for 24 hours.

This study also reports finding no significant increase in the frequency of CAs in bone marrow cells of male and female CD-1 mice treated with cyclophosphamide (dosages of either 5, 25, or 50 mg/kg b.w.) and exposed to a magnetic field intensity of 7.3 G for 24 hours, as compared to male and female CD-1 mice treated with cyclophosphamide (same dosages), but sham exposed for 24 hours.

In addition, the results show that in regards to the frequency of CB/CF (Figure 9) and TAM (Figure 14), the frequency of occurrence was significantly (P<0.05) lower in male and female CD-1 mice treated with cyclophosphamide at a 25 mg/kg b.w. dose and exposed to 7.3 G magnetic field intensity, as compared to male and female CD-1 mice treated with cyclophosphamide at a 25 mg/kg dose and sham exposed.
CHAPTER V

DISCUSSION AND CONCLUSION

Mutagenicity of Extremely Low Frequency Electromagnetic Fields

There are a number of epidemiological studies that have reported positive statistical correlations between exposure to ELF EMFs and the increased incidence of certain types of cancer (Feychting et al., 1993; Lin et al., 1994; London et al. 1991; Lovely et al., 1994; Tomenius, 1986; Wertheimer et al., 1979, 1982). Since carcinogenesis involves the damage of chromosomes (DNA) as a prerequisite to the formation of transformed cancer cells, it is very important to know if ELF EMF has mutagenic and/or clastogenic effects on living cells. It is also important to know if ELF EMFs can enhance the mutagenic and/or clastogenic effects of physical or chemical agents that are known to be mutagenic, clastogenic, and carcinogenic. The problem addressed in the present study was to determine whether or not ELF EMFs are mutagenic and/or clastogenic to bone marrow cells of CD-1 mice. The present study was also concerned with determining the ability of ELF EMFs to enhance the mutagenic and/or clastogenic effects of cyclophosphamide, a known mutagen, clastogen, and carcinogen (Povirk and Shuker, 1994)(IARC, 1981, 1982, 1987), in bone marrow cells of mice.
Although the majority of the research studies suggest that electric and/or magnetic fields do not cause significant DNA damage, there are a few studies that suggest that ELF EMFs may cause DNA damage. Table 15 and Table 16 respectively display the results of the in vitro and in vivo studies investigating the mutagenicity of ELF EMFs.

The results of the present study are in agreement with the majority of studies cited in the two tables which report finding no mutagenic and/or clastogenic effects in cells exposed to ELF EMFs. The present study reports finding no statistically significant increase in the frequency of CAs in bone marrow cells of CD-1 mice that were administered a single i.p. injection of vehicle only (0.9% NaCl solution) and exposed to a magnetic field intensity of 7.3 G for 24 hours, as compared to CD-1 mice administered a single i.p. injection of vehicle, but sham exposed for 24 hours.

This study also reports finding no statistically significant increase in the frequency of CAs in bone marrow cells of CD-1 mice treated with a single i.p. injection of cyclophosphamide (dosages of either 5, 25, or 50 mg/kg b.w.) and exposed to a magnetic field intensity of 7.3 G for 24 hours, as compared to CD-1 mice treated with a single i.p. injection of cyclophosphamide (same dosages), but sham exposed for 24 hours.

Uniqueness of Study

The present study is unique in that it is one of a very few in vivo
<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism/Cell Type</th>
<th>Exp. Time</th>
<th>ELF EMF</th>
<th>End-point</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nordenson et al.</td>
<td>human/lymphocytes</td>
<td>(10)3 μs</td>
<td>(EC) 1 mA/cm²</td>
<td>CA</td>
<td></td>
</tr>
<tr>
<td>(1984)</td>
<td>sparks</td>
<td>OR</td>
<td></td>
<td>CA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(EF) 2.5 kV/cm</td>
<td>CA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(EF) 3.0 kV/cm</td>
<td>CA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(EF) 3.5 kV/cm</td>
<td>CA</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>d'Ambrosio et al.</td>
<td>bovine/lymphocytes</td>
<td>48/72 h</td>
<td>(EC) 2.4 μA/cm²</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>(1985)</td>
<td></td>
<td>SCE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(MF) 1 or 2 G</td>
<td>CA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohen et al.</td>
<td>human/lymphocytes</td>
<td>69 h</td>
<td>(EC) 30 μA/cm²</td>
<td>CA</td>
<td></td>
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<tr>
<td>(1986a)</td>
<td></td>
<td>(MF) 1 or 2 G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohen et al.</td>
<td>human/lymphocytes</td>
<td>69 h</td>
<td>(EC) 30 μA/cm²</td>
<td>SCE</td>
<td></td>
</tr>
<tr>
<td>(1986b)</td>
<td></td>
<td>(MF) 1 or 2 G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takahashi et al.</td>
<td>Chinese hamster/V79</td>
<td>24 h</td>
<td>PEMF</td>
<td>25 μs pulses</td>
<td></td>
</tr>
<tr>
<td>(1987)</td>
<td></td>
<td></td>
<td>(MF) 1.8-25 G</td>
<td>SCE</td>
<td></td>
</tr>
<tr>
<td>Reese et al.</td>
<td>CHO</td>
<td>1 h</td>
<td>(EF) 1 or 38 V/m</td>
<td>SSB</td>
<td></td>
</tr>
<tr>
<td>(1988)</td>
<td></td>
<td></td>
<td>(MF) 1 or 20 G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosenthal et al.</td>
<td>human/lymphocytes</td>
<td>48/72 h</td>
<td>(MF) 50 G</td>
<td>CA</td>
<td></td>
</tr>
<tr>
<td>(1989)</td>
<td></td>
<td>OR</td>
<td></td>
<td>SCE</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(MF) 75 G &amp;</td>
<td></td>
<td>MNU or TRN</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garcia-Sagredo et al.</td>
<td>human/lymphocytes</td>
<td>48 h</td>
<td>PEMF</td>
<td>26 μs pulses</td>
<td></td>
</tr>
<tr>
<td>(1990)</td>
<td></td>
<td></td>
<td>(MF) 10/20/40 G</td>
<td>SCE</td>
<td></td>
</tr>
<tr>
<td>Garcia-Sagredo et al.</td>
<td>human/lymphocytes</td>
<td>24 h</td>
<td>PEMF</td>
<td>26 μs pulses</td>
<td></td>
</tr>
<tr>
<td>(1991)</td>
<td></td>
<td></td>
<td>(MF) 10/20/40 G</td>
<td>CA</td>
<td>++</td>
</tr>
<tr>
<td>Reference</td>
<td>Organism/ Cell Type</td>
<td>Exp. Time</td>
<td>ELF EMF</td>
<td>End-point</td>
<td>Effect</td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------------</td>
<td>-----------</td>
<td>--------------------------</td>
<td>-----------</td>
<td>--------</td>
</tr>
<tr>
<td>Khalil et al. (1991)</td>
<td>human/ lymphocytes</td>
<td>24/48/or 72 h</td>
<td>PEMF-10 ms pulses (MF) 10.5 G</td>
<td>SCE</td>
<td></td>
</tr>
<tr>
<td>Livingston et al. (1991)</td>
<td>human/ lymphocytes or CHO</td>
<td>72 h</td>
<td>(MF) 2.2 G (EC) 3/30/300/ or 3000 μA/cm²</td>
<td>SCE</td>
<td>MN</td>
</tr>
<tr>
<td>Fiorani et al. (1992)</td>
<td>human/ tumor cell line #562</td>
<td>1/4/6/ or 24 h</td>
<td>(EF) 0.2-20 kV/m (MF) 0.002-2 G</td>
<td>SSB</td>
<td></td>
</tr>
<tr>
<td>Fiorio et al. (1993)</td>
<td>Chinese hamster/V79</td>
<td>2/5/7/or 10 days</td>
<td>(MF) 2 G</td>
<td>HGPRT</td>
<td></td>
</tr>
<tr>
<td>Fairbairn et al. (1994)</td>
<td>HL-60 cells, Raji cells, Hela cells, or human lymphocytes</td>
<td>24 h</td>
<td>(MF) 50 G</td>
<td>SSB</td>
<td></td>
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<tr>
<td>Tabrah et al. (1994)</td>
<td>Salmonella typhimurium/ TA 100 cells</td>
<td>48 h</td>
<td>(EF) 21 mV/cm revert. (EC) 0.21 μA/cm²</td>
<td>colonies</td>
<td></td>
</tr>
</tbody>
</table>

++ = positive results and statistically significant
- = negative results; no difference between treated and controls
CA = chromosome aberrations
SCE = sister chromatid exchange
MN = micronucleus
HGPRT = a specific mutation locus
SSB = single strand breaks
(EF) = electric field intensity
(EC) = electric current density
(MF) = magnetic field intensity
MNU = methylnitrosourea; TRN = trenimon
<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism/Cell Type</th>
<th>Exp. Time</th>
<th>ELF EMF</th>
<th>End-point</th>
<th>Effect</th>
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</thead>
<tbody>
<tr>
<td>Diebolt et al.</td>
<td>Fruit flies; Drosophila melanogaster</td>
<td>24 h</td>
<td>(MF) 9.226 G (EF) 0.3 kV/cm</td>
<td>recessive lethal</td>
<td></td>
</tr>
<tr>
<td>(1978)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bauchinger et al.</td>
<td>human/lymphocytes</td>
<td>&gt; 20 yrs.</td>
<td>MF and EF @ 380 kV switchyard</td>
<td>SCE</td>
<td>CA -</td>
</tr>
<tr>
<td>(1981)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Nordenson et al.</td>
<td>human/lymphocytes</td>
<td>1-8 wks</td>
<td>MF and EF @ 400 kV switchyard</td>
<td>CA</td>
<td>++</td>
</tr>
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<td>(1984)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nahas et al.</td>
<td>mice/polychromatic erythrocytes</td>
<td>24 h</td>
<td>(EF) 170/200/ or 290 kV/m</td>
<td>MN ++</td>
<td></td>
</tr>
<tr>
<td>(1989)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ma et al.</td>
<td>Tradescantia plant/pollen cells</td>
<td>6 h</td>
<td>(MF) 10 G</td>
<td>MN 3 fold increase</td>
<td></td>
</tr>
<tr>
<td>(1992)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haider et al.</td>
<td>Tradescantia plant/pollen cells</td>
<td>30 h</td>
<td>(EF) 1-170 V/m (MF) .01-.11 A/m.</td>
<td>MN ++</td>
<td></td>
</tr>
</tbody>
</table>

++ = positive results and statistically significant
- = no effect seen; no difference between treated and controls
(MF) = magnetic field intensity
(EF) = electric field intensity
SCE = sister chromatid exchange
MN = micronucleus
CA = chromosome aberration

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studies (see Table 16) that has looked at the mutagenic and/or clastogenic effects of ELF EMFs on research animals, as compared to a large number of in vitro studies that have investigated the mutagenicity of ELF EMFs on cultured cells.

The present study is also unique in that at the time it was performed, this investigator found no other study cited in the literature which attempted to look at the possible synergistic effects of ELF EMFs with a known clastogen/mutagen/carcinogen, which in the present study was the clinical drug cyclophosphamide.

Conclusion

The first hypothesis tested in the present study was that exposure of mice to a 7.3 G magnetic field for 24 hours, would significantly increase the frequency of clastogenic and/or mutagenic events in the bone marrow cells of the mice exposed to the magnetic field. The results show there was not a statistically significant increase in the frequency of CAs in bone marrow cells of CD-1 mice administered the vehicle and exposed to a 7.3 G magnetic field for 24 hours, as compared to CD-1 mice administered the vehicle but sham exposed for 24 hours. The results failed to support the hypothesis.

The second hypothesis tested in this study was that exposure of mice treated with cyclophosphamide and then exposed to a 7.3 G magnetic field for 24 hours would significantly increase the clastogenic and/or mutagenic
effects of cyclophosphamide in the bone marrow cells of mice. The results show there was not a statistically significant increase in the frequency of CAs in bone marrow cells of CD-1 mice treated with cyclophosphamide (dosages of either 5, 25, or 50 mg/kg b.w.) and exposed to a 7.3 G magnetic field for 24 hours, as compared to CD-1 mice treated with a single i.p. injection of cyclophosphamide (same dosages), but sham exposed for 24 hours. The results failed to support the hypothesis that the ELF EMF would increase the mutagenicity and clastogenicity of cyclophosphamide and hence may contribute to the initiation of cancer by cyclophosphamide.
Figure 15. Layout of ELF EMF Exposure Hardware.
Appendix B

Monitoring Positions of Gauss Detector in Animal Cage
Monitoring Positions of Gauss Detector in Animal Cage.

Gauss Readings at Six Positions in Animal Cage

<table>
<thead>
<tr>
<th>Voltage to Coil</th>
<th>Amperage to Coil</th>
<th>Gauss Readings at Six Positions in Animal Cage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pos. 1</td>
</tr>
<tr>
<td>7.35 V</td>
<td>0.85 A</td>
<td>1.6 G</td>
</tr>
<tr>
<td>12.23 V</td>
<td>1.52 A</td>
<td>2.6 G</td>
</tr>
<tr>
<td>14.72 V</td>
<td>1.82 A</td>
<td>3.2 G</td>
</tr>
<tr>
<td>17.07 V</td>
<td>2.13 A</td>
<td>3.7 G</td>
</tr>
<tr>
<td>19.52 V</td>
<td>2.42 A</td>
<td>4.2 G</td>
</tr>
<tr>
<td>21.67 V</td>
<td>2.67 A</td>
<td>4.7 G</td>
</tr>
<tr>
<td>24.11 V</td>
<td>2.98 A</td>
<td>5.2 G</td>
</tr>
<tr>
<td>26.52 V</td>
<td>3.27 A</td>
<td>5.8 G</td>
</tr>
<tr>
<td>29.10 V</td>
<td>3.60 A</td>
<td>6.4 G</td>
</tr>
<tr>
<td>31.40 V</td>
<td>3.88 A</td>
<td>6.9 G</td>
</tr>
<tr>
<td>32.88 V (Max.)</td>
<td>4.05 A (Max.)</td>
<td>7.3 G</td>
</tr>
</tbody>
</table>

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Appendix C

Institutional Animal Care and Use Committee Form
WESTERN MICHIGAN UNIVERSITY
INSTITUTIONAL ANIMAL CARE
AND USE COMMITTEE (IACUC)

Application to use Vertebrate Animals for Research or Teaching

The use of any vertebrate animals in research and/or teaching without prior approval of the Institutional Animal Care and Use Committee (IACUC) is a violation of Western Michigan University policies and procedures. This Committee is charged with the institutional responsibility for assuring the appropriate care and treatment of vertebrate animals.

Mail the signed original and five (5) copies of the typed application and any supplements to Research and Sponsored Program, Room A-221 Ellsworth hall, (616) 387-3670.

Any application that includes use of hazardous materials, chemicals, radioisotopes or biohazards must be accompanied with SUPPLEMENT A. (Radiation will be at another organization than WMU. The mice will not contain hazardous material.)

Any application that includes survival surgery must be accompanied with SUPPLEMENT B.

Principal Investigator/Instructor: Kevin K. Block
Signature: __________________________ Date: 2-11-94
Department: Biological Sciences Campus Phone: (616) 387-5630
Responsible Faculty Member: Gyula Ficsor
Signature: __________________________ Date: 2-11-94
Department: Biological Sciences Campus Phone: (616) 387-5630
Title of Project/Course: Synergistic Effect of Cyclophosphamide and DMSO.
Check one: Teaching: X Research: Other: __

I. ANIMAL USE CATEGORIES (check ONLY one category)

A. ______ Projects that involve little or not discomfort (including injections).
B. ______ Projects that may result in some discomfort or pain, but of short duration. Anesthetics, analgesics or tranquilizers will be used.
C. ______ Projects that may result in significant discomfort or pain. Anesthetics, analgesics, or tranquilizers will not be used.
II. ANIMAL USE FACILITIES

The animals(s) will be housed and maintained in accordance with the WMU Human Care and Use of Animals Policies and Procedures.

Yes [X] No _____

If no, give explanation.

Please indicate the building and room(s) where the animals(s) will be housed and cared for as well as the location of the experiments and procedures if different from where housed.

The mice will housed in the approved McCracken Hall animal rooms following the rules and procedures of said animal facility. Same sex mice will be housed in mouse breeding cages not exceeding 6 mice per cage up to one year.

III. ANIMAL USE SUMMARY

In language understandable to a layperson, summarize your primary aims and describe the proposed use of animals as concisely as possible. Bear in mind that the IACUC is primarily interested in the responsible, necessary, humane use of animals. Include a description of procedures designed to assure that discomfort and pain to animals will be minimized. It should include method of restraint; method of dosing with test compound; and methods of euthanasia or disposition of the animal after the experiment.

Groups of 12 mice (6 d & 6 p) will be exposed to cyclophosphamide via single i.p. injection at 0mg/kg b.wt., 5 mg/kg. b.wt., and 50mg/kg b.wt. doses\(^2\). The animals (housed in their own cage @ 6 mice per cage) will be placed within a Helmholtz coil that generates non-ionizing radiation. The animals will remain within the electrically energized coil for 24 hours, give food and water ad libitum. Except for the single i.p. injection the mice should suffer no discomfort. The non-ionizing radiation will not be felt. At 24 hours post treatment, the animals will be sacrificed by cervical dislocation. Both femurs will be surgically removed from dead animals and used for preparation of bone-marrow slides for scoring of bone marrow cell aberrations.


IV. JUSTIFICATION FOR ALL ANIMAL EXPERIMENTS

Please provide a narrative with reference sources which addresses each of the following:

A. What assurance can be provided to indicate that the procedure is not duplicative?

To date, this investigator has found no reference in the literature that has investigated synergistic effects of cyclophosphamide and non-ionizing radiation.

B. Have non-live animal techniques (e.g. in vitro biological systems, computer simulation, audiovisual demonstration) been considered? Explain why they have not been utilized?

Humans are exposed to chemotherapeutic dosages of cyclophosphamide, and also to strong magnetic fields at home and work. Since the nature of the responses is unknown in-vivo, it is impossible to design an in-vitro or computer simulation experiment. The only sensible experimental system is an in-vivo animal model which we propose.
C. Why has this species been selected for this procedure: For two reasons: 1) mice are traditionally used in genetic experiments, and 2) they will more easily fit into the Helmholtz radiation coil.

D. How many animals will be used in this project? How often will its procedures be done and over what duration?

In total 94 mice will be needed: (12 @ 0mg/kg cyclo + 8 Gauss (G) EMF) (12 @ 5mg/kg cyclo + 8 G EMF) (12 @ 50 mg/kg cyclo + 8 G EMF) (12 @ 0 mg/kg cyclo) (12 @ 5mg/kg cyclo) (12 @ 50mg/kg cyclo) for the main experiment, and two mice for each of the six aforementioned groups for a pilot experiment.

E. In light of concern to minimize the number of animals used in experimentation, how will you determine the number of animals to be used?

We chose the minimum of 6 animals of each sex per dose to assure sufficient statistical power for each of the sex experimental groups.

NOTE: Items, F, G, H and I require the approval of the Consulting Veterinarian

F. What is the anticipated pain or distress response of the animals; and what is the duration of discomfort? (Injections not included).

Except for the single i.p. injection with a 25 gauge syringe needle, the mice should suffer no discomfort during the 24 hour treatment period (Personal communication, Michael Higgins, The Upjohn Co.). L.C. Becker et al., (Mutation Res., 203:317-330, 1988) reported on the results of a study where mice were injected i.p. with 100 mg/kg cyclophosphamide and sacrificed the mice 12 days later with no reported ill effects within this time period. This dose is twice as high and the duration of exposure is 12 times longer than in our study.

G. How will the pain in the animal be monitored?

Mice will be monitored within their cage during treatment period of cyclophosphamide and/or non-ionizing radiation. No overt toxicity shown at these doses and time period. (per Mike Higgins @ Upjohn)

H. What sedative, analgesic, or anesthetics will be used, if any? Include dose, route and frequency of administration.

None is necessary

I. What is the justification if pain relieving drugs are not used?

We expect to see no pain experienced by animals except for injection

Signature: Consulting Veterinarian

Date
Title of Project: Induction of Chromosome Aberrations in Bone Marrow Cells of Mice by Cyclophosphamide and/or Non-Ionizing Electromagnetic Radiation.

The information included in this IACUC application is accurate to the best of my knowledge. All personnel listed recognize their responsibility in complying with university policies governing the care and use of animals.

I declare that all experiments involving live animals will be performed under by supervision or that of another qualified scientist. Technicians or students involved have been trained in proper procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in the project.

If this project is funded by an extramural source, I certify that this application accurately reflects all procedures involving laboratory animal subjects described in the proposal to the funding agency noted above.

Any proposed revision to or variations from the animal care and use data will be promptly forwarded to the IACUC for approval.

___ Disapproved ___ Approved ___ Approved with the provisions listed below

Provisions or Explanation

________________________________
IACUC Chairperson

Acceptance of Provisions

Signature: Principal Investigator/Instructor Date Date

IACUC Chairperson Final Approval Date

Approved IACUC Number 94-02-01
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