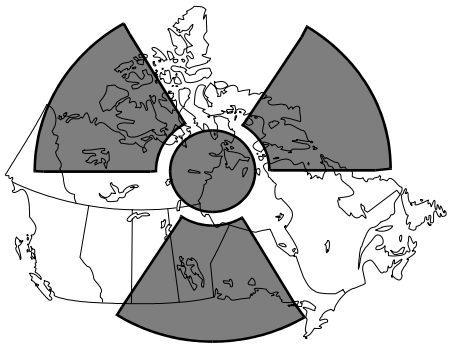

HEALTH EFFECTS AND EXPOSURE GUIDELINES RELATED TO EXTREMELY LOW FREQUENCY ELECTRIC AND MAGNETIC FIELDS - AN OVERVIEW

Prepared by

The ELF Working Group

of

The Federal-Provincial-Territorial Radiation Protection Committee - Canada

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ABOUT THE COMMITTEE

The Federal-Provincial-Territorial Radiation Protection Committee was established to support Federal, Provincial and Territorial government radiation protection agencies in Canada with their respective mandates.

The Mission of the Committee is to advance the development and harmonization of practices and standards for radiation protection within Federal, Provincial and Territorial jurisdictions.

The Committee comprises a forum of delegates from each of the following government organizations:

1. Canadian Nuclear Safety Commission
2. Health Canada - Radiation Protection Bureau
3. Provincial and Territorial Radiation Protection Programs

To assist in fulfilling its mandate, the Committee may establish Sub-committees or Working Groups to address issues of concern that are brought to the Committee's attention. The work of the Committee may involve the preparation of public documents such as Position Statements and Technical Reports; supporting the development and review of National Standards, Guidelines and Codes of Practice; and briefing member organizations and their governing bodies.

For further information on the Committee or its documents, please contact any of the representative agencies listed on the inside back cover of this report. Comments on all documents are welcome.

PREFACE

There exist concerns about the possibility that exposure to electric and magnetic fields (EMF) at extremely low frequencies (ELF), such as those from high-voltage transmission lines and power transformers, may present health effects to the general public. These concerns have arisen as a result of the controversial and contradictory findings in scientific research, especially from epidemiological studies (statistically based studies of disease patterns). Throughout the world, a large number of research papers and overview reports have been produced along with numerous scientific conferences over the past three decades. Unfortunately, the findings remain controversial and contradictory and seem only to exasperate rather than resolve public concerns.

Health problems, thought to be associated with ELF exposure, were first reported in a group of Russian electrical switchyard workers in the 1960's and created widespread scientific interest. In the late 1970's and early 1980's, reports suggesting a link between ELF residential exposures and childhood and adult cancers heightened public anxiety. Following these early reports, extensive national and international research programs were initiated.

In Canada, concerns emerged during the mid 1980's which stimulated the formation of a national Working Group on Electric and Magnetic Fields, co-ordinated by the former Federal Department of National Health and Welfare. Representatives on this group were drawn from Canadian labour, utility companies, academia as well as federal and provincial government scientists. A report from the Working Group published in 1989, reached a number of conclusions based on the status of knowledge at that time. It made recommendations regarding the need for further research as well as the necessity to inform workers and the general public about this matter.

Since EMF and health continues to be a subject of public concerns, the Federal-Provincial-Territorial Radiation Protection Committee (FPTRPC) has established an ELF Working Group to carry out periodic reviews of scientific literature and exposure guidelines and recommend appropriate actions to address this subject. As a result of an initial review, the Committee issued its first document titled “Health effects and exposure guidelines related to extremely low frequency (ELF) 50/60 Hz electric and magnetic fields - an overview” in 1998. The document included the Committee’s position statement for the general public on the health effects of these fields, which reflects a common opinion of the Canadian federal, provincial and territorial health authorities on the EMF-health issue.

The present document incorporates scientific data in refereed journals between 1998 and 2002, and replaces the 1998 report.

The draft of this document was prepared on behalf of the ELF Working Group by Dr. A. Thansandote of the Consumer and Clinical Radiation Protection Bureau (CCRPB), Health Canada. A significant portion of the document was contributed by Dr. J.R.N. McLean through a contract. The working group would like to thank Mr. W. Gorman and Mr. E. Lemay of the CCRPB and Mr. D.W. Lecuyer for their assistance in the preparation of this document. Thanks also go to Mr. G. Gajda of the CCRPB for his review and to Mr. R.P. Bradley of the CCRPB for his support.

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There were a number of comprehensive studies on the risk analysis of power frequency magnetic fields and cancer. All found evidence for weak, but inconsistent, association between exposure to extremely-low-frequency (ELF) electromagnetic fields and some types of cancers.

In this review, data was assessed from epidemiology, animal pathology and *in vitro* laboratory studies to determine if there was any new evidence to strengthen or weaken the possible link between power frequency fields (PFF) and adverse health effects. The main findings were a suggested association between PFF and childhood leukemia, and two new studies that found a possible association between PFF and miscarriage.

The controversy of whether PFF can produce adverse health effects continues to arise almost exclusively from the uncertainties in the epidemiological data. The weakness of epidemiological evidence comes from the confusion caused by unidentified or uncontrolled risk factors that are associated with ELF magnetic fields (so-called confounders), or from the effects of bias, which depend on the way evidence is collected, or from the size of a study, which, if small, limits statistical power. The impact of bias can be limited by careful experimental design, whereas a small sample size can be overcome by conducting a meta-analysis on data pooled from many similar studies. Three recent meta-analyses provide evidence that suggests a link between exposure to PFF, at high magnetic flux densities, and an increase in the risk of developing childhood leukemia. It was concluded that these results were unlikely to be due to chance. Such conclusions leave only confounding, bias and causality as possible reasons for the observed results.

The possible impact of traffic density as a confounder seems less likely as a recent study found no association between traffic density and childhood leukemia. Traffic density has often been cited as a possible confounder in studies of PFF, because of benzene in automobile exhaust. However, the search for a possible confounder continues, as contact currents from low frequency voltages have recently been proposed as a possible component of the ‘EMF mixture’ that contributes to the development of childhood leukemia.

In laboratory studies, animals are exposed to magnetic fields in insulated plastic cages, which eliminates any contribution contact current could make to a carcinogenic or co-carcinogenic endpoint. Animal experiments continue to provide little support for magnetic fields as a risk factor for cancer, or for adverse reproductive outcomes, which justifies the continued search for a possible confounder. An exception comes from recent German studies, which have found evidence that magnetic fields, under rigorously defined experimental conditions, act as a promoter of mammary cancer in a sub-line of Sprague-Dawley rats. American investigators, using a different sub-line of

this species, have been unable to confirm this finding. This issue has yet to be resolved.

Recently, two studies have suggested that exposure to PFF can contribute to spontaneous abortion. These studies suggest that maximum fields (the highest exposure during the day), or the average change between consecutive exposures, may be the relevant exposure metric. Miscarriages occur in about 10% of pregnancies, and these studies suggest that the added risk for an EMF-exposed pregnant women could be as high as an additional 10%. The types of exposure implicated by these two studies may come from proximity to electrical appliances, wiring in walls, and grounded plumbing. These types of exposures are unavoidable in modern life. More studies will be required to confirm these findings.

The *in vitro* studies do not indicate any consistent mode of action.

In Canada, there are no national standards for occupational and general public exposure at frequencies below 3 kHz. However, a number of governmental and non-governmental organizations worldwide have issued exposure standards for ELF fields. These standards were derived from biological effects data since the epidemiological studies have not produced conclusive evidence. There are variations among the guidelines formulated by standard setting organizations, especially dissimilarities between eastern European and western safety standards. These variations could be attributed to differences in the scientific data, philosophy and methodology used for standard development. There have been increasing requests from concerned citizens worldwide that the precautionary principle be used in a number of areas, including exposure to ELF fields. Some countries have incorporated PP into their exposure standards, while others have different points of view. These actions suggest that there is confusion about what PP means and how it should be applied.

2. INTRODUCTION

Electricity plays a vital role in modern society, as it is used to light homes, prepare food, run computers and operate household devices, such as washers, dryers, air-conditioners, computers, TVs and radios. Equipment utilizing electricity is also found in industry and the workplace. Examples of such equipment are radio and TV transmitters, radars, fax machines, photocopiers, food processors, welding machines, electric furnaces, electric heaters and electric tools in machine shops. The increasing demand of electric power for industrial, workplace and home use has led to the extensive developments of the generation, transmission and distribution of electricity. As a result, high-voltage transmission lines and prominent distribution lines have become common outdoor features.

In Canada, alternating current (AC) electricity operates at a power frequency of 60 hertz (Hz), which is in the extremely low frequency (ELF) range. The term "extremely low" is used to describe any frequency below 300 Hz.

The use of AC electricity results in the production of electric and magnetic fields (EMFs), which oscillate at 60 Hz. These fields are emitted by transmission and distribution lines, power transformers, service wires, electrical panels, indoor wires and household electrical appliances. The electric field changes with the voltage, while the magnetic field varies with the current. An electric field is produced whenever two objects are at a different potential (voltage). The strength of the electric field is described in terms of volts per metre (V/m) or kilovolts per metre (kV/m). For example, 1 kV/m means that there is a difference of 1 kV (1000 V) between two points, 1 metre (m) apart.

A magnetic field is produced by electric current in a conductor such as a power cord for appliances. The strength of the magnetic field depends on the current in the conductor and the distance from the conductor, and is usually measured in amperes per metre (A/m). At a power frequency of 60 Hz, the magnetic field is frequently expressed in Tesla (T) or Gauss (G). A smaller unit such as microtesla (μT), which is one millionth of a Tesla, is also used. It should be noted that 1 A/m corresponds to 1.257 μT or 12.57 milligauss (mG). As an example, 1 μT is the magnetic flux density (field strength) produced at the centre of a single-turn circular loop of wire with 1 m diameter when approximately 1.5 amperes (A) of current flows in the loop, or the magnetic flux density at 1 m away from a long wire carrying 5 A.

Both electric and magnetic fields are strongest when close to their source. As we move away from the source, the strength of the fields fades rapidly. For example, the magnetic field from typical 500 kV transmission lines is of the order of 3 μT at the edge of the line corridor and drops to 0.1 μT at 70 m farther (Bonneville Power

Administration, 1993). Information about EMF exposures in homes and workplaces has been compiled and made available to the general public (NIEHS, 2002).

EMFs can cause interference effects on the operation of some electronic equipment, e.g. cardiac pacemakers and radio receivers. In some cases, these effects are perceptible. For example, office workers may notice image movement (jitter) on their computer monitors if placed in an area where magnetic fields are $\geq$0.5 μT (Sandström et al., 1993), which is slightly above typical levels found in offices. Some sources that generate these slightly elevated levels are the cables that bring electrical power into an office area, and common electrical equipment, such as power transformers. Magnetic fields that cause jitter on computer screens are well below the levels that would cause human health effects.

Physical objects such as vegetation, buildings, fences and towers can reduce electric fields to a lower level. On the other hand, magnetic fields can pass through most objects. As such, EMF shielding works well for power-frequency electric fields but not usually for power-frequency magnetic fields.

Like populations in other countries, Canadians are becoming increasingly aware of and concerned about the possible health effects of power frequency EMFs. The awareness and concerns have been reflected in media reports and increased scientific research over the years. Collaboration has been made at local, national and international level in an attempt to address these concerns. While scientific research is ongoing, standards and guidelines have been developed for some EMF sources and exposures.

In this document, all relevant scientific information about ELF epidemiological and laboratory (biological) studies reported in refereed journals during the 1998-2002 period is reviewed. A synopsis and comment of each relevant publication concerning its potential relevance to the production of adverse health effects is included. The comments are focussed on the adequacy of the study design and methods used to generate and present the data, together with the authors' conclusions. A summary of recent ELF exposure standards that are based on established effects is also presented. Since the topics on electromagnetic interference and EMF shielding are beyond the mandate of FPTRPC’s ELF Working Group, they are not further discussed in this document.

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National Institute of Environmental Health Sciences (NIEHS). Questions and Answers about EMF. NIEHS, Research Triangle Park, North Carolina, USA, 2002 (available at the NIEHS website: www.niehs.nih.gov/emfrapid/home.htm).

Sandström M, Mild KH, Sandström K and Berglund A. External power frequency magnetic field-induced jitter on computer monitors. Behaviour and Information Technology. 1993;12(6):359-363.

3. ANALYTICAL FRAMEWORK

3.1 Introduction

Evidence from laboratory studies have found limited evidence in support of the hypothesis that power-frequency fields (PFF) can interact with biological systems. However, careful scrutiny of the literature indicates that many of those reported effects have never been verified by independent confirmation or replication studies. The epidemiology is also equally vague, with the strength of the association between PFF and adverse health effects ranging from very weak to nonexistent. However, the association between PFF fields and human disease continues as a health issue for both the public and workers in electrical industries.

3.2 Review Objectives

The objective of this review is to examine the scientific literature published between 1998 and 2002 inclusive, to determine if there is any new evidence to support the existence of an association between PFF and adverse health effects, including cancer and neurodegenerative disease. Relevant studies were identified from MEDLINE and PubMed databases, and from review articles on this topic. The criteria used to assess the relevant literature is outlined in the Analytical Framework that follows.

3.3 Analytical Framework

3.3.1 Epidemiology

For this review, some of the criteria used to assess the association of PFF with adverse health effects in humans included:

(i) whether the study adequately controlled for sources of confounding and bias (residential mobility, recall bias, non-participation bias, socioeconomic status, age, sex, presence of chronic infections, etc.),

(ii) whether the study design was clearly stated (the study design being defined as the procedures for conducting a study, including choices of variables, their magnitudes, and the quality assurance procedures used to verify the validity of those choices),

(iii) how “exposure cut points” were determined, arbitrarily *post hoc* (after-the-fact), or *a priori,*

(iv) the criteria used to select the exposure metric,

(v) whether the, quantity and quality of the data were adequate for a rigorous statistical analysis, including whether the power of the test was disclosed, and, where applicable, whether p-values were adjusted to reflect multiple comparisons, and

(vi) additional criteria was applied as needed.

3.3.2 Meta-Analysis

For a meta-analysis, or pooled studies, the analytical framework included an examination of,

(i) whether the study designs were similar enough to justify combining data,

(ii) whether the results were consistent enough among studies so that combining them could be justified (that is, did the studies show similar associations between PFF fields and the same types of cancer, etc.),

(iii) whether the individual studies were internally consistency (that is, whether a study showed positive or negative results for all measures of exposure).

(iv) additional criteria were applied as needed.

3.3.3 Statistical Analysis

For the statistics, the analytical framework included an examination of:

(i) whether the use of parametric or non-parametric tests were justified,

(ii) whether the p-values were adjusted for multiple comparisons, when applicable, that is, for multiple exposure metrics, multiple exposure “cutoff” points, multiple cancer sites, or other multiple endpoints,

(iii) whether the positive or negative findings could have been due to faulty statistics, and

(iv) the potential biological relevance of statistically significant differences.

3.3.4 Laboratory Studies

Laboratory studies were assessed on:

(i) the ability of the experimental design to control all significant assignable causes of variation,

(ii) the precision of measured endpoints (i.e. the degree of mutual agreement among individual measurements),

(iii) the ruggedness of the design (i.e. did the design have the ability to withstand small uncontrolled changes in operating conditions), and,

(iv) the practicality of the design (i.e. did the experiment require any exotic equipment, reagents etc, which would make independent replication difficult).

Laboratory studies were also assessed for their ability to contribute to defining a 'mode of action' for PFF, regardless of whether or not a plausible mechanism of action was known. This is important when the epidemiological evidence supporting an association between PFF and adverse health effects is weak and inconsistent. Laboratory experiments can be used to identify a plausible mode of action**,** which can be used to either confirm or reject the plausibility of the epidemiological evidence. A mode of action is defined as the series of biological changes (key events) that precede the development of symptoms or tumors (Wiltse et al., 2000). For PFF, defining a mode of action would involve using laboratory studies to identify a sequence of molecular, biochemical, cellular, physiological, tissue, or organ changes that would be necessary to precede tumor development, or the onset of neurodegenerative disease. The molecular and cellular events involved in carcinogenesis are reasonably well understood (not understood as well for neurodegenerative diseases), and the key events that precede tumor formation have been generally established by years of fundamental research in cancer biology.

For purposes of this review, key events will be classified as being either genotoxic or epigenetic (nongenotoxic) in nature (Trosko, 2000). In the context of the multistage model of carcinogensis, which are defined as initiation, promotion and progression, a key event will be considered genotoxic if it causes initiation (the irreversibly alteration of a stem cell's ability to terminally differentiate), or enables malignant conversion (the immortalization of a precancerous cell). Examples of key genotoxic events are DNA or chromosome damage or mutations. Promotion, the presumed second stage of carcinogenesis involves key epigenetic events that effect clonal expansion of an "initiated"cell.

Defining a mode of action for PFF would enable one to predict the existence of a response threshold, and to infer the shape of a dose-response relationship (linear or nonlinear). For example, showing that PFF in the laboratory produced DNA or chromosomal damage at any level of exposure, in any recognized assay for genotoxicity, would provide support for those epidemiological studies that suggest a weak association between PFF and cancer. This support would be strengthened

considerably if the results were independently replicated by a number of investigators and confirmed through a battery of related assays. Alternatively, if the mode of action involved key events such as, an effect on the functional integrity of gap junctions, or the modulation of gene expression or, possibly, the inhibition of repair or apoptosis, then one might expect a nonlinear dose-response relationship and an action threshold (Wiltse et al., 2000) (Trosko, 2000). In this context, laboratory studies are important adjuncts to epidemiological evidence in the identification, assessment and management of the risks, if any, associated with exposure to PFF.

Therefore, if a mode of action cannot be defined after an exhaustive search of all possibilities, this would strengthen the argument that the observed association between PFF and adverse health effects was due to some other non-identified factor. On the other hand, if a genotoxic or non-genotoxic mode of action could be identified and repeatedly verified by confirmation and/or replication, then the search for a plausible biophysical mechanism would be necessary to focus the epidemiology.

3.3.4.1 Analytical Framework Involving Key Events Related to Genotoxicity

For carcinogenesis, key events will be considered genotoxic if the outcome is cell death or mutagenesis, for example structural changes in DNA and chromosomes. Such events are considered to have a linear dose response without an action threshold and are most often associated with initiation and progression in the multistage model of carcinogenesis.

3.3.4.2 Analytical Framework Involving Key Events Related to Epigenetic Toxicity

Key events will be considered epigenetic if they involve the alteration in the expression of genetic information at the level of transcription or translation (actions that turn genes “on” or “off”, or stabilize or destabilize the genetic message), and in the post-translational modifications that alter gene products (Wiltse et al., 2000). For example, epigenetic events can alter cell signal transduction and the integrity of gap junctions, which can affect cell proliferation, differentiation, transformation, survival and adaptive responses, or lead to changes in hormone secretion or the status of the immune system. Epigenetic carcinogens, unlike genotoxic carcinogens, usually follow nonlinear dynamics and have an action threshold, require persistent exposure, and can be species or tissue specific.

This analytical framework also accommodated modes of action with both genotoxic and nongenotoxic components, as indicated by key events that measure the inhibition of the repair of genotoxic injury, increase the susceptibility of DNA or nucleosomes to attack by genotoxins, enhance the survival of stem cells with genotoxic injury (inhibition of apoptosis), or stimulate damaged cells to proliferate. The use of key events to establish a mode of action for PFF is not without uncertainty or controversy, and is used

conservatively in this review to either strengthen or disparage the plausibility of an association that has been suggested by epidemiology.

References

Trosko JE. Human health consequences of environmentally-modulated gene expression: potential roles of ELF-EMF induced epigenetic versus mutagenic mechanisms of disease. Bioelectromagnetics 2000;21:402-406.

Wiltse JA and Dellarco VL. U.S. Environmental Protection Agency's revised guidelines for carcinogen risk assessment: evaluating a postulated mode of carcinogenic action in guiding dose-response extrapolation. Mutation Res 2000;464:105-115.

4. BRAIN CANCER

4.1 Review of Brain Cancer and Power- Frequency Fields

Power-frequency fields (PFF) were first associated with brain cancer in a study by Wertheimer and Leeper in 1979 (Wertheimer, 1979), who showed that children living in high current configuration homes had a 2.4-fold increase in the risk of dying from brain cancer. A subsequent study, also by Wertheimer, showed this increased risk was applicable to adults. A number of studies since then have found little, if any, support for this contention, regardless of whether exposures were defined as wire codes, distance from source, or measured or calculated PFF. Occupational studies, however, have found a weak, but inconsistent, link between exposure to PFF and brain cancer.

Summary tables of relevant studies (1998 to 2002) are given below.

Table 1. Brain cancer - residential exposure

Study	year	Location	Excess Risk	Weakness
Wrensch	1999	USA	no	SB, EA, SSS

Abbreviations:
EA = Exposure Assessment Shortcomings
SB = (possible) Selection Bias
SSS = Small Sample Size.

Table 2. Brain cancer - occupational exposure

Study	Year	Location	Excess Risk	Weakness
Johansen	1998	Denmark	no	SSS, C
Cocco	1998	USA	no	SSS, EA, MC
Rodvall	1998	Sweden	no	SSS, EA,
Cocco	1999	USA	no	EA, MC, DM, SSS
Savitz*	2000	USA	yes	EA, MC
Minder	2001	Switzerland	yes**	EA, SSS, DM, C, 16.7 Hz
Villeneuve	2002	Canada	yes	EA, SSS, RB

Abbreviations:
EA = Exposure Assessment Shortcomings
SSS = Small Sample Size
C = (possible) Confounding
MC = Multiple Comparisons
DM = (possible) Diagnostic Misclassification
RB = (possible) Recall Bias
* Same results as a 1995 study by Savitz and Loomis, which found evidence of an association in one high exposure category. The present 2000 study was the result of applying the 1995 data to a refined job-exposure matrix (no new data).
** Only shunting yard engineers had a positive OR, and the highest percentage of smokers.

4.2 Summary of Study Findings

Two recent occupational studies merit summary, Johansen (Johansen, 1998) on Danish utility workers, and Villeneuve (Villeneuve, 2002) on Canadian utility workers. The first study reported the incidence of brain cancer in a large cohort of employees and compared it with the appropriate rate of brain cancer in the Danish population. Exposure assessment was done by a dedicated job-matrix (Johansen, 2002) that distinguished between 25 different job titles and 19 different work areas within the companies. Each of the 475 combinations of job title and work area was assigned an exposure, based partly on direct 24-hour measurements and partly on expert opinion. These were then distributed among 5 exposure categories, background (<0.09 μT), low (0.1 - 0.29 μT), medium (0.3 - 0.99 μT), high (> 1.0 μT) and unknown. The results, expressed as the ratio of observed to expected cancers, found no increased risk for brain cancer (ratio = 0.79, 95% CI = 0.6-1.0). This is consistent with the studies of utility workers in California (Sahl, 1993), in France and Canada (Theriault, 1994), and in the United Kingdom (Harrington, 1997), but contrary to the findings of Savitz and Loomis (1995).

The Danish study of 32,006 individuals, found only 72 brain tumors, 57 in men and 15 in women, which limits its statistical power. Confounders, other than asbestos, were not considered. There was some indication of disease misclassification, since no distinction was made between benign and malignant brain tumors, no mention of whether the diagnosis was verified by a follow-up histological examination, and there was no information on whether a brain tumor was the result of metastatic spread.

The study by Villeneuve (2002) was undertaken to investigate the relationship between occupational magnetic field exposures and different histological types of brain cancer. Using data collected through the Canadian National Enhanced Cancer Surveillance System (CNECSS), the author examined this relationship using several different measures of exposure. It was postulated that elevated risks for the more aggressive

subtype of brain cancer (glioblastoma multiforme) would support the premise that magnetic fields had acted as a tumor promoter. The study was restricted to males.

The Villeneuve analysis was based on 543 brain cancer cases (213 astrocytomas, 198 glioblastomas, 115 other brain cancers, and 16 unknowns) and 543 age-matched controls. All brain cancer cases were confirmed by histological examination, and benign tumors were excluded from the analysis. Participants were sent a detailed questionnaire to determine their residential and occupational history, ethnicity, education, income, smoking, height, weight, physical activity, job-title, company name, work location, duties, the start and end dates of employment, workplace odors, and tobacco smoke, diet 2 years before interview, changes in diet from 20 years ago, and exposure to specific occupational carcinogens, including pesticides, herbicides, ionizing radiation, and vinyl chloride. Telephone interviews were conducted to clarify responses. Expert review was used to assign each occupation to one of three exposure categories: < 0.3 μT, 0.3 to < 0.6 μT, and > 0.6 μT. The resulting odds ratios (OR) were calculated for a large number of different exposure scenarios, and corrections were made for several potential confounders, including self-reported exposures to vinyl chloride, herbicides, pesticides and ionizing radiation.

A statistically non-significant increase in risk of brain cancer was observed for subjects who had ever held a job having average magnetic field exposures of > 0.6 μT, relative to those with a level of < 0.3 μT, OR=1.33, 95% CI = 0.75-2.36, or when exposure was based on the longest held job with an exposure > 0.6 μT. However, when restricted to the highest level of exposure ever received, the OR for glioblastoma multiforme was 5.36, 95% CI = 1.16 - 24.78 for 18 cases and 6 controls, but no increased risk was detected when a similar comparison was made for astrocytomas, or any other type of brain cancer. A significant risk for glioblastoma multiforme was also detected when the analysis was based on an occupational exposure of > 0.6 μT at the last job held, OR=12.59, 95% CI = 1.50 -105.6 for 8 cases and 1 control, but not for the first job held, OR=4.81, 95% CI = 0.94 - 24.71 for 10 cases and 3 controls. This implies PFF had acted as a tumor promoter, since a glioblastoma multiforme will often progress from an astrocytomas, which is considered a less aggressive form of brain cancer.

Of concern is the exclusion of cases that had died within the reference period, since aggressive and rapidly growing brain cancers would be excluded from the study. Furthermore, the work history depended on the recall accuracy of the participant, and errors of omission could have resulted in exposure misclassification. Also, the highest exposure categories (0.3 - 0.6 μT and > 0.6 μT) contained small numbers of subjects, only 16.1% of subjects were in the 0.3 - 0.6 μT category and 4.1% were in the > 0.6 μT. Prudence dictates these results be discounted until a larger study, with greater statistical power, can verify the findings. It is a study worth repeating.

4.3 Detailed Review of Individual Studies

Malignancies of the central nervous system (CNS) are rare, occurring at a rate of about 6 per 100,000 people per year, and representing about 1.4% of all malignant neoplasms. Brain tumors can be either benign (non-cancerous) or malignant (cancerous). Primary brain tumors (i.e. brain cancer) are comprised of two main types: gliomas and malignant meningiomas. Gliomas are a general classification of malignant tumors that include a variety of types: astrocytomas, oligodenrogliomas and ependymomas. Meningiomas arise from the meninges, which are the tissues that surround the outer part of the spinal cord and brain. Although meningiomas are not technically brain tumors, as they occur outside of the brain, they account for 50% of all brain and spinal cord tumors (the majority, about 85% are benign). In addition to these main types, there are a number of rare brain tumors including medulloblastomas, which develop from the primitive stem cells of the cerebellum and are most often seen in children. The brain is also a site where both primary and secondary tumors can arise; a secondary brain tumor generally originates elsewhere in the body and then metastasize to the brain. Brain and spinal cord cancers account for over 20% of all cancers diagnosed in children aged 0-14. About half of all childhood brain tumors are astrocytomas and 25% are medulloblastomas. In adults, the most frequent brain tumors are astrocytic (mainly astrocytomas and glioblastoma multiforme). Astrocytomas and glioblastoma multiforme account for approximately 80% of all gliomas. It is generally accepted that astrocytic gliomas that are classified as grades 1 or 2 are classified as astrocytomas, and the more aggressive forms, grades 3 and 4, are classified as glioblastoma multiforme. Glioblastoma multiforme tumors often evolve from the less malignant astrocytomas, although some can arise *de novo*. The risk of brain cancer increases steadily with age.

Despite numerous scientific and medical investigations, the risk factors for brain cancers in occupational settings are still largely unknown. Possible risk factors include exposure to ionizing radiation, electromagnetic fields, certain work-related chemicals (lead, vinyl chloride, solvents), N-nitrosos compounds (from smoking and consumption of cured meats), head trauma, exposure to farm and domestic animals (veterinarians and farmers because of their association with bacteria, pesticides, herbicides, and certain animal oncogenic viruses), alcohol consumption, infectious diseases (tuberculosis and chicken pox), and certain genetic disorders (neurofibromatosis, types 1 and 2), Li-Fraumeni syndrom and tuberous sclerosis). These genetic disorders account for less than 5% of all brain cancers. Recently, 60 Hz PFF (30 to 100 mT) was shown *in vitro* to cause a time- and dose-dependant increase in proliferation of astrocytoma cells, and to strongly potentiate the effect of two agonists. In contrast, PFF had no effect on rat cortical astrocytes, which are similar to astrocytoma cells but are nontransformed (Wei, 2000). Such studies, if verified by confirmation and replication, strongly suggest that PFF could be a tumor promoter in brain cancers. Tumor promoters are characterized by the existence of an action threshold, prolonged exposure and reversibility of effects.

4.4 Summary of Past Studies

With few exceptions, past studies have found little support for a relationship between adult brain cancer and exposure to residential PFF (Gurney, 1999). Occupational studies, however, have found a weak, but inconsistent, link between exposure to PFF and brain cancer (Kheifets, 2001). A cohort study from the United States of 138,000 male electric utility workers employed at five electric power companies, found an association between PFF and an increased risk of mortality from brain cancer, and a dose-response relationship as well (Savitz, 1995). However, another cohort study from the United States of 36,000 electrical utility workers found no increase in mortality from brain cancer after adjustment for confounders (Sahl, 1993). A study by Harrington et al. (Harrington, 1997) of 84,000 employees in the British National Utility Company also found no association between PFF and brain cancer. Some possible sources of error in these, and other studies, include, inappropriate comparison populations, misclassified exposures, failure to adjust for relevant confounders, small sample size, incomplete occupational history, the use of decedent rather than incident data, and, in some cases, the possibility that the disease could have been misclassified or misdiagnosed. The evidence as of 1997 does not allow a conclusion to be made, either way, on the relationship between PFF and brain cancer.

4.4.1 Residential Exposure and Brain Cancer

Wrensch M, Yost M, Miike R, Lee G and Touchstone J. Adult glioma in relation to residential power frequency electromagnetic field exposures in the San Francisco Bay area. Epidemiology 1999;10(5),523-527.

The principal author is with the Department of Epidemiology and Biostatistics, University of California, San Francisco 94143-1215, USA.

Abstract: In a population-based study, we examined residential power frequency electromagnetic field exposures for 492 adults newly diagnosed with histologically confirmed glioma between August 1, 1991 and April 30, 1994, in the San Francisco Bay area and 462 controls, obtained through random-digit dialing frequency, matched to cases for age, gender, and race. Residential exposure assessment consisted of spot measures with EMDEX (Enertech Consultants, Campbell, CA) meters and wire codes based on characterization and location of nearby power lines. We considered the index residence at the time of the case's diagnosis or the control's interview and all other California residences of each subject for 7 years before study entry. We obtained wire codes for eligible residences of 76% and for index residences of 99% of subjects. Using the Kaune-Savitz wire code classification, the relative risk for longest held residences coded as "high" compared with "low" was 0.9 [95% confidence interval (CI): 0.7-1.3], while relative risk and 95% CIs for front door spot measures of 1.01-2 milligauss (mG), 2.01-3 mG, and higher than 3 mG compared with

less than or equal to 1 mG were 1.0 (0.7-1.4), 0.6 (0.3-1.1), and 1.7 (0.8-3.6). Adjustment for age, gender, race, and whether the subject owned the residence did not meaningfully alter these findings, nor did comparisons using index or highest coded residence. Because of potential exposure misclassification and the unknown pertinent exposure period, these data cannot provide strong support against, but clearly do not support an association between, adult glioma and residential power frequency electromagnetic field exposures.

Comment: This is a case-control study of adult glioma that assessed exposure to residential PFF through spot measurements, wire codes and distances from electrical facilities. Other relevant sources of residential exposure, such appliance or electric blanket use, were not considered, nor were exposures incurred outside the home during leisure activities or work-related activities. The RR estimate for the longest residence occupied coded as high current configuration was 0.9, 95% CI = 0.7 - 1.3, when compared with longest occupied low current configuration residences. The risk estimates corresponding to spot measurements of 1-2 mG (0.1 - 0.2 μT), 2-3 mG (0.2 - 0.3 μT), or > 3 mG (> 0.3 μT), relative to < 1 mG (< 0.1 μT) were 1.0, 95% CI = 0.7 - 1.4; 0.6, 95 % CI = 0.3 - 1.1; and 1.7, 95% CI = 0.8 - 3.6, respectively. Only a small number of cases were assigned to the highest exposure categories, which would lower the power of the study to detect real differences. The study does not support an association between residential exposure to magnetic fields and increased risk for brain cancer. However, since outside sources of exposure were not considered, the validity of this study is questionable and its contribution to the body of knowledge on brain cancer and magnetic fields is therefore somewhat limited.

4.4.2 Occupational Exposure and Brain Cancer

Johansen C and Olsen JH. Risk of cancer among Danish utility workers-a nationwide cohort study. American Journal of Epidemiology. 1998(a);147:548-555.

The principal author is with the Division for Cancer Epidemiology, Danish Cancer Society, Copenhagen.

Abstract: The principal author report the incidence of cancer in a large cohort of employees identified from all 99 Danish utility companies. Personal data and information on employment and exposure to magnetic fields and asbestos were obtained from manual files at the companies, the Danish Supplementary Pension Fund, and the public payroll administration. A total of 32,006 individuals with more than 3 months of employment were linked with the files of the Danish Cancer Registry. The period of follow-up for cancer occurrence among the employees was from April 1968 through December 1993 in the study conducted from 1994 to 1997. Overall, 3,008 cancers were observed, with 2,825 expected, yielding a small but significantly increased risk of 1.06 (95% CI: 1.03-1.10) among the utility workers in comparison with

the general population. No excess was observed for all leukemias or for cancers of the brain or breast among men or women. There was no association of electromagnetic field exposure with risk of these cancers, even when the level and length of exposure to magnetic fields were taken into account. Increased risks for cancers of the lung and pleural cavity were seen mainly for workers whose jobs involved exposure to asbestos. The results from this study do not support the hypothesis of an association between occupational exposures to magnetic fields in the electric utility industry and the risk for cancer.

Comment: This study reports the incidences of cancer in a large cohort of employees in Danish electric utility companies and compares them with the appropriate rates of cancer in the general population. The pattern of cancers were examined for exposure to PFF at work and for the likelihood of exposure to asbestos. Exposure assessment was by a dedicated job-matrix (Johansen, 2002, for abstract see A.1 in Appendix) that distinguished between 25 different job titles and 19 different work areas within the companies. Each of the 475 combinations of job title and work area was assigned an average level of exposure to 50 Hz PFF during a working day, based partly on direct 24-hour measurements of exposure and partly on subjective opinion. These in turn were grouped into 5 exposure categories, background exposure (< 0.09 μT), low exposure (0.1 to 0.29 μT), medium exposure (0.3 to 0.99 μT), high exposure (> 1.0 μT) and unknown exposure. Individual exposure assignments were based on the characteristics of the first job held by an employee (the proportion of employees changing jobs during the study period was < 2%). It was further assumed that category-specific exposure levels remained unchanged during the study period. A job exposure matrix was subjectively established for asbestos, and consisted of two categories, no exposure and above average exposure at work. No direct measurements were made of asbestos concentrations. The use of job history and data from the matrices provided rough estimates of historical exposures to PFF and asbestos. About 19% of male and 9% of female employees had more than 20 years of service.

The results were expressed as standard incidence ratio (SIR), defined as the ratio of observed to expected cancers (3008 observed to 2825 expected). A small but significant increased risk was observed for all cancers (SIR=1.06, 95% CI = 1.03-1.10). However, no association was found between exposure to PFF and brain cancer, SIR = 0.79, 95% CI = 0.6-1.0, which is consistent with results of a study by Sahl et al. (Sahl, 1993) of California utility workers, the nested case-control study of utility workers in France and Canada, and a UK study by Harrington et al. (Harrington, 1997) of utility workers in England. These studies, like the one by Johansen and Olsen (Johansen, 1998) included magnetic field measurements, as part of their exposure assessment protocol, as well as adjustments for potential confounders in the workplace. A meta-analysis of 29 studies by Kheifets et al. (Kheifets, 1995) also found no such elevated risk for brain cancer.

One weakness of the study by Johansen and Olsen is that the job histories of all workers were not known precisely, and consequently in the assignment of average exposures to single job categories individuals might have been assigned to the wrong exposure category. Under these conditions, some degree of exposure misclassification is possible, which for cohort studies, such as Johansen's, biases the estimate of RR towards unity. A later evaluation of the job-exposure matrix used in this study (Johansen, 2002) found that misclassification errors would be introduced mainly between adjacent categories (i.e. between medium (0.1-0.29 μT) and high (0.3-0.99 μT) exposure categories. However, the low (< 0.1 μT) and very high (> 1.0 μT) exposure categories should be satisfactorily separated (see abstract as A.1 in Appendix). The principal author concluded that, if the sample size of the extreme categories were sufficiently large, a real association between exposure to PFF and brain cancer would be detected if it existed. In the present study of 32,006 individuals, only 72 brain tumors were found, 57 in men and 15 in women. Confounders other than asbestos were not considered. There was some indication of the misclassification of disease, since no apparent distinction was made between benign and malignant brain tumors, and no mention of whether or not the diagnosis was verified by a follow-up histological examination. Also, there is no information on whether or not a brain tumor was the result of metastatic spread. Therefore, the present study probably does not have sufficient power to provide a definitive answer to the nature of the relationship, if any, between PFF and the incidence of brain cancer.

Cocco P, Dosemeci M and Heineman EF. Occupational risk factors for cancer of the central nervous system: a case-control study on death certificates from 24 U.S. states. American Journal of Industrial Medicine 1998;33:247-255.

The principal author is with the Institute of Occupational Medicine, University of Cagliari, Italy.

Abstract: The risk of cancer of the central nervous system (CNS) by industry and occupation was investigated with a case-control analysis of the death certificates of 28,416 cases and 113,664 controls, selected from over 4.5 million deaths in 24 U.S. states between 1984 and 1992. Industries showing consistent increases in risk by gender and race included textile mills, paper mills, printing and publishing industries, petroleum refining, motor vehicles manufacturing, telephone and electric utilities, department stores, health care services, elementary and secondary schools, and colleges and universities. CNS cancer risk was increased for administrators in education and related fields, secondary school teachers, and other education- and health-related occupations. The application of job-exposure matrices to the industry/occupation combinations revealed a modest increase in risk for potential contact with the public at work and exposure to solvents. Occupational exposure to electromagnetic fields (EMF) was not associated with CNS cancer, although an association was observed with a few EMF-related occupations and industries. Agricultural exposures were associated with significant risk increases among white

women and white men. Further work is required to investigate in more detail specific occupational exposures or possible confounders responsible for the observed associations.

Comment: This study surveys the relationship between the risk of brain cancer and a wide spectrum of occupational exposures, including exposure to PFF in electric utilities and related industries. This data was used to calculate the risk of brain cancer by occupation, industry and specific workplace exposures among both men and women and among whites and African-Americans. The subjects were aged 25 years, or more, during the period 1984 to 1992. An occupational physician and an experienced industrial hygienist were used to develop, *a priori,* a job-exposure matrix for PFF, solvents, herbicides, other pesticides, contact with the public, and contact with animals. The matrices were based on three-digit occupation and industry codes from the 1980 Census of the Population. A binary, yes/no, exposure code for each risk factor was assigned to every three-digit industry and occupation, based on literature information, computerized exposure databases and the subjective opinions of the hygienist and the physician. Odds ratios were calculated for each of the six study groups and for each industry and occupation with at least 18 subjects (including cases and controls) or at least 3 cases. When a significant risk was detected for a given occupation or industry within a study group, the risk for the same occupation or industry in the other study groups were also calculated, even when represented by only two cases. Variates included in the model were, marital, and socio-economic status, and age at death.

The use of death certificates in studies of brain cancer mortality can lead to errors of disease misclassification, since it is not clear if the brain tumor originated from the metastatic spread of a tumor existing in another anatomical location, or if it was benign or malignant, or if it was the cause of death. Also, in this study, there was no information on the duration of employment, and only one occupational category was available per industry. The broad nature of this survey results in small numbers of brain tumors in each industry category, which reduces the statistical power to detect real differences. Finally, the survey undertakes multiple statistical comparisons, which increases the likelihood of generating false positives and negative results and creating inconsistencies across races, gender and industries. This study found no association between exposure to PFF and brain cancer, but there were significant associations for brain cancers and some industries and occupations related to the manufacture, use, maintenance, and sale of electrical devices and telephones. This survey contributes little to our understanding of the relationship between PFF and brain cancer, but does identify some non-PFF risk factors for future verification.

Rodvall Y, Ahlbom A, Stenlund C, Preston-Martin S, Lindh T and Spannare B. Occupational exposure to magnetic fields and brain tumors in central Sweden. European Journal of Epidemiology 1998;14(6):563-569.

The principal author is with the Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden.

Abstract: Occupations with exposure to magnetic fields were studied in a population-based case-control study of male glioma and meningioma in Central Sweden. The study included 84 cases of glioma, 20 cases of meningioma and 155 controls. Information about job titles was obtained by means of a questionnaire. Three different methods were used to classify exposure: (1) “electrical occupations”, (2) assessment of magnetic fields by an electrical engineer, and (3) job values based on magnetic field measurements at work sites for occupational groups. When analyses were based on electrical occupations, a relative risk (RR) of 1.0 (95% CI: 0.4-2.4) was seen for glioma and 1.8 (95% CI: 0.3-3.6) for meningioma. When analyses were based on measurements, a relative risk of 1.9 (95% CI: 0.8-5.0) was seen for glioma and 1.6 (95% CI: 0.3-10.2) for those ever in an exposed job of an average mean value of > 0.4 μT. A larger number of individuals was classified as exposed, when exposure was based on measurements. Information was available regarding several potential confounders, but none of them seemed to be of any importance. Our conclusion is that the results based on magnetic field measurements give some support to the hypothesis that magnetic fields exposure may play a role in the development of brain tumours.

Comment: Cases included all newly-diagnosed histologically confirmed intracranial gliomas and meningiomas. Controls were contacted by telephone and were identified from parish population registers. Controls were matched to cases on the basis of age, month of birth, and parish. Cases were asked to participate at their neurosurgery clinic. This study used 3 methods to classify PFF exposures: (i) a subjective classification based on “electrical occupation” defined job titles, (ii) a subjective assignment of exposure by review of work histories, and (iii) an objective measure of exposure that was linked to job titles in a job-exposure matrix, which gave 4 exposure categories from < 0.2 μT to > 0.4 μT. The subjective methods of exposure assessment identified similar numbers of cases in each exposure category. Slightly more exposed individuals were identified when exposure was assessed by the job-exposure matrix. When the two subjective methods were used to categorize participants none of the RR were significant for either gliomas or meningiomas. When exposure categories were based on the job-exposure matrix, the RR for glioma was elevated, but non-significantly, at 1.9, 95% CI = 0.8-5.0, and similarly, for individuals ever having been exposed to a mean value of > 0.4 μT (RR=1.6, 95% CI = 0.3-10.2). Therefore, the RR for all of the 3 exposure classification methods detected only non-significant relationships between PFF and brain tumors. A major shortcoming of this study is the relatively small number of cases. For exposure based on electrical occupation, there were only 11 of 84 cases with gliomas that were ever exposed to PFF, and of the 11, only 5 were exposed for > 5 years. The cases with meningiomas were even smaller, only 5 of 15 ever exposed, and of the 5, only 4 were exposed for > 5 years. The principal author stated that there was a tendency for an increase in gliomas among those ever in a job with mean values above 0.4 μT, which supports the results of Floderus et al. (Floderus, 1992). As a

consequence of its small size, and multiple comparisons, the study results must be accepted with caution. This study by Rodvall contributes little to clarifying the relationship between exposure to PFF and the incidence of brain tumors.

Cocco P, Heineman EF and Dosemeci M. Occupational risk factors for cancer of the central nervous system (CNS) among US women. American Journal of Industrial Medicine 1999;36(1):70-74.

The principal author is with the Institute of Occupational Medicine, University of Cagliari, Italy.

Abstract: *Background:* In a recent report, we found an elevated risk of cancer of the central nervous system (CNS) in several occupations and industries, and a modest association with exposure to solvents and to contact with the public. *Methods:* To further explore the occupational risk of CNS cancer among women, we extended the analysis of the previous death certificate-based case-control study, including 12,980 female cases (ICD-9 codes 191 and 192) in 24 US states in 1984-1992 and 51,920 female controls who died from diseases other than malignancies and neurological disorders. We applied newly designed job-exposure matrices for 11 occupational hazards, previously reported as brain cancer risk factors, to the occupation and industry codes in the death certificates. We also conducted a separate analysis of 161 meningioma cases (ICD-9 codes 192.1 and 192.3), a tumor more frequent among women, particularly in the postmenopausal age group. *Results:* Overall, CNS cancer risk showed a 20-30% increase among women exposed to electromagnetic fields (EMF), methylene chloride, insecticides and fungicides, and contact with the public. Risk for meningioma was elevated among women exposed to lead (OR = 1.9; 95% CI: 1.0-3.9). CNS cancer did not show a clear pattern of risk increase by probability and intensity of exposure to any of the explored risk factors. Cross-classification by probability and intensity of exposure did not reveal any significant trend. Cases were too few to explore trends of meningioma by probability and intensity of exposure to lead. *Conclusions:* We did not find evidence of a strong contribution of 11 occupational hazards to the etiology of CNS cancer. However, limitations of the occupational information might have reduced our ability to detect clear patterns of risk.

Comment: The present study focused exclusively on women and brain cancer (particularly meningiomas) in the workplace. The application of a newly designed job-exposure matrix was supposed to eliminate some of the exposure misclassification that was known to have occurred in a previous study. The results now found a non-significant 20-30% excess of brain tumors in women exposed to electromagnetic fields, methylene chloride, insecticides, fungicides and contact with the public. The risk of meningioma was significantly associated with exposure to lead, which might be another risk factor for brain cancer. The number of cases in each occupation and industry limits the statistical power of this study. It is also not clear if the tumors were primary or secondary, or were confirmed by autopsy. Inconsistencies between the results of this

and the previous study (Cocco, 1998) still exist, which limits the contribution of both to the body of knowledge needed to clarify the relationship between exposure to PFF and brain cancer.

Savitz DA, Cai J, van Wijngaarden E, Loomis D, Mihlan G, Dufort V, Kleckner RC, Nylander-French L, Kromhout H and Zhou H. Case-cohort analysis of brain cancer and leukemia in electric utility workers using a refined magnetic field job-exposure matrix. American Journal of Industrial Medicine 2000; 38:417-425.

The principal author is with the Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill 27599-7400, USA.

Abstract: *Background:* The potential association between occupational electric and magnetic field exposure and cancer is well documented in the literature, but there is uncertainty regarding a causal relation. *Methods:* Using data from a completed cohort study, we sought to refine the job-exposure matrix in a case-cohort analysis by regrouping jobs into more homogeneous groups, but without making additional measurements. From the original cohort, we selected the 164 men who died of leukemia, 145 men who died of brain cancer, and a random subcohort of 800 men (0.6% of the cohort). Erroneous job assignments were corrected and job groups were subdivided based on differences in work environments or tasks performed. *Results:* Magnetic field exposure remained unrelated to leukemia mortality and positively associated with brain cancer mortality based on both cumulative and average magnetic field indices. Although not monotonic across the middle intervals, increased risk of brain cancer was found in relation to career exposure, with risk ratios of 1.8 (95% CI: 0.7-4.7) and 2.5 (95% CI: 1.0-6.3) in the uppermost categories for cumulative and average exposure, stronger for exposure 2-10 years past. *Conclusions:* Improvements in exposure assignment based only on reassignment of job titles to occupational categories had little impact on the measured associations of magnetic fields with leukemia or brain cancer.

Comment: This is a follow up of a study by Savitz et al. (Savitz, 1995) which used a refined job-exposure matrix that redefined jobs into more homogeneous groups, thereby eliminating some of the erroneous job assignments that might have occurred in the original study. An increased risk of brain cancer mortality was positively associated with average exposure, RR=2.5, 95% CI = 1.0 - 6.3, and non-significantly for cumulative exposure, RR=1.8, 95% CI = 0.7 - 4.7. The improvements in exposure assignment, based only on reassignment of job titles to occupational categories, did not change the overall conclusion of the previous study (Savitz, 1995).

Minder CE and Pfluger DH. Leukemia, brain tumors, and exposure to extremely low frequency electromagnetic fields in Swiss railway employees. American Journal of Epidemiology 2001;153(9);825-840.

The principal author is with the Institute for Social and Preventive Medicine, University of Berne, Berne, Switzerland.

Abstract: Railway engineers provide excellent opportunities for studying the relation between exposure to extremely low frequency magnetic fields and leukemia or brain tumors. In a cohort study of Swiss railway personnel with 2.7 x 10(5) person-years of follow-up (1972--1993), The principal author compared occupations with high average exposures (line engineers: 25.9 µT) to those with medium and low exposures (station masters: 1 µT). The mortality rate ratio for leukemia was 2.4 (95% CI: 1.0-6.1) among line engineers (reference category: station masters). The mortality rate ratio for brain tumors was 1.0 (95% CI: 0.2-4.6) among line engineers and 5.1 (95% CI: 1.2-21.2) among shunting yard engineers (compared with station masters). Two exposure characteristics were evaluated: cumulative exposure in µT-years and years spent under exposure to magnetic fields of greater than or equal to 10 µT. There was a significant increase in leukemia mortality of 0.9% (95% CI: 0.2-1.7) per µT-year of cumulative exposure to extremely low frequency magnetic fields. The increase by years spent under exposure of greater than or equal to 10 µT was even stronger: 62% per year (95% CI: 15-129). Brain cancer risk did not show a dose-response relation. This study contributes to the evidence for a link between heavy exposure to extremely low frequency magnetic fields and leukemia. Its strengths include reliable measurements and reliable historical reconstruction of exposures.

Comment: While this study does not directly relate to 50/60 Hz PFF, it does involve a detailed examination of the effect of low frequency (16.7 Hz) magnetic fields on incidence of brain cancer. The Minder study is included in this review of the literature for completeness, since, some laboratory studies have found empirical evidence to support a phenomenon, termed “resonance” in cells exposed to 15 Hz electromagnetic fields. Resonance has been postulated to change the pattern of intracellular calcium ion fluxes, which may or may not lead to biological effects. If resonance effects were observed at 16.7 Hz, then they would also be relevant at, or near multiples of that frequency, namely 50 and 60 Hz. Calcium resonance is not considered by physicists to be a plausible mechanism to explain the interaction between PPF and biological systems.

Two hypotheses, formulated *a priori*, were tested in Minder’s study. *Hypothesis one:* Among four groups of railway employees (line engineers, shunting yard engineers, train attendants, and station masters), the groups with the higher exposures to ELF magnetic fields would have a higher mortality from leukemia and brain tumors than groups with lower exposures. *Hypothesis two:* Independent of job description, there would be a dose-response relation between leukemia and brain cancer mortality, and cumulative exposure (µT-years) to ELF magnetic fields and to the time spent exposed to ELF magnetic fields of greater than 10 µT.

The study cohort was established using Swiss Federal Railways personnel and pension records, and consisted of all male employees and living retirees from 1972 to 1993. Only four jobs were considered (see hypothesis one). Mortality follow-up was for the same period and consisted of linking personnel or pension records to death certificates, with follow-up cross-checks with the Cancer Registry.

Exposures of railway engineers were accurately measured, since the position of the train's driver was fixed and the EMF characteristics remained stable over the service lifetime of the engine. Swiss trains run on 16.7 Hz alternating current but measurements could record magnetic flux densities in the range of 0 to 100 Hz every 10 seconds. A measurement series covered the complete driving cycle (starting, acceleration, driving, braking and stopping). Measurements were recorded at the level of the head, thorax and feet. The magnetic flux densities varied considerably within a specific type of train engine; coefficients of variation of magnetic flux density within one driving cycle was less than or equal to 26%. For estimation of historical exposures, engine-specific exposure characteristics for all electric type engines, in operation from 1905 to 1993, were evaluated and then adjusted to reflect the proportion of time used relative to steam plus diesel-electric engines, which operates on DC. Exposure for train attendants and station masters was assessed by spot measurements, lasting 2 to 20 minutes, at various locations within their respective work spaces (thereby differing significantly from more accurate measurements made on engineers). Exposure was then estimated as a time-weighted average of location-specific field strengths. Historical exposures for train attendants and station masters were not available and were linearly interpolated between 0 for 1900 and the exposure level for 1993. The proportion of smokers among the groups were noted as, 8% of line engineers, 12% of station masters, 29% of train attendants, and 38% of shunting yard engineers; but, in this study, smoking was not considered to be a potential confounder (smoking was not considered as a risk factor for brain cancer).

Of the total cohort of 18,070 men, 23 deaths were from brain tumors (4 for line engineers out of 6879, 5 for shunting yard engineers out of 1314, 11 for train attendants out of 5720, and 3 for station master out of 4157). The RR for brain tumors (adjusted for age and calendar period) as a cause of death, relative to station masters (the lowest exposed sub-population) were 1.02, 95% CI = 0.23-4.55 for line engineers; 5.06, 95% CI = 1.21-21.2 for shunting yard engineers; and 2.67, 95% CI = 0.75-9.62 for train attendants. The principal author concluded that overall the risk of brain tumors did not appear to be related to magnetic fields alone, and suggested some role for electric fields. A dose-response relationship between exposure and brain tumors was not established and both hypotheses were rejected. The magnitudes of the RR for each job category paralleled the percentage of smokers in each group, so smoking might have contributed to the results observed for shunting yard engineers (i.e. brain cancers could have been secondary tumors arising from the lungs). Other limitations of this study include failure to assess the magnitude of electric fields, and exposure misclassification, since measurements were done in different ways for the four groups

of workers. Also, errors in ascertaining death could have occurred during the process of linking personnel and pension records to death certificates (i.e. problems of false positives, such as false links, and false negatives, such as missed links. The use of death certificates to identify brain cancer cases can also lead to some degree of disease misclassification, since the tumor could represent metastatic spread from other anatomical sites, or they could have been benign and death could have been the result of medical complications associated with treatment. A study of this small size in terms of cases has only a limited power to detect a real difference. In summary, no conclusive link was found between brain cancer and exposure to magnetic fields, except for shunting yard engineers, and the possibility of a spurious result cannot be ruled out.

Villeneuve PJ, Agnew DA, Johnson KC and Mao Y. Brain cancer and occupational exposure to magnetic fields among men: results from a Canadian population-based case-control study. International Journal of Epidemiology 2002;31(1):210-217.

The principal author is with the Canadian Cancer Registries Epidemiology Research Group, Environmental Risk Assessment and Case Surveillance Division, Laboratory Centre for Disease Control, Health Canada, Ottawa, Ontario, Canada K1A 0L2.

Abstract: *Background:* The relationship between occupational exposure to magnetic fields and brain cancer in men was investigated using population-based case-control data collected in eight Canadian provinces. Emphasis was placed on examining the variations in risk across different histological types. *Methods:* A list of occupations was compiled for 543 cases and 543 controls that were individually matched by age. Occupations were categorized according to their average magnetic field exposure through blinded expert review (< 0.3, 0.3-<0.6, and ≥ 0.6 μT). In total, 133 cases (14%) and 123 controls (12%) were estimated to have at least one occupation whereby magnetic field exposures exceeded 0.3 μT. Odds ratios (OR) were generated using conditional logistic regression, and were adjusted for suspected occupational risk factors for brain cancer. *Results:* A non-significantly increased risk of brain cancer was observed among men who had ever held a job with an average magnetic field exposure > 0.6 μT relative to those with exposures < 0.3 μT (OR = 1.33, 95% CI: 0.75-2.36). A more pronounced risk was observed among men diagnosed with glioblastoma multiforme (OR = 5.36, 95% CI: 1.16-24.78). Moreover, a cumulative time weighted index score of magnetic field exposure was significantly related to glioblastoma multiforme (p = 0.02). In contrast, magnetic field exposures were not associated with astrocytoma or other brain cancers. *Conclusions*: Our findings support the hypothesis that occupational magnetic field exposure increases the risk of glioblastoma multiforme.

Comment: This study was undertaken to investigate the relationship between occupational magnetic field exposures and different histological types of brain cancer. Using data collected through the Canadian National Enhanced Cancer Surveillance

System (CNECSS), the principal author examined this relationship using several different measures of exposure. It was postulated that elevated risks for the more aggressive subtype of brain cancer would support the premise that magnetic fields could act in the tumor promotion stage of carcinogenesis. The study was restricted to males.

The analysis was based on 543 brain cancer cases made up of astrocytoma, 213 cases, glioblastoma, 198 cases, others, 115 cases, and unknown, 16 cases, and 543 age-matched controls. Steps were taken to enroll the cases early in their disease to minimize the loss of subjects due to severe illness or death. Data was not collected for those identified subjects that died, or whose physician would not agree to participate (a total of 33.3%). Of the remainder, 63% completed a questionnaire, which was similar to the 65% completion rate for controls. All brain cancer cases were confirmed by histological examination. Benign tumors were excluded from the analysis. Controls were selected from provincial health insurance plans, provincial Departments of Finance, or by random digit-dialing, to achieve a similar age and sex distribution to all cancer cases. The size of the study was considered sufficiently large to perform risk assessment across different histological types of brain cancer.

Mailed questionnaires were used to obtain information on subjects' residential and occupational histories, and on other putative occupational risk factors for brain cancer. When necessary, telephone interviews were conducted to clarify responses. Data was collected on ethnicity, education, income, smoking, height, weight, physical activity, diet 2 years before interview, changes in diet from 20 years ago, exposure to specific occupational carcinogens, including pesticides, herbicides, radiation sources, and vinyl chlorides. Each subject was asked to list all jobs held for at least one year, and all residences occupied for at least one year in Canada. Participants were asked to describe their job-title, company name, work location, duties, the start and end dates of employment, workplace odors, and tobacco smoke. Residential data included address, occupancy period, the source of water, type of heating and the number of smokers in the residence.

The assessment of occupational magnetic field exposures was performed by expert review that was blinded to the case-control status of the subjects. The study derived magnetic field exposure indices that took into account the complete occupational history of each subject. Each occupation was assigned an exposure value based on a presumed time-weighted mean flux density. The categories of average exposure were <0.3, 0.3 - < 0.6, and > 0.6 μT. The lower cut-point of 0.3 μT was chosen to assure that occupational exposures in the upper two categories were greater than residential background levels. Information on residential exposures were obtained from a study by Green et al. (Green, 1999). The 0.3 μT cut-point was estimated to correspond to the 82nd percentile for adult exposures (Ontario Hydro, 1989). The resulting OR were calculated for a number of different magnetic field exposure scenarios: (i) the highest average occupational exposure, (ii) the magnetic field exposure received in the job of

longest duration, (iii) the exposure in first held job, (iv) the exposure held in the last job, and (v) the cumulative time-weighted occupational magnetic field exposure, which was calculated from the estimated exposure at each job, the duration of the job, and whether the job was full-time. Several variables were also examined as potential confounders, including self-reported exposures to vinyl chloride, herbicides, pesticides, and ionizing radiation. An index of exposure for ionizing radiation was constructed on the basis of expert review, and cases and controls were classified as having an annual exposure of < 1 or > 1 mSv.

For cases, it was estimated that 86% held jobs which had exposure levels < 0.3 μT, and for controls the value was 88%. A statistically non-significant increase in risk of brain cancer was observed for subjects who had ever held a job having average magnetic field exposures of > 0.6 μT, relative to those with a level of < 0.3 μT, OR=1.33, 95% CI = 0.75-2.36. When risk analysis was restricted to the highest level of exposure ever received, the OR for glioblastoma multiforme was 5.36, 95% CI = 1.16 - 24.78 for 18 cases and 6 controls, after adjustment for exposure to ionizing radiation and vinyl chloride. No significant differences were detected when a similar comparison was made for those with astrocytomas or any other type of brain cancer, or when exposure was based on the longest held job with an exposure > 0.6 μT. A significant risk for glioblastoma multiforme was detected when the analysis was based on an occupational exposure of > 0.6 μT at the last job held, OR=12.59, 95% CI = 1.50 -105.6 for 8 cases and 1 control, but not for the first job held, OR = 4.81, 95% CI = 0.94 - 24.71 for 10 cases and 3 controls. Finally, the relationship between glioblastoma multiforme and the cumulative index of occupational exposure was modeled by regression analysis. The change in the risk of glioblastoma multiforme, per unit increase in cumulative index of magnetic field exposure, was significantly different from control (p = 0.02), and the OR for the change in risk of cancer, per unit increase in the cumulative index, was significant at 1.04, after adjustment of model parameters for exposure to ionizing radiation and vinyl chloride.

As a whole, brain cancer was not significantly related to occupational magnetic fields. However, when the analysis was restricted to histological subtypes, the highest exposure during the last job and the cumulative exposure index were positively associated with glioblastoma multiforme. The differences between the two risk estimates for the first and last job held could be due to the small size of the samples. For example, the OR for the first job held was based on 10 cases and 3 controls, while the OR of the last job held was based on 8 cases and 1 control. The statistical power for such small samples would be low (as suggested by their wide confidence intervals), and therefore the possibility of a spurious correlation cannot be ruled out. Another area of concern is the exclusion of cases that were known to have died, which could result in missed cases of aggressive and rapidly growing brain cancers. This error of omission would result in an attenuation of the observed OR because the data set would consist of less aggressive tumors types. The weak correlation between residential and workplace exposures suggests that residential sources would not have a significant

impact on exposure classification. However, much of the work histories depended on the ability of the participant to accurately recall past events, this could be a potential source of exposure misclassification, as could the reliance placed on the subjective method used to assign participants to exposure categories. Also, the exposure categories that defined the cut-points 0.3 - 0.6 μT and > 0.6 μT contained small numbers of subjects, only 16.1% of subjects were in the 0.3 - 0.6 μT category and 4.1% were in the > 0.6 μT. The small sample sizes and the limited statistical power of this study, as reflected in the wide confidence intervals, suggest the results be interpreted with caution. It is a study worth repeating with greater numbers of cases.

4.5 Conclusions - Brain Cancer

Studies of the relationship between the exposure of adults and children to residential PFF and brain tumors have produced inconsistent results, regardless of whether exposure was defined as wire codes, distance from source, or measured or calculated PFF. The meta-analysis of occupational studies indicates a slightly elevated risk of brain tumors for electrical workers without any discernible dose-response trend. Some well designed studies have also suggested a small increase in brain cancer risk while others have not. The inconsistency among the studies has been related to exposure misclassification.

A recent meta-analysis of occupational studies indicated a slightly elevated risk of brain tumors for electrical workers, without a discernible dose-respond trend (Kheifets, 1995). An updated analysis by Kheifets (2001) to include nine new studies gave a pooled OR of 1.16, 95% CI = 1.08 - 1.24, which is similar to the results of the 1995 meta-analysis. Of interest is the finding that none of the nine new studies individually found an increase in the risk for brain cancer, OR = 1.00, 95% CI = 0.99 - 1.01. The lack of compatible study designs and standardized methods of exposure assessment make cross-study comparisons difficult and, consequently, the results of any meta-analysis somewhat problematic.

Brain Cancer Appendix

A.1 Johansen C, Raaschou-Nielsen O, Skotte J, Thomsen BL and Olsen JH. Validation of a job-exposure matrix for assessment of utility worker exposure to magnetic fields. Appl Occup Environ Hyg 2002;17(4):304-310.

The principal author is with the Institute of Cancer Epidemiology, the Danish Cancer Society, Copenhagen.

Abstract: The aim of this study was to evaluate a 50-Hz electromagnetic field job-exposure matrix used in epidemiological studies of a nationwide cohort of utility workers in Denmark. We compared a job-exposure matrix that distinguished four categories of exposure to 50-Hz time-weighted average (TWA) magnetic fields: low (<

0.1 μT), medium (0.1-0.29 μT), high (0.3-0.99 μT) and very high (> 1.0 μT) of utility company employees with 196 measurements of 8-h exposure for 129 workers in this industry. The 129 workers were selected from the following five main work environments: generation facilities, transmission lines, distribution lines, substations, and other electrically and non-electrically relates jobs. This study shows that the job-exposure matrix can be expected to introduce misclassification mainly between adjacent categories of exposure. Thus, the distribution of measurements of exposure to 50-Hz magnetic fields was similar for workers in the medium and the high exposure matrix categories. But the two extreme categories satisfactorily separate low and very highly exposed workers. The study shows that epidemiological use of this job-exposure matrix might combine the two intermediate categories of exposure. If the sample size in extreme categories provides enough power, a study in which this job-exposure matrix is used should allow detection of a true association between exposure to 50-Hz magnetic field and disease.

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Johansen C, Raaschou-Nielsen O, Skotte J, Thomsen BL and Olsen JH. Validation of a job-exposure matrix for assessment of utility workers exposure to magnetic fields. Appl Occup Environ Hyg. 2002;17(4):304-310.

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Savitz DA and Loomis DP. Magnetic field exposure in relation to leukemia and brain cancer mortality among electric utility workers. American Journal of Epidemiology 1995;141:123-134.

Savitz DA, Cai J, van Wijngaarden E, Loomis D, Mihlan G, Dufort V, Kleckner RC, Nylander-French L, Kromhout H and Zhou H. Case-cohort analysis of brain cancer and leukemia in electric utility workers using a refined magnetic field job-exposure matrix. American Journal of Industrial Medicine 2000;38:417-425.

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Wei M, Guizzetti M, Yost M and Costa LG. Exposure to 60-Hz magnetic fields and proliferation of human astrocytoma cells *in vitro*. Toxicol Appl Pharmacol 2000;162:166-176.

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Wrensch M, Yost M, Miike R, Lee G and Touchstone J. Adult glioma in relation to residential power frequency electromagnetic field exposures in the San Francisco Bay area. Epidemiology 1999; 10(5), 523-527.

5. NEURODEGENERATIVE DISEASES

5.1 Introduction

The only established mode of interaction between PFF and humans is by means of induced currents from strong magnetic fields. In the case of environmental exposure, these fields are usually too weak to induce currents whose magnitudes are above the signal to noise ratio produced by the normal functioning of the body's nervous system. Hypotheses relating PFF to neurodegenerative disease are a relatively new aspect of EMF research. For a number of methodological reasons, the study of neurodegenerative diseases is more difficult than the study of cancer. For example, there are fewer registries for neurodegenerative diseases than for cancer, and identifying cases from death certificates is less reliable than for cancer, since there is a lack of consensus on diagnostic criteria. For some diseases, such as Alzheimer's Disease, diagnosis must await autopsy for verification, but a study's publication often precedes this, rendering it prone to errors of disease misclassification. To-date, studies have focused on the relationship between PFF and amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and, to a lesser extent, multiple sclerosis (MS), Parkinson's disease (PD), depression and suicide, and cognitive impairment. ALS is a rare, fatal neurodegenerative disease which has an annual incidence rate of about 1/100,000, with men being twice as susceptible as women. The etiology of ALS is unknown; however, identification of a gene associated with familial ALS has been reported on chromosome 21 (Rosen ,1993). In a number of previous studies, the number of episodes of electric shock before development of ALS was significantly higher among cases than controls [(Gawel, 1983) and (Gunnarsson, 1992)]. However, the few studies that have examined the relationship between PFF and the neurodegenerative diseases generally have many methodological shortcomings and, invariably, small sample sizes, which together makes their interpretation somewhat problematic. The nature of the relationship, if any, will have to remain highly speculative until methodological issues can be resolved.

5.2 Summary of Study Findings

In a series of studies, Johansen (1998a&b, 2000) examined a cohort of 30,631 persons employed in the Danish utility industry for evidence of an association between occupational exposure to PFF and the incidence of senile dementia, presenility, including AD, other demyelinating CNS neuropathies, Parkinson's disease, cerebral palsy, epilepsy, motor neuron disease, including ALS, progressive bulbar palsy, progressive muscular atrophy, and diseases of the spinal medulla. Medical records were obtained for ALS and other motor neuron diseases to verify diagnosis, and to obtain information on episodes of electric shock. Since the cohort was linked to nationwide population-based patient registries, the use of death certificates as the sole source of reporting mortality was avoided, thereby reducing the possible impact of

disease misclassification on results. Exposure was assessed by a job-exposure matrix (Johansen, 2002).

The incidence rates of the diseases in the cohort were compared with the corresponding rates found in the general population. The results were expressed as a standardized incidence ratio (SIR = Observed/Expected). Men had an increased risk for all motor neuron diseases (including ALS) with an SIR of 1.89, 95% CI = 1.16 - 2.93, based on only 20 cases. Individually, the CNS diseases were non-significantly related to PFF exposure, but the observed associations could have been spurious, since the small sample sizes would have limited the statistical power of the tests. For women in the cohort, the number of identified positives was too few to be analyzed.

Johansen (1999) also used the same cohort of Danish utility workers to investigate the hypothesis that exposure to PFF, or electric shock, could cause neuronal degeneration, which could lead to various chronic neurologic diseases, including MS. The incidence of MS was assessed by data linkage to a unique nationwide Danish MS registry and compared with the appropriate rates of MS in the general population. Only verified cases of MS were included in the study. A dedicated EMF job-exposure matrix (Johansen, 2002) was used to assign exposure. The calculated SIR found no excess risk for MS in either men or women. Although limited by a small sample size, the results does not support the hypothesis that PFF is one of the environmental agents responsible fo MS. Larger studies will be needed to confirm this result.

A summary table of relevant studies is given below.

Table 3. Neurodegenerative disease

Author	Year	Country	Disease	Increased Risk ?	Weakness
Johansen	1998b	Denmark	ALS; AD; D&S; AD; SD; CI	ALS yes others no	SSS; MC; DM; RB
Savitz	1998	USA	ALS; AD; PD	inconclusive	SSS; MC
Feychting	1998	Sweden	SD; AD	D&S yes AD no	SSS; DM; MC
Johansen	1999	Denmark	MS	no	SSS; DM
Graves	1999	USA	AD	no	EA; RB
Johansen	2000	Denmark	ALS; AD; PD; SD	ALS yes Others no	SSS; MC
Li	2002	Taiwan	CI	no	EA; SSS

Abbreviations:

AD = Alzheimer's Disease
ALS = Amyotrophic Lateral Sclerosis
CI = Cognitive Impairment
DM = (possible) Diagnostic Misclassification
D&S = Depression and Suicide
EA = (possible) Exposure Assessment issues
MC = Multiple Comparisons
MS = Multiple Sclerosis
RB = (possible) Recall Bias
SSS = Small Sample Size
PD = Parkinson's Disease
SD = Senile Dementia

5.3 Detailed Review of Individual Studies

Johansen C and Olsen JH. Mortality from amyotrophic lateral sclerosis, other chronic disorders, and electric shocks among utility workers. American Journal of Epidemiology 1998b;148(4):362-368.

The principal author is with the Division for Cancer Epidemiology, Danish Cancer Society, Copenhagen.

Abstract: Above-average exposure to electromagnetic fields has been associated with certain nonmalignant medical conditions such as amyotrophic lateral sclerosis, other neurologic diseases, depressive symptoms, and suicide. The principal author conducted a nationwide mortality study in Denmark of 21,236 men employed in utility companies between 1900 and 1993. The causes of death were ascertained for January 1, 1974, through December 31, 1993, and cause-specific mortality was analyzed by latency and estimated levels of exposure to 50-Hz electromagnetic fields. Overall, 3,540 deaths were observed as compared with 3,709 expected from national mortality rates, yielding a standardized mortality ratio of 0.96 (95% CI = 0.93-0.99). A slight excess in mortality from cancer was due to deaths from cancers of the lung and pleural cavity, probably because of exposure to asbestos. A twofold increase in mortality from amyotrophic lateral sclerosis and a tenfold increase in mortality from electrical accidents were seen on the basis of 14 and 10 deaths, respectively, the former increasing with time since first employment in a utility company. The excess mortality from amyotrophic lateral sclerosis seems to be associated with above-average levels of exposure to electromagnetic fields and may be due to repeated episodes with electric shocks.

Comment: This is a cohort study of male employees in Danish utility companies employed during the period 1974 to 1993. Cases of death from ALS were obtained from a mortality registry. With the use of a well defined cohort and death registry to identify

cases, selection bias would be small. There were only 9 exposed cases, making this a relatively small study. Exposure to magnetic fields was by measurement-based job titles. Job histories were used to determine the duration of exposure. The overall standardized mortality ratio (SMR), calculated as the ratio of observed to expected deaths, was 2, 95% CI = 1.1 - 3.4 for 14 observed (6.9 expected) cases ($p < 0.05$). A non-significant rising trend in risk was seen with increasing time since first employment, with an SMR of 2.7, 95% CI = 1.0 - 6.0 (6 cases only) for a follow-up period of 30 or more years ($p < 0.05$). An increasing trend in mortality from ALS was seen with increasing level of estimated exposure to PFF, although neither trend nor any of the individual SMR reached significance. When the high (> 1 μT) and medium (0.3 - 0.99 μT) exposure levels were combined, a significantly increased mortality from ALS was observed, based on 9 observed deaths (3.6 expected), SMR = 2.5, 95% CI = 1.1 - 4.8. It was hypothesized that the increased number of episodes of electric shock may be somehow linked to the increase in risk of death from ALS. This study also found no increased mortality from other neurological disease that previously had been associated with above average exposures to PFF, including senile dementia and Alzheimer's disease. There were no indication of excess mortality from suicide, based on 133 cases. In addition, there were no excess deaths from cardiovascular or respiratory diseases associated with elevated exposures to PFF. This is a wide-ranging, well designed study that examined a large number of endpoints. However, the portion of the study relating to ALS has limited statistical power due to its small size, and therefore provides only limited support to the hypothesis that exposure to PFF increases the risk of death from ALS.

Savitz DA, Checkoway H and Loomis DP. Magnetic field exposure and neurodegenerative disease mortality among electric utility workers. Epidemiology 1998a;9(4):398-404.

The principal author is with the Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill 27599-7400 USA.

Abstract: Several recent reports indicate that occupational exposure to electric and magnetic fields may be associated with increased risk of neurodegenerative diseases. To address that hypothesis, we analyzed data from a cohort study of electric utility workers. We examined exposure to magnetic fields, assessed as duration of work in exposed jobs and through an index of cumulative exposure based on magnetic field measurements, in relation to mortality from Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, considering both underlying and all mentioned causes of death. Adjusted mortality rate ratios based on Poisson regression models indicate no association between magnetic fields and Parkinson's disease and little support for an association with Alzheimer's disease mortality. Mortality from amyotrophic lateral sclerosis was positively associated with duration of work in exposed jobs [rate ratio = 2.0, 95% confidence interval (CI)= 0.7-6.0; and rate ratio = 3.1, 95% CI = 1.0-9.8, based on underlying cause for 5 - < 20 years and > or = 20 years vs < 5

years, respectively], as well as with cumulative magnetic field exposure with a > or = 20-year lag (rate ratio = 2.3, 95% CI = 0.8-6.6; and rate ratio = 3.0, 95% CI = 1.0-9.2, for exposure in the middle and upper intervals relative to the lowest interval, respectively).

Comment: This is a cohort study of male utility workers, employed from 1950 to 1988, that investigated the association between PFF and the risk of neurodegenerative diseases such as ALS, Alzheimer's disease (AD) and Parkinson's disease (PD). Cases were selected from mortality registries to keep the risk of selection bias small. Exposure was assessed by job classification, field measurements, and duration of employment. Despite the nominal size of the cohort, however, the number of exposed cases was small, only 9 cases with more than 20 years in exposed occupations. The small sample size, together with the possibility of misdiagnosis makes it difficult to interpret the results. For ALS, the RR = 2.4; 95% CI = 0.8 - 6.7. The RRs for AD and PD were also non-significant.

Savitz DA, Loomis DP and Tse C-KJ. Electrical occupations and neurodegenerative disease: analysis of U.S. mortality data. Archives of Environmental Health 1998b;53(1): 71-74.

The principal author is with the Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill 27599-7400, USA.

Abstract: Investigators have hypothesized that occupations involving electric and magnetic field exposure are associated with a variety of health problems, including neurological disease. The principal author conducted a case-control study, and they used U.S. death certificates with occupational coding to compare male cases of Alzheimer's disease (n = 256), Parkinson's disease (n = 168), and amyotrophic lateral sclerosis (n = 114) with controls matched for age and calendar time. The principal author selected controls in a 3:1 ratio to cases from persons who died of causes other than leukemia, brain cancer, and breast cancer. Overall associations with electrical occupations were modest (i.e., adjusted odds ratios of 1.2, 1.1, and 1.3 for Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, respectively). Individual electrical occupations were associated more strongly with disease than overall electrical occupations, particularly amyotrophic lateral sclerosis, for which relative risks ranged from 2 to 5 across several job categories. The largest associations with all three diseases occurred for power plant operators.

Comment: Cases were selected from death certificates and only job titles were used to assess exposure. The strengths of this study are in the small risk of selection or recall bias, and the relatively large sample size. The major weakness was that exposure assessment relied only on job title, without verification by field measurements. In addition, work history was not considered, only job title at one point in time. When

cases with electrical occupations were taken in the aggregate, ALS had an RR of 1.3; 95% CI = 1.1 - 1.6 , others were non-significant.

Feychting M, Pedersen NL, Svedberg P, Floderus B and Gatz M. Dementia and occupational exposure to magnetic fields. Scandinavian Journal of Work, Environment & Health 1998;24(1):46-53.

The principal author is with the Institute of Environmental Medicine, The Karolinska Institute, Stockholm, Sweden.

Abstract: *Objectives:* The purpose of the present report was to assess whether occupational magnetic field exposure is a risk factor for dementia, in particular for Alzheimer's disease. *Methods:* Case-control analyses were applied to 77 dementia cases, 55 of whom had Alzheimer's disease, ascertained from the population-based Swedish twin register. Two reference groups were derived, with 228 and 238 persons, respectively. Occupations were linked to a job-exposure matrix based on magnetic field measurements. Primary occupation, last occupation before reference date, and the occupation with the highest magnetic field exposure during the subject's lifetime were evaluated. *Results:* For primary occupation, all relative risk estimates were close to unity. For last occupation, at the exposure level > or = 0.2 μT, a relative risk was found for dementia estimated at 3.3 [95% confidence interval (95% CI): 1.3-8.6] and 3.8 (95% CI: 1.4-10.2) for reference groups 1 and 2, respectively. The relative risk for Alzheimer's disease was estimated at 2.4 (95% CI: 0.8-6.9) and 2.7 (95% CI: 0.9-7.8), respectively. For the occupation with the highest magnetic field exposure, the relative risk estimates were close to unity for reference group 1 and slightly elevated for reference group 2. The relative risk estimates were greater for the subjects who were younger at onset (< or =75 years). *Conclusions:* These results only partially support previous findings, but they indicate that occupational magnetic field exposure may possibly influence the development of dementia.

Comment: The conclusion of this study, that occupational magnetic field exposures may possibly influence the development of dementia, is speculative, as suggested by the wide confidence intervals. The prospect of misdiagnosis of AD can not be ruled out, because the study was published before diagnoses were confirmed by autopsy. Therefore, it is equally speculative to conclude that there was no association between exposure to PFF and the increased risk of Alzheimer’s. The small size and the inconsistent findings (positive for dementia and negative for Alzheimer’s) adds to confusion concerning the nature of the relationship between PFF and neurodegenerative diseases.

Johansen C, Koch-Henriksen N, Rasmussen S and Olsen JH. Multiple sclerosis among utility workers. Neurology 1999;52:1279-1282.

The principal author is with the Institute of Cancer Epidemiology, the Danish Cancer Society, Copenhagen, Denmark.

Abstract: The incidence of MS was assessed in a nationwide cohort study of 31,990 employees of Danish utility companies between 1900 and 1993. Overall, 32 cases of MS were diagnosed, as compared with 23.7 expected from national incidence rates, to yield a standardized incidence ratio of 1.35 (95% confidence interval, 0.92 to 1.91). We found no support for the hypothesis of an association between occupational exposure to electromagnetic fields and the risk of MS.

Comment: This study tested the hypothesis that exposure to PFF, or electric shock, could cause neuronal degeneration, which could lead to various chronic neurologic diseases, including multiple sclerosis. The study was based on the same nation-wide cohort of men and women employed in Danish utility companies, as discussed previously for brain and other cancers (Johansen and Olsen, 1998a), and neurodegenerative conditions. The incidence of MS was assessed by data linkage to a unique nationwide Danish register of MS and compared with the appropriate rates of MS in the general population. The risk pattern for MS was examined by duration and estimated average level of exposure to PFF at work. A dedicated EMF job-exposure matrix (Johansen, 2002) was designed to distinguish 25 different job titles held by utility company employees and 19 work areas within the industry. The current study 31,990 employees (26,124 men and 5,866 women) with at least 3 months of employment, between 1968 and 1993. Only verified cases of MS were included in the study. The expected number of MS cases was derived by applying the appropriate age-, sex-, and calendar-specific rates of MS derived from the Danish Multiple Sclerosis Society. Standardized incidence ratios (ratio of observed to expected cases) and confidence intervals were calculated. Overall, 32 cases of MS were observed, with 23.7 expected, the SIR was 1.4 and the 95% CI = 0.9 - 1.9. For men, 21 cases were observed (17.3 expected) with an SIR of 1.2, 95% CI = 0.8 - 1.9. For women, the SIR was 1.7, 95% CI = 0.9 - 3.1. No overall trend in risk for MS was seen with time since first employment, or by number of years employed in a utility company. Although, MS is relatively common in young and middle-aged adults, its cause remains largely unknown. The evidence from some studies seems to be consistent with an environmental influence occurring on a background of genetic susceptibility. The results of the study by Johansen does not support the hypothesis that PFF is one of the environmental agents responsible for MS.

Graves AB, Rosner D, Echeverria D, Yost M and Larson EB. Occupational exposure to electromagnetic fields and Alzheimer disease. Alzheimer Dis Assoc Disord 1999;13(3):165-170.

The principal author is with the Department of Epidemiology and Biostatistics, University of South Florida, Tampa 33612-3805, USA.

Abstract: The association between occupational exposure to electromagnetic fields (EMF) and Alzheimer disease (AD) was examined. Subjects were identified from a large health maintenance organization in Seattle, Washington, and matched by age, sex, and proxy type. A complete occupational history was obtained from proxies and controls. Following the interview, two industrial hygienists (IHs) rated exposures to EMF for each job blinded to case-control status. Exposures to EMF were rated as probable intermittent exposure or probable exposure for extended periods to levels above threshold. Conditional logistic regression was used to calculate the risk of AD given EMF exposure stratified by IH. The odds ratios for ever having been exposed to EMF were 0.74 [95% confidence interval (CI): 0.29-1.92] and 0.95 (95% CI: 0.27-2.43) for each IH, adjusting for age and education. No dose-response effect was noted. Agreement between the two IHs for ever having been exposed to EMF was good (kappa = 0.57, $p < 0.0001$). This study was unable to support an association between EMF and AD.

Comment: This is a clinical-based study so the probability of misdiagnosis is low. The weakness is in the exposure assessment, which used proxies to obtain occupational histories, and industrial hygienists to estimate the probable levels of exposure for the jobs held by cases and controls.

Johansen C. Exposure to electromagnetic fields and risk of central nervous system disease in utility workers. Epidemiology 2000;11(5):539-543.

The principal author is with the Institute of Cancer Epidemiology, the Danish Cancer Society, Copenhagen, Denmark.

Abstract: Occupational exposure to electromagnetic fields has been associated with neurological diseases such as amyotrophic lateral sclerosis, senile dementia, Parkinson disease, and Alzheimer disease. I studied the incidence of central nervous system diseases in 30,631 persons employed in Danish utility companies between 1900 and 1993. I linked the cohort to the nationwide, population-based Danish National Register of Patients and compared the numbers of cases of these diseases observed between 1978 and 1993 with the corresponding rates in the general population. In addition I fit to the data on utility workers a multiplicative Poisson regression model in relation to estimated levels of exposure to 50-Hz electromagnetic fields. Overall, there was an increase in risk for senile dementia and motor neuron diseases combined. The incidences of Parkinson disease, Alzheimer disease, and other diseases of the central nervous system were essentially unrelated to exposure to electromagnetic fields. A decreased risk of epilepsy compared with the general population probably reflects a healthy worker effect; I observed an increased risk of epilepsy based on internal comparisons. The increased risk for senile dementia and motorneuron diseases may be associated with above-average levels of exposure to electromagnetic fields.

Comment: The hypothesis tested was that exposure to PFF is associated with an increase in mortality from central nervous system diseases, defined here as ALS, AD, senile dementia and PD. The study is related to 3 previous reports by Johansen (Johansen, 1998a&b, 1999). As in the previous studies, the cohort consisted of persons employed in the Danish utility industry between 1900 and 1993. The cohort was linked to a nationwide population-based patient registry. Data on the utility workers included duration of employment and estimated average level of exposure to PFF at work. The average exposure was calculated from a job-exposure matrix (described in Johansen, 2002). Five categories of exposure were distinguished, background (<0.09 μT), low exposure (0.1 to 0.29 μT), medium (0.3 to 0.99 μT) and high (> 1.0 μT). The CNS diseases examined were senile dementia, presenility, including AD, other demyelinating CNS neuropathies, PD, cerebral palsey, epilepsy, motor neuron disease, including ALS, progressive bulbar palsy, progressive muscular atrophy, and diseases of the spinal medulla. Medical records were obtained for ALS and other motor neuron diseases to verify diagnosis and to obtain information on episodes of electric shock. The use of death certificates, as a sole source of reporting mortality from neurodegenerative diseases, was avoided in this study because they can be a source of disease misclassification, since the reliability of the information they provide depends on the quality of the diagnosis, which for AD would mean follow-up confirmation at death by autopsy. Instead, this study assessed the incidence rates by data linkage to the nationwide register of patients and compared the rates found in the cohort with the corresponding rates of these diseases in the general population, and expressed the results as standardized incident rates (SIR). Men had an increased risk for all motor neuron diseases combined, SIR = 1.89, 95% CI = 1.16 - 2.93, based on only 20 cases, most of which were for ALS, and a non-significant increasing trend with dose. A similar non-significant increasing trend with dose was also noted for senile dementia and presenility. Other CNS diseases were non-significantly related to PFF exposure. For women, a number of positive results were obtained, but the number of cases were small and the results unstable, as reflected by the wide confidence intervals. When a large number of multiple comparisons are made, a number of spurious associations would be expected. Consequently, the results of this study must be considered as inconclusive.

Li CY, Sung FC and Wu SC. Risk of cognitive impairment in relation to elevated exposure to electromagnetic fields. Journal of Occupational and Environmental Medicine 2002;44(1):66-72.

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Abstract: Occupational exposure to power-frequency electromagnetic fields (PF-EMF) has been suspected of being associated with adverse neurological outcomes. We

performed a case-control study to assess the relationship between exposure to PF-EMF and the risk of cognitive impairment, an indication of certain adverse neurological diseases such as Alzheimer's disease and dementia. Among 2198 elderly individuals aged 65 years or older, 290 persons with score-based cognitive impairment were compared with 580 sex-matched controls to assess the risk of cognitive impairment in relation to PF-EMF exposure. Participants who were former electrical workers or living within 100 meters of high-voltage transmission lines were considered to have higher exposure. Compared with background exposure, the risk was equal or close to unity for participants with higher exposure from a previous occupation (odds ratio [OR], 1.3; 95% confidence interval [CI], 0.7 to 2.3), higher residential exposure (OR, 0.9; 95% CI, 0.3 to 2.6), or higher exposure in both occupation and residential environments (OR, 1.0; 95% CI, 0.2 to 4.6). Our findings provide little support for the link between PF-EMF and cognitive impairment. Nevertheless, the study results do not
preclude the possible association between PF-EMF and any specific neurodegenerative disease previously investigated.

Comment: This is a preliminary study into the relationship, if any, between exposure to PFF and the presence of pre-clinical symptoms (cognitive impairment) related to the onset of AD, or organic brain syndrome (dementia). It is not possible to reconcile the results of Li with previous studies because Li used SPMSQ (short portable mental status questionnaire) scores as a basis for measuring cognitive functioning, whereas previous studies used specific diagnoses of AD or dementia as their endpoints [(Sobel, 1995), (Sobel, 1996) and (Savitz, 1998a and 1998b)]. Li used two similar cohorts in a longitudinal study, one cohort (1583 individuals) from a study beginning in 1993 and ending in 1997, and a similar second cohort (615 individuals) beginning in 1996 and ending 1997. The SPMSQ tests were administered every year during the study periods, and participants with 5 errors on the 10 question test were considered to be impaired. Also, those with at least one episode of impairment over the study period were considered impaired. Of the 290 cases of impairment detected in the combined cases, 42 were considered to be prevalent (having more than one impaired score) and 248 were considered incident (one impaired score over the study period). The validity of the relationship between SPMSQ scores and the diagnosis of organic brain syndrome have been fairly well established [(Pfeiffer, 1975), (Fillenbaum, 1980) and (Albert, 1991)]. When SPMSQ scores are compared with the clinical diagnosis of organic brain syndrome, the sensitivity ranged from 26-56% and the specificity from 94-96%. Sensitivity is the percentage of individuals correctly identified by SPMSQ as having AD or organic brain syndrome, and specificity is the percentage of normal individuals correctly identified by the SPMSQ test as not having the disease.

For exposure assessment, data on lifetime occupations were collected by interview or by proxy (191 individuals). Participants work histories were independently categorized by longest held job and last job held, and assigned an exposure based on job title from standard occupational classification system of Taiwan. Information on proximity to high voltage power lines was identified on a residential map and calculated to within an

accuracy of 10 m of the residence. Since participants were being examined for memory impairment, it is not hard to visualize some serious questions regarding the accuracy of the exposure assessment. Accuracy of recall could also be a problem for the proxy interviewees, since they may not have been familiar enough with the individual's history to accurately recount past events. Therefore, the major problems with this study are the poor sensitivity of the SPMSQ test and the weakness of the exposure assessment. No association was found between exposure to PFF and cognitive impairment, but more efficiently designed studies will be needed to verify these findings.

5.4 Meta-Analysis of Neurodegenerative Diseases

Ahlbom A. Neurodegenerative diseases, suicide and depressive symptoms in relation to EMF. Bioelectromagnetics 2001;Suppl 5:S132-S143.

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Abstract: In 1979 the first study was published which indicated that environmental exposure to power frequency, electric and magnetic fields (EMF), might increase the risk of chronic disease. This was a study on cancer. However, this research area has gradually evolved and come also to include outcomes other than cancer. The purpose of this paper is to provide a better understanding of the literature on neurodegenerative diseases and on suicide and depressive symptoms in relation to EMF by using a meta-analysis technique. It is concluded that for amyotrophic lateral sclerosis, there are relatively strong data indicating that electric utility work may be associated with an increased risk. However, EMF exposure is only one of several possible explanations to this. For Alzheimer's disease the combined data on an association with EMF are weaker than that for ALS. For suicide an overall assessment yields the conclusion that the support for an association is weak. For depressive symptoms the assessment is more complex, but the overall conclusion is nevertheless that the evidence is relatively weak. For other diseases, such as Parkinson's, there is not enough information for an assessment.

5.4.1 Amyotrophic Lateral Sclerosis

This meta-analysis examined the strength of the relationship between exposure to PFF and ALS, AD, suicide and depression. To be included in the review, a study had to present a quantitative risk estimate (with confidence intervals) for a population with elevated PFF exposure relative to a reference population. For ALS, seven studies met these criteria [(Deapen and Hendersen, 1986), (Gunnarsson, 1991), (Gunnasrron, 1992), (Davanipour, 1997), (Savitz, 1998a), (Savitz, 1998b) and (Johansen and Olsen, 1998)]. The designs of these seven studies were then examined for their susceptibility to selection bias, which included defining the relationship between the study population and the methods used to select cases and controls, their corresponding responses or

participation rates, and the diagnostic criteria used to identify the disease. Information was examined on whether job titles, job-exposure matrices, or direct measurements, or some combination thereof, were used to estimate exposure. Pooling, that is the estimation of the average effect across studies, was done using a fixed effects model with weights that were proportional to the inverse of the variance estimates of individual studies. The RR for all seven studies was estimated at 1.5, 95% CI = 1.2 -1.7. Studies were then divided into three groups for meta-analysis, according to their experimental design, (i) clinical and ALS society-based information, three studies, (ii) mortality registry and census based information, two studies, and (iii) utility-based cohort, two studies.

Of the three studies considered to be clinical, two of them identified cases from neurological clinics or from the ALS Society. These studies did not have a specified population from which to identify cases, and randomly selected controls from friends and relatives of patients [(Deapen and Hendersen, 1986) and (Davanipour, 1997)], and therefore would be susceptible to selection bias. The third study in this group (Gunnarsson, 1992) had a clearly defined population from which to select cases and controls, but used only a crude questionnaire to gather information on exposure. The pooled RR for these three studies was 3.3, 95% CI = 1.7 - 6.7. In conclusion, despite some strong features, such as diagnostic accuracy, the shortcomings of these studies limit there usefulness and their contribution to the body of evidence supporting an association between PFF and ALS.

The second group of studies used death certificates for identification of cases and job titles to assess exposure [(Gunnarsson, 1991) and (Savitz, 1998b)]. The strength of these studies are in the large sample size and the small risk of selection or recall bias, which is the result of collecting information from a registry. Potential sources of error include (i) misdiagnosis, which could arise from the use of death certificates to identify cases, and (ii) exposure misclassification, which could arise from the use of job titles to assess exposure without verification by direct measurements. Work history was not considered, only a job title at one point in time. The overall RR for these two studies was 1.3, 95% CI = 1.1 - 1.6.

The third group consisted of two utility-based cohort studies [(Johansen and Olsen, 1998) and (Savitz, 1998a)]. Both studies began with well defined cohorts, and searched mortality registries for information on deaths, which would minimize selection bias. Both studies also used detailed procedures for exposure assessment that included classification of jobs based on measurements and on job duration. Also, the misclassification of diagnosis did not appear to be a source of error. However, despite their strengths, the effective numbers of exposed cases were modest in both studies. These two studies should carry more weight than the others in the overall analysis. Their report combined RR was 2.7, 95% CI = 1.4 - 5.0.

In summary, the three studies with the highest likelihood of selection bias also had the highest combined estimate of RR, and the two studies with the crudest exposure assessment had the lowest combined RR. The two utility-based studies had the most valid designs and most thorough exposure assessments, and provided a combined RR in between the other two. Only a few studies have investigated the link between PFF and the incidence of ALS, and the RR > 1 may, like the early studies on PFF and cancer, require time and effort to improve study design and identify potential confounders and control for their effects (such as exposure to electric shocks). Until more studies are completed, the current information can not rule out a putative association between exposure to PFF and ALS. More confirmation studies are needed to verify these findings.

Table 4. Meta-analysis summary for ALS

Author	Year	Country	Sub-Group	Increased Risk?	Weakness
Deapen	1986	USA	CL	yes	SB; EA
Gunnarsson	1991	Sweden	CC	no	EA; DM
Gunnarsson	1992	Sweden	CL	no	SSS; EA; CR
Davanipour	1997	USA	CL	no	SSS; EA; SB
Savitz	1998b	USA	CC	yes	EA; DM
Savitz	1998a	USA	cohort	no	SSS
Johansen	1998	Denmark	cohort	yes	SSS

Abbreviations:
ALS = Amyotrophic Lateral Sclerosis
AD = Alzheimer's Disease
CC = Case-Control Study
CI = Cognitive Impairment
CL = Clinically-Based Case-Control Study
CR = Contradictory Results
DM = Diagnostic Misclassification
D&S = Depression and Suicide
EA = (possible) Exposure Assessment Issues
SD = Senile Dementia
MC = Multiple Comparisons
MS = Multiple Sclerosis
PD = Parkinson's Disease
RB = (possible) Recall Bias
SB = (possible) Selection Bias
SSS = Small Sample Size

Table 5. Pooled results across sub-groups of studies on PFF and ALS

Pooled Studies	Number of Studies	RR	95% CI
All	7	1.5	1.2 - 1.7
Clinical	3	3.3	1.7 - 6.7
Case-Control	2	1.3	1.1 - 1.6
Cohort	2	2.7	1.4 - 5.0

5.4.2 Alzheimer's Disease

Five studies fulfilled the criteria for meta-analysis [(Sobel, 1995 and 1996), (Feychting, 1998) and (Savitz, 1998a and 1998b)]. These studies varied considerably in design and homogeneity. Nevertheless, their combined RR was calculated and found to be 2.2, 95% CI =1.5 - 3.2. Sub-grouping by study design allowed the refinement of relative risks. The two clinical studies by Sobel had high diagnostic specificity (diagnosis confirmed by autopsy), good exposure classification and reasonable sample sizes. These two studies also controlled for genetic confounders in that they were able to explore, in detail, family histories of the disease. The main weakness was the lack of a specified study population from which to select controls and therefore the potential for differential selection bias. Their combined RR was 3.2, 95% CI = 1.5 - 5.4.

A third study (Feychting, 1998) was based on the Swedish twin registry. Exposure was assessed through interviews that included job histories. Jobs were also classified according to EMF exposures from an earlier study on PFF and cancer (Floderus, 1996). Both primary and last occupations were used as an acceptable assessment of exposure. Diagnostic specificity was acceptable, but was not confirmed by autopsy (the only method to firmly verify a diagnosis). The size and definition of the study population was acceptable. The main weakness was the small sample size for cases, as reflected by the wide confidence intervals, and poor control of genetic confounders. This may have been the reason for a non-significant risk (RR = 0.9, 95% CI = 0.3 - 2.8). However, when exposure was based only on the last occupation, the result was significant. This inconsistency, which was not explained, makes validity of this study somewhat questionable.

The fourth and fifth studies (Savitz, 1998a and 1998b) examined both ALS and Alzheimer's. Weakness of the fourth and fifth studies was the use of death certificates to select AD cases, which may not always include autopsy results. Also, death certificates provide only crude data upon which to assess EMF exposure. When the third, fourth and fifth studies were combined, the RR was 1.2, 95% CI = 0.7 - 3.1.

Overall, this sub-group of studies does not provide convincing support for an association between PFF and AD.

Table 6. Meta-analysis summary (AD)

Author	Year	Country	Sub-Group	Increased Risk?	Weakness
Sobel	1995	USA & Finland	CL	yes	SB
Sobel	1996	USA	CL	yes	SB
Feychting	1998	Sweden	CC	CR	SSS
Savitz	1998(a)	USA	CC	no	EA
Savitz	1998(b)	USA	Cohort	no	SSS

Abbreviations:
AD = Alzheimer's Disease
CC = Case-Control Study
CL = Clinically-Based Case-Control Study
CR = Contradictory Results
EA = (possible) Exposure Assessment Issues
SB = (possible) Selection Bias
SSS = Small Sample Size

Table 7. Pooled results across sub-groups (AD)

Pooled Studies	Number of Studies	RR	95% CI
All	5	2.2	1.5 - 3.2
Clinical	2	3.2	1.9 - 5.4
Population-based (Case-Control and Cohort)	3	1.2	0.7 - 2.3

5.4.3 Suicide

Seven studies were examined for suicide in this portion of the meta-analysis [(Reichmanis, 1979), (Perry, 1981), (McDowall, 1986), (Baris and Armstrong, 1990) (Baris, 1996a and 1996b) and (Johansen and Olsen, 1998)]. The paired studies by Reichmanis (Reichmanis, 1979) and Perry (Perry, 1981) were based on 589 suicide cases and controls. Reichmanis based exposure in terms of the distance between residence and power lines, whereas the study by Perry based exposure on direct

measurement of PFF. These studies found an association between PFF and suicide. However, all of the subsequent studies did not verify this finding. Differences in study design would have made a meta-analysis invalid. Taken together, the support for the hypothesis that occupational exposure to PFF increases the risk of suicide is very weak to non-existent. More studies will be needed to clarify the nature of the postulated association.

5.4.4 Depressive Disorders

Six studies examined the relationship between exposure to PFF and depressive states. The study designs however were too diverse to allow for a meta-analysis [(Dowson, 1988), (Perry, 1989), (Poole, 1993), (Savitz, 1994), (McMahan, 1994) and (Verkasalo, 1997)]. The studies examined populations in England, the United States and Finland. Two studies used what was considered to be invalid methods for selecting study subjects and did not use validated scales to identify depressive symptoms [(Dowson, 1988) and (Perry, 1989)]. The remaining studies used validated scales to identify depressive states, and used accepted methods for selecting cases and controls. The study by Poole (Poole, 1993) detected a positive association between PFF and depression, but three other studies did not [(McMahan, 1994), (Savitz, 1994) and (Verkasalo, 1997)]. While the study by Verkasalo detected no overall association between exposure to PFF and depression, it did detect a clear excess risk for severe depression among those living within 100 meters of power lines. There was no acceptable reason given for this lack of internal consistency. In summary, the body of evidence to-date does not convincingly support the existence of an association between PFF and depression. Once again, more studies will be needed to clarify this relationship.

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6. BREAST CANCER

6.1 Review of Breast Cancer and Power-Frequency Fields

Stevens and coworkers (Stevens, 1987, 1992) were the first to suggest an association between power-frequency electric and magnetic fields (PFF) and breast cancer. They postulated that exposure to PFF, especially at night, might suppress the level of melatonin circulating in blood and cause the level of estrogens to rise (estrogens are known risk factors for breast cancer). To date, the epidemiological evidence in support of such a hypothesis is weak to nonexistent.

All studies on PFF and breast cancer are subject to three sources of error: (i) inaccuracies in assessing long-term exposures, (ii) small sample sizes, which limit statistical power, and (iii) the impact of confounders, covariates and unidentified risk factors that could influence breast cancer development. In this regards, four risk factors have been recently identified that could be important modulators of breast cancer in women, and failure to account for them could distort the results of studies on PFF and breast cancer. Three of the factors appear to protect against breast cancer: the number of births (parity), the cumulative length of time spent breast feeding offspring, and the regular use of oral anti-inflammatory drugs. The fourth factor, the use of estrogen replacement therapy (ERT), is now accepted as a potential risk factor for breast cancer. Furthermore, if melatonin levels and the risk of breast cancer are related, then the extended use of medications that suppress melatonin production (beta blockers, calcium channel blockers and certain psychotropic drugs) could be potential sources of error in studies of PFF and breast cancer. The use of these expensive prescription drugs could also be related to socioeconomic status (SES), since their cost might be prohibitive for those on low or fixed incomes.

In summary, the lack of agreement on what constitutes the correct exposure metric, the low statistical power due to small samples, the failure to adjust for all potential confounders, covariates or risk factors, the role of light-at-night in breast cancer development, and the potential effect of exposure misclassification, leads to the conclusion that the estimated measures of risk from all past studies, including those from 1998 to 2002, could as easily be artifactual as real. More occupational studies of female breast cancer will be required to resolve this impasse. To broaden the scope of the investigations, studies should focus on female occupations in industries other than electric power generation and distribution. Simplified summaries of the reviewed articles are provided in the following tables.

A summary of relevant studies (1998 to 2002) is given in the following tables.

Table 8. Breast cancer - residential exposure

Study	year	Location	Excess Risk	Weakness
Coogan	1998	USA	no	SSS, EAS
Johansen	1998	Denmark	no	SSS
Feychting	1998	Sweden	no	SSS at high exposure
Davis	2002	USA	no	SSS at high exposure

Abbreviations:
SSS = Small Sample Size
EAS = Exposure Assessment Shortcomings

Table 9. Breast cancer - occupational exposure, male

Study	year	Location	Excess Risk	Weakness
Cocco	1998	USA	no	SSS, EAS, MC
Feychting	1998	Sweden	no	SSS
Koc	2001	Turkey	yes	SSS; EA; MD

Abbreviations:
EAS = Exposure Assessment Shortcomings
MC = Multiple Comparisons
MD = (possible) Misdiagnosis
SSS = Small Sample Size

Table 10. Breast cancer - occupational exposure, female

Study	year	Location	Excess Risk	Weakness
Petralia	1998	China	no	SSS, EAS, MC
Kliukiene	1999	Norway	yes	SSS, EAS
Pollan	1999	Sweden	no	SSS, EAS
Forssen	2000	Sweden	no	SSS

Abbreviations:
EAS = Exposure Assessment Shortcomings
MC = Multiple Comparisons
SSS = Small Sample Size

Table 11. Breast cancer - electric blanket use

Study	year	Location	Excess Risk	Weakness
Gammon	1998	USA	no	AB
Zheng	2000	USA	no	EA, RB
Laden	2000	USA	no	MC, SSS, C
McElroy	2001	USA	no	C

Abbreviations:
AB = (possible) Age Bias
C = (possible) Confounding Errors
MC = Multiple Comparisons
RB = (possible) Recall Bias

6.2 Review of Individual Breast Cancer Studies

Coogan PF and Aschengrau A. Exposure to power frequency magnetic fields and risk of breast cancer in the Upper Cape Cod Cancer Incidence Study. Environ. Health. 1998;53(5):359-367.

The principal author is with the Department of Epidemiology and Biostatistics, Boston University School of Public Health, Massachusetts, USA.

Abstract: Investigators used a population-based case-control study to evaluate the relationship between breast cancer risk and exposure to 60-Hz magnetic fields from various sources. There was no increase in breast cancer risk associated with (a) holding a job with high (odds ratio [OR] = 1.2; 95% confidence interval [CI] = 0.4, 3.4) or medium (OR = 0.9; 95% CI = 0.5, 1.7) exposure to magnetic fields; (b) living in a home heated electrically (OR = 1.0; 95% CI = 0.7, 1.4); or (c) sleeping with an electric blanket (OR = 1.0; 95% CI = 0.7, 1.4). There was a nonsignificant 50% increase in risk for subjects who lived within 152 m (500 ft) of an electricity transmission line or substation (OR = 1.5; 95% CI = 0.6, 3.3). Although limited by small numbers and exposure misclassification, the data in this study did not support the hypothesis that exposure to 60-Hz magnetic fields increases the risk of breast cancer in women.

Comment: The Upper Cape Cod Incidence Study assessed residential exposures from data collected by in-person interviews, which included residential history from 1943 to 1986, and whether or not the home was electrically heated. Controls were selected by random digit dialing (RDD), and medicare lists. Exposure assessment was by job title and expert judgement, supplemented by measurements. There was no information on length of employment for those participants working outside of the home, which would underestimate historical exposures and lead to some exposure misclassification. The study adjusted for the following potential covariates: vital status, age at diagnosis, family and personal history of breast disease, age at first live- or stillbirth, marital status, and education. No increased risk for breast cancer was found for any of the exposure categories, nor was an increased risk found in women who were exposed to electric heat in their present or past residences. In addition, this study investigated whether participants used an electric blanket, electric heating pad, electric mattress pad, or electric water bed heater, and the total years of use. Once again, there was no increase in risk for women who regularly used these devices when compared to those with very low exposures.

Gammon MD, Schoenberg JB, Britton JA, Kelsey JL, Stanford JL, Malone KE, Coates RJ, Brogan DJ, Potischman N, Swanson CA and Brinton LA. Electric blanket use and breast cancer risk among younger women. Am. J. Epidemiol. 1998;148:556-563.

The principal author is with the Division of Epidemiology, Columbia School of Public Health, New York, USA.

Abstract: To investigate whether use of electric blankets, one of the largest sources of electromagnetic field exposure in the home, is associated with the risk of female breast cancer, the authors analyzed data from a population-based US case-control study. The 2,199 case patients were under age 55 years and had been newly diagnosed with breast cancer between 1990 and 1992. The 2,009 controls were frequency-matched to cases by 5-year age group and geographic area. There was little or no risk associated with ever having used electric blankets, mattress pads, or heated water beds among women under age 45 years (adjusted odds ratio = 1.01, 95% confidence interval 0.86-1.18) or among women aged > or = 45 years (adjusted odds ratio = 1.12, 95% confidence interval 0.87- 1.43). There was no substantial variation in risk with duration of use; with whether the appliance was used only to warm the bed or used throughout the night; with menopausal status; or with the cases' hormone receptor status or stage of disease. Potential breast cancer risk factors that were associated with electric blanket use did not substantially confound the associations under investigation. These data do not support the hypothesis that electric blanket use increases breast cancer risk among women under age 55 years.

Comment: This is a population based case-control study of younger women in 3 regions of the United States. All 2199 cases were under the age of 55, and were

diagnosed with breast cancer during the period 1990 to 1992. For the controls, 2009 women were identified by random digit dialing. Data was collected by in-person interviews. Information on the age or make of the electric blanket was not collected and other possible sources of PFF exposure were not investigated. The results were adjusted to account for menopausal status and for the presence of estrogen-receptor positive cancer, but not for number of births, breastfeeding patterns, or the use of hormone replacement therapy or other prescription drugs. No significant association was found between electric blanket use and breast cancer.

Feychting M, Forssen U, Rutqvist LE and Ahlbom A. Magnetic fields and breast cancer in Swedish adults residing near high-voltage power lines. Epidemiology 1998;9(4):392-397.

The principal author is with the Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

Abstract: We conducted a case-control study to test the hypothesis that residential magnetic field exposures increase the incidence of breast cancer. The study was based on people who had lived within 300 m of 220- or 400-kV power lines in Sweden at any time between 1960 and 1985. We identified 699 cases of breast cancer in women and 9 cases in men. One matched control per female case and eight per male case were selected at random. Estrogen receptor information was available for a subset of female cases. We assessed magnetic field exposure through calculations of the magnetic fields generated by the power lines before diagnosis. For calculated magnetic field levels > or = 0.2 microtesla (μT) closest in times before diagnosis, we estimated the relative risk to be 1.0 [95% confidence interval (CI) = 0.7-1.5] for women and 2.1 (95% CI = 0.3-14.1) for men. Women younger than 50 years of age at diagnosis had a relative risk of 1.8 (95% CI = 0.7-4.3). For women with estrogen receptor-positive breast cancer, the relative risk was estimated at 1.6 (95% CI = 0.6-4.1), using the exposure cutoff point > or = 0.1 μT. Among estrogen receptor-positive women younger than 50 years at diagnosis, the relative risk increased to 7.4 (95% CI = 1.0-178.1).

Comment: This case-control study, nested within a cohort, was drawn from a Swedish population of about 400,000 women and men, aged 16 years or older, that lived within 300 m of 220 or 400 kV power lines for more than one year, within the period 1960 and 1985. Exposure to PFF was estimated by calculation. Cases consisted of 699 breast cancer patients. Controls were selected at random from the study base and were matched on age, location of residence during the year of case diagnosis, and proximity to the same power line. The study controlled for age, and socioeconomic status. A non-significant increased risk for breast cancer was observed in women exposed to PFF in the 6 years preceding diagnosis, as well as in younger women under the age of 50 years and in women whose breast cancers were estrogen-receptor positive. Among women who had estrogen-receptor-positive breast cancers, and were less than age 50 years, the OR was 7.4 (95% CI = 1.0-178). However, the significance of this finding is

questionable since it was based on only 6 exposed cases. The study contained no information on other possible sources of exposure or on other risk factors for breast cancer. This study also examined the risk of male breast cancer and PFF exposure. Breast cancer is rare in men and accounts for only 0.35 to 1.5% of all male cancers (Sasco, 1993). A nonsignificant two-fold increase in risk of breast cancer was detected for men exposed to PFF. However, a valid conclusion is not possible since only 9 cases of male breast cancer were found, which limits the statistical power of the study.

Petralia SA, Chow WH, McLaughlin J, Jin F, Gao YT and Dosemeci M. Occupational risk factors for breast cancer among women in Shanghai. American Journal of Industrial Medicine 1998;34(5):477-483.

The principal author is with the Occupational Epidemiology Branch, National Cancer Institute, Bethesda, Maryland, USA.

Abstract: Although female breast cancer rates are lower in China than in Western countries, rates have been rising rapidly in China. This increase may be due to changes in established breast cancer risk factors, but it is possible that exposure to occupational and environmental carcinogens in Shanghai also have contributed to the rise in incidence. We used data collected by the Shanghai Cancer Registry and the Chinese Third National Census to study the risk of breast cancer by occupation and by occupational exposures. Standardized incidence ratios (SIRs) were used to compare observed cases to expected numbers of cases, based on the incidence rates for Shanghai and the number of women in each occupation according to the 1982 census. Statistically elevated SIRs for breast cancer were seen for a number of professional occupational categories, with the greatest risk seen among scientific research workers (SIR = 3.3). Administrative clerks, political and security personnel, and makers of rubber and plastics products also had significant excesses. Significant deficits of risk were seen for the categories of production and related workers, construction workers, and transportation equipment operators. For specific occupations, the highest SIRs were observed among doctors of Chinese-Western medicine (SIR = 14.7, 95% CI = 5.9-30.3) and doctors of Chinese medicine (SIR = 7.2, 95% CI = 4.4-11.4). We also found excesses among teachers at each level of education, librarians, clerical workers, electrical and electronic engineers, nurses, lab technicians, accountants and bookkeepers, rubber manufacturing products makers, weavers, and knitters. SIRs were significantly elevated for high probability of exposure to organic solvents (SIR = 1.4). For benzene exposure, we found significant excesses for overall exposure (SIR = 1.1) and for medium level of exposure (SIR = 1.3). There was no evidence of an association between risk and electromagnetic fields (EMF) exposure. Based on a small number of exposed, SIRs were elevated for both medium probability and high level of exposure to pesticides. The elevations in occupations reported here support some previous reports. Our finding of an increased risk associated with benzene also has been reported previously; the finding for organic solvents is new. However, the literature on the risk of breast cancer related to occupational exposures is limited and there is no consistent

body of literature for any of the exposures studied here. Further, many comparisons were made and the problem of multiple hypothesis testing cannot be ignored in a survey such as ours.

Comment: The focus of this survey is not specifically on PFF and breast cancer. Cases were identified through the Shanghai Cancer Registry from 1980 through 1984 and included women 30 or more years of age. Control incident rates were obtained for women living in Shanghai, and the number of women in each occupation was obtained from the 1982 census. Exposure was assessed from a job exposure matrix, which included an estimate for level and probability of exposure. Only 130 cases of breast cancer were found that were heavily exposed to PFF. Data was adjusted for age, time period and socioeconomic status. When observed incidence rates were compared against expected rates, the standardized incidence ratio (SIR) was 1.0, 95% CI = 0.8-1.2). As pointed out by the authors, multiple comparisons of multiple endpoints could have resulted in false positive or spurious associations. Other shortcomings are in exposure assessment and in the adjustment for other possible risk factors.

Cocco P, Figgs L, Dosemeci M, Hayes R, Linet MS and Hsing AW. Case-control study of occupational exposures and male breast cancer. Occupational and Environmental Medicine 1998;55(9):599-604.

The principal author is with the Institute of Occupational Medicine, University of Cagliari, Italy.

Abstract: *Objective:* To investigate whether risk of male breast cancer is associated with workplace exposures. *Methods:* A case-control study of 178 cases of male breast cancer and 1041 controls was carried out with data from the United States national mortality follow-back survey, which collected questionnaire information from proxy respondents of a 1% sample of all 1986 United States deaths among subjects aged 25-74 years. Occupational exposure to electromagnetic fields, high temperatures, polycyclic aromatic hydrocarbons (PAHs), herbicides, other pesticides, and organic solvents was assessed by applying job-exposure matrices, based on the 1980 United States census occupation and industry codes, to the longest job held by study subjects as reported by the informants. A socioeconomic status index was created by combining information on annual family income, education, assets, and occupation to assess the association of socioeconomic status with male breast cancer. Relative risks were derived from logistic regression modeling, which included age, socioeconomic status, marital status, and body mass index, as well as occupational exposures. *Results:* Risk for male breast cancer increased significantly with increasing socioeconomic status index (test for trend: $p < 0.01$), but the risks associated with individual socioeconomic status variables were smaller and the trends were not significant. A significant increase in risk of male breast cancer was associated with employment in blast furnaces, steel works, and rolling mills (odds ratio (OR) 3.4; 95% confidence interval (95% CI) 1.1 to 10.1, based on six cases), and motor vehicle manufacturing

(OR 3.1; 95% CI 1.2 to 8.2, based on seven cases). However, exposures to electromagnetic fields, high temperature, PAHs, herbicides, other pesticides, and organic solvents were not associated with risk of male breast cancer. *Conclusions:* The role of workplace exposures in increasing risk of breast cancer among men employed in motor vehicle manufacturing and in blast furnaces, steel works, and rolling mills deserves further investigation. The finding on socioeconomic status suggests that as well as reproductive factors, other lifestyle factors such as diet that may be related to high socioeconomic status in men should be investigated further.

Comment: This study examined the relationship between a number of occupations (including occupations with significant exposure to PFF) and the incidence of male breast cancer. Exposure was assessed through a job-exposure matrix. No significant association between PFF and male breast cancer was detected. However, the risk factors for male breast cancer are not well understood, which makes it difficult, if not impossible, to identify and adjust for these variables. In addition, the use of proxy respondents could also have resulted in misleading or unreliable information about the cases. The study was more like a survey that examined a large number of occupations with few breast cancers in each category. These small numbers imply that the probability of detecting a real change would be small, and any conclusion about an association between occupation and the incidence of male breast cancer would be of doubtful validity.

Kliukiene J, Tynes T, Martinsen JI, Blaasaas KG and Andersen A. Incidence of breast cancer in a Norwegian cohort of women with potential workplace exposure to 50 Hz magnetic fields. Am J Ind Med 1999; 36(1):147-154.

The principal author is with the Cancer Registry of Norway, Institute of Epidemiological Cancer Research, Oslo, Norway.

Abstract: *Background:* The risk of breast cancer was investigated in a large dynamic population-based cohort of all 1.1 million economically active women in Norway with potential exposure to 50 Hz magnetic fields at the censuses of 1960, 1970, and 1980. *Methods:* The follow-up period for the cohort was 1961-1992. For each woman, date of birth and census information on occupation and socioeconomic status were ascertained. These data were linked to the breast cancer morbidity information in the Cancer Registry of Norway. Exposure to magnetic fields was assessed a priori using two different approaches. In the first approach, hours per week in a potential magnetic field above background level (0.1 μT) were classified by an expert panel. In the second approach, measured magnetic fields from a separate study of men at work were allocated to the women's census job titles. In both approaches, exposure was cumulated over the years of employment (work hours and microT-years, respectively). *Results:* The Poisson regression analysis showed a risk ratio (RR) of 1.14 (95% confidence interval (CI) = 1.10-1.19) in the highest exposure category compared to the lowest when using the first approach, and the corresponding RR was 1.08 (95% CI =

1.01-1.16) when using the second approach. For women younger than 50 years, RR was 1.20 (95% CI = 1.11-1.29) and 1.12 (95% CI = 0.98-1.28), respectively. *Conclusions:* The results give some support to the hypothesis that exposure to 50 Hz magnetic fields may increase the risk of breast cancer. However, since no direct information on exposure was available, no firm conclusions can be drawn.

Comment: This is a cohort study of the Norwegian female population that found, at best, weak support for an association between PF fields and breast cancer in women below 50 years of age. The relative risk (RR) was 1.14 (95% confidence interval (CI) = 1.10-1.19) in the highest exposure category when compared to the lowest. Exposure was assessed by job title and expert opinion. The study adjusted for a number of covariates, including, age, time period, and socioeconomic status. However, the RR was close to 1.0 and any error in exposure classification, or failure to adjust for potential covariates, could mask a real effect. The tentative positive association is therefore of questionable validity.

Pollan M and Gustavsson P. High-risk occupations for breast cancer in the Swedish female working population. American Journal of Public Health 1999;89(6):875-881.

The principal author is with the Cancer Epidemiology Unit, National Centre for Epidemiology, Carlos III Institute of Health, Madrid, Spain.

Abstract: *Objectives:* The purpose of this study was to estimate, for the period 1971 through 1989, occupation-specific risks of breast cancer among Swedish women employed in 1970. *Methods:* Age-period standardized incidence ratios were computed. Log-linear Poisson models were fitted, with geographical area and town size taken into account. Risks were further adjusted for major occupational group, used as a proxy for socioeconomic status. Risk estimators were also calculated for women reporting the same occupation in 1960 and 1970. *Results:* Most elevated risks among professionals, managers, and clerks were reduced when intragroup comparisons were carried out, indicating the confounding effect of socioeconomic status. Excess risks were found for pharmacists, teachers of theoretical subjects, schoolmasters, systems analysts and programmers, telephone operators, telegraph and radio operators, metal platers and coaters, and hairdressers and beauticians, as well as for women working in 1960 and 1970 as physicians, religious workers, social workers, bank tellers, cost accountants, and telephonists. *Conclusions:* While the high risks observed among professional, administrative, and clerical workers might be related to lower birth rates and increased case detection, excess risks found for telephone workers and for hairdressers and beauticians deserve further attention.

Comment.: This study involved Swedish women employed during the 1970 census who lived in Sweden during the 1960 census, and were between 24 and 64 years of age as of 1/1/71. More than one million women were followed for 19 years.

Cases accrued through the Swedish Environment Cancer Registry. To be included in the study, an occupational category had to contain at least 200 women and at least 10 breast cancer cases. A total of 19,284 breast cancer cases, aged 26 to 64 years, were found and compared to expected number of cases using age and time period-specific reference rates. After adjustment for age, period of diagnosis, geographical category (urban vs rural) and town size, 25 occupations were identified with RR > 1.0, including electrical engineers, RR of 1.40 (95% CI = 0.88-2.23), telegraph and radio operators, RR = 1.40 (95% CI = 1.04-1.88), and telephone operators, RR=1.27 (95% CI = 1.08-1.48), when compared to all other occupations as the reference group. The definition of exposure to PFF was severely limited in this study. The study also found that breast cancer rates varied markedly between Swedish counties, from 161 per 100,000 residents in a southern county to 110 per 100,000 residents in a northern county. The same was true for urban and rural areas. These two factors proved to be confounders in Pollan's study, which illustrates the need to determine the distribution of localized breast cancer rates when the study population resides in a large and diverse geographical area. The conclusions from this study are limited by the lack of real data on PFF exposures, and from failure to account for other postulated covariates and potential confounders.

Zheng T, Holford TR, Mayne ST, Owens PH, Zhang B, Boyle P, Carter D, Ward B, Zhang Y and Zahm SH. Exposure to electromagnetic fields from use of electric blankets and other in-home electrical appliances and breast cancer risk. American Journal of Epidemiology 2000;151(11):1103-1111.

The principal author is with Yale University School of Medicine and Yale Cancer Center, New Haven, Connecticut, USA.

Abstract: Exposure to electromagnetic fields (EMFs) from use of electric blankets and other in-home electrical appliances has been hypothesized to increase breast cancer risk. To test the hypothesis, the authors analyzed data from a case-control study of female breast cancer conducted in Connecticut in 1994-1997. A total of 608 incident breast cancer patients and 609 age frequency-matched controls, 31-85 years old, were interviewed by trained study interviewers using a standardized, structured questionnaire to obtain information on lifetime use of various in-home electrical appliances. A total of 40% of the cases and 43% of the controls reported regular use of electric blankets in their lifetime, which gave an adjusted odds ratio of 0.9 (95% confidence interval (CI) = 0.7 - 1.1). For those who reported using electric blankets continuously throughout the night, the adjusted odds ratio was 0.9 (95% CI = 0.7 - 1.2) when compared with never users. The risk did not vary according to age at first use, duration of use, or menopausal and estrogen receptor status. The authors also did not find an association between use of other major in-home electrical appliances and breast cancer risk. In conclusion, exposure to EMFs from in-home electrical appliance use was not found to increase breast cancer risk in this study.

Comment: In this study, the primary measure of exposure was the use of an electric bed-warming device, and the duration the participant used it. PFF were not measured in the bedroom, nor were other possible sources of exposure considered. The duration of electric blanket use, or in-house appliance use, was determined by a standardized questionnaire conducted by an interviewer, which could result in considerable recall error and errors in exposure classification. Failure to fully account for historical exposure would also be important if a "window of opportunity" existed whereby the action of PFF could significantly influence the rates of tumor promotion or progression. Other important risk factors for breast cancer were not examined including the use of certain prescription drugs, birth number (parity) and length of time offspring were breastfed. These potential sources of error would be cause for questioning the validity of the study's conclusions.

Forssen UM, Feychting M, Rutqvist LE, Floderus B and Ahlbom A. Occupational and residential magnetic field exposure and breast cancer in females. Epidemiology 2000;11(1):24-29.

The principal author is with the Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

Abstract: The purpose of this study was to evaluate the effect of occupational magnetic field exposure on breast cancer in females and to combine residential and occupational magnetic field exposure to reduce misclassification. The study was conducted as a case-control study within a population living within 300 m of transmission lines in Sweden. We identified cases of breast cancer in females from the national cancer registry, and we selected one matched control per case at random. Residential exposure was estimated through calculations of the magnetic fields generated by power lines. We obtained information about occupation from censuses, and the occupations were linked to a job-exposure matrix that was based on magnetic field measurements. For occupational exposure to magnetic fields over 0.25 μT closest in time before diagnosis, the estimated relative risk was 1.0 [96% confidence interval (CI) = 0.6-1.7]. Women below age 50 years at diagnosis had a relative risk of 1.5 (95% CI = 0.6-3.5). For women below 50 years of age who had estrogen receptor-positive breast cancer, there was a relative risk of 3.2 (95% CI = 0.5-18.9). The results for residential and occupational exposures combined showed similar results.

Comment: This case-control study involved 215,820 Swedish women over the age of 16 y who lived within 300 m of a power line for more than one year in the period from 1960 to 1985. Controls were selected at random from the study base. Both residential and occupational exposures were partly based on a job exposure matrix and on magnetic field measurements. Leisure and travel time to and from the workplace were not considered in the exposure calculations. Such omissions could lead to errors in exposure classification. The study accounted for a number of postulated covariates, including age, neighborhood (geographical location), socioeconomic status, exposure

to motor fuel or exhaust fumes, benzene, oil products, solvents, welding fumes and estrogen receptor status of the breast cancer. The results from this study appear to support the conclusions of Feytching et al., (Feytching, 1998) who suggested a weak association between PFF and breast cancer in women below age 50 y, and in particular, for those with estrogen-receptor positive breast cancers. However, none of the estimated RRs are convincing (all have wide confidence intervals that span 1.0), and the positive association could be due to the presence of unknown confounders.

Laden F, Neas LM, Tolbert PE, Holmes MD, Hankinson SE, Spiegelman D, Speizer FE and Hunter DJ. Electric blanket use and breast cancer in the Nurses' Health Study. American Journal of Epidemiology 2000;152(1):41-49.

The principal author is with the Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA.

Abstract: Electric and magnetic fields (EMFs) have been hypothesized to increase the risk of breast cancer, and electric blankets represent an important source of exposure to EMFs. The authors examined the relation between electric blanket use and invasive breast cancer in the Nurses' Health Study. On the biennial questionnaire in 1992, 87,497 women provided information on this exposure during three consecutive time periods. In a prospective analysis with 301,775 person-years of follow-up through 1996 (954 cases), the relative risk for any electric blanket use was not elevated (relative risk (RR) = 1.08, 95% confidence interval (CI) = 0.95-1.24) after controlling for breast cancer risk factors. There was a weak association between breast cancer and electric blanket use at least 16 years before diagnosis and long-term use in age-adjusted analyses but not in multivariate models. In a retrospective analysis of 1,318,683 person-years of follow-up (2,426 cases), the multivariate relative risk associated with use before disease follow-up began was null (RR = 1.05, 95% CI = 0.95-1.16). Similar results were obtained in analyses stratified by menopause and restricted to estrogen receptor-positive breast cancers. While 95% confidence intervals for these estimates did not exclude small risks, overall, results did not support an association between breast cancer risk and exposure to EMFs from electric blankets.

Comment: This study did not consider exposures incurred in the workplace, the residence or during leisure time. Light-at-night was also not considered. Multiple analyses are presented with sometime inconsistent results. The RR when just menopause and estrogen-receptor breast cancers were considered borders on 1.00 and appears to support the work of Feychting (Feychting, 2000) and Forssen (Forssen, 2000). However, these results are questionable since many other risk factors were not taken into account.

Koc M and Polat P. Epidemiology and aetiological factors of male breast cancer: a ten years retrospective study in eastern Turkey. European Journal of Cancer Prevention 2001;10(6): 531-534.

The principal author is with the Department of Radiation Oncology, Medical School of Ataturk University, Erzurum, Turkey.

Abstract: The aim of this study was to assess the epidemiological and aetiological factors of male breast carcinoma in eastern Turkey. For this purpose we evaluated breast carcinoma patients admitted to our regional hospital from 1990 to 2000. A total of 196 patients were admitted during that time, 11 of whom were male (5%). The average age at presentation was 60.7± 7.5. Infiltrating ductal carcinoma was the most frequent histopathological type; lobular carcinoma was detected in only one of our cases. Right-sided male breast carcinoma was seen in 7 of 11 cases, left-sided in four cases. We detected gynaecomastia in two patients. Other factors were excessive alcohol consumption for 35 years in one patient, family history in one patient and exposure to electromagnetic fields (EMFs) and light at night in four patients. We demonstrated no risk factor in the other three cases. Of the patients in our study, the youngest was 45 years old--the patient with post-pubertal gynaecomastia. The overall rate of male breast carcinoma seen among people who had worked for the Turkish Institution of Electricity in eastern Turkey was 0.3%. In our study we demonstrated a close relation between exposure to EMFs and light at night and male breast carcinoma in eastern Turkey. We also supposed that not only exposure to EMFs but also the duration of the exposure could affect the risk of development of male breast carcinoma.

Comment: This is a small study (11 cases) that examined a number of presumed risk factors for male breast cancer, including PFF and light-at-night. There appeared to be no systematic attempt to estimate exposures to PFF other than asking the patient if they worked in an electrical occupation and for how long, or were exposed to light-at-night (presumably, shift work). Only 11 cases of male breast cancer were observed out of 196 breast cancer patients. The incidence of male breast cancer was 5.4 %, far to high to be believable, which suggests the possibility of misdiagnosis. Four of 11 cases worked for the Turkish Institute of Electricity and were exposed to light-at-night. However, the quality of exposure data, the small number of cases, the lack of proper controls, and possibility of misdiagnosis makes the results of questionable value.

McElroy JA, Newcomb PA, Remington PL, Egan KM, Titus-Ernstoff L, Trentham-Dietz A, Hampton JM, Baron JA, Stampfer MJ and Willett WC. Electric blanket or mattress cover use and breast cancer incidence in women 50-79 years of age. Epidemiology 2001;12(6):613-617.

The principal author is with the University of Wisconsin Comprehensive Cancer Center, Madison, Wisconsin, USA.

Abstract: Previous research has demonstrated inconsistent associations between electromagnetic radiation, especially from electric blanket use, and breast cancer. Breast cancer risk according to electric blanket or mattress cover use was examined as part of a multicenter population-based case-control study. Breast cancer patients 50-79 years of age (N = 1949) were identified from statewide tumor registries in Massachusetts, New Hampshire, and Wisconsin from the period June 1994 to July 1995. Women of similar age were randomly selected from population lists as controls. Information regarding electric blanket and mattress cover use and breast cancer risk factors was obtained through telephone interviews. After adjustment for age, body mass index, and other breast cancer risk factors, the risk of breast cancer was similar among ever-users (relative risk = 0.93; 95% confidence interval = 0.82-1.06) and lower among current users than among never-users (relative risk = 0.79; 95% confidence interval = 0.66-0.95). There was no evidence of a dose-response relation with increasing number of months that electric blankets had been used. This study provides evidence against a positive association between electric blanket or mattress cover use and breast cancer.

Comment: This is another case-control study of the relationship between electric blanket use and the risk of breast cancer. Data pertains to women 50 to 79 years of age. Information was obtain on newly diagnosed breast cancer cases reported between 1992 and 1994 from the cancer registries of 3 northeastern US states. According to the cancer registry reports, 98% of the cases had confirmation of invasive breast carcinoma through histologic or cytologic analysis, or other means. Only cases with listed phone numbers, a valid driver's licence, and without previous breast cancer were eligible. Of the 6,839 eligible, 5,685 (83.1%) agreed to participate. Personal information was solicited from cases through a structured telephone interview, including information on known or suspected risk factors such as reproductive and menstrual history, history of alcohol use, mammogram history, familial breast cancer, and personal data. Histories of electric blanket or mattress cover use, and duration of use, were obtained by questionnaire. To determine the reliability of the data obtained by questionnaire, a significant sample of both cases and controls were re-interviewed 2 to 6 months after the original assessment. A similar proportion of women with breast cancer reported electric blanket or mattress use (43%) as age-matched controls (44%). The RR for breast cancer in women who had always used electric blankets compared with never users was 0.93, 95% CI = 0.82 - 1.06. The RR for former users was 1.01, 95% CI = 0.87 - 1.17, and the RR for current users was 0.79, 95% CI = 0.66 - 0.95. Thus, there was no meaningful association between electric blanket or mattress cover use and breast cancer risk. The study controlled for education (a surrogate of socioeconomic status) body mass index, and other risk factors, although such adjustments did not meaningfully alter the RR estimates. The study did not consider exposures incurred in the workplace, the residence as a whole or during leisure time. Light-at-night was not considered.

Davis S, Kaune WT, Mirick DK, Chen C and Stevens RG. Residential magnetic fields, light-at-night, and nocturnal urinary 6-sulfatoxymelatonin concentration in women. American Journal of Epidemiology 2001;154(7):591-600.

The principal author is with the Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.

Abstract: Exposure to 60-Hz magnetic fields may increase breast cancer risk by suppressing the normal nocturnal rise in melatonin. This 1994-1996 Washington State study investigated whether such exposure was associated with lower nocturnal urinary concentration of 6-sulfatoxymelatonin in 203 women aged 20-74 years with no history of breast cancer. Each woman was interviewed and provided data on the following for a 72-hour period at two different seasons of the year: 1) magnetic field and ambient light measured every 30 seconds in her bedroom, 2) personal magnetic field measured at 30-second intervals, and 3) complete nighttime urine samples on three consecutive nights. Lower nocturnal urinary 6-sulfatoxymelatonin level was associated with more hours of daylight, older age, higher body mass index, current alcohol consumption, and current use of medications classified as beta blockers, calcium channel blockers, or psychotropics. After adjustment for these factors, higher bedroom magnetic field level was associated with significantly lower urinary concentration of 6-sulfatoxymelatonin during the same night, primarily in women who used these medications and during times of the year with the fewest hours of darkness. These results suggest that exposure to nighttime residential 60-Hz magnetic fields can depress the normal nocturnal rise in melatonin.

Comment: This paper tests the hypothesis that exposure to PFF might perturb normal physiological rhythms by suppressing the nighttime production of melatonin, and, as a consequence, raise the level of estrogens, which are known risk factors for breast cancer. Participants were women aged 20 to 74 years selected from a group of 591 who had participated as controls in a case-control study of breast cancer and exposure to PFF. The women were initially identified by random digit dialing (RDD). Both personal and bedroom exposures to PFF were obtained. Personal exposures were monitored continuously over a 24 h period, while bedroom measurements were made only during the night. Magnetic field strength was also inferred from wire code configurations. Ambient light was measured with a light sensor placed at the head of the bed, by self-reporting of the number of times a light was turned on during the night, and length of darkness (season). Two samples of approximately equal size were selected from the pooled group of participants, one, consisting of 101 women with the highest magnetic field exposures, and 110 women with the lowest. Nighttime urine samples were collected on 3 consecutive nights and assessed for 6-sulfatoxymelatonin (6-SM), the primary metabolite of melatonin. Factors known to affect melatonin levels were specified *a priori* and used in covariate adjustment, including participant age, menopausal status and duration of darkness. Suspected, or secondary covariates, were also accounted for including alcohol consumption, electric blanket use, smoking,

body mass index and nightly use of certain prescription drugs, including beta blockers, calcium channel blockers and psychotropic drugs (but not for estrogen replacement therapy). Urine and exposure data were collected for each participant on three consecutive days during two different sessions. The authors concluded, exposure to higher magnetic field strength, as measured in a women's bedroom during the night, was associated with a lower concentration of 6-SM in urine. Regular ingestion of prescription drugs (beta blockers, calcium channel blockers and psychotropics) all suppressed melatonin output, and women with these lowered baseline 6-SM levels were more likely to have greater melatonin suppression than women with higher baseline levels. The study is inconsistent because wire code configurations, the 24-hour personal exposure measurements, PFF variability in the bedroom, and two measures of light-at-night (sensor detection and self-reporting) were non-significantly associated with the suppression of nocturnal urinary 6-SM concentration. Given these inconsistencies, the study provides only weak evidence that PFF can suppress melatonin levels.

Davis S, Mirick DK and Stevens RG. Residential magnetic fields and the risk of breast cancer. American Journal of Epidemiology 2002;155(5):446-454.

The principal author is with the Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.

Abstract: Chronic exposure to 60-Hz magnetic fields may increase the risk of breast cancer by suppressing the normal nocturnal production of melatonin. This population-based case-control study investigated whether such exposure is associated with an increased risk of breast cancer in women aged 20-74 years from the greater Seattle, Washington, area. Cases were diagnosed between November 1992 and March 1995 (n = 813); controls were identified by random digit dialing and were frequency matched by 5-year age groups (n = 793). Exposure was estimated using magnetic field measurements in the home at diagnosis, wiring configuration of all homes occupied in the 10 years prior to diagnosis, and self-reported measures of at-home electric appliance use. Odds ratios and 95% confidence intervals were estimated using conditional logistic regression with adjustment for other potential risk factors. Risk did not increase with measured nighttime bedroom magnetic field level, wiring configuration of the home at diagnosis, weighted summary wire codes of all homes occupied 5 and 10 years prior to diagnosis, or reported use of common household appliances, including bed-warming devices. These data do not support the hypothesis that exposure to residential magnetic fields is associated with an increased risk of developing breast cancer.

Comment: Exposures to PFF were estimated from (i) magnetic field measurements in the home (including the bedroom) at the time of breast cancer diagnosis, (ii) wiring codes of all homes for 5 and 10 years prior to diagnosis, (iii) self-reported use of in-house electrical appliances, including electric blankets, and (iv) in-house interviews on

the potential for exposure to PFF in the workplace. Less than 1% of participants worked in jobs associated with significant exposure to PFF. This study differed from all past ones in that magnetic field exposures were directly measured in the home (and bedroom) at the time of diagnosis. The in-house 24-h exposure measurements did not agree with estimated values obtained from wire codes, an alternative surrogate for in-house exposure. The error in assessing past exposure could be a significant shortcoming of this study, particularly if a temporal “window” exists in the exposure spectrum within which PFF could exert some critical action on the promotion or progression stages of tumor development. The study also had relatively few participants with high levels of exposure (> 90% in both cases and controls had mean nighttime PFF of < 0.16 μT). In contrast, the study by Feychting, calculated RR for participants with exposures ≥ 0.2 μT, and for Forssen, the RR was based on exposure ≥ 0.25 μT (Feychting, 1998) (Forssen, 2000). In study by Kliukiene, background was considered to be 0.1 μT (Kliukiene, 1999). The study by Davis et al. found no association between risk of breast cancer and exposure to magnetic fields in premenopausal women or in premenopausal women with estrogen-receptor positive breast cancer, unlike the studies of Feychting and Forssen, that found some evidence of increased risk for these subgroups (Feychting, 1998) (Forssen, 2000). However, in the Davis study, the smallest RR that could be detected with 80% power was 1.9 for the premenopausal subgroup, and 2.3 for the subgroup of premenopausal estrogen-receptor positive subjects. It remains unclear whether the small measurable association between higher nighttime bedroom magnetic field levels (found by Davis in the companion study, (Davis et al., 2001) and lower nocturnal melatonin concentrations is substantial enough to affect the risk of developing breast cancer. The association between nocturnal melatonin levels and magnetic field exposure was observed in subjects that reported taking beta blockers, calcium channel blockers or psychotropic drugs, medications associated with reduced melatonin levels. The present study, however, did not adjust for these potential covariates, but did adjust for the effect of estrogen replacement therapy. Therefore, the results of this study are also inconclusive.

6.3 Overall Conclusions - Breast Cancer

To date, the epidemiological evidence in support of an association between PFF and breast cancer is weak to nonexistent. If recent reports identifying new risk factors for breast cancer can be verified by confirmation or replication, then the value of previous epidemiological studies on PFF and breast cancer could be seriously questioned. Two sources of error have been identified in these studies: (i) inaccuracies in assessing long-term exposures, and (ii) failure to adjust for potential covariates and confounders.

6.3.1 Inaccuracies in Assessing Long-term Exposures

In workplace studies, the reliance on job title as the sole source of exposure information, assumes non-occupational exposures to be trivial. In a study by Lindgren

(Lindgren, 2002, see also abstract A.1 in Appendix A**)** stray fields within a city environment averaged ~ 0.2 μT, with some areas as high as 1 μT. These exposures were incurred while shopping or traveling to and from work, and were similar to those found in occupational settings. Failure to account for these exposures could result in a participant being assigned to the wrong exposure category. Such exposure misclassification could result in spurious associations or in missed real effects. The correct exposure metric has still not been defined, and it is not clear whether a cumulative nighttime exposure, total exposure (including historical exposure), a rapidly fluctuating exposure level, or a maximum exposure level is the critical exposure metric. The contribution of ground currents to the exposure milieu must also be considered in future studies (Kavet, 2000).

In another recent study, (Deadman and Infante-Rivard, 2002, abstract A.6 in Appendix A), typical exposures were measured for a number of occupations commonly held by women in work environments other than in electric utilities and telephone facilities. The study consisted of 491 mothers of acute lymphoblastic leukemia cases and an equal number of mothers of healthy children. For 61 job categories, exposures ranged from 0.03 to 0.68 μT. The most highly exposed jobs (> 0.23 μT) included bakery worker, cashier, cook and kitchen worker, electronic worker, residential and industrial sewing machine operator, and textile machinery operator. By work environment, the most highly exposed job categories were electronic workers in an assembly plant (0.70 μT) and sewing machine operators in a textile factory (0.68 μT) and shoe factory (0.66 μT). If the results of this study can be validated, then, it has been suggested, future epidemiological studies on magnetic fields and breast cancer should focus on work environments other than electric utilities. This would expand considerably the opportunities to study the relationship between PFF exposure and breast cancer in women. The Deadman study gathered self-reported information about occupations, and detailed descriptions of general and specific work environments, tasks and their durations, and electric equipment used. Information was collected on 111 sources of exposure and 59 work environments. The list of job titles were then standardized into a set of 61. This information, together with the published data on the intensity of the magnetic fields associated with work equipment or work environments, were then combined into a semi-quantitative estimate of exposure for a wide variety of jobs commonly held by women. This approach provides (i) new information on expected exposures in a range of jobs commonly held by women, (ii) exposures that are based on individually reported exposure data, and (iii) practical indications of exposure as matrices by source of exposure, work environment and job title. Two issues in all the present studies on occupational exposure and breast cancer in women have been the paucity of jobs for women working in electrical occupations and the incompleteness of exposure data for women working in non-electrical occupations. The exposure matrix of Deadman might provide a starting point to resolve these issues. Therefore, the relationship between magnetic fields and breast cancer in women will require still more studies that will extended to working environments other than electric utilities.

6.3.2 Potential Covariates

Recently, three studies have identified four risk factors for breast cancer, one positive (Rossouw, 2002, abstract A.2 in Appendix A) and three negative (Beral, 2002, abstract A.3 in Appendix A)(Coogan 1999, abstract A.4 in Appendix A). The study by Beral (2002) identified the number of births and the cumulative length of time a women breastfed her offspring as negative risk factors for breast cancer, and the study by Rossouw (2002) identified hormone replacement therapy as a positive risk factor for cancer. A third study (Coogan, 1999) implied the regular use of anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAID), might have a mild protective effect against breast cancer, which is not unexpected given the close association between chronic inflammation and cancer. More importantly, if anti-inflammatory agents generally, including acetylsalicylic acid (ASA), are found to be protective, then their use would also have to be considered. The results of past studies of PFF and breast cancer could be compromised, since they did not consider the impact of these risk factors. Furthermore, if the melatonin-breast cancer hypothesis is correct, then results could be further compromised by the failure to account for the use of drugs that affect melatonin levels (beta blockers, calcium channel blockers, and psychotropic drugs). The relevance of light-at-night (LAN) to breast cancer development must also be clarified, since this hypothesis has found limited support among some animal experiments, but little, if any, support from epidemiological studies.
A theoretical study by Goodman, on the possible effect of confounders in studies on breast cancer and occupational exposure to PFF, found that failure to account for the effects of even one confounder could result in odds ratios (OR) in the range of 1.2 - 1.3 (Goodman, 2002, abstract A.5 in Breast Cancer Appendix A). Interestingly, Goodman et al. found that continent of birth (Asia or Africa) could be a significant confounder in breast cancer studies, and if an occupational study included a relatively large number of recently immigrated women from these countries, it was possible to detect a spurious negative association. This fits nicely with the study by Beral (2002) which found that relative to women in western countries, women in third world countries were protected against breast cancer because they were likely to have more children, to breastfeed their offspring, and to breastfeed them longer, not only out of necessity, but because of its cultural acceptability. A study by Rossouw (2002) (abstract included as A.5 in Appendix A) found that estrogen replacement therapy (ERT) increased a women's risk of developing breast cancer. While ERT is a popular measure for disease prevention in western countries, it is not widely used in third world countries. Only one study (Davis, 2002) considered ERT as a potential covariate. It is also possible that the use of ERT by western women is related to socioeconomic status, since its cost might be prohibitive for those on a low or fixed income.

In summary, the lack of agreement on what constitutes the correct exposure metric, the low statistical power due to small samples, the failure to adjust for all potential risk factors, especially the new ones discussed above, the role of light-at-night in breast

cancer development and the potential effect of exposure misclassification lead to the conclusion that the calculated OR or RR or SIR could as easily be artifactual as real. Therefore, it is still premature to conclude that there is a real association between exposure to PF fields and incidence of breast cancer in males or females. To resolve this issue, more studies will be required of individuals in workplace environments other than electric utilities.

6.4 Meta-Analysis of Breast Cancer Studies

Erren TC. A meta-analysis of epidemiologic studies of electric and magnetic fields and breast cancer in women and men. Bioelectromagnetics 2001;Suppl 5:S105-119.

The author is with the Institute and Polyclinic for Occupational and Social Medicine, University of Cologne, Germany.

Abstract: This paper reviews 43 publications that provide information about possible associations between exposure to electric and magnetic fields (EMF) at work or at home and risks of breast cancer in women and men. Estimation of relative risk associated with exposure was possible for 24 studies among women and 15 among men. The data are grouped in relation to gender of study subjects, type of study, geographical location, and method used to assess exposure, with corresponding precision-weighted estimates of pooled relative risks (RRs). The chi(2) statistics are used to assess the degree to which differences between studies, within subgroups, may be attributable simply to sampling variability. The pooled RR from studies in women was 1.12 (95% CI = 1.09-1.15), but variations between the contributing results are not easily attributable to chance (P = 0.0365). A fairly homogeneous increased risk was found for men (a pooled RR of 1.37, with 95% confidence limits of 1.11-1.71, and homogeneity P-value = 0.1101). However, in both genders, results from individual studies are very variable and in part contradictory. The paramount methodological problem inhibiting valid conclusions about an association between EMF and breast cancer is the probable misclassification of exposure and the possible misclassification of the disease itself.

Comment: Individual studies were stratified according to measured exposure, or assumed intensity, and then the estimated RR for the most heavily exposed category was extracted for use in the meta-analysis. Quantitative information required to estimate RR and 95% CI was available in 24 of 25 studies of women and 15 of 23 studies of men. In the meta-analysis, RR was grouped by sex, type of study (case-control or cohort), country, and the method used to assess exposure. For the 24 studies of women, the pooled estimate of RR was 1.12. For case-control studies, the pooled estimate of RR was 1.13, and for cohort studies, it was 1.11. However, the RR from individual studies ranged from 0.6 to 1.64: five studies had RR < 0.9, fourteen studies had RR between 0.9 and 1.2 and five had RR > 1.35. Of the 24 study of

women, 5 had RR that excluded unity. However, the variations in RR that occurred between the 24 studies were not likely due to random error (χ^2 test), indicating that some studies did not account for all of the important risk factors.

For the 15 studies of PF fields and male breast cancer, the RR ranged from 0.5 to 6.5: four studies had RR < 1, nine studies had RR between 1 and 2.2, one study had an RR of 4.9 and another of 6.5. The pooled estimate of RR for all 15 studies was 1.37. Case-control and cohort studies gave pooled estimates of 1.31 and 1.40, respectively.

All studies differed in design, methods, and data presentation, which imposed limitations on what could be extracted for use in the meta-analysis. The precision and accuracy of exposure assessment also varied between studies. Other differences were in the length of follow-up, which is important, since there must be sufficient time between exposure and the appearance of clinical symptoms. Exposure misclassification was another source of variability in combining the studies. When exposure to PFF was assessed by job title, the RR for women was 13% above the pooled average, while for men similarly classified, the risk was elevated by 76%. This discrepancy could have come from (i) differences in the average exposure for men and women, (ii) some systematic error in assigning exposures to women on the basis of job titles that were designed for men, and (iii) differences between men and women in their susceptibility to PFF-induced breast cancer. Failure to consider factors that preferentially protect women against breast cancer, such as birthing and breastfeeding, could also have contributed to the observed differences, as could the more prevalent use by women of anti-inflammatory medication.

In addition, the 6 studies that used exposure matrices based on more than one source of exposure information yielded a pooled RR of 1, which is in contrast to a pooled RR of 1.76 for studies based only on job title. This illustrates the need to identify the proper exposure metric that could be related to the carcinogenic potential of PFF. For example, if the light-at-night hypothesis is plausible, then both light and an exposure metric for PFF would have to be considered together. Also, given the 20 to 30 year latency period for breast cancer and the pervasive nature of PFF, total exposure, present and past, might be more important than simply trying to categorize exposures as being either residential or occupational (see Lindgren 2001). The study by Forssen et al. (Forssen, 2000) combined both residential and occupational exposures in their analysis. Studies that measure only partial exposures, such as residential or occupational, run the risk of introducing classification errors into the analysis. Such misclassification, if random and similar across all sub-groups being compared, will tend to bias the results towards the null.

All of the studies reviewed by Erren in this meta-analysis were limited by unknowingly excluding adjustments for potential covariates and risk factors that have been recently documented, as discussed above**.** Three studies adjusted their results for estrogen-receptor (ER) status of the breast cancer [(Gammon, 1998), (Feychting, 1998) and

(Forssen, 2000)]. The RR of Gammon remained unaltered when the data was stratified by ER status, which differed from the results of both Feychting and Forssen, who found a positive association between exposure to PFF and ER positive breast cancers. A recent study of PFF and breast cancer by Scott (2002) found no such association, in agreement with Gammon. It remains unclear whether or not women with ER positive breast cancer are more sensitive to the effects of PFF. Mode of action experiments have found melatonin to be cytotoxic to ER positive tumor cells in vitro [(Hill, 1998), (Cos, 1990) and (Shallard, 1989)]. The results of a study by Scott et al. (Scott, 2001) found a weak, but inconsistent negative association in the melatonin levels of women exposed to PFF and extended hours of darkness (light-at-night). This suggests that future studies might have to incorporate the measurement of melatonin levels into their methodology in order to adjust for this potential confounder.

In summary, the meta-analysis of Erren (2001) sets the pooled estimate of risk for women (24 studies) at 1.12 (95% CI = 1.09-1.15) with a range of 0.6 to 1.64, and for men (15 studies) at 1.37 (95% CI = 1.11-1.71, with a range 0.5 to 6.5. However, the lack of consistency in the RR between individual studies, along with their differing indices of exposure and failure to adjust for relevant covariates, does not exclude the possibility that the results are artifactual rather than real.

Appendix A. Supplementary References and Abstracts on Breast Cancer Studies

A.1 Lindgren M, Gustavsson M, Hamnerius Y and Galt S. ELF magnetic fields in a city environment. Bioelectromagnetics 2001;22(2):87-90.

The principal author is with the Department of Electromagnetics, Chalmers University of Technology, Goteborg, Sweden.

Abstract: Some epidemiological studies indicate an association between extremely low frequency electromagnetic field (ELF-EMF) exposure and cancer risks. These studies have mainly taken residential and occupational exposure into consideration. Outdoor environments are often considered as low level areas, but in this paper we show that this is not true in a city environment. We have mapped the ELF magnetic flux densities along certain stretches of sidewalk in central Goteborg City, Sweden. About 50% of the investigated street length shows flux densities of the same order of magnitude (0.2 μT and above) as those associated with increased risks of cancer in epidemiological studies. We conclude that the outdoor exposures in a city environment also should be considered in exposure assessments and risk evaluations. These elevated flux densities are probably due to stray currents. We also found strong magnetic flux densities (> 1.0 μT) close to ordinary distribution pillars, power substations, shoplifting alarms, and other electrical devices.

A.2 Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM

and Ockene J. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 2002;288(3): 321-333.

The principal author is with the Division of Women's Health Initiative, National Heart, Lung, and Blood Institute, Bethesda, Maryland, USA.

Abstract: Despite decades of accumulated observational evidence, the balance of risks and benefits for hormone use in healthy postmenopausal women remains uncertain. *Objective:* To assess the major health benefits and risks of the most commonly used combined hormone preparation in the United States. *Design:* Estrogen plus progestin component of the Women's Health Initiative, a randomized controlled primary prevention trial (planned duration, 8.5 years) in which 16608 postmenopausal women aged 50-79 years with an intact uterus at baseline were recruited by 40 US clinical centers in 1993-1998. *Interventions:* Participants received conjugated equine estrogens, 0.625 mg/d, plus medroxyprogesterone acetate, 2.5 mg/d, in 1 tablet (n = 8506) or placebo (n = 8102). *Main Outcomes Measures:* The primary outcome was coronary heart disease (CHD) (nonfatal myocardial infarction and CHD death), with invasive breast cancer as the primary adverse outcome. A global index summarizing the balance of risks and benefits included the 2 primary outcomes plus stroke, pulmonary embolism (PE), endometrial cancer, colorectal cancer, hip fracture, and death due to other causes. *Results*: On May 31, 2002, after a mean of 5.2 years of follow-up, the data and safety monitoring board recommended stopping the trial of estrogen plus progestin vs placebo because the test statistic for invasive breast cancer exceeded the stopping boundary for this adverse effect and the global index statistic supported risks exceeding benefits. This report includes data on the major clinical outcomes through April 30, 2002. Estimated hazard ratios (HRs) (nominal 95% confidence intervals [CIs]) were as follows: CHD, 1.29 (1.02-1.63) with 286 cases; breast cancer, 1.26 (1.00-1.59) with 290 cases; stroke, 1.41 (1.07-1.85) with 212 cases; PE, 2.13 (1.39-3.25) with 101 cases; colorectal cancer, 0.63 (0.43-0.92) with 112 cases; endometrial cancer, 0.83 (0.47-1.47) with 47 cases; hip fracture, 0.66 (0.45-0.98) with 106 cases; and death due to other causes, 0.92 (0.74-1.14) with 331 cases. Corresponding HRs (nominal 95% CIs) for composite outcomes were 1.22 (1.09-1.36) for total cardiovascular disease (arterial and venous disease), 1.03 (0.90-1.17) for total cancer, 0.76 (0.69-0.85) for combined fractures, 0.98 (0.82-1.18) for total mortality, and 1.15 (1.03-1.28) for the global index. Absolute excess risks per 10 000 person-years attributable to estrogen plus progestin were 7 more CHD events, 8 more strokes, 8 more PEs, and 8 more invasive breast cancers, while absolute risk reductions per 10 000 person-years were 6 fewer colorectal cancers and 5 fewer hip fractures. The absolute excess risk of events included in the global index was 19 per 10 000 person-years. *Conclusions*: Overall health risks exceeded benefits from use of combined estrogen plus progestin for an average 5.2-year follow-up among healthy postmenopausal US women. All-cause mortality was not affected during the trial. The risk-benefit profile found in this trial is not consistent with the requirements for a viable

intervention for primary prevention of chronic diseases, and the results indicate that this regimen should not be initiated or continued for primary prevention of CHD.

A.3 Beral V, Bull D, Doll R, Peto R and Reeves G. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50,302 women with breast cancer and 96,973 women without the disease. Lancet 2002;360:187-195.

The principal author is with the Collaborative Group on Hormonal Factors in Breast Cancer.

Abstract: *Background:* Although childbearing is known to protect against breast cancer, whether or not breastfeeding contributes to this protective effect is unclear. *Methods:* Individual data from 47 epidemiological studies in 30 countries that included information on breastfeeding patterns and other aspects of childbearing were collected, checked, and analysed centrally, for 50 302 women with invasive breast cancer and 96 973 controls. Estimates of the relative risk for breast cancer associated with breastfeeding in parous women were obtained after stratification by fine divisions of age, parity, and women's ages when their first child was born, as well as by study and menopausal status. *Findings:* Women with breast cancer had, on average, fewer births than did controls (2.2 vs 2.6). Furthermore, fewer parous women with cancer than parous controls had ever breastfed (71% vs 79%), and their average lifetime duration of breastfeeding was shorter (9.8 vs 15.6 months). The relative risk of breast cancer decreased by 4.3% (95% CI = 2.9-5.8; $p<0.0001$) for every 12 months of breastfeeding in addition to a decrease of 7.0% (5.0-9.0; $p<0.0001$) for each birth. The size of the decline in the relative risk of breast cancer associated with breastfeeding did not differ significantly for women in developed and developing countries, and did not vary significantly by age, menopausal status, ethnic origin, the number of births a woman had, her age when her first child was born, or any of nine other personal characteristics examined. It is estimated that the cumulative incidence of breast cancer in developed countries would be reduced by more than half, from 6.3 to 2.7 per 100 women by age 70, if women had the average number of births and lifetime duration of breastfeeding that had been prevalent in developing countries until recently. Breastfeeding could account for almost two-thirds of this estimated reduction in breast cancer incidence. *Interpretation:* The longer women breast feed the more they are protected against breast cancer. The lack of or short lifetime duration of breastfeeding typical of women in developed countries makes a major contribution to the high incidence of breast cancer in these countries.

A.4 Coogan PF, Rao SR, Rosenberg L, Palmer JR, Strom BL, Zauber AG, Stolley PD and Shapiro S. The relationship of nonsteroidal anti-inflammatory drug use to the risk of breast cancer. Prev Med 1999;29(2):72-76

The principal author is with the Slone Epidemiology Unit, Boston University School of Medicine, Brookline, Massachusetts, USA.

Abstract: *Background:* The effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on the risk of breast cancer is unclear. We assessed the association in a hospital-based case-control study. *Methods:* The cases (n = 6558) were compared with cancer controls (n = 3296) and noncancer controls admitted for trauma or acute infection (n = 2925). Odds ratios were estimated using multivariate logistic regression models. *Results:* For women who used NSAIDs regularly beginning at least 1 year before admission, the odds ratios (OR) were 0.8 (95% CI 0.7, 1.0) with cancer controls and 0.7 (95% CI 0.6, 0.9) with noncancer controls. With noncancer controls, there was a statistically significant decreasing trend in the odds ratios as duration of use increased, whereas with cancer controls there was not. The reduction in risk for regular use was accounted for largely by a reduced odds ratio for one study center (Boston), which contributed 9% of the cases. *Conclusions:* The data are compatible with a small reduction in risk associated with regular NSAID use. However, inconsistencies in the data detract from a causal interpretation.

A.5 Goodman M, Kelsh M, Ebi K, Iannuzzi J and Langholz B. Evaluation of potential confounders in planning a study of occupational magnetic field exposure and female breast cancer. Epidemiology 2002;13(1):50-58.

The principal author is with the Exponent Health Group, Alexandria, Virginia, USA.

Abstract: We examined potential confounding factors that, if unaccounted for, could possibly produce a spurious association in a study of breast cancer among women occupationally exposed to magnetic fields. For each risk factor, we estimated strength of association, prevalence in the general population, and prevalence of the risk factor in the exposed group required to explain completely hypothetical odds ratios between occupational exposure to magnetic fields and breast cancer. We performed similar analyses for two, three, four, and five confounding factors acting simultaneously. Factors numerically capable of substantial confounding included obesity, continent of birth, family history of breast cancer in a first-degree relative, densities on the mammogram, benign proliferative breast disease, history of cancer in one breast, and consumption of at least two alcoholic drinks per day. Nevertheless, only continent of birth, history of cancer, obesity, and consumption of alcohol could potentially be related to occupation. Uncontrolled confounders, either alone or in combination, could possibly account for odds ratios in the 1.2-1.3 range but were very unlikely to produce an odds ratio of more than 1.5. A spurious negative association between magnetic fields and breast cancer could occur if the exposed group included a large number of immigrants from Asia and Africa.

A.6 Deadman JE and Infante-Rivard C. Individual estimation of exposures to extremely low frequency magnetic fields in jobs commonly held by women. American Journal of Epidemiology 2002 Feb 15;155(4):368-378.

The principal author is with the Joint Departments of Epidemiology, Biostatistics, and Occupational Health, Faculty of Medicine, McGill University, Montreal, Quebec, Canada.

Abstract: Exposures to extremely low frequency (ELF) magnetic fields have not been documented extensively in occupations besides the work environments of electric or telephone utilities. A 1980-1993 study of childhood acute lymphoblastic leukemia (ALL) in Quebec, Canada, gathered detailed information about the occupations of 491 mothers of ALL cases and mothers of a similar number of healthy controls. This information was combined with published data on the intensities of ELF magnetic fields associated with sources or work environments to estimate ELF magnetic field exposures for a wide range of jobs commonly held by women. Estimated exposures for 61 job categories ranged from 0.03 to 0.68 μT; the 25th, 50th, and 75th percentiles were 0.135, 0.17, and 0.23 μT, respectively. By job category, the most highly exposed jobs (>0.23 μT) included bakery worker, cashier, cook and kitchen worker, electronics worker, residential and industrial sewing machine operator, and textile machine operator. By work environment, the most highly exposed job categories were electronics worker in an assembly plant (0.70 μT) and sewing machine operators in a textile factory (0.68 μT) and shoe factory (0.66 μT). These results provide new information on expected levels of exposure in a wide range of jobs commonly held by women.

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7. LEUKEMIA

7.1 Study Designs

Three epidemiologic designs have been used to study the relationship between power-frequency fields (PFF) and childhood and adult leukemia. The first is the standard case-control design, in which cases are identified from hospital or population based registries of disease, and controls are selected from other registries, such as birth certificates, through random digit dialing or other means. The second is referred to as a nested case-control design, in which cases and controls and exposed and less exposed individuals are members of a cohort. Cases and controls are independently selected from the cohort and compared on the basis of their exposures. The third is the historical cohort from which an incidence rate is calculated for all cases and this rate is then compared with the national incidence rate for all children within the specified age bracket.

7.2 Effect of Bias on Study Results

In case-control studies, bias or systematic error can arise from a number of sources. Errors due to disease misclassification can occur from improper diagnosis. Exposure misclassification can occur when information on exposure is lacking, incomplete, or otherwise compromised. For example, exposures can come from sources inside and outside of the home, the magnitude and characteristics of which can vary widely between homes. Exposure misclassification can also arise from the measurement technique used to assess exposure and from the way exposure data is arbitrarily categorized (i.e. selection of exposure cut points). Exposure misclassification is usually non-differential, affecting both cases and controls equally, which would weaken the ability of a study to detect any true association between exposure and leukemia.

Selection bias arises from some systematic difference in the way cases and controls are selected which compromises their comparability. Differences between cases and controls can arise from the differential assignment of socioeconomic status, as for example, from the careless or improper use of random digit dialing to select controls and a population-based register to select cases, or by the selection of friends of cases to act as controls, which is considered a non-random process. To minimize or avoid the possibility of selection bias, controls must be similar to, and selected from, the same population as cases. In general, the procedures used to select controls are not well enough documented to allow a critical evaluation of their potential effect on the study results. Bias in the selection of controls has often been suggested as a possible explanation for the observed association between wire codes, magnetic fields and childhood leukemia; however, there is not enough data to support or validate this claim.

Participation (or non-participation) bias is due to differences in the non-participation rates of cases and controls, where the influence of non-participants or excluded individuals could modify the results. The odds ratio (OR) will not be biased if non-participation rates differ by exposure status only or by disease status only. However, if the non-participation rates differ by both exposure status and disease status, a substantial bias can exist in the resulting OR. Therefore, it is important that the numbers of individuals that refuse to participate in the study be known. It is also necessary to set the exclusion criteria used to determine who is refused entry into the study.

Yet another type of bias can occur when there is a difference in residential mobility, or stability, of cases and controls. Some studies provide information on how many residences have been occupied by each study subject, but others do not. Higher residential mobility has been associated with high current configuration wire codes, and lower socioeconomic status, so differences in the mobility of cases and controls could influence the size of the resulting OR. To minimize this type of bias, controls must be resident in the study area at the same time cases were diagnosed. For example, in some studies, the residence for cases was restricted to the area where the hospital was located, but the controls were not. Differential residential mobility is a potential source of bias whose affect on OR cannot be readily predicted.

7.3 Childhood Leukemia

Six studies on residential exposure and childhood leukemia were examined. All were well designed, but all have limitations that make critical evaluation somewhat problematic. The results of Green (1999) and Schuz (2001) have some internal inconsistencies. Green found a significant increase in risk at an exposure level where others have repeatedly found no excess risk. Schuz found an increased risk for the median exposure at night but not for the 24 hour exposure. McBride and Green used a version of the Wertheimer and Leeper wire codes without validating their use for studies in 1998/1999 in Canada (the wire codes of Wertheimer and Leeper were designed for 1979 metropolitan Denver). The results are summarized in the tables next page.

Table 12. Childhood leukemia, summary of relevant studies

Author	Year	Country	Increased Risk?	Weakness
Dockerty	1998	New Zealand	no	SSS; EA; MC
McBride	1998	Canada	no	M; NPD; MC; EA
Green	1999	Canada	yes (note 1)	CR; SB; M
UK (study group)	1999	UK	no	SSS
Sorahan (note 2)	1999	UK	no	EA; SSS; NPB; DM, M
Schuz	2001	Germany	yes (note 2)	SSS; RB

Abbreviations:
CR = Contradictory Results
DM = (possible) Diagnostic Misclassification
EA = (possible) Exposure Assessment issues
IB = (possible) Information Bias
M = Mobility (possible differential mobility Bias)
MC = Multiple Comparisons
NPD = (possible) Differential Non-Participation bias
SB = (possible) Selection Bias
SSS = Small Sample Size
RB = (possible) Recall Bias

Note 1: OR = 4.5; 95% CI = 1.3-15.9 for exposure $\geq$ 0.14 μT adjusted for confounders and power consumption (externally inconsistent, most studies find no significance at this exposure level). Greatest risk < 6 y
Note 2: Maternal occupational exposure and childhood leukemia.
Note 3: OR = 3.36; 95% CI = 1.35-8.37 (11 cases and 12 controls) confirms previous smaller study, greatest risk < 4 y, but is also has internal inconsistencies.

Table 13. Childhood leukemia, infants exposed at birth to magnetic fields inside infant incubators

Author	year	Country	Increased Risk?	Weakness
Soderberg	2000	Sweden	No	IB; RB; EA

Abbreviations:
EA = (possible) Exposure Assessment issues
IB = (possible) Information Bias
RB = (possible) Recall Bias

7.3.1 Study Summaries - Childhood Leukemia

Dockerty JD, Elwood JM, Skegg DC and Herbison GP. Electromagnetic field exposures and childhood cancers in New Zealand. Cancer Causes Control 1998;9(3):299-309.

The principal author is with the Department of Preventive and Social Medicine, University of Otago, Dunedin, New Zealand.

Abstract: *Objectives:* To assess childhood cancer risks for electromagnetic field (EMF) exposures. *Methods:* A case-control study was conducted in New Zealand. Cases (aged from zero to 14 years) were ascertained from national databases including the New Zealand Cancer Registry; 303 took part (participation rate, 88 percent). The 303 age- and gender-matched controls were selected randomly from birth records (participation, 69 percent). Mothers were interviewed about appliance exposures (all cases and controls), and 24 hour residential measurements of EMFs were made (leukemia cases and matched controls). *Results:* For the various appliance exposures, there were some odds ratios (OR) above 1.0 and others below 1.0. For electric blanket use by the child before diagnosis, the adjusted ORs were: leukemia, 2.2 (95 percent confidence interval [CI] = 0.7-6.4); central nervous system cancers, ORs = 1.6 (CI = 0.4-7.1); and other solid cancers, OR = 2.4 (CI = 1.0-6.1). Leukemia risk was increased for the highest category of the mean measured bedroom magnetic field (> or = 0.2 μT cf < 0.1 μT), with an adjusted OR of 15.5 (CI = 1.1-224). A gradient in OR with exposure was not shown (middle category: OR 1.4, CI = 0.3-7.6), and there was no association with exposure categorized into thirds based on controls' exposure. The adjusted OR for leukemia in relation to the measured daytime room magnetic field (> or = 0.2 μT cf < 0.1 μT) was 5.2 (CI = 0.9-30.8). *Conclusions:* This was a small study and multiple comparisons were made. The positive findings thus should be interpreted cautiously.

Comment: The population of New Zealand is less than 4 million, making it difficult to obtain large samples for case-control studies of rare diseases, such as childhood leukemia. Therefore, the objective of this study was to collect data in such a way, and with such rigor, that results could be incorporated into future meta-analyses. All cases of leukemia were identified through three registry sources in NZ. Children 0 to 14 y of age and diagnosed between 1990 and 1993 were eligible for inclusion in the study. Inclusion criteria were residency in NZ at the time of diagnosis, and birth in NZ. Exclusions were all adoptive children. All cases of childhood leukemia were confirmed by independent review. A total of 131 cases with leukemia were identified. The controls were selected at random from national birth records and matched 1:1 to cases on age and gender. Like cases, control children were subject to the same inclusion and exclusion criteria. Information from both cases and controls were obtained by in-depth interviews. Interviews were obtain for 121 of the 131 eligible cases (participation rate of 92%). A list of age and gender-matched controls was obtained from the Birth Register and from telephone books and electoral rolls, and satisfactory participation rates were

achieved. The interview also included questions on appliance use. Social class was based on the occupation of the parents, with the higher of the two being assigned to the child. Magnetic field measurements were made for 24 hours at the time of interview in the two rooms most frequented by the child over the two years prior to diagnosis. Measurements were made for 115 of the 121 eligible leukemia cases and for 117 of 121 eligible matched controls. Questionnaire data was checked for consistency and error. Cut points were selected *a priori* at 0.1 and 0.2 μT so as to be comparable with similar studies. However, few houses had mean magnetic field exposures above 0.2 μT, so cut points at 0.017 and 0.055 μT were also examined. A potential confounder was adjusted for if adding it to the model changed the OR by more than 10%. Only social class required adjustment in this study. Overall, the results of this study are unequivocal. No positive association was detected for childhood leukemia and measured magnetic field exposures at levels > 0.2 μT in the child's bedroom (OR = 8.8, CI = 0.6 - 126), the wide confidence interval reflecting the imprecision of the OR due to small sample size (4 cases and 1 control). There were non-significant associations at lower cut points. Also, there was no evidence of an exposure-response relationship. When 0.017 and 0.055 μT cut points were used to divide the exposure spectrum in the bedroom, no significant increase in risk was detected for the highest third. Multiple comparisons were conducted and some positive findings could be expected due to chance variation. Other weaknesses included low statistical power due to small sample size at the higher levels of exposure, and the fact that magnetic fields were monitored for each child only in the house occupied at interview. It is not clear whether children with Down's syndrome were excluded (they have an increased risk for leukemia). In terms of strength, the study was population-based, and achieved satisfactory participation rates, reducing the potential for selection bias. The objective of the author was to produce a study that could be integrated into a subsequent meta-analysis. This was achieved.

McBride ML, Gallagher RP, Theriault G, Armstrong BG, Tamaro S, Spinelli JJ, Deadman JE, Fincham S, Robson D and Choi W. Power-frequency electric and magnetic fields and risk of childhood leukemia in Canada. Am J Epidemiol. 1999;149(9):831-842.

The principal author is with the Cancer Control Research Programme, British Columbia Cancer Agency, Vancouver, Canada.

Abstract: In a case-control study of childhood leukemia in relation to exposure to power-frequency electric and magnetic fields (EMF), 399 children resident in five Canadian provinces who were diagnosed at ages 0-14 years between 1990 and 1994 (June 1995 in British Columbia and Quebec) were enrolled, along with 399 controls. Exposure assessment included 48 hour personal EMF measurement, wire coding and magnetic field measurements for subjects' residences from conception to diagnosis/reference date, and a 24 h magnetic field bedroom measurement.

Personal magnetic fields were not related to risk of leukemia (adjusted odds ratio (OR) = 0.95, p for trend = 0.73) or acute lymphatic leukemia (OR = 0.93, p for trend = 0.64). There were no clear associations with predicted magnetic field exposure 2 years before the diagnosis/reference date or over the subject's lifetime or with personal electric field exposure. A statistically nonsignificant elevated risk of acute lymphatic leukemia was observed with very high wiring configurations among residences of subjects 2 years before the diagnosis/reference date (OR = 1.72 compared with underground wiring, 95% confidence interval 0.54 -5.45). These results provide little support for a relation between power-frequency EMF exposure and risk of childhood leukemia.

Comment: Exposure assessment consisted of calculated, 48 h time-weighted average fields, wire codes, and personal monitoring. Risk of childhood leukemia was calculated for contemporaneous exposures, predicted exposures 2 years before diagnosis, and for lifetime predicted exposures. No significant increased risk of leukemia was detected. When measured exposures were categorized, < 16.7 % of cases and controls had exposures > 0.2 μT, 7.4% had exposures > 0.3 μT, and 4.2% had exposures > 0.4 μT. The results were similarly non-significant when wire codes were used as a surrogate of exposure.

Wire codes were adapted from the 1979 wire codes of Wertheimer and Leeper in their study of childhood leukemia in the Denver metropolitan area. Risk assessment was based on a comparison of the sum of the Very High Current Code and Ordinary High Current Code categories (the exposed group) with the sum of the Ordinary Low Current Code, Very Low Current Code, and Under Ground Code categories (the unexposed group). On the basis of this cut point, there were 122 exposed cases and 128 exposed controls, and 229 unexposed cases and 234 unexposed controls. However, the wire codes used by McBride were designed specifically for the Denver metropolitan area as of 1979, and, unless extensively customized, may not be applicable to the diverse geographical areas of contemporary Canada. Wertheimer and Leeper have repeatedly said that their wire codes are not generally applicable to other geographical locations (Savitz and Poole, 2001). In addition, the Very High Current Code used by McBride was associated with an average magnetic field measurement of < 0.3 μT (Savitz and Poole, 2001), an exposure category that has been shown repeatedly to have a non-significant association with risk of childhood leukemia. Wertheimer and Leeper also regarded ground currents as an essential part of wire coding, since they may be responsible for creating exposure ‘hot spots’ within a home (Kavet, 2000) (Kavet, 2002). In their Denver study, Wertheimer and Leeper verified that most of the plumbing was conductive (Savitz and Poole, 2001). Ground currents contribute to exposure in homes with underground and Very Low Current Code (VLCC), but not to exposures in homes with Very High Current Code (VHCC) (Savitz and Poole, 2001). The conventional wisdom is that successive studies have improved study designs and lower risk estimates for childhood leukemia. However, it is now recognized that the adequacy of wire codes, as a predictor of exposure, has gotten worse over time (Savitz and Poole, 2001), because they have been applied generally, instead of being modified to reflect the uniqueness of the

geographical area under study (for more detailed comment on the role of contact currents see Kavet, abstract in the Appendix).

The effect of participation bias may be another issue in the McBride study. In terms of participation rates, of the 449 cases, 11% could not be located or contacted, while for the 627 controls, 39% could not be located or contacted. The mobility of cases was also higher than for controls. The combination of exposure misclassification that could have arisen from the questionable use of wire codes, the presence of bias due to differential non-participation and mobilities rates, could all have an impact on the observed OR. Overall however, this study is considered to be well designed and executed.

Green L, Miller AB, Agnew DA, Greenberg ML, Li J, Villeneuve PJ and Tibshirani R. Childhood leukemia and personal monitoring of residential exposures to electric and magnetic fields in Ontario, Canada. Cancer Causes Control 1999;10(3):233-243.

The principal author is with the Department of Public Health Sciences, University of Toronto, Ontario, Canada.

Abstract: *Objectives:* To evaluate the risk of childhood leukemia in relation to residential electric and magnetic field (EMF) exposures. *Methods:* A case control study based on 88 cases and 133 controls used different assessment methods to determine EMF exposure in the child's current residence. Cases comprised incident leukemias diagnosed at 0-14 years of age between 1985-1993 from a larger study in southern Ontario; population controls were individually matched to the cases by age and sex. Exposure was measured by a personal monitoring device worn by the child during usual activities at home, by point-in-time measurements in three rooms and according to wire code assigned to the child's residence. *Results:* An association between magnetic field exposures as measured with the personal monitor and increased risk of leukemia was observed. The risk was more pronounced for those children diagnosed at less than 6 years of age and those with acute lymphoblastic leukemia. Risk estimates associated with magnetic fields tended to increase after adjusting for power consumption and potential confounders with significant odds ratios (OR) (OR: 4.5, 95% confidence interval (CI): 1.3-15.9) observed for exposures > or = 0.14 μT). For the most part point-in-time measurements of magnetic fields were associated with non-significant elevations in risk which were generally compatible with previous research. Residential proximity to power lines having a high current configuration was not associated with increased risk of leukemia. Exposures to electric fields as measured by personal monitoring were associated with a decreased leukemia risk. *Conclusions:* The findings relating to magnetic field exposures directly measured by personal monitoring support an association with the risk of childhood leukemia. As exposure assessment is refined, the possible role of magnetic fields in the etiology of childhood leukemia becomes more evident.

Comment: Controls were selected randomly from a marketing list and then contacted by phone. This is more problematic than random digit dialing (RDD) since it starts with a selected and probably biased list in terms of socioeconomic status, and then encounters many of the same access limitations as RDD. Random digit dialing is a method used to identify a set of controls from a designated geographical area. In RDD, the sampling unit is the residence rather than the individual, which is different from the procedure used to select cases (cases are individually sampled or selected from incidence registries). Also, RDD samples only homes with telephones, which may under-represent individuals of low SES. Finally, the residence may have more than one eligible child, but only one would be eligible to participate as a control in the study. Another limitation is lack of information of residences that do not respond to the RDD call, since it is not known how these non-participants would have responded, had they been included. The effort required to reach the owner of a phone line has increased substantially and the representativeness of those reached through RDD as a random sample has become increasingly questionable. The effect is unlikely to be large but it can result in differences between the cases and controls in terms of socioeconomic status. Differential mobility between cases and controls is also evident in the study by Green. Residential mobility has been associated with higher wire codes, which can introduce bias into the results, but the magnitude of the Impact on the estimated OR is unknown. The comment on the effect of ground currents, as discussed in comments on McBride, also applies to Green (see abstract in the Appendix by Kavet et al.).

Sorahan T, Hamilton L, Gardiner K, Hodgson JT and Harrington JM. Maternal occupational exposure to electromagnetic fields before, during, and after pregnancy in relation to risks of childhood cancers: findings from the Oxford Survey of Childhood Cancers, 1953-1981 deaths. Am J Ind Med 1999;35(4):348-357.

The principal author is with the Institute of Occupational Health, University of Birmingham, Edgbaston, United Kingdom.

Abstract: *Background:* The concern that maternal exposure to electromagnetic fields (EMF) might be related to childhood cancer risks, particularly leukemia risks. *Methods:* Maternal occupational data already collected as part of the Oxford Survey of Childhood Cancers have been reviewed. Information on occupations held before, during, and after the pregnancy was sought for 15,041 children dying of cancer in Great Britain in the period 1953-1981, and for an equal number of matched controls. Each period of working was classified under one of five headings: (1) sewing machinist; (2) textile industry workers (other than sewing machinists) with likely exposures to EMF; (3) other machinists and other jobs with likely "higher" EMF exposure; (4) other jobs with likely exposure to some EMF, and (5) jobs with little potential for EMF exposure. *Results:* Relative to risks in the children of mothers who held occupations with little potential for EMF exposure during pregnancy (a category that included housewives), risks of all childhood cancers were close to unity both for the

children of sewing machinists (22 case and 31 control mothers, RR 0.72, 95% CI 0.42 to 1.25) and for the children of other machinists with likely "higher" EMF exposures (44 case and 47 control mothers, RR 0.93, 95% CI 0.61 to 1.41). Corresponding risks for all childhood leukemias and for all childhood brain cancers were similarly unexceptional. Simultaneous adjustment for social class, maternal age at birth of child, and sibship position had little effect. *Conclusions:* The study findings did not indicate that maternal occupational exposure to EMF during pregnancy is a risk factor for childhood leukemias, childhood brain cancers, or the generality of all childhood cancers.

Comment: Participants were identified from birth certificates and data that was originally collected from questionnaires and interviews conducted between 1951 and 1981. Data was obtained from only 68% of those identified. Most questionnaires did not obtain a full occupational history. Matched controls were selected from birth registries in the geographical area in which the parents of cases were living at the time of interview. There were 6153 unique job titles among the interviewed mothers and categorizing them into job areas with potential for exposure to PFF was arbitrary. Exposure assessment was also questionable since exposures had to be assigned to jobs that existed years in the past. Other limitations included an unknown affect on results of non-participants. Adjustment for possible confounders were also arbitrary, since socioeconomic status had to be inferred from questionnaires conducted years ago. Mobile families were also under-represented in controls. With all these limitations, no significant association between a mother's exposure during pregnancy and childhood leukemia was detected.

United Kingdom Childhood Cancer Study Investigators. Exposure to power-frequency magnetic fields and the risk of childhood cancer. Lancet 1999;354(9194):1925-1931.

Abstract: *Background:* Previous studies have suggested an association between exposure to power-frequency electromagnetic fields (EMF) and the development of childhood malignant disease, especially leukaemia and tumours of the central nervous system. We investigated the relation between all childhood cancer and exposure to power-frequency magnetic fields. *Methods:* The UK Childhood Cancer Study was a population case-control study covering the whole of England, Wales, and Scotland. All children with a confirmed malignant disorder were potentially eligible. For each case, we matched two controls on date of birth and sex, randomly chosen from the list of the Family Health Services Authority in England and Wales or Health Board in Scotland. In the main study, 3838 cases and 7629 controls were interviewed. The EMF part of the study included only one control per case, and household EMF measurements and school measurements where relevant were taken on 2226 matched pairs. These measurements, adjusted for historical line load and appliance fields, were used to estimate average exposure in the year before the date of diagnosis, or an equivalent date for controls. Analyses were by conditional logistic regression, incorporating a census-derived deprivation index used as a measure of socioeconomic status. *Findings:* For children with mean exposures of more than 0.2 μT compared with children with

mean exposures of less than 0-1 μT, the adjusted odds ratios were 0.92 (95% CI 0.47-1.79) for acute lymphoblastic leukemia, 0.90 (0.49-1.63) for all leukaemia, 0.46 (0.11-1.86) for central-nervous-system tumours, 0.97 (0.46-2.05) for other malignant disease, and 0.87 (0.56-1.35) for all malignant disease combined. Higher exposures (>0.4 μT) were recorded for only 17 (<0.4%) individuals (eight cases, nine controls). *Interpretation:* This study provides no evidence that exposure to magnetic fields associated with the electricity supply in the UK increases risks for childhood leukaemia, cancers of the central nervous system, or any other childhood cancer.

Comment: The main weakness of this study is the number of cases (8) and controls (9) in the high exposure category (> 0.4 μT). This is the range where other studies have reported a statistical significant association between exposure and leukemia. Overall, the results are based on a total of 21 exposed cases and 23 exposed controls, relative to 1051 unexposed cases and 1051 unexposed controls.

Schuz J, Grigat JP, Brinkmann K and Michaelis J. Residential magnetic fields as a risk factor for childhood acute leukemia: results from a German population-based case-control study. Int J Cancer 2001;91(5):728-735.

The principal author is with the Institute of Medical Statistics and Documentation, University of Mainz, Mainz, Germany.

Abstract: Our objective was to investigate whether exposure to residential power-frequency (50 Hz) magnetic fields above 0.2 μT increases a child's risk of leukemia and to confirm or reject a finding from a previous German study on this topic, which reported increased leukaemia risk with exposure to stronger magnetic fields during the night. A population-based case-control study was used, covering the whole of the former West Germany. Residential magnetic fields were measured over 24 h for 514 children with acute leukaemia identified by the German Childhood Cancer Registry and 1,301 control children taken from population registration files. Magnetic fields above 0.2 μT were relatively rare in Germany (only 1.5% of the study population). Childhood leukemia and 24 h median magnetic fields were only weakly related (OR = 1.55, 95% CI 0.65-3.67). A significant association was seen between childhood leukaemia and magnetic field exposure during the night (OR = 3.21, 95% CI 1.33-7.80). A dose-response-relationship was observed after combining the data of all German studies on magnetic fields and childhood leukaemia. The evidence for an association between childhood leukaemia and magnetic field exposure in our study comes from a measure of exposure during the night. Despite the large size of our study, the results are based on small numbers of exposed children. If the observed association stands, the effect on a population level in Germany would be small.

Comment: This large population-based study was undertaken to confirm or reject the findings of a much smaller, earlier one, that found an association between night time exposure to PFF and childhood leukemia. Cases were identified from a nationwide

cancer registry, and controls from residents' registration offices. Information was collected by self-administered questionnaire and follow-up interviews. There were 847 cases and 2127 controls selected for final study. The address where the child lived the longest before the date of diagnosis was used for the exposure assessment. Exposure was assessed by 24 h measurements (under the child's mattress and in the living room). Spot measurements were used to identify hot spots within the home. The median of the 24 h measurements in the child's bedroom was used as the proxy for the child's average exposure. The cut off point between exposed and non-exposed cases and controls was set at 0.2 μT. The possibility of a dose-response was examined using 4 exposure categories, with the highest being > 0.4 μT. Results were adjusted for a large number of possible confounders, including socioeconomic status, degree of urbanization, residential mobility. When the calculated results were based on 24 h exposure, the association between PFF and leukemia was non-significant (OR = 1.35; 95% CI = 0.6 - 3.07), based on 10 cases and 22 controls (504 unexposed cases and 1279 unexposed controls). However, when exposure was based on the median nigh time value, the OR = 3.21; 95% CI = 1.33 - 7.80, based on 12 cases and 12 controls (502 unexposed cases and 1289 unexposed controls), and when restricted to children under the age of 4 y, the OR = 3.36; 95% CI= 1.35 - 8.37, based on 11 cases and 12 controls. When trend was examined, the OR at > 0.4 μT was 5.53; 95% CI = 1.15 - 2.26, based on only 5 cases and 4 controls. Adjustment for confounders made little difference in the final results. Only 1.3% of the cases and controls had exposures > 0.2 μT. In conclusion, the results of the larger study confirmed those of the smaller one, and further suggests children under 4 maybe at highest risk. The main weakness of this study is the number of highly exposed children. While this may be a problem for the epidemiologists, it is not for the children of Germany.

Soderberg KC, Naumburg E, Anger G, Cnattingius S, Ekbom A and Feychting M. Childhood leukemia and magnetic fields in infant incubators. Epidemiology 2002;13(1):45-49.

The principal author is with the Institute of Environmental Medicine, Karolinska Institutet, Institute, Stockholm, Sweden.

Abstract: In studies of magnetic field exposure and childhood leukemia, power lines and other electrical installations close to the children's homes constitute the most extensively studied source of exposure. We conducted a study to assess whether exposure to magnetic fields in infant incubators is associated with an increased leukemia risk. We identified all children with leukemia born in Sweden between 1973 and 1989 from the national Cancer Registry and selected at random one control per case, individually matched by sex and time of birth, from the study base. We retrieved information about treatment in infant incubators from medical records. We made measurements of the magnetic fields inside the incubators for each incubator model kept by the hospitals. Exposure assessment was based on measurements of the magnetic field level inside the incubator, as well as on the length of treatment. For acute

lymphoblastic leukemia, the risk estimates were close to unity for all exposure definitions. For acute myeloid leukemia, we found a slightly elevated risk, but with wide confidence intervals and with no indication of dose response. Overall, our results give little evidence that exposure to magnetic fields inside infant incubators is associated with an increased risk of childhood leukemia.

Comment: A peak in the incidence of acute lymphoblastic leukemia occurs between 2 and 4 years of age, which implies that pre- and perinatal risk factors may be important for disease development. Within this context, one source of potential exposure to PFF would be inside infant incubators. These exposures would occur earlier in life, at higher levels, and for shorter durations than those experienced later in life at home. The study was population-based and consisted of all infants born in Sweden between 1973 and 1989 (1.7 million). Infants were identified from the Medical Birth Registry and deaths from Cause of Death Registry. A total of 726 eligible children were identified. Matched controls were selected at random to minimize selection bias, and excluded those with Down's syndrome, a known risk factor for childhood leukemia. Information on incubator treatment of cases and controls were obtained from hospital medical records. In all 647 matched pairs were analyzed. A standardized set of magnetic field measurements were made inside of most of the incubators in use within the period 1973 to 1989. Medical records did not however match individuals to incubators so precise exposures could not be assigned. Exposures were categorized into highly exposed and moderately exposed using a cut point of 0.6 μT. Finally, cumulative exposure for each individual was estimated from the mean magnetic flux density measurements and the length of treatment, expressed as μT-h. The exposure cut point for the cumulative exposure was set at 10 μT-h.

No significant association between childhood leukemia or measured or cumulative fields was detected. Adjustment for a variety of potential confounders did not appreciably alter the results. Therefore, there was no evidence of an increased risk for leukemia among children neonatally exposed for short durations to high levels of magnetic fields in infant incubators. There was no reason to assume any systematic difference between cases and controls in terms of completeness of information about incubator treatment. All exposure information was collected without knowledge of case or control status to avoid differential exposure misclassification. However, there were some uncertainties in exposure assessment from recall bias, since the nursing staff had to recall information on different incubators in use 11 to 27 years ago. In addition, it was learned that some hospitals routinely placed all new born infants into incubators for a short periods of time without notifying medical records. The results cannot be used to draw conclusions generally about residential exposure to PFF and childhood leukemia because of differences in the nature of the two exposures.

7.3.2 Meta-Analysis - Childhood Leukemia

The purpose of a meta-analysis is to provide a systematic, rigorous and quantitative review of a body of literature (Wartenberg, 2001). It is a statistical method to summarize and simplify a complex set of study results. The goals are (i) to identify and review all relevant studies, (ii) to assess the consistency and comparability (homogeneity) of study results and methods, and if studies are sufficiently similar, and (iii) to combine their results into a composite which has a greater statistical power than each individual study alone.

The meta-analysis must specify *a priori*, a set of inclusion criteria to identify acceptable studies. The body of literature being combined often includes studies with a diversity of designs and methods, each with its own set of shortcomings and biases. A set of statistical methods must also be selected *a priori* to determine how average risk will be estimated, and how the heterogeneity of the data will be tested and whether all of the studies can be combined to provide a single estimate of risk or whether the analysis must be restricted to specific sub-groups of studies. Potential confounders must be identified and a strategy to control their effects must be formulated. To ensure the validity of the meta-analysis the investigator must also select an exposure metric by which all component studies can be compared. After a summary analysis, the relative weight each study contributes to the final pooled result has to be determined. Finally, the possible effect of publication bias must be assessed. To estimate this, the investigator calculates the so-called fail safe number, which is the number of null studies that would have had to be included in the meta-analysis to reduce the observed statistical significance of the pooled analysis to non-significance. Alternatively, the investigator can calculate how large the risk estimate of a similar hypothetical study would have to be in order to reduce the observed pooled result to null.

The pooled odds ratio, or relative risk estimate, and confidence intervals are calculated in the meta-analysis using either (i) the fixed-effects statistical model, or (ii) the random-effects statistical model. When the fixed effect model is used, all individuals in the component studies are considered the population of interest, and any conclusion based on this model is only valid for the studies being combined. The certainty of the results, as reflected in the width of the confidence interval, depends only on the variation within an individual study. In a meta-analysis that uses a random-effects model, the resulting odds ratio is influenced by both the variation within each component study and the variation between studies. The choice of which model to use in a meta-analysis is often based on the results of a test for homogeneity (Chi-squared or Q-test) that assess the constancy of the treatment effects. If the test indicates the results from all the studies are sufficiently similar (i.e. a non-significant p value), then the fixed effect model is valid. However, if the test is statistically significant ($p < 0.05$, or some other acceptable cut point), homogeneity is rejected, and the results of individual studies are too variable for use in the fixed effect model. In this latter instance, the random effect model may be useful.

7.3.3 Summary of Previous Meta-Analyses

The first meta-analysis of residential magnetic field exposure and childhood leukemia was presented by the National Radiological Protection Board of the United Kingdom (NRPB, 1992). The combined results from three studies [(Fulton, 1980), (Tomenius, 1986) and (Savitz, 1988)] were presented in three separate analyses using alternative exposure metrics of wire codes, spot measures and distance from source. Only wire codes were found to be significantly associated with childhood leukemia with an OR of 1.4; 95% CI = 1.1 - 1.8.

The second meta-analysis was conducted by the authors of three Scandinavian studies [(Feychting and Ahlbom, 1993), (Olsen, 1993) and (Verkasalo, 1993)]. In these studies, calculated magnetic field exposures were based on proximity to electric power transmission lines and historical electrical loads on these lines. Nationwide cohorts were used to select subjects. The authors found a significant association (OR = 2.1; 95% CI = 1.1 - 4.1).

The third meta-analysis was conducted by Washburn (Washburn, 1994) using six studies [(Colman, 1989), (Lin and Lu, 1989), (London, 1991), (Lowenthal, 1991), (Fajardo-Gutierrez, 1993) and (Petridou, 1993)]. The author could not decide whether the results suggested non-differential exposure misclassification or that an exposure-response relationship had been detected.

Miller et al. conducted a fourth meta-analysis that combined seven studies [(Wertheimer, 1979), (Fulton, 1980), (Tomenius, 1986), (Savitz, 1988), (Coleman, 1989), (London, 1991) and (Feychting and Ahlbom, 1993)]. Rather than pooling results across exposure metrics, a separate analysis was done for each (wire codes, distance, spot measures and calculated index of exposure). The use of a sub-group analysis improved the consistency of exposure metrics and cut points across studies, but decreased the sample size. The results for wire codes, distance and calculated fields gave statistically significant associations with childhood leukemia, but the spot measures did not.

In 1996, Meinert and Michaelis conducted a meta-analysis using the same study set as Washburn, but excluded two studies [(Lin and Lu, 1989) and (Lowenthal, 1991)]. They found significantly elevated OR for wire codes and magnetic field strength, but the heterogeneity among studies was not adequately addressed, so the validity of the pooled result is questionable.

In 1996, the U.S. National Academy of Sciences (NAS) conducted a comprehensive meta-analysis that included all but two of Washburn's study set, all of the set used by NRPB, Ahlbom, Miller, Meinert and Michaelis (National Research Council, 1997). A series of sub-group analyses were conducted that assessed both publication bias and the influence of individual studies on the summary results. There was limited to

moderate heterogeneity among studies, and the OR for individual studies were elevated for wire codes, wire codes and distance, and calculated fields. However, spot measures showed a slightly protective effect. The pooled results also showed a positive association with leukemia when the highest exposure category was compared with all other categories. The influence analysis showed that no single study had a disproportionate effect, and publication bias analysis indicated that many null studies would have had to have been unpublished to explain the observed results as being due to random fluctuations. When this meta-analysis was revised to account for four additional studies [(Linet, 1997), (Peridou, 1997), (Tynes and Haldorsen, 1997) and (Michaelis, 1997)], none of the results were significantly altered from the original result (Wartenberg, 1998a).

The EMF RAPID program identified 22 studies for meta-analysis, but excluded seven for inadequate data and design. The selected studies for meta-analysis were the same ones as those used by Wartenberg in his revised NAS study (Wartenberg, 1998a), except more consideration was given to the comparability of exposures across studies. Effort was made to isolate sources of heterogeneity by sub-group analysis. When exposure was categorized, the OR for wire codes, calculated and measured fields combined, and proximity to electrical facilities, showed a statistically significant, but unconvincing, association between exposure and childhood leukemia.

7.3.4 Recent Meta-Analyses

Ahlbom A, Day N, Feychting M, Roman E, Skinner J, Dockerty J, Linet M, McBride M, Michaelis J, Olsen JH, Tynes T and Verkasalo PK. A pooled analysis of magnetic fields and childhood leukaemia. Br J Cancer 2000;83(5):692-698.

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Abstract: Previous studies have suggested an association between exposure to 50-60 Hz magnetic fields (EMF) and childhood leukemia. We conducted a pooled analysis based on individual records from nine studies, including the most recent ones. Studies with 24/48 h magnetic field measurements or calculated magnetic fields were included. We specified which data analyses we planned to do and how to do them before we commenced the work. The use of individual records allowed us to use the same exposure definitions, and the large numbers of subjects enabled more precise estimation of risks at high exposure levels. For the 3,203 children with leukemia and 10,338 control children with estimated residential magnetic field exposures levels < 0.4 μT, we observed risk estimates near the no effect level, while for the 44 children with leukemia and 62 control children with estimated residential magnetic field exposures >/= 0.4 μT the estimated summary relative risk was 2.00 (1.27-3.13), P value = 0.002. Adjustment for potential confounding variables did not appreciably change the results. For North American subjects whose residences were in the highest wire code category,

the estimated summary relative risk was 1.24 (0.82-1.87). Thus, we found no evidence in the combined data for the existence of the so-called wire-code paradox. In summary, the 99.2% of children residing in homes with exposure levels < 0.4 μT had estimates compatible with no increased risk, while the 0.8% of children with exposures >/= 0.4 μT had a relative risk estimate of approximately 2, which is unlikely to be due to random variability. The explanation for the elevated risk is unknown, but selection bias may have accounted for some of the increase.

Comment: A pooled analysis was conducted on primary data from nine studies on EMF and childhood leukemia risk. Three questions were addressed: (i) is there an association between PFF and childhood leukemia risk which is larger than could be expected from random variability?, (ii) does adjustment for confounding change the results?, and (iii) is there a stronger association between proxy measures of PFF exposures (i.e. wire codes) and leukemia than between direct measures of exposure and leukemia? Results were pooled from six European studies, including one from the UK [(Feychting and Ahlbom, 1993; Olsen et al., 1993; Verkasalo et al., 1993; Tynes and Haldorsen, 1997; Michaelis et al., 1998) and (UK Childhood Cancer Study, 1999)], one from the USA (Linet et al., 1997), one from New Zealand (Dockerty et al., 1998), and one from Canada (McBride et al., 1999). These studies were selected because all had based their exposure assessments on 24 or 48 h magnetic field measurements, or on calculated fields. The analyses of individual studies were set out *a priori*, and included an examination of diagnostic categories, exposure definitions, time period for evaluation, selection of cut points, confounders, and statistical methods. To make the studies more consistent, the data from particular studies were sometimes adjusted to better fit the criteria for a pooled analysis, and therefore were sometimes different from that found in the original manuscript. For example, arithmetic means from all studies were replaced by geometric means, because they were less likely to be affected by outliers. These adjustments were deemed to have negligible effect on the final pooled result. Wire codes were assessed for the two North American studies (according to the original Wertheimer-Leeper protocol). The average exposure during the last year prior to diagnosis was selected as the reference time for exposure. The summary RR for each study was adjusted for potential confounders such as socioeconomic status, residential mobility, level of urbanization, dwelling type, level of traffic exhaust. In each of the studies, exposures were categorized into four levels: < 0.1 μT ; 0.1 - <0.2 μT; 0.2 < 0.4 μT; and > 0.4 μT. The two wire code studies were treated similarly. Exposure was also treated as a continuous variable and results were reported as RR at 0.2 μT intervals. There were 3,247 cases (83% were acute lymphocytic leukemia) and 10,400 controls In the final pooled analysis. In the highest exposure category (> 0.4 μT) there were 44 cases and 62 controls.

The study was further sub-divided into two categories before final pooling, one with exposure levels based on direct field measurements [(McBride, 1998), (Dockert, 1998), (Linet, 1997), (UK, 1999) and (Michaelis, 1998)], and the other based on calculated fields [(Feychting and Ahlbom, 1993), (Olsen, 1993), (Verkasalo, 1993) and (Tynes

and Haldorsen, 1997)]. This sub-division allowed for a more consistent analyses, as calculated fields reflect the exposure from sources outside of the residence, such as high voltage power lines, and measured fields reflect exposure from internal sources, such as appliances, ground currents, household wiring, etc. For studies using measured fields, the pooled relative risk (RR) for 36 cases and 25 controls was 1.87; 95% CI = 1.10 - 3.18 for the highest exposure category (> 0.4 μT). For studies using calculated fields, the comparable RR for 8 cases and 37 controls was 2.13; 95% CI = 0.93 - 4.88). When all studies were pooled, the final RR for the highest exposure category was 2.00; 95% CI = 1.27 - 3.13, based on 44 cases and 62 controls. In addition, alternative exposure cut points were tested to confirm the robustness of the final pooled results. The RR for the lower exposure categories did not differ significantly from 1.0.

When exposure was treated as a continuous variable, the studies using a measured value had a pooled RR of 1.17; 95% CI = 1.02 - 1.34; the studies using a calculated value had a pooled RR of 1.11; 95% CI = 0.94 - 1.30; and for all studies combined, the pooled RR was 1.15; 95% CI = 1.04 - 1.27.

The studies that carried most weight in the pooled analysis were those of Linet, McBride and the UK. If the study of Linet was excluded from the pooled analysis, the RR for the highest exposure category (> 0.4 μT) was reduced to 1.68; 95% CI = 1.00 - 2.83 (considered as non-significant). The exclusion of the McBride study increased the pooled estimate for the highest exposure category to 2.14; 95% CI = 1.27 - 3.61. Exclusion of the UK study increased the RR for the highest exposure category to 2.29; 95% CI = 1.41 - 3.74.

When the analysis was further restricted to only acute lymphocytic leukemia, which made up 83% of total leukemia cases in this meta-analysis, the pooled RR at the highest exposure category (> 0.4 μT) for studies using measured exposures was 1.95; 95% CI = 1.14 - 3.35 for 34 cases and 25 controls; for studies using calculated exposures, the RR at the highest exposure category was 2.23; 95% CI = 0.88 - 5.65) for 6 cases and 37 controls; for all studies combined, the RR at the highest exposure category was 2.08; 95% CI = 1.30 - 3.33) for 40 cases and 62 controls.

The adjustment for putative confounders, such as age, sex, socioeconomic status, type of dwelling, traffic exhaust, urban/rural residency, residential mobility, resulted in only minor changes in the RR. These confounders are not risk factors for childhood leukemia (risk factors for leukemia are largely unknown) and so most, with exception of age, sex and socioeconomic status, were not important considerations. For studies that based exposure on direct measurement, there was evidence in some studies, Linet in particular, of selection bias due to non-participation. In these studies there were apparent differences in the distribution of socioeconomic status between cases and controls, with controls generally characterized by higher socioeconomic status than cases. In a study by Hatch et al. (Hatch, 2000, abstract appended in attached

Appendix), lower socioeconomic status tends to be associated with a higher exposure to magnetic fields, and this is confirmed in the UK study, which found a moderate association between a 'deprivation index' and measured magnetic fields. According to Hatch, this type of bias is unlikely to change the RR by more than 15% in individual studies, and therefore to have only a limited, but undetermined, effect on the pooled estimate of RR.

The finding of this pooled analysis did not support the so-called "wire code paradox" which was observed in some earlier studies that found wire codes were more strongly associated with leukemia risk than measured fields. When the wire code results for the two North American studies (Linet and McBride) were combined, the RR for the very high current configuration, adjusted for age, sex and socioeconomic status, was 1.24; 95% CI = 0.82 - 1.87, much smaller than the pooled estimate for measured exposures, RR 1.95; 95% CI = 1.14 - 3.35.

In summary, the meta-analysis by Ahlbom et al. did not find any evidence of an increased risk of childhood leukemia at residential magnetic field levels of < 0. 4 μT. There was strong evidence to support a RR of 2 for childhood leukemia in children living with residential exposures of > 0.4 μT during the year prior to diagnosis. Less than 1% of subjects were in this exposure category. This result did not change after adjustment for potential confounders. The inconsistent findings between studies that used measured fields and those that used calculated fields is likely due to the relatively small number of cases and controls in the latter, as suggested by the relatively wide confidence intervals. However, the final pooled risk estimate, according to the authors of this meta-analysis, clearly indicates that the excess risk at high levels of exposure is not likely due to chance.

Greenland S, Sheppard AR, Kaune WT, Poole C and Kelsh MA. A pooled analysis of magnetic fields, wire codes, and childhood leukemia. Epidemiology 2000;11(6):624-634.

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Abstract: We obtained original individual data from 15 studies of magnetic fields or wire codes and childhood leukemia, and we estimated magnetic field exposure for subjects with sufficient data to do so. Summary estimates from 12 studies that supplied magnetic field measures exhibited little or no association of magnetic fields with leukemia when comparing 0.1-0.2 μT and 0.2-0.3 μT) categories with the 0-0.1 μT category, but the Mantel-Haenszel summary odds ratio comparing >0.3 μT to 0-0.1 μT was 1.7 (95% confidence limits = 1.2, 2.3). Similar results were obtained using covariate adjustment and spline regression. The study-specific relations appeared consistent despite the numerous methodologic differences among the studies. The association of wire codes with leukemia varied considerably across studies, with odds ratio estimates for very high

current vs low current configurations ranging from 0.7 to 3.0 (homogeneity P = 0.005). Based on a survey of household magnetic fields, an estimate of the U.S. population attributable fraction of childhood leukemia associated with residential exposure is 3% (95% confidence limits = -2%, 8%). Our results contradict the idea that the magnetic field association with leukemia is less consistent than the wire code association with leukemia, although analysis of the four studies with both measures indicates that the wire code association is not explained by measured fields. The results also suggest that appreciable magnetic field effects, if any, may be concentrated among relatively high and uncommon exposures, and that studies of highly exposed populations would be needed to clarify the relation of magnetic fields to childhood leukemia.

Comment: The objective of this meta-analysis was to answer the following question: are magnetic fields or wire codes consistently associated with childhood leukemia? Records for 16 studies were obtained, including the eight of the nine studies that were evaluated by Ahlbom, and eight additional ones [(Coghill, 1996), (Fulton, 1980), (Green, 1999), (Savitz, 1988), (Wertheimer, 1979), (Fajardo-Gutierrez, 1997) and (Tomenius, 1986)]. All studies were case-control except Verkasalo's, which was a nested case-control study (Verkasalo, 1993). All had some geographical restrictions on their source populations. The four Nordic studies [(Feychting, 1993), (Olsen, 1993), (Tynes, 1997) and (Verlasalo, 1993)] supplied calculated exposures from proximity to power lines and historical current supply data. A variety of measurement techniques were used to gather exposure data in the eight studies that measured the fields directly, including one or more of measurements at the front door of the residence, in the child's bedroom, in rooms of the residence, and average personal measures [(Coghill, 1996), (Linet, 1997), (London, 1991), (Michaelis, 1998), (Savitz, 1988), (Tomenius, 1986), (McBride, 1999) and (Dockerty, 1999)]. Some studies supplied more than one set of measurements, including spot measurements and 24 h or 48 h measurements. To avoid multiple comparison issues, the target measure was defined as a child's time-weighted average exposure up to 3 months before diagnosis. In addition, all North American studies supplied wire codes as an alternative surrogate of exposure [(McBride, 1999), (Green, 1999), (Savitz, 1988), (Fajardo-Gutierrez, 1997), (Fulton, 1980), (London, 1991), (Wertheimer, 1979) and (Linet, 1997)]. All studies varied in the availability of data on covariates, and in completeness of exposure data. To avoid the exclusion of cases and controls from the meta-analysis, the investigator used an unmatched analysis, with control for matched covariates. This approach maximized the number of cases and controls in the higher exposure categories, thereby minimizing the bias away from the null that would be the inevitable result of a small sample size.

In the categorical analysis, exposures were defined as < 0.1 μT; 0.1 - < 0.2 μT; 0.2 < 0.3 μT; and > 0.3 μT. There were differences among studies in the distribution of subjects among exposure categories. At the extremes, the study by Olsen had only 0.5% of cases and controls with exposures > 0.1 μT while Linet had > 30%. Values > 0.3 μT were infrequent in all of the pooled studies. A full 90% of surveyed homes in the US (and probably Canada) had exposures in the range of 0 to 0.2 μT (exclusive of

personal use sources such as electric blankets, razors, hair dryers etc.). The North American studies also tended to have a greater proportion of cases in the higher exposure categories, which is probably due to differences in the European and North American power distribution systems, per capita consumption, grounding procedures etc. There was no evidence of an association between exposure and leukemia below 0.3 μT. Without adjustments for covariates, the OR at exposures > 0. 3 μT was 1.87; 95% CI = 1.35 - 2.60, and with covariate adjustment, 2.06; 95% CI = 1.30 - 3.01. A trend analysis, which treated exposure as a continuous variable, gave a similar result. Neither single study deletions nor alternative choices for measures of exposure altered the results qualitatively. The Nordic studies based their exposure estimates on sources external to the residence (i.e. power lines), and after a correction for exposure from internal sources, their OR were adjusted towards the null. However, since the Nordic studies contributed so few cases at the higher exposure levels, this correction had only a small effect on the pooled risk estimates.

There were extensive differences among the North American studies when wire codes were used as a surrogate of exposure, ranging from a low in the Linet study of 15% of residences with ordinary high current codes + very high current codes (OHCC + VHCC) to nearly 50% in the London study. To make the wire code studies more amenable to pooling, the two earliest studies were arbitrarily dropped for the meta-analysis [(Wertheimer, 1979) and (London, 1994)]. For OHCC, the pooled OR was 1.02; 95% CI = 0.87-1.22 and for VHCC, the pooled OR was 1.50; 95% CI = 1.17-1.92 from five studies [(Fajardo-Gutierrez, 1997), (Linet, 1997), (Savitz, 1988), (McBride, 1999) and (Green, 1999)]. The adjustment for confounders had little effect on the wire code estimates of risk. It has been postulated that wire codes show a more consistent association with childhood leukemia than do measured fields, but for this meta-analysis, the opposite was found, and the so-called “wire code paradox” was not supported.

The confounding effects of socioeconomic status, residential mobility, residence type, viral contacts and traffic density could be responsible for the observed association between PFF and childhood leukemia. However, a confounding explanation requires the presumed covariate(s) to have an association with childhood leukemia that is considerably stronger than the observed association with exposure, and to also have a strong positive association with exposure. In this study, adjustment for socioeconomic status and housing factors resulted in only small changes in the association between PFF and leukemia. However, the risk factors for childhood leukemia are relative unknown, and so it is possible that some unforseen agent or condition will be identified in the future that will be able to explain the apparent association between PFF and childhood leukemia. It is also possible that selection bias influenced the results of this meta-analysis, but there is no way to determine either its magnitude or direction. The findings of this meta-analysis are weakened somewhat by the process of pooling data whose compatibility were not known with certainty.

Wartenberg D. Residential EMF exposure and childhood leukemia: meta-analysis and population attributable risk. Bioelectromagnetics 2001;Suppl 5:S86-104.

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Abstract: The controversy over the possible association between magnetic field exposure and childhood leukemia has led several researchers to summarize the literature using meta-analysis. This paper reviews these previous meta-analyses and extends them by adding results from four studies published since the most recent analysis. The analyses include odds ratio calculations based on both dichotomous and continuous exposure models, heterogeneity analysis including subgroup summaries and meta-regression, "leave one out" influence analyses, and publication bias assessments. In addition, there is a review of some of the considerations of the exposure assessments used in the studies and their implications for cross-study comparisons. Finally, the results of the analyses using dichotomous and continuous exposure model are combined with national exposure data to estimate the population attributable risk of childhood leukemia among children in the US. If an association exists, as many as 175 -240 cases of childhood leukemia in the US may be due to magnetic field exposure.

Comment: Nineteen studies were included in this meta-analysis [(Coleman, 1989), (Dockerty, 1998), (Fajardo, 1993), (Feychting and Ahlbom, 1993), (Fulton, 1980), (Green, 1999), (Linet, 1997), (Tynes and Haldorsen, 1997), (London, 1991), (McBride, 1999), (Michaelis, 1998), (Myers, 1990), (Olsen, 1993), (Petridou, 1997), (Savitz, 1988), (Tomenius, 1986), (UK, 1999), (Verkasalo, 1993) and (Wertheimer, 1979)]. Four exposure metrics were chosen for study: (i) calculated historical transmission line fields, (ii) measured magnetic fields, (iii) wire codes, and (iv) proximity to electrical facilities.

Six studies used calculated time-weighted averages as an exposure metric. This metric is applicable to only a small number of children that live near high voltage transmission lines, where the exposure from these lines is high enough to overshadow exposures from other sources, and cumulative exposure estimates can be developed using historical line load data [(Feychting and Ahlbom, 1993), (Olsen, 1993), (Verkasalo, 1993) (Tynes and Haldorsen, 1997)]. One study (Myers, 1990) calculated peak exposure over a period of several years and this was adjusted to reflect an average to make it comparable with other studies in this sub-group. Similarly, the exposure data from the two UK studies [(UK, 1999) and (UK, 2000)] were re-calculated to reflected a time-weighted average.

Nine studies reported the use of spot or point-in-time measurements as an exposure metric [(Tomenius, 1986), (Savitz, 1988), (Feychting and Ahlbom, 1993) and (Green, 1999)], or 24 or 48 h measurements [(London, 1991), (Linet, 1997), (Dockerty, 1998),

(McBride, 1999), and (Green, 1999)]. This metric is often chosen because it may be useful in averaging diurnal variations in exposure. However, this advantage is largely lost when the error associated with estimating long-term historical exposures is considered. The data from personal exposure monitors [(McBride, 1999) and (Green, 1999)] were not used in this meta-analysis. Some studies selected exposure cut points *a priori* some selected them *post hoc* and some reported data for both (an a priori selection is considered to have less opportunity for bias).

Wire codes categorize magnetic field exposures on the basis of the size of the electric power lines outside a residence, as a proxy for the electrical load on the lines, and the distance these lines are from the residence. The original 2-level code developed by Wertheimer and Leeper for the Denver childhood study was also used by Savitz in a similar 1988 Denver study. Fulton (1980) used a revised 4 level wire code for a study on Rhode Island. To make these studies comparable, the 3 lowest categories of exposure in Fulton's study (75% of controls residences) were aggregated and compared with Wertheimer's Lowest Current Code (LCC) class, which accounted for 78% of control homes, and Fulton's very high exposure category (25% of residences) was compared with Wertheimer's High Current Code (HCC) category (22% of control homes). The Linet study (Linet, 1997) used wire codes that included nine states in the U.S., and this raises questions of both internal and external comparability. For example, Savitz (Savitz, 1988) found 60% of the Very High Current Code (VHCC) homes in Denver gave low spot magnetic field measurements, whereas Linet found only 40% of VHCC homes across a wide geographical area gave low spot magnetic field measurements. Furthermore Linet found that 24 h spot measurements at VHCC homes over eight states differed by more than 300%, ranging from 0.082 μT to 0.267 μT. Therefore, wire coding protocols applied to different geographical regions may not correspond to similar magnetic field levels between regions. Both the McBride and Green studies used wire codes based on the Wertheimer method.

Proximity to electrical facilities is an imprecise indicator of magnetic field exposure, but despite this limitation, a distance metric was used by Feychting and Ahlbom (1993), Tynes and Haldorsen (1997), Myers (1990), Coleman (1989) and Fajardo-Gutierrez (1993). This meta-analysis used a distance cut point of 15-20 m for distribution lines and transformers, and 50 m for transmission lines and sub-stations. In contrast, wire codes are based on both distance from, and type of distribution line, but distance captures a large proportion of the information. Therefore, to pool the data from wire code studies with the cruder proximity studies, the meta-analysis combined the highest wire code categories with proximity data from residences within 20 m of distribution systems or 50 m of transmission systems. Residences with the lowest wire code categories were combined with those from the proximity studies that were outside of the 20 m and 50 m cut points. *Results:* This meta-analysis calculated results for two sub-groups of studies: (i) those that used calculated and measured magnetic fields as an exposure metric, and (ii) those that used wire codes and proximity measures as an

exposure metric. Results were calculated as (i) a simple average of all combined (unstratified) studies, (ii) a fixed effect model, and (iii) a random effect model.

Table 14. Studies using calculated and measured fields

Study Type	Exposed Cases / Controls	Unexposed Cases / Controls	Odds Ratio	95% Confidence Interval
Combined	241 / 298	3697 / 6000	1.31	1.09 - 1.59
Fixed effect	241 / 298	3697 / 6000	1.32	1.09 - 1.59
Random Effect	241 / 298	3697 / 6000	1.34	1.07 - 1.67

When these studies were considered as a single unstratified group, the Q-test for homogeneity was 0.3, indicating moderate homogeneity, and therefore meeting the criteria of this meta-analysis for pooling. Six factors were further examined to determine sources of heterogeneity; study design, country, age of subjects, year of publication, magnetic field strength, and control selection. Study design contributed most to the observed heterogeneity, possibly because of the inclusion of the four Scandinavian studies [(Feychting and Ahlbom, 1993), (Olsen, 1993), (Tynes and Haldorsen, 1997) and (Verkasalo, 1993)]. These four differed in study design, used calculated fields as an exposure metric and had higher odds ratios than the other studies. Influence analysis (i.e. eliminating one study at a time from the meta-analysis) indicated that Linet (1997) was most influential, but its removal increased the odds ratio by only 10%. The Fail Safe N was 24 (the number of null studies needed to reduce the observed result to non-significance) with a required sample size of 8900 cases and controls.

Table 15. Studies using proximity measures

Study Type	Exposed Cases / Controls	Unexposed Cases / Controls	Odds Ratio	95% Confidence Interval
Combined	527 / 610	1535 / 2906	1.64	1.43 - 1.87
Fixed Effect	527 / 610	1535 / 2906	1.18	1.02 - 1.37
Random Effect	527 / 610	1535 / 2906	1.24	0.99 - 1.56

When the proximity studies were considered as a single unstratified group, the Q-test for homogeneity was 0.03, indicating considerable heterogeneity. Even after stratification into sub-groups considerable heterogeneity was still apparent. Stratification of studies by year of publication explained some of the heterogeneity, but not all

(studies published before 1993 tended to find a positive association between exposure and childhood leukemia, while those published after 1993 did not). The non-significant results for the random effect model was not considered an adequate representation of the combined studies because of the heterogeneity. The results from the unadjusted study and the fixed effect model were not sensitive to the deletion of single studies, and publication bias was unlikely, given the Fail Safe Number (> 20 studies to reduce the observed results to non-significance) and the number of cases and controls needed (> 4400 each).

All meta-analyses, except the random-effects model, detected a statistically significant association for childhood leukemia at the highest levels of exposure. For those studies that present sufficient data, no dose-response trend was detected, possibly due to insufficient numbers. However, in the final interpretation of results, four factors need to be considered: the number of studies in the meta-analysis, their heterogeneity, the effect size, and the sensitivity (robustness) of results. Five of the six analyses detected a small to moderately sized association with childhood leukemia when the highest and lowest levels of exposure were compared. These results were not affected by study deletion. Also, these results are unlikely to be changed by additional studies, unless they are large and produce markedly different results. However, the studies underlying this meta-analysis contain considerable heterogeneity that is not easily rationalized. Therefore, the limitations due to design, confounding or other biases might still suggest alternative interpretations other than the ones presented here.

7.4 Adult Leukemia

Table 16. Adult leukemia and occupational exposures, summary of relevant studies (1998 to 2002)

Author	Date	Country	Increased Risk?	Weakness
Harrington	2001	UK	No	EA; DM
Bjork (note 1)	2001	Sweden	Yes (note 2)	EA; RB; CR
Bethwaite	2001	New Zealand	Yes	SSS; EA
Oppenheimer	2002	USA	No	SSS; EA; SB; RB

Abbreviations:
CR = Contradictory Results
DM = (possible) Diagnostic Misclassification
EA = (possible) Exposure Assessment Issues
IB = (possible) Information Bias
M = Mobility (Possible Differential Mobility Bias)
MC = Multiple Comparisons

NPD = (possible) Differential Non-Participation Bias
SB = (possible) Selection Bias
SSS = Small Sample Size
RB = (possible) Recall Bias

Note 1: Study involved residential and occupational exposures and only chronic myeloid leukemia (Ph^+)
Note 2: Internally inconsistent, significant only when exposure was based on 15 to 20 y duration, OR = 2.3; 95%CI = 1.2 - 4.5 (35 cases and 22 controls).

Study Summaries - Adult Leukemia

Harrington JM, Nichols L, Sorahan T, and van Tongeren M. Leukemia mortality in relation to magnetic field exposure: findings from a study of United Kingdom electricity generation and transmission workers, 1973-97. Occupational Environmental Medicine 2001;58(5):307-314.

The principal author is with the Institute of Occupational Health, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Abstract: *Objective:* To investigate whether risks of leukemia are related to occupational exposure to magnetic fields. *Methods:* The mortality experienced by a cohort of 83,997 employees of the former Central Electricity Generating Board of England and Wales was investigated for the period 1973-97. All employees were employed for at least 6 months with some employment in the period 1973-82. Computerized work histories were available for 79,972 study subjects for the period 1971-93. Detailed calculations were performed by others to enable a novel assessment to be made of exposures to magnetic fields. Two analytical approaches were used,
indirect standardisation (n=83 997) and Poisson regression (n=79 972). RESULTS: Based on serial mortalities for England and Wales, the standardised mortality ratio of 84 for all leukemias (observed 111, expected 132.3) was similar to that of 83 for all causes (observed 14 845, expected 17 918). No significant positive trends were found for the risks of various types of leukemia (chronic lymphatic leukemia, acute myeloid leukemia, chronic myeloid leukemia, all leukemia) either with lifetime cumulative exposure to magnetic fields or with such exposures received in the most recent 5 years. *Conclusions:* There are no discernible excess risks of leukemia as a consequence of occupational exposure to magnetic fields in United Kingdom electricity generation and transmission workers.

Comments: The cohort consisted of some 129,000 employees that worked in the UK electric generating industry from 1973-1997. The utilities provided job titles and employment records for each eligible member of the cohort. Exposures were assessed

by a complicated scheme that calculated exposure for an area within a power station work space, taking into account time and line load factors. For transmission workers, a complex series of survey measurements was used to estimate exposures for 8 job categories. Cumulative exposures were expressed in μT-y. Results were expressed as a standardized mortality ratio (SMR) of the observed numbers of leukemia in the cohort relative to expected number in the national population as a whole, after adjustment for sex, calendar year, and age. A model, based on Poisson regression, was used to account for a number of variables that were considered to influence mortality within the sub-groups of workers comprising the cohort. For the SMR analysis, there was no significant association between exposure to magnetic fields and leukemia mortality. There was also no significant association between cumulative exposure and leukemia mortality. Overall, the study found no evidence that UK electricity generation and transmission workers have an excess risk of leukemia as a consequence of occupational exposure to magnetic fields.

Bethwaite P, Cook A, Kennedy J and Pearce N. Acute leukemia in electrical workers: a New Zealand case-control study. Cancer Causes Control 2001;12(8):683-689.

The principal author is with the Medical Laboratory Wellington, New Zealand.

Abstract: *Objectives:* To assess the risks for adult-onset acute leukemia associated with electrical employment in New Zealand. *Methods:* The occupational and environmental exposures histories of 110 incident leukemia cases and 199 general population controls were compared. The cases were recruited through referrals to treatment centers in New Zealand between 1989 and 1991. For subjects classified as having worked in one or more of the "electrical occupations," the degree of exposures to extremely low frequency electromagnetic fields (ELF-EMFs) was assessed in detail using a job-exposure matrix. *Results:* An odds ratio of 1.9 (95% CI 1.0-3.8) was found for subjects who had ever worked in an electrical occupation. Significantly increased risks for leukemia are seen amongst welders/flame cutters (OR = 2.8 (95% CI 1.2-6.8)) and telephone line workers (OR = 5.81 (95% CI 1.2-27.8)). The excess leukemia risk appeared to be confined to acute non-lymphocytic leukemia (OR=2.31 (95% CI 1.2-4.6)), in comparison to acute lymphoblastic leukemia (OR = 0.9 (95% CI 0.3-2.9)) but for the latter category the numbers were very small. A dose-response effect was also found, with acute leukemia risk rising with increasing occupational magnetic field exposure, based on both current and historical occupational field exposure estimates. *Conclusions:* The findings of the current study indicate a significantly elevated risk of acute leukemia for electrical workers overall, and for the specific occupational categories of welders/flame cutters and telephone line workers. A dose-response effect was also found, indicating that acute leukemia risk was related to historical and current magnetic field exposures in an occupational context.

Comment: This was a small study with 100 cases and 199 controls. Subjects were identified as those every having worked in one or more 'electrical' occupations. Exposures were assessed by measurement, historically calculated fields and by job-exposure matrix. Increased risks were detected for welders (OR = 2.8; 95% CI = 1.2 - 6.8), and for telephone linesmen (OR = 5.81; 95% CI = 1.2 - 27.8). The risk was confined to Acute non-lymphocytic leukemia (ANLL), OR = 2.31; 95% CI = 1.2 - 4.6. For acute lymphocytic leukemia (ALL), the OR was non-significant. The main weakness with this study is the small number of cases and controls, which is reflected in the wide confidence intervals. Some misclassification of exposure was present. Diagnostic misclassification is unlikely as diagnoses were verified independently.

Bjork J, Albin M, Welinder H, Tinnerberg H, Mauritzson N, Kauppinen T, Stromberg U, Johansson B, Billstrom R, Mikoczy Z, Ahlgren T, Nilsson PG, Mitelman F and Hagmar L. Are occupational, hobby, or lifestyle exposures associated with Philadelphia chromosome positive chronic myeloid leukemia?
Occup Environmental Medicine 2001;58(11):722-727.

The principal author is with the Department of Occupational and Environmental Medicine, Lund University Hospital, SE-221 85 Lund, Sweden.

Abstract: *Objectives:* To investigate a broad range of occupational, hobby, and lifestyle exposures, suggested as risk factors for Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML). *Methods:* A case-control study, comprising 255 Ph+CML patients from southern Sweden and matched controls, was conducted.
Individual data on work tasks, hobbies, and lifestyle exposures were obtained by telephone interviews. Occupational hygienists assessed occupational and hobby exposures for each subject individually. Also, occupational titles were obtained from national registries, and group level exposure-that is, the exposure proportion for each occupational title-was assessed with a job exposure matrix. The effects of 11 exposures using individual data and two exposures using group data (organic solvents and animal dust) were estimated. *Results:* For the individual data on organic solvents, an effect was found for moderate or high intensity of exposure (odds ratio (OR) 3.4, 95% confidence interval (95% CI) 1.1 to 11) and for long duration (15-20 years) of exposure (OR 2.1, 95% CI 1.1 to 4.0). By contrast, the group data showed no association (OR 0.69, 95% CI 0.27 to 1.8; moderate or high intensity versus no exposure). For extremely low frequency electromagnetic fields (EMFs), only individual data were available. An association with long occupational exposure to EMFs was found (OR 2.3, 95% CI 1.2 to 4.5). However, no effect of EMF intensity was indicated. No significant effects of benzene, gasoline or diesel, or tobacco smoking were found. OR estimates below unity were suggested for personal use of hair dye and for agricultural exposures. *Conclusions:* Associations between exposure to organic solvents and EMFs, and Ph+CML were indicated but were not entirely consistent.

Comment: Almost all cases of chronic myeloid leukemia (CML) are also Philadelphia chromosome positive (Ph^+), that is having the translocation t(9;22)(q34;q11). The etiology of CML is not known with certainty, but exposure to ionizing radiation is likely to be a risk factor. Other risk factors have been proposed, including treatment with DNA topoisomerase II inhibitors, smoking, benzene and other organic solvents, ELF magnetic and electric fields, viruses, and pesticides. The object of this study was to investigate a broad range of occupational, hobby and lifestyle exposures that have been suggested as risk factors for CML.

The cases consisted of 255 adult patients with Ph^+ CML from Southern Sweden. Three controls were selected for each case, matched for age, sex, county of living at the time the case was diagnosed with CML. All cases and respective controls were interviewed. If a subject was dead or too ill to participate, a next of kin was selected for interview. Information had to obtained from next of kin more often for cases than controls. For ethical reasons, the interviewer knew *a priori* who were cases and who were controls.

Individual exposure data were determined subjectively by expert opinion, and group level exposure estimates were made through a job exposure matrix. Exposure to magnetic fields were classified according 8 hour means values reported for different occupations, with the following categories, 0.23 - 0.30 μT, > 0.30 - 0.50 μT and > 0.50 μT (Floderus, 1996). No consistent relationship between exposure to PFF and CML was detected. When the risk estimate was based on duration of exposure, only the 15 to 20 y category was statistically significant (OR = 2.3; 95% CI = 1.2 - 4.5), based on 35 cases and 22 controls. A spurious association cannot be ruled out.

Oppenheimer M[(1)] and Preston-Martin S[(2)]. Adult onset acute myelogenous leukemia and electromagnetic fields in Los Angeles County: Bed-heating and occupational exposures. Bioelectromagnetics 2002;23:411- 415.

The authors are with [(1)]the Clinical Studies Department, House Ear Institute, Los Angeles, California and [(2)]the Department of Preventive Medicine, University of Southern California, Los Angeles, California.

Abstract: In a large matched population-based case-control study of acute myelogenous leukemia (AML), we did not find incident AML in Los Angeles County (1987-1994) to be associated with previous exposure to electric blankets, electrically heated waterbeds, or occupations with presumed high exposure to electromagnetic fields.

Comment: This is a relatively small case control study on the association between magnetic field exposure (based on the use of electric blankets and electrically heated water beds, and on electrical occupations) and acute myelogenous leukemia (AML). The study consisted of 412 cases and 412 controls, matched on the basis of age, race and gender. Controls were identified by canvassing the case's neighborhood.

Stratification by age, gender, race or socioeconomic status did not reveal any sub-groups where magnetic field exposures may have been higher in AML cases than in controls. Only 51 % of cases had direct personal interviews and exposure misclassification by proxy data is a possibility (there is a high fatality from AML). The control participation rate was only 55% of the potentials, and the possibility exists that their exposure to magnetic fields may not have been representative. While this was a population-based study, the possibility of selection bias remains due to the relatively low participation rates of both cases and controls. The study was also prone to exposure misclassification since only job title and whether the participant used an electric bed heating device were known. The actual exposure was not known. The study results must be considered as inconclusive.

Leukemia Appendix - Supplementary References

Hatch EE, Kleinerman RA, Linet MS, Tarone RE, Kaune WT, Auvinen A, Baris D, Robison LL and Wacholder S. Do confounding or selection factors of residential wiring codes and magnetic fields distort findings of electromagnetic fields studies? Epidemiology 2000;11(2):189-198.

The principal author is with the Radiation Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD 20892, USA.

Abstract: In contrast with several previous studies, our recent large case-control study found little association between childhood acute lymphoblastic leukemia (ALL) and electric-power-line wire codes. Here we examine internal evidence from our study to assess the possibility that selection bias and/or confounding may have affected the findings. We compared the relation between childhood ALL and wire codes and direct measurements of magnetic fields in subjects who participated in all phases of the study with the relation in all subjects, including those who declined to allow access inside the home. We found that the odds ratio for ALL among those living in homes with very high current configurations increased by 23% when 107 "partial participants" were excluded. We found similar, but slightly smaller, increases in the odds ratios when we performed the same comparisons using direct measurements of magnetic fields, excluding subjects who allowed only a measurement outside the front door. "Partial participants" tended to be characterized by lower socioeconomic status than subjects who participated fully, suggesting possible selection bias. We also examined the relation between a large number of potential confounding variables and both proxy and direct measurements of magnetic fields. Univariate adjustment for individual variables changed the odds ratio for ALL by less than 8%, while simultaneous adjustment for several factors reduced the estimate by a maximum of 15%. We conclude that while confounding alone is unlikely to be an important source of bias in our own and previous studies of magnetic fields, selection bias may be more of a concern, particularly in light of the generally low response rates among controls in case-control studies.

Kavet R, Zaffanella LE, Daigle JP and Ebi KL. The possible role of contact current in cancer risk associated with residential magnetic fields. Bioelectromagnetics 2000;21(7):538-553.

The principal author is with the Electric Power Research Institute **(**EPRI), Palo Alto, California 94303, USA.

Abstract: Residential electrical wiring safety practices in the US result in the possibility of a small voltage (up to a few tenths of a volt) on appliance surfaces with respect to water pipes or other grounded surfaces. This "open circuit voltage" (V(OC)) will cause "contact current" to flow in a person who touches the appliance and completes an electrical circuit to ground. This paper presents data suggesting that contact current due to V(OC) is an exposure that may explain the reported associations of residential magnetic fields with childhood leukemia. Our analysis is based on a computer model of a 40 house (single-unit, detached dwelling) neighborhood with electrical service that is representative of US grounding practices. The analysis was motivated by recent research suggesting that the physical location of power lines in the backyard, in contrast to the street, may be relevant to a relationship of power lines with childhood leukemia. In the model, the highest magnetic field levels and V(OC)s were both associated with backyard lines, and the highest V(OC)s were also associated with long ground paths in the residence. Across the entire neighborhood, magnetic field exposure was highly correlated with V(OC) ® =0.93). Dosimetric modeling indicates that, compared to a very high residential level of a uniform horizontal magnetic field (10 μT) or a vertical electric field (100 V/m), a modest level of contact current (approximately 18 μA) leads to considerably greater induced electric fields (> 1 mV/m) averaged across tissue, such as bone marrow and heart. The correlation of V(OC) with magnetic fields in the model, combined with the dose estimates, lead us to conclude that V(OC) is a potentially important exposure with respect to childhood leukemia risks associated with residential magnetic fields. These findings, nonetheless, may not apply to residential service used in several European countries or to the Scandinavian studies concerned with populations exposed to magnetic fields from overhead transmission lines.

Kavet R[(1)] and Zaffanella LE[(2)]. Contact voltage measured in residences: Implications to the association between magnetic fields and childhood leukemia. Bioelectromagnetics 2002;23:464-474.

The authors are with [(1)]the Environment Department, EPRI, Palo Alto, California, USA and [(2)]Enertech Consultants, Lee, Massachusetts, USA.

Abstract: We measured magnetic fields and two sources of contact current in 36 homes in Pittsfield, MA. The first source, VP-W, is the voltage due to current in the grounding wire, which extends from the service panel neutral to the water service line. This voltage can cause contact current to flow upon simultaneous contact with a metallic part of the

water system, such as the faucet, and the frame of an appliance, which is connected to the panel neutral through the equipment-grounding conductor. The second is VW-E, the voltage between the water pipe and earth, attributable to ground currents in the water system and magnetic induction from nearby power lines. In homes with conductive water systems and drains, VW-E can produce a voltage between the faucet and drain, which may produce contact current into an individual contacting the faucet while immersed in a bathtub. VP-W was not strongly correlated to the magnetic field (both log transformed) ® = 0.28; P < 0.1). On the other hand, VW-E was correlated to the residential magnetic field (both log transformed) ® = 0.54; P < 0.001), with the highest voltages occurring in homes near high voltage transmission lines, most likely due to magnetic induction on the grounding system. This correlation, combined with both frequent exposure opportunity for bathing children and substantial dose to bone marrow resulting from contact, lead us to suggest that contact current due to VW-E could explain the association between high residential magnetic fields and childhood leukemia.

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8. MISCARRIAGE, FERTILITY AND REPRODUCTIVE CAPACITY

8.1 Human Studies

Reports of miscarriages among women who used video display terminals (VDT) prompted the initial concern about electric and magnetic fields and miscarriage. Most of the past studies of miscarriage and the use of VDT, electric blankets and electric bed heaters used surrogates, rather than personal measures, to evaluate the magnetic field exposure. Therefore, the resulting inconsistent risk estimates for miscarriage could have been due to non-differential exposure misclassification arising from the use of surrogates, rather than personal measures, to characterize exposure (Wilson, 1996).

Retrospective studies of magnetic fields and adverse health effects rely on wire codes or present day field measurements to infer an individual's past exposure. The error inherent in this procedure is considerable, and substantial exposure misclassification can result, which, if non-differential, can lessen the chances of detecting an effect. Also, the nature of the etiologically relevant time period is unknown, or unidentifiable, making it difficult to determine how far a predicted exposure should extend into the past. Furthermore, only a small proportion of the general population may be susceptible to the effects of magnetic fields. Taken together, these uncertainties make it difficult for a retrospective study to detect any real effect of magnetic fields. The ideal study to resolve this issue would be a prospective one, involving a 'susceptible' population, where magnetic fields could be measured contemporaneously for a definable, etiologically relevant time period of short duration. While such an experiment can be easily designed for animals, it is ethically difficult to justify for humans. The issue of PFF and miscarriage is one area where a prospective study of humans has been published recently (Li D-K, 2002), and where a 'susceptible' population was available (i.e. in this instance, women that had a history of miscarriage).

Table 17. Summary of reproductive health and power-frequency fields human studies

Author	Date	Country	Increased Risk ?	Weakness
Belanger	1998	USA	no	EA; RB
Li	2002	USA	yes	NPB; SSS; EA
Lee	2002	USA	yes	EA; NPB; CR; RB

Abbreviations:
EA = Exposure Assessment shortcomings
CR = Conflicting Results

NPB = (possible) Non-Participation Bias
RB = (possible) Recall Bias
SSS = Small Sample Size

8.2 Study Summaries - Miscarriage

Belanger K, Leaderer B, Hellenbrand K, Holford TR, McSharry J, Power ME and Bracken MB. Spontaneous abortion and exposure to electric blankets and heated water beds. Epidemiology 1998;9(1):36-42.

The principal author is with the Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06510-8055, USA.

Abstract: We conducted a prospective study (N = 2,967) to evaluate the relation of spontaneous abortion with use of electrically heated beds (electric blankets and heated water beds) during pregnancy. At interview, 61.5% of women were at less than 12 weeks gestation, and 38.5% were between 13 and 16 weeks; thus, very early pregnancy losses would have been excluded. Information regarding exposure
to electric beds was obtained for the month of conception and the 7 days before interview. Electric blanket use at conception was associated with an increased risk of spontaneous abortion in the unadjusted analysis [relative risk (RR) = 1.84; 95% confidence interval (CI) = 1.08-3.13], but adjustment for other factors reduced the risk slightly (odds ratio (OR) = 1.74; 95% CI = 0.96-3.15). Heated water bed use was not associated with an increased risk of spontaneous abortion at conception (OR = 0.59; 95% CI = 0.33-1.07) or at interview (OR = 0.63; 95% CI = 0.36-1.12). Measures of dose response (daily use, hours of use, or temperature setting) were not associated with increased risk. Wire code data were obtained for the first, or only, house lived in during pregnancy. Women living in homes classified as "very high" or "ordinary high" current configuration were not at greater risk than women living in homes with buried wires. Nor was there any trend for increased risk of spontaneous abortion by wire code category. This study does not support the hypothesis that use of electric beds or residence in a high current configuration home increases the risk of spontaneous abortion; however, it indicates that electric blanket use at the time of conception and in early pregnancy may be associated with a slight increase in risk of pregnancy loss.

Comment: In this study only 61% of participants were at less than 12 weeks of gestation, which is the time D-K Li (see below) found the strongest association between magnetic fields and risk of miscarriage. Therefore, it is conceivable that the 39 % that were at longer gestation times diluted any potentially observable effect of the magnetic field on the rate of miscarriage.

Li D-K[(1)], Odouli R[(1)], Wi S[(1)], Janevic T[(1)], Golditch I[(2)], Bracken TD[(3)], Senior R[(3)], Rankin R[(4)] and Iriye R[(5)]. A Population-Based Prospective Cohort Study of

Personal Exposure to Magnetic Fields during Pregnancy and the Risk of Miscarriage. Epidemiology 2002;13(1):9-20.

The authors are with [(1)]the Division of Research, Kaiser Foundation Research Institute, Kaiser Permanente, Oakland, CA; [(2)]the Department of Obstetrics and Gynecology, Kaiser San Francisco, San Francisco, CA; [(3)]T. Dan Bracken, Inc, Portland, OR; [(4)]Oregon Applied Research Services, Lake Oswego, OR; and [(5)]Enertech Consultants Inc, Campbell, CA.

Abstract: To study the effect of magnetic fields on the risk of miscarriage, we conducted a population-based prospective cohort study among pregnant women within a large health maintenance organization. All women with a positive pregnancy test at less than 10 weeks of gestation and residing in the San Francisco area were contacted for participation in the study. We conducted in-person interviews to obtain information on risk factors for miscarriage and other potential confounders. All participants were also asked to wear a magnetic field-measuring meter for 24 h and to keep a diary of their activities. Pregnancy outcomes were obtained for all participants by searching the health maintenance organizations' databases, reviewing medical charts, and telephone follow-up. We used the Cox proportional hazard model for examining the magnetic field-miscarriage association. A total of 969 subjects were included in the final analyses. Although we did not observe an association between miscarriage risk and the average magnetic field level, miscarriage risk increased with an increasing level of maximum magnetic field exposure with a threshold around 16 milligauss (mG). The rate ratio (RR) associated with magnetic field exposure 16 mG (vs <16 mG) was 1.8 (95% confidence interval (CI) = 1.2-2.7). The risk remained elevated for levels (in tertiles) of maximum magnetic field exposure 16 mG. The association was stronger for early miscarriages (<10 weeks of gestation) (RR = 2.2, 95% CI = 1.2-4.0) and among "susceptible" women with multiple prior fetal losses or subfertility (RR = 3.1, 95% CI = 1.3-7.7). After excluding women who indicated that their daily activity pattern during the measurements did not represent their typical daily activity during pregnancy, the association was strengthened; RR = 2.9 (95% CI = 1.6-5.3) for maximum magnetic field exposure 16 mG, RR = 5.7 (95% CI = 2.1-15.7) for early miscarriage, and RR = 4.0 (95% CI =1.4-11.5) among the susceptible women. Our findings provide strong prospective evidence that prenatal maximum magnetic field exposure above a certain level (possibly around 16 mG) may be associated with miscarriage risk. This observed association is unlikely to be due to uncontrolled biases or unmeasured confounders.

Comment: This was a prospective cohort study to examine the association between 24 h personal magnetic field exposures and miscarriage. A previous related study (Lee, 2002) found that a time-weighted average (TWA) magnetic field exposure above 2 mG (0.2 µT) increased the risk for miscarriage. This present study was to confirm and extend this finding by examining the effect of both the TWA exposure and the maximum magnetic field (MMF) exposure encountered during the day. The study was prospective and clinic-based, opened to all members of a large health maintenance organization

(HMO) in the San Francisco bay area, and who also had a positive pregnancy test at the HMO clinic. The final analysis consisted of 969 eligible women. Since the relevant exposure metric was unknown, this study examined the risk of miscarriage in relation to a 24 h maximum magnetic field (MMF) exposure as well as a 24 h TWA exposure. This was to test the hypothesis that a magnetic field could have an effect threshold for miscarriage.

The risk of miscarriage was evaluated with the 24 h TWA at a cut point of $\geq$ 0.3 µT (3 mG); RR = 1.2; 95% CI = 0.7-2.2. This result failed to confirm a previous retrospective study, which had found an effect at this cut point (Lee, 2002). Examination of the data indicated that the MMF level appeared to be associated with an increased rate of miscarriage starting around 12-18 mG (1.2 to 1.8 µT). Therefore, a cut point was selected *post hoc* at 16 mG (1.6 µT). Under these conditions, the RR = 2.2; 95% CI = 1.2 - 4.0 was estimated for fetuses at or under 10 weeks of gestation. If a fetus survived more than 10 weeks, the risk of miscarriage was non-significant, RR = 1.4; 95% CI = 0.8 - 2.5. Women that were 'susceptible' to miscarriage (i.e. had a history of two or more miscarriages) also had an increased risk of miscarriage, RR = 3.1; 95% CI = 1.3 - 7.7 at the $\geq$ 16 mG (1.6 µT) cut point. The risk was further increased in this sub-group if the pregnancy was at less than 10 weeks of gestation, RR = 4.7; 95% CI = 1.4 - 15.9.

The limitations in this study include the selection of the 16 mG cut point *post hoc.* Exposure misclassification could also have been present since a single 24 h measurement may not have been representative of the exposure during the entire gestational period. However, another study (Bracken, 1994) concluded that the MF level was stable and a single 24 h measurement was a good indication of average exposure (the MMF metric was not examined specifically in this study). Another limitation is the subjective definition of a 'typical' day during pregnancy. Participants were asked to judge whether or not the measurement day was made on a 'typical' day during pregnancy. When women who judged their measurement day as non-typical were excluded from the analysis, the RR was significantly associated with exposure, RR = 2.9; 95% CI = 1.6 - 5.3 (women who were excluded had a non-significant association with exposure). Other limitations could be due to non-participation (only 39% of eligible women participated). However, the rates of miscarriage among non-participants and participants were comparable, which would limit the impact of non-participation bias. Small sample size is another limitation of this study, as reflected in the wide confidence intervals surrounding some risk estimates. The results suggest that MF exposure was strongly related to early miscarriage (< 10 weeks of gestation). However, due to its potential shortcomings, this study will require extensive validation.

Lee GM[(1)], Raymond R[(1)], Janevic N[(1)], Hristova L[(1)],Yost M[(2)] and Hiatt RA[(3)]. A Nested Case-Control Study of Residential and Personal Magnetic Field Measures and Miscarriages. Epidemiology 2002;13(1):21-31.

The authors are with [1]the Environmental Health Investigations Branch California Department of Health Services, Oakland, CA, [2]the Department of Environmental Health, University of Washington, Seattle, WA, and [3] the Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, MD.

Abstract: We conducted a nested case-control study (177 cases, 550 controls) to assess the relation between retrospective magnetic field measures and clinical miscarriage among members of the northern California Kaiser Permanente medical care system. We also conducted a prospective substudy of 219 participants of the same parent cohort to determine whether 12 week and 30 week exposure assessments were similar. We evaluated wire codes, area measures, and three personal meter metrics: (1) the average difference between consecutive levels (a rate-of-change metric), (2) the maximum level, and (3) the time-weighted average. For wire codes and area measures, we found little association. For the personal metrics (30 weeks after last menstrual period), we found positive associations. Each exposure was divided into quartiles, with the lowest quartile as referent. Starting with the highest quartile, adjusted odds ratios and 95% confidence intervals were 3.1 (95% CI = 1.6- 6.0), 2.3 (95% CI = 1.2- 4.4), and 1.5 (95% CI = 0.8-3.1) for the rate-of-change metric; 2.3 (95% CI = 1.2-4.4), 1.9 (95% CI = 1.0-3.5), and 1.4 (95% CI = 0.7-2.8) for the maximum value; and 1.7 (95% CI = 0.9-3.3), 1.7 (95% CI = 0.9-3.3), and 1.7 (95% CI = 0.9-3.3) for the time-weighted average. The odds ratio conveyed by being above a 24 hour time-weighted average of 2 milligauss was 1.0 (95% CI = 0.5-2.1). Exposure assessment measurements at 12 weeks were poorly correlated with those taken at 30 weeks. Nonetheless, the prospective substudy results regarding miscarriage risk were consistent with the nested study results.

Comment: This is a case-control study nested within a larger prospective reproductive study. Cases were defined as women who had miscarriages before 20 weeks of gestation, and controls were defined as women who had live births. The hypothesis tested was that cases were more likely than controls to: (i) live near high current power lines, (ii) have higher residential magnetic field area (spot) measures, and (iii) have higher personal magnetic field exposures. Subjects were recruited from a cohort of 3402 pregnant women at < 13 weeks gestation that were members of a large HMO in California.

The full nested case-control study assessed magnetic fields at 30 weeks after the last menstrual period, and assumed this would be the same as those experienced at 10 weeks of gestation (during the first trimester), before a miscarriage, if any, had occurred. To validate this assumption, a small prospective sub-study was conducted to determine how closely the 30 week measurements agreed with those measured at 10 weeks of gestation, and to determine if the sub-study gave risk estimates similar to those of the full nested case control retrospective study (i.e. the 30 week study). In the sub-study, 18 cases and 201 controls allowed measurements at 12 weeks, and 10 of 18 cases and 166 of 201 controls allowed measurements at 30 weeks. In the fully nested case-control

retrospective study, 167 of 328 eligible cases and 384 of 806 eligible controls, allowed measurements at 30 weeks. Both studies combined resulted in a total of 177 cases and 550 controls. Miscarriage was defined as the loss of a conceptus before 20 weeks of gestation.

Four measures of exposure were examined. The home of each participant was coded according to Wertheimer-Leeper code categories. Three personal magnetic field measures were selected *a priori* (i) time-weighted average (TWA), (ii) rate of change metric (RCM), and (iii) the maximum level (ML) encountered during the 24 hour measurement period. Personal and residential area (spot) measurements were taken at waist level, with both field worker and participant blinded to the exposure levels. The 24 h measurement partitioned exposure between home, work, outside of home and work, and at night while sleeping. The different exposure metrics captured different aspects of a person's total exposure. The effects of a large number of confounders on risk estimates were also examined.

The correlation between magnetic field measurements at 12 weeks did not correlate well with those made at 30 weeks. Wire codes showed a non-significant association with risk of miscarriage. For the sub-study, the TWA was associated with an increased risk for miscarriage (OR = 3.0; 95% CI = 1.1 - 8.4) whereas the RCM and ML metrics were not. However, in the full study, the opposite was true, the TWA was non-significantly associated with miscarriage whereas the RCM and ML were significant. For RCM, significance was reached at exposures > 0.62 μT, OR = 2.4; 95% CI = 1.3 - 4.5 (37 cases and 118 controls). At > 0.94 μT the OR = 3.3; 95% CI = 1.8 - 6.0 (46 cases and 109 controls). For ML exposures > 2.3 μT, the OR = 2.1; 95% CI = 1.2 - 3.4 (38 cases and 115 controls).

8.3 Relevant Animal Studies

Theoretically, magnetic fields could effect reproductive health by several mechanisms, (i) by genetic mutations in the maternal or paternal germ cells, (ii) preimplantation loss, (iii) during early prenatal development, and (iv) by changing fetal or maternal physiology. However, most of the studies on magnetic fields and reproductive health have found little evidence of teratogenic or embryotoxic effects (Huuskonen, 1998(a) for mini-review).

Table 18. Summary of reproductive health relevant animal studies

Author	Year	Country	Increased Risk?	Weakness
Huuskonen	1998	Finland	no	none apparent
Ryan	1999	USA	no	none apparent
Ryan	2000	USA	no	none apparent
Huuskonen	2001	Finland	no	SAE
Huuskonen	2001	Finland	no	none apparent
Al-Akhras	2001	Jorden	yes	SSS; CD
Elbetieh	2002	Jordan	no	none apparent
Negishi	2002	Japan	no	none apparent

Abbreviation:
CD = (possible) Compromised Study Design(housing stress possible)
SAE = Subjective Assessment of Endpoint
SSS = Small Sample Size

Huuskonen H, Juutilainen J, Julkunen A, Maki-Paakkanen J and Komulainen H. Effects of low-frequency magnetic fields on fetal development in CBA/Ca mice. Bioelectromagnetics 1998(b);19(8):477- 485.

The principal author is with the National Public Health Institute, Division of Environmental Health, Laboratory of Toxicology, Kuopio, Finland.

Abstract: Effects of alternating magnetic fields (MFs) on the embryonic and fetal development in CBA/Ca mice were studied. Mated females were exposed continuously to a sinusoidal 50 Hz (13 μT or 0.13 mT root mean square) or a sawtooth 20 kHz (15 μT peak-to-peak) MF from day 0 to day 18 of pregnancy for 24 h/day until necropsied on day 18. Control animals were kept under the same conditions without the MF. MFs did not cause maternal toxicity. No adverse effects were seen in maternal hematology and the frequency of micronuclei in maternal bone marrow erythrocytes did not change. The MFs did not increase the number of resorptions or fetuses with major or minor malformations in any exposure group. The mean number of implantations and living fetuses per litter were similar in all groups. The corrected weight gain (weight gain without uterine content) of dams, pregnancy rates, incidences of resorptions and late fetal deaths, and fetal body weights were similar in all groups. There was, however, a statistically significant increase in the incidence of fetuses with at least three skeletal variations in all groups

exposed to MFs. In conclusion, the 50 Hz or 20 kHz MFs did not increase incidences of malformations or resorptions in CBA/Ca mice, but increased skeletal variations consistently in all exposure groups.

Comment: The exposure system and related quality assurance protocols were adequately described. Animal handing and exposure protocols were adequate. The number of animals were sufficient for a statistically acceptable analysis, and the statistical procedures used to compare samples were acceptable and well known. Parametric and non-parametric statistics were applied according to acceptable criteria. In this study, the only effect clearly related to magnetic field exposure was an increased incidence of fetuses with skeletal variations. There are no reasons to question the conclusion that exposure of mice to magnetic fields has no adverse effect on fetal development, birth number, or any other aspect of reproductive health.

Ryan BM, Symanski RR, Pomeranz LE, Johnson TR, Gauger JR and McCormick DL. Multigeneration reproductive toxicity assessment of 60 Hz magnetic fields using a continuous breeding protocol in rats. Teratology 1999;59(3):156-162.

The principal author is with the Life Sciences Department, IIT Research Institute, Chicago, Illinois 60616, USA.

Abstract: Male and female reproductive functions have been proposed as possibly sensitive targets for the biological effects of 60 Hz (power frequency) magnetic fields (MF). However, experimental data relevant to this hypothesized association are very limited. In the present study, the "reproductive assessment by continuous breeding" design was used to identify possible effects of MF exposure on reproductive performance, fetal development, and early postnatal growth in rats. Groups of age-matched Sprague-Dawley rats (40 breeding pairs/group) were exposed continuously (18.5 h per day) to linearly polarized, transient-free 60 Hz MF at field strengths of 0 Gauss (G; sham control), 0.02 G, 2.0 G, or 10.0 G. An additional group of 40 breeding pairs received intermittent (1 h on/1 h off) exposure to 10.0 G fields. F0 breeding pairs were exposed to MF or sham fields for 1 week prior to mating, during a 14 week period of cohabitation, and during a 3 week holding period after cohabitation. The duration of the cohabitation period was selected to be sufficient for the delivery of five litters in the sham control group. Pups from the final F1 litter from each breeding pair were exposed to MF or sham fields until sexual maturity, were cohabitated in MF or sham fields for 7 days with nonsiblings from the same exposure group, and were held in the MF or sham fields for 22 days to permit delivery of F2 pups for evaluation. No evidence of exposure-related toxicity was identified in any rat in the F0, F1, or F2 generations. Fetal viability and body weights in all litters of groups exposed to MF were comparable to those of sham controls. No significant differences between sham controls and MF-exposed groups were seen in any measure of reproductive performance (litters/breeding pair, percent fertile pairs, latency to parturition, litter size, or sex ratio) in either the F0 or F1 generation. Exposure of

Sprague-Dawley rats to 60 Hz MF strengths of up to 10.0 G either during their peak reproductive period (F0) or during gestation and throughout their life span (F1) has no biologically significant effects on reproductive performance. These results do not support the hypothesis that exposure to pure, linearly polarized 60 Hz MF is a significant reproductive or developmental toxicant.

Comment: This was a large well designed study with adequate exposure systems and appropriate quality assurance protocols to ensure reproducible and reliable operation of the exposure system over the experimental period. The animal handling and exposure protocols were adequately described. Stress due to housing or handling is unlikely to be a factor in the observed outcomes. Well known statistical procedures were used to analyze the data, and sample sizes were adequate. There was no evidence that exposure to magnetic fields affected reproductive capacity of either males or female rats or the reproductive capacities of their offspring. There is no reason to reject these findings.

Ryan BM, Polen M, Gauger JR, Mallett E Jr, Kearns MB, Bryan TL and McCormick DL. Evaluation of the developmental toxicity of 60 Hz magnetic fields and harmonic frequencies in Sprague-Dawley rats. Radiat Res 2000;153(5 Pt 2):637-641.

The principal author is with the Life Sciences Department, IIT Research Institute, 10 West 35th Street, Chicago, Illinois 60616, USA.

Abstract: Experimental data suggest that exposure to the 50 and 60 Hz sinusoidal components of power-frequency magnetic fields (MFs) does not have an adverse impact on fetal development. However, the possible developmental toxicity of MF harmonics has not been investigated. This study was designed to determine whether exposure to 180 Hz MFs (third harmonic), alone or in combination with 60 Hz MFs, induces birth defects in Sprague-Dawley rats. Groups of sperm-positive dams (> or = 20/group) were exposed for 18.5 h per day from gestation days 6 through 19 to (1) ambient MFs only (<0.0001 mT; sham controls); (2) 60 Hz MFs at 0.2 mT; (3) 180 Hz MFs at 0.2 mT; or (4) 60 Hz + 180 Hz MFs (10% third harmonic; total field strength = 0.2 mT). Litter size, litter weight, percentage live births, sex ratio, and number of resorption sites were determined for each dam, and gross external, visceral, cephalic and skeletal examinations were performed on all fetuses. MF exposure had no significant effects on litter size, litter weight, or fetal development. With the exception of common rib variants, the incidence of fetal anomalies was comparable in all groups. A small increase in the incidence of rib variants was seen in the group exposed to 60 Hz and 180 Hz MFs; however, the incidence of rib variants in this group was similar to that in historical controls from our laboratory. These data extend the existing database on developmental toxicity of MFs by demonstrating that exposure to 180 Hz MFs, either alone or superimposed on an underlying 60 Hz signal, does not induce biologically significant

developmental toxicity. These data do not support the hypothesis that exposure to power-frequency MFs is an important risk factor for fetal development.

Comment: This is a large well described study with an adequate exposure system and appropriate quality assurance protocols. Animal handling and exposure protocols are adequate, and housing stress should not be factor in the observed outcomes. Standard statistical techniques were used to analyze the data and samples sizes are sufficient to minimize false negative outcomes. There is no reason to reject the conclusion of the authors that exposure to 60 Hz magnetic fields, either alone or in combination with a well defined 180 Hz harmonic, had any adverse effect on the reproductive capacity of either male or female rats.

Huuskonen H, Juutilainen J, Komulainen H. Development of preimplantation mouse embryos after exposure to a 50 Hz magnetic field in vitro. Toxicol Lett 2001(a)20;122(2):149-155.

The principal author is with the National Public Health Institute, Department of Environmental Health, Laboratory of Toxicology, P.O. Box 95, FIN-70701, Kuopio, Finland.

Abstract: Effect of sinusoidal 50 Hz magnetic field (MF) on development of preimplantation CBA/S mouse embryos *in vitro* was studied. Superovulated and *in vivo* fertilized preimplantation embryos were collected at one cell stage and divided to control and MF-exposed groups. Sinusoidal 50 Hz MF with field strength of 10 A/m r.m.s., corresponding a flux density of 13 μT r.m.s., was used to expose the embryos in culture at 37 degrees C in a CO_2-incubator. The developmental stage and abnormalities were recorded twice daily except once daily during weekends. The vitality and developmental stages of the embryos were similar in both groups although slightly more dead embryos were found during the 1st day in MF-exposed group ($P<0.05$) and the development of MF-exposed embryos was slightly impaired. In conclusion, the exposure to sinusoidal 50 Hz MF at field strength of 10 A/m did not significantly disturb the development of the mouse embryos in vitro up to the blastocyst stage.

Comment: This is a study to determine the effect of 50 Hz magnetic fields on embryonic development from zygote to the blastocyst stage. No adverse effects were noted. However, the criteria used to assess the microscopic development of embryos were subjective, and operator-dependent, which makes replication or confirmation difficult. The implication of this study are that MF produced no significant effect on early embryonic development. Whether the results of this *in vitro* study are relevant to development *in vivo* is not known.

Huuskonen H, Saastamoinen V, Komulainen H, Laitinen J and Juutilainen J. Effects of low-frequency magnetic fields on implantation in rats. Reprod Toxicol 2001(b);15(1):49-59.

The principal author is with the Laboratory of Toxicology, Division of Environmental Health, National Public Health Institute, P.O. Box 95, FIN-70701, Kuopio, Finland.

Abstract: Effects of 50-Hz sinusoidal magnetic fields (MFs) on embryo implantation, serum 17beta-estradiol, progesterone, testosterone, and melatonin levels, and on estrogen receptor (ER) and progesterone receptor (PgR) densities in the uterus were studied during the preimplantation and implantation periods in rats. Pregnant Wistar rats were exposed to magnetic r.m.s. field strengths of 10 or 100 A/m (13 or 130 microT) or sham-exposed (controls) from day 0 of pregnancy for 24 h/day and killed during light and dark periods between 70 h and 176 h after ovulation. MFs did not influence the mean total number of implantations. The nocturnal mean serum melatonin concentration decreased by 34 and 38% at 10 and 100 A/m, respectively. At the same time, the first embryos, at an early developmental stage, arrived in the uterus in the MF-exposed groups. Serum estradiol and progesterone levels did not significantly change. Nuclear PgR and ER densities in the uterus decreased before implantation and there was an increased incidence of early stage embryos and fewer hatched embryos were found in the uterus at 100 A/m. During the early implantation period, the uterine cytosolic ER/PgR-ratio was increased at 100 A/m and no implants were concomitantly found in uterus. The nuclear ER/PgR-ratio decreased during implantation in both MF-groups due to decreased nuclear ER density. At the same time, 19% and 15% of the embryos (calculated from the corpora luteae) at 10 and 100 A/m, respectively, were yet morulae and not implanted. In summary, the results show that MFs do not impair implantation in rats although there may be some borderline changes in the transport and development of embryos and associated endocrinologic parameters.

Comment: This laboratory study shows that exposure of pregnant rats to 13 and 130 µT magnetic fields had a minimal effect on implantation (these doses very approximately scale to 2 to 25 µT in humans). Presumably, at these environmental levels, there would be no effect on human fertility. However, there are many difficulties in attempting to extrapolate data from rats experiments to humans. Other endpoints of interest is the decrease in mean serum concentrations of melatonin that occurred in response to the magnetic field, although the decrease was not dose-related. Whether these responses would occur under non-laboratory conditions is not known, and neither is the biological relevance of small changes in the levels of melatonin.

Al-Akhras MA, Elbetieha A, Hasan MK, Al-Omari I, Darmani H and Albiss B. Effects of extremely low frequency magnetic field on fertility of adult male and female rats. Bioelectromagnetics 2001;22(5):340-344.

The principal author is with the Biophysics Laboratory, Department of Physics, Jordan University of Science &Technology, Irbid, Jordan.

Abstract: To investigate the effects of an extremely low-frequency (ELF) magnetic field on their fertility, adult male and female Sprague-Dawley rats were exposed to a 50 Hz sinusoidal magnetic field of approximately 25 μT (rms) for 90 days before they were mated with unexposed counterparts. Exposure to a 50 Hz field reduced male rat fertility. The number of pregnant females was reduced when mated with exposed males, and the number of resorptions increased. The effects of magnetic field on male fertility were shown to be partly reversible, when the same exposed group of males were remated 45 and 90 days after being removed from the fields. Exposure of adult female rats to 50 Hz magnetic fields for 90 days before mating significantly reduced their fertility. The mean numbers of implantations and living fetuses per litter were statistically significantly decreased in the 50 Hz group. These results suggest that low frequency magnetic fields have some adverse effects on fertility of male and female rats.

Comment: The descriptions of the exposure system, and the quality assurance protocols used to ensure its reliable and stable operation are adequately given. However, neither the animal handling nor exposure protocols are adequate. The rat exposure cages were overcrowded, 10 females being exposed for 90 days in a single cage 48 cm x 24 cm x 15 cm. Males rats were slightly less crowded at 5 per cage. At the end of 90 days, exposed males were immediately mated with virgin females (of unspecified age) which were then killed after 10 days for necropsy. This procedure was repeated 45 days and 90 days after cessation of their exposure. Not surprisingly there was a drop in the number of pregnant females in the immediately after, and 45 day after groups. The authors attribute this decrease to the magnetic field, but it could just as easily have been due to housing stress. It is not clear whether the control males, to which exposed males were compared, were also sham exposed. If the controls were sham exposed, then the argument for the observed affect being due to the field would be strengthened. The exposed females and control females (no indication whether the controls were also sham exposed) were then mated with males of known reproductive capacity, and no significant effect was observed. This was not equivalent to the protocol that was used to test the reproductive capacity of exposed males (that protocol used virgin females instead of females of known reproductive capacity). Despite an adequate exposure system, and an adequate statistical treatment of the small numbers of animals, the inadequacy of the animal handling protocols (or its description in the paper), compromises the author's conclusion that magnetic field exposure compromised the reproductive capacity of male rats.

Elbetieha A, AL-Akhras MA and Darmani H. Long-term exposure of male and female mice to 50 Hz magnetic field: effects on fertility. Bioelectromagnetics 2002;23(2):168-172.

The principal author is with the Department of Applied Biological Sciences, and Technology (JUST), Irbid, Jordan.

Abstract: The effect of an extremely low frequency (ELF) magnetic field on the fertility of adult male and female Swiss mice was investigated. Adult male and female mice were exposed to a 50 Hz sinusoidal magnetic field at approximately 25 μT (rms) for 90 days before they were mated with unexposed counterparts. There were no exposure related effects on the fertility of male or female mice. The number of implantation sites, viable fetuses, and the total number of resorptions were not significantly affected in females impregnated by males exposed to the 50 Hz magnetic field as compared with the control group. The number of implantation sites, viable fetuses and the total number of resorptions in exposed females were also not statistically different from the control group. There were no significant effects on the weights of the testes, seminal vesicles, preputial gland or body weights of males exposed to 50 Hz magnetic field. Furthermore, body and uterine weights were not affected in females exposed to 50 Hz field; however, ovarian weight was significantly increased in females exposed to the same field. These results suggest that exposure of male and female mice to low frequency magnetic field had no adverse effects on fertility and reproduction in mice.

Comment: The exposure system was described previously by Juutilainen (1987) and Al-Akhras (2002) and was acceptable, as described. Calibration and quality assurance protocols were acceptable. Staff were blind to the exposure status of mice. Two experiments were completed. In the first, male and female Swiss Webster mice were exposed for 90 days to ~ 25 μT (50 Hz) magnetic fields. At the end of exposure, the males were mated to unexposed females and the females mated with unexposed males and then 20 days later the females were killed and examined for pregnancy rate, and reproductive capacity (number of viable fetuses and resorptions). There was no difference between mice exposed to magnetic fields and those exposed to sham conditions in terms of pregnancy rate or in reproductive capacity. In the second experiment, male and female mice were exposed or sham exposed to magnetic fields for 90 days, and then their body and organ weights were assessed. There were no significant differences between magnetic field exposed mice and mice exposed to sham conditions in any of the parameters measured. This study does not support the hypothesis that magnetic fields can affect reproductive outcomes in mice.

Negishi T, Imai S, Itabashi M, Nishimura I and Sasano T. Studies of 50 Hz circularly polarized magnetic fields of up to 350 μT on reproduction and embryo-fetal development in rats: Exposure during organogenesis or during preimplantation. Bioelectromagnetics 2002;23(5):369-389.

The principal author is with the Bio-Science Department, Abiko Laboratory, Central Research Institute of Electric Power Industry (CRIEPI), Abiko, Chiba, Japan.

Abstract: Groups of mated female Sprague-Dawley rats were simultaneously exposed to 0 (sham exposed), 7, 70, or 350 μT (rms) circularly polarized 50 Hz magnetic fields (MF) for 22 h/day on gestational day 8-15, the period of rat fetal organogenesis (organogenesis study) or from day 0 to day 7 of gestation, the rat preimplantation period (preimplantation study). Developmental toxicity was assessed on gestational day 20. Identical experiments were repeated to confirm reproducibility of both studies. In both studies, statistically significant differences between exposed and sham exposed animals were observed in several measured parameters; however, these differences only appeared in one, but not both replicate experiments and generally at only an isolated exposure level. Because these differences were not reproducible and did not show a dose response relationship, they were not considered related to MF exposure. In the organogenesis study, lower kidney weights of dams were seen at 70 and 350 μT in Experiment 1. Lower dam liver weights and lower mean body weights of viable female and male fetuses were seen at 70 μT in Experiment 2. Otherwise, there were no differences in these parameters or in group means for fetal loss after implantation, number of viable fetuses, fetal body weight and sex ratio, incidences of external, visceral, and skeletal abnormalities or variations, or tissue abnormalities after histopathological examination. In the preimplantation study, dam health and indices for reproduction and embryo-fetal development, including pre or postimplantation loss, number and body weight of live fetuses, and sex ratio, external, skeletal abnormalities and variations, and skeletal ossification did not differ. Dam inorganic phosphorous concentration at 350 μT was elevated in one experiment and depressed in another. In one experiment, visceral abnormalities, primarily thymic remnant in neck and accessory liver lobe, were increased in the 7 μT group. Based on these results from two studies, we conclude that circularly polarized 50 Hz MF exposure of up to 350 μT during the fetal organogenesis or during the preimplantation period does not affect reproduction and embryo-fetal development in Sprague-Dawley rats.

Comment: In this two part study, the effect of magnetic fields was assessed on (i) organogenesis in fetal rats, and (ii) reproductive success (pre-implantation). The experiments were independently replicated twice. The study was large, about 35 rats per group. Rats were pre-screened for viral infections or the presence of pathogenic bacteria. The exposure system was adequate, as described, as were the calibration and quality assurance protocols. Temperature control was not a problem. Staff were blind to the exposure status of the rats. No significant differences were detected between rats exposed to magnetic fields and those exposed to sham conditions. The study does not support an association between exposure to magnetic fields and adverse pregnancy outcomes.

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Negishi T, Imai S, Itabashi M, Nishimura I and Sasano T. Studies of 50 Hz circularly polarized magnetic fields of up to 350 μT on reproduction and embryo-fetal development in rats: Exposure during organogenesis or during preimplantation. Bioelectromagnetics 2002;23(5):369-389.

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9. SEARCH FOR GENOTOXIC MODES OF ACTION

9.1 Analytical Framework for Laboratory Studies

Laboratory studies were assessed on the (i) ability of the experimental design to control all significant assignable causes of variation, (ii) precision of measured endpoints (i.e. the degree of mutual agreement among individual measurements), (iii) ruggedness of the design (i.e. had the ability to withstand small uncontrolled changes in operating conditions), and (iv) practicality of the design (i.e. did not require any exotic equipment, reagents, etc., which would make independent replication difficult). However, most papers examined did not contain sufficient information to determine in detail their strengths and shortcomings, factors which are essential to establish the credibility of the findings.

The analytical framework for the statistics, included an examination of:
(i) whether the use of parametric or non-parametric tests were justified,
(ii) whether the p-values were adjusted for multiple comparisons,
(iii) whether the positive or negative findings could have been due to faulty statistics, and
(iv) the potential biological relevance of statistically significant differences.

9.2 Objective of Experimental Studies

For this review, laboratory studies were evaluated on their capacity to contribute towards defining a ‘mode of action’ for magnetic fields. Magnetic fields, rather than electric fields, have been postulated as the cause of many observed biological effects, because at environmental levels, the electric current induced in the biological target by the magnetic field is too small to effect change. If studies repeatedly show magnetic fields can’t produce some critical ‘key event’ along the pathway leading to disease, then the argument for an alternative explanation (a so-called confounder effect) would be strengthened. Therefore, defining a mode of action for magnetic fields would affirm the direction of present research, whereas not finding one would stimulate interest in identifying possible confounders. The confounder, in this instance, would be strongly associated with both the magnetic field and the observed biological effect. Kavet (2000 and 2002) has proposed an electric current (the ground current) as an important exposure variable that has been ignored in past epidemiological studies. Johansen (1998) as well suggested that repeated electric shocks, rather than the magnetic field, could be a risk factor for amytrophic lateral sclerosis (ALS). One possible exposure variable that is now being considered is the concept of the ‘open circuit voltage’ or Voc (Kavet, 2000). Voc is a small power frequency voltage, up to a few tenths of a volt, that may appear on electrical appliances or equipment. This voltage can cause a contact current to flow directly into a person in manual contact with the appliance. There would be no equivalent to a ‘contact current’ in either *in vitro* or *in vivo* studies, since cells are cultured in insulated plastic dishes within a grounded incubator, and rodents are exposed in plastic cages. This could explain why so many *in vitro* and *in vivo* magnetic field

experiments have failed to find biological effects. So, the absence of effects in most laboratory experiments, could be due to the absence of an equivalent to the 'contact current'. When one critically examines the laboratory literature on magnetic fields, only a few of the studies that report positive biological effects, at environmentally relevant exposures, have ever been subjected to rigorous verification, and those that have usually can not withstand careful scrutiny. So defining a mode of action for magnetic fields could help clarify the paradoxical positions often taken by the experimentalists on one hand and some epidemiologists on the other.

As used in this review, a mode of action for magnetic fields would be defined by repeatedly showing its capacity to disrupt some regulatory pathway in a cell or organism. The 'key event' responsible for this dysfunction would contribute to, but would not necessarily be essential for, the development of disease. For example, the processes involved in carcinogenesis are reasonably well understood, and the key events that precede tumor formation have been generally established by years of fundamental research in cancer biology. Key events, as used here, will be broadly classified as being either genotoxic or epigenetic (nongenotoxic) (Trosko, 2000). In the context of the multistage model of carcinogensis (initiation, promotion and progression), a key event will be considered genotoxic if it causes initiation (the irreversibly alteration of a stem cell's ability to terminally differentiate) or enables malignant conversion (the immortalization of a precancerous cell). Examples of key genotoxic events are DNA or chromosome damages, which lead to cell death or mutations. Promotion, the presumed second stage of carcinogenesis, involves key epigenetic events that effect clonal expansion of an "initiated"cell. Defining a possible mode of action for magnetic fields would enable one to predict the existence of a response threshold, and to infer the shape of a dose-response relationship. For example, showing magnetic fields produce DNA or chromosomal damage (a genotoxic key event), at any level of exposure, in any recognized assay for genotoxicity, would support those epidemiological studies that suggest an association between magnetic fields and cancer. This support would be strengthened considerably if the results were independently replicated by a number of investigators and confirmed through a battery of related assays. A linear dose-response could be expected if magnetic fields were genotoxic. The likelihood of these effects extrapolating to environmental levels of exposure is another issue. Alternatively, if the mode of action involved an effect, for example, on the functionality of gap junctions, or the modulation of gene expression or, possibly, the inhibition of cellular repair or apoptosis, then one might expect a nonlinear dose-response relationship and an action threshold (Wiltse, 2000) (Trosko, 2000). In this context, laboratory studies can be important adjuncts to risk assessment in that they can strengthen or discredit the plausibility of epidemiological findings, or stimulate the search for alternative explanations.

This review also faced the problem of how to treat studies reporting effects for magnetic fields at low levels of exposure, where the possibility existed for spurious, inconsistent or biased results being misinterpreted as real effects (and vice versa). Early studies to define an action spectrum for ionizing radiation used doses large enough to produce measurable and verifiable effects. The effect sizes was then extrapolated to lower doses where their relevance to human health could be established. This review concentrated on those studies that used a high enough exposure to establish the existence or non-existence of a biological effect, regardless of how relevant the exposure was to environmental levels. Studies that used this approach were singled out for closer examination, especially if their results agreed with other studies. If these interrelated studies had experimental designs that were clearly defined, and without omission of important details, they were assigned full weight. If there were obvious design flaws, important omissions or vague descriptions, the studies were given no weight or relegated to an inconclusive category.

9.3 Key Events Related to Genotoxicity

For the purposes of this review, a treatment will be considered genotoxic if the result is a key event, such as chromosome damage, suppression of DNA repair, an increase in reactive oxygen species, or impairment of their detoxification. For carcinogenesis, a key event will be considered genotoxic if the outcome is mutagenesis. Such events are most often associated with initiation and progression in the multistage model of carcinogenesis. The formation of sister chromatid exchanges (SCE) will also be considered genotoxic, since their formation involves breakage and rejoining of DNA at homologous sites on the two chromatids of a single chromosome (Perry and Evans, 1975). The breakage and rejoining process may not always be error-free, and mutations may be the end result (Latt, 1981). For example, the rates of SCE formation and single gene mutations are directly related (Carrano and Thompson, 1982).

9.4 Key Events Related to Epigenetic Toxicity

A treatment will be considered epigenetic (or non-genotoxic) if the result is a change in gene expression at the level of transcription or translation (actions that turn genes “on” or “off”, or stabilize or destabilize the genetic message), or in post-translational modification of a gene product (Trosko, 2000). Epigenetic events can alter signal transduction, the functionality of gap junctions, apoptosis, cellular transport, etc., processes that are essential for the maintenance of such diverse activities as cell proliferation, differentiation, transformation, survival, homeostasis and adaptation. Epigenetic carcinogens, unlike genotoxic carcinogens, usually follow nonlinear dynamics, have an action threshold, require persistent exposure, and can be species or tissue specific. Non-genotoxicity will be considered in modes of action - part II.

This analytical framework also accommodates modes of action with both genotoxic and nongenotoxic components, as indicated by key events that increase the susceptibility of

DNA or nucleosomes to attack by genotoxins, or increase survival of stem cells with genotoxic injury, or stimulate damaged cells to proliferate. The use of key events to establish a mode of action for magnetic fields is not without uncertainty or controversy, and its use is conservative in this review to either strengthen or disparage the plausibility of the association that has been suggested by some of the epidemiological studies.

9.5 Search for Genotoxic Modes of Action

As used in this review, sensitivity describes the probability of an assay generating a positive response when a specified lesion or endpoint is present in a cell or organism. Specificity describes the probability of an assay producing a negative response in the absence of a specified lesion or endpoint. In the experimental design, these definitions are related to the possibilities of false negative or false positive outcomes and to the possibilities of committing Type I and Type II errors. In the design of laboratory experiments, these concepts are often ignored, possibly because some of the assays are so new that not enough data has been accumulated to fully characterize their sensitivities and specificities. Alternatively, assuming a dose-response relationship, the exposure may be too low to produce sufficient endpoint for the assay to reliably detect. Therefore, whether a study properly incorporates positive and negative controls into its design will be used as a surrogate for issues of sensitivity and specificity. In this instance, the proper use of a positive control would at least allow an investigator to determine what the postulated response would look like. As an example, in the paper by Singh and Lai (1998), the authors infer how DNA-protein cross-links should affect fluorescence migration patterns on their slides in response to electrophoresis, but then do not incorporate an appropriate positive control into the study to actually demonstrate the behavior of this lesion. In this review, such a deficiency in design will be considered equivalent to publishing the results of an assay whose sensitivity and response characteristics would be unknown.

Table 19. Genotoxicity summary

Author	Date	End Point	Indicates MOA?	Relevance to Risk Management	Weakness
Tateno	1998	ChA	No	None	SSS; ED(-PC)
Maes	2000	ChA SCE	No No	Inconclusive	MC
Nordenson	2001	ChA	No	None	MC; ED(-PC)
Skyberg	2001	ChA Rep.	Indirectly Yes	None None	SSS; ED(-PC);BR
Singh	1998	SB	No	None	ED(-PC)
Ivancsits	2002	SB	No	None	ED(-PC)
McNamee	2002	SB	NC	NC	NC
Simko	1998	MN	No	None	ED(-PC)
Simko	1998	MN	No	None	ED(-PC)
Svedenstal	1999	SB	No	None	ED(-PC)
Svedenstal	1999	SB	No	None	ED(-PC)
Simko	2001	MN	No	None	ED(-PC)
Abramsson	2001	MNRBC	No	None	LD; ED(-PC); & improper sham
Walleczek	1999	Mut	Yes	Yes	None Apparent
Nakasono	2000	Mut	Indirectly	Yes	None Apparent
Ansari	2000	Mut	Indirectly	Yes	None apparent
Ding	2000	Mut	Yes	Yes	ED*
Ding	2001	Mut	Yes	Yes	None Apparent
Ding	2001	Mut	Yes	Yes	None Apparent
Yaguchi	1999	SCE	Yes	Yes	None Apparent
Heredia-Rojas	2001	SCE	No	None	Numerous Design Flaws
Robison	2002	Apop. Rep.	No No	None None	Numerous Design Flaws

Abbreviations:

BR = Biological Relevance of differences between exposed and non-exposed
ChA = Chromosome Aberrations.
Ctd = Chromatid Aberrations.
ED(-PC) = Experimental Design shortcomings (including No Positive Control)
ED* = of interest but Experimental Design to complex to confirm or replicate
LD = (unrealistically) Low Dose
MN = Micronucleus
MNRBC = Micronuclei in Red Blood Cells.
MOA = Mode OF Action.
NC = No Comment (conflict of interest).
Rep = Repair
SB = (DNA) Strand Breaks
SCE = Sister Chromatid Exchange
SSS = Small Sample Size

9.6 Summary Findings

The studies were rigorously vetted, using criteria discussed in the Introductory Analytical Framework. The experimental design was the weakest part of many studies, in particular, the lack of a positive control [ED(-PC)] [Tateno (1998), Singh (1998), Abramsson (1999), Ivancsits (2002), Simko (1998a, 1998b, 2001), Heredia-Rojas (2001) and Robison (2002)], or the lack of an internal laboratory standard [Nordenson (2001) and Skyberg (2001)]. Some were deficient in sham exposures and blinding protocols [Robison (2002), Abramsson (2001) and Heredia-Rojas (2000)], and in culture conditions (Heredia-Rojas, 2000). Without a proper negative and positive controls, a study's negative result is meaningless at worst and inconclusive at best. One study used an unacceptably low dose that would almost certainly guarantee a negative result [Abramsson (1999)]. Many studies reported statistical differences as if they were also biologically significant or relevant. Despite inconclusive data, some studies 'reached' for answers or indulged in over-interpretation [Singh (1998), Simko (1998a, 1998b, 2001), Nordensen (2001), Heredia-Rojas (2001) and Robison (2002)].

In the chromosome studies, the guidelines for analyzing aberrations and micronuclei include quality assurance protocols, but only Nordenson provided independent verification of results. For many of the studies involving the comet assay, guidelines for data acquisition were subjective and operator-dependent, and procedures to ensure quality data were almost never used. For example, many studies based their results on the analysis of 50 cells selected at random from a slide, with atypical cells being excluded. Such procedures are, by definition, non-random, and the arbitrary exclusion of cells could substantially bias the final results. To overcome this deficiency, quality assurance protocols would have to be incorporated into the experimental design for it to be considered adequate, for example, by reporting intra-slide variability after acquisition of two or three sets of data from a substantial portion of the total number of slides being

analyzed, or by having another qualified analyst verify the data. The use of subjective analytical criteria, and operator-dependent collection routines, especially under conditions that may not be blinded, makes replication or confirmation of results difficult or impossible. Despite these shortcomings, it is probable that magnetic fields did not produce an increased in micronuclei at the levels of the exposures studied by Simko. However, without a positive control one can not be sure. Maes, with two exceptions, reported that magnetic fields produced no increase in either chromosome aberrations or sister chromatid exchanges, with the exceptions probably being spurious because of multiple comparisons. But, once again, without proper controls, one can not be sure. Many studies used exposures that were probably too low to produce an effect, and together with inadequate experimental designs, one can not be sure if results were real or spurious. These uncertainties are the reason for rejecting the findings of these studies, and fully discounting their results in weighting the evidence needed to establish a credible mode of action for magnetic fields. Providing solid evidence for a mode of action would help to confirm or reject the credibility of epidemiological studies that find an association between magnetic fields and adverse health effects.

The studies that were not rejected [Walleczek (1999), Yaguchi (1999), Nakasono (2000), Ansari (2000) and Ding (2001a & 2001b)] had fully acceptable experimental designs and exposure systems, and results that could not be discounted. The study by Ding (2000) on NF-kβ would require a ‘heroic’ effort to independently verify, so these findings are considered ‘interesting’, but unverifiable at this time.

Using the HPRT assay with Chinese hamster ovary cells, Walleczek found that a 12 hour exposure to 1 mT (60 Hz) magnetic field enhanced the mutation frequency of a 2 Gy dose of gamma rays. The HPRT assay detects mutations resulting from DNA base pair substitutions, large and small deletions, and DNA inversions (basically strand breaks). The exposures were large enough to produce a measurable effect (x1.8 increase over gamma ray alone) under well defined experimental conditions. The magnetic field was some 2500 times the level (0.4 μT) where some epidemiological studies have detect increases in the incidence of adverse health effects (the gamma ray dose is ~600 x the background level). The Walleczek study confirms one by Miyoshi (1999, see abstract in Appendix - the original article was in Japanese) which used an exposure of 5 mT (60 Hz) and a similar CHO cell line.

Nakasono found that a 14 mT (50 Hz) magnetic field was neither mutagenic nor co-mutagenic in a battery of bacterial tester strains that detect point and frameshift mutations (but not mutations produced by strand breaks) from chemicals that were DNA reactive mutagens, promutagens, base analogs, and reactive oxygen generators. This implies magnetic fields do not produce point or frameshift mutations in mammalian cells. There was also no effect when S9 mix was used to activate procarcinogens, which implies mammalian cytochrome P450 dependent electron transport was unaffected by strong magnetic fields.

The exposure system used by Yaguchi and collaborators (Ding, Miyakoshi et al. are described in the paper by Yaguchi (2000) and by Miyakoshi (1994), see Appendix for abstract). Yaguchi (2000) exposed mouse cells to 400 mT (50 Hz) magnetic fields in order to produce an increase in SCE, relative to sham exposed controls, but exposure to 50 mT or 5 mT magnetic fields produced no such increase. This is consistent with a previous study by Antonopoulos (1995) that found no increase in SCE at 5 mT or less. The rate of SCE formation in cells co-exposed to MMC and 400 mT magnetic were not significantly different from cells exposed to MMC alone. Yaguchi's results indicate that at high flux densities, magnetic fields cause chromatid breaks, most likely through an induced current mechanism. The magnetic fields did not induce cytotoxicity or alter the proliferative index of these cells. This exposure was ~10^6 times higher than epidemiologically relevant exposures (0.4 μT). Lower levels of exposure (5 and 50 mT) that had no effect are respectively ~1.25×10^4, and ~1.25×10^5 times environmentally relevant exposures.

Ansari (2000) used a magnetic field exposure of 100 μT (60 Hz) and found it non-cytotoxic and non-mutagenic by itself, or when applied to cells treated in combination with ionizing radiation or MNNG. This indicates at low flux densities, relative to the studies of Yaguchi, Ding, and Walleczek, magnetic fields do not increase the rate of mutations, possibly because (i) a true action threshold exists, (ii) the effects were below the assay's limit of detection, or more likely, because (iii) the current induced in the target by the magnetic field was at, or below, the level of electrical background noise in the cell. The studies by Ansari (2000) and Nakasono (2000) provide indirect support (by showing the absence of an effect at lower levels of exposure) for an effective action threshold, regardless of whether its shape is linear or non-linear. Further, they speculate the effective threshold probably the result of induced currents becoming progressively weaker as the flux density of the magnetic field approaches environmentally relevant levels of exposure, until the induced current is finally lost in the background electrical noise of the cell. This scenario suggests adverse effects of magnetic fields on cells would be unlikely at environmental levels. While, it is not possible for this limited number of studies to reach a consensus, they do point in the general direction of DNA damage as one possible mode of action for magnetic fields, and speculating further, the induced current, rather than the magnetic field per se, could be the agent responsible for observed effects. In order to properly define a possible mode of action, a comprehensive review of all past experiments would be required.

Of the 3 studies by Ding et al., one study (Ding, 2000) was too complex for easy verification, and its findings remain interesting, pending further study. However, the other two (Ding, 2001a & 2001b) used adequate exposure systems and experimental designs to obtain their results. In the first study (Ding, 2001a), an exposure chamber was specifically constructed to deliver an electric current to cell culture medium. Ding found an increase in mutation rate at the HPRT gene locus in CHO cells. These findings complement those of (Miyakoshi, 1996) that found a 400 mT (50 Hz) magnetic fields increased HPRT detectable mutants in a transformed cell line. In the second, Ding

(2001b), studied the effect of magnetic fields on MCF-7, a breast cancer derived cell line. Alone, the magnetic field, 5 mT (60 Hz) did not affect cell growth or the progression of cells through the cell cycle. The magnetic field also failed to induce p21, BAX or Bcl-2 expression, confirming the results of Loberg (2000). However, when cells pretreated with X-rays were subsequently exposed for 24 h to the magnetic field, X-ray-induced apoptosis was transiently decreased, relative to cells pretreated with X-rays and sham exposed. This decrease in X-ray-induced apoptosis was due to a magnetic field-dependent inhibition of BAX expression and an increased expression of Bcl-2. These results confirm a previous study by Sakakura (1998).

Recently, a paper by Wan (Wan, 2000 - see Genotoxicity Appendix for abstract) describes the pico-second transfer of electrons between the stacked bases within supramolecular assemblies of DNA. Bixon (1999) also defines a mechanism for long-range electron transfer in DNA. These processes have relevance for DNA damage or its repair, since magnetic fields, or the induced current, might modulate or interfere with the intra- or inter-strand electron transfers that occur during chemical reaction involving DNA base pairs.

9.7 Individual Study Summaries

9.7.1 Chromosome and Chromatid Aberrations (*In Vitro* and *In Vivo*)

The studies grouped under this heading involve a wide range of exposure scenarios, including humans exposed to workplace magnetic fields, human blood cells, spermatozoa, and cell lines exposed in vitro. However, they all have a common perspective, to accumulate a body of knowledge on the genotoxic potential of magnetic fields, regardless of whether the perspective is directed towards cancer (DNA damage, chromosome aberrations and mutations in somatic cells) or reproductive health (DNA damage, chromosome aberrations, and mutations in germ cells).

Tateno H, Iijima S, Nakanishi Y, Kamiguchi Y and Asaka A. No induction of chromosome aberrations in human spermatozoa exposed to extremely low frequency electromagnetic fields. Mutat Res 1998;414(1-3):31-35.

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Abstract: Clastogenic effects of extremely low frequency electromagnetic fields (ELF-EMFs) on human sperm chromosomes were studied using an interspecific in vitro fertilization system with zona-free golden hamster oocytes. Semen samples from healthy men were exposed to ELF-EMFs (50 Hz, 20 mT) for 2 h at 37 degrees C under 5% CO2 in air. The samples were then cryopreserved in liquid nitrogen for shipment to a cytogenetic laboratory. After thawing the samples, motile spermatozoa were collected using a continuous Percoll density gradient centrifugation and then capacitated for in

vitro fertilization with hamster oocytes. Sperm-derived chromosomes were analyzed at first cleavage metaphase. The present experiment was performed twice using semen samples from two different donors. In test-1, incidence of spermatozoa that displayed structural chromosome aberrations was 17.0% (35/206) in the exposed group and 20.8% (55/264) in the control group. In test-2, structural chromosome aberrations were observed in 11.1% (13/117) of exposed spermatozoa and 13.8% (13/94) of spermatozoa in the control group. In both tests, there was no significant difference in the incidence of chromosomally abnormal spermatozoa between the exposed group and the control group. Types of aberrations observed and their incidences per spermatozoon in the exposed group were similar to those of the control group. Despite the small sample size, the present results suggest that ELF-EMFs have no clastogenic effect on human sperm chromosomes.

Comment: This is a small study (2 human sperm donors) involving the use of an experimental interspecies *in vitro* fertilization system (human spermatozoa and hamster oocytes). A preliminary study showed that cryopreservation of samples did not alter the chromosome aberration rate in spermatozoa, nor did freezing alter the rate of chromosome aberrations induced by exposure to 1.9 Gy of X-rays, nor did these treatments alter the spermatozoa's survivability, motility, or ability to fertilize hamster ova. The study samples were processed for cryopreservation, divided into two portions, which were then either exposed to 20 mT (50 Hz) magnetic fields or sham exposed before being frozen. When convenient, sperm samples were thawed, activated with ionophore, and then fused with golden hamster oocytes. At times specified by a published protocol, the ova were collected and fixed for chromosome analysis. The χ^2 test was used to determine if the exposure produced a significant increase in the percentage of spermatozoa with structural chromosome aberrations. The frequency of chromosome aberrations per spermatozoa for all samples was compared using a 2-tailed t-test. Neither the frequency of chromosome aberrations per spermatozoa, nor the percentage of aberrant spermatozoa, differed significantly (p ,0.05) from sham exposed controls. Weakness in experimental design included, the lack of a positive control to document assay performance, no quality assurance procedures to verify scoring, scoring was done in a non-blinded fashion, the sham exposure protocol, and the sample size (two individuals) and the number of cell scored (~100/ sample) were inadequate.

Maes A, Collier M, Vandoninck S, Scarpa P and Verschaeve L. Cytogenetic effects of 50 Hz magnetic fields of different magnetic flux densities. Bioelectromagnetics 2000;21(8):589-596.

The principal author is with VITO, Environmental Toxicology, Mol, Belgium.

Abstract: Cytogenetic investigations were performed in human peripheral blood lymphocytes following exposure to 50 Hz magnetic fields alone or in combination with the chemical mutagen mitomycin C or with X-rays. It was found that magnetic fields up to 2500 µT did not significantly influence the chromosome aberration and

sister chromatid exchange frequency. Also, the combined treatments failed to indicate the presence of any synergistic, potentiating, or antagonistic effect between the ELF magnetic fields and the mutagens. However, there were two exceptions: cells exposed to 504 μT magnetic fields before and during cultivation displayed a statistically significant decrease in sister chromatid exchange frequency. Also, when cells were cultivated in the presence of 88.4 μT magnetic fields following X-ray exposures there was a significant increase in chromosome aberration frequency compared to X-ray exposure alone.

Comment: The construction and operation of the exposure chamber was described in detail and temperature control during exposure was not a concern. The chamber was calibrated and subject to monitoring during operation. Blood was collected using accepted techniques. Some samples were exposed to various levels of magnetic fields alone, before or after exposure to X-rays, and some were exposed continuously to Mitomycin.C (MMC) alone or to various combinations of MMC and magnetic fields. The objective of the combination treatments were to determine if any synergy existed between the agents. Samples were treated in triplicates, and processed for chromosomes aberrations (magnetic fields and/or X-rays) or for sister chromatid exchanges (magnetic fields and/or MMC), using accepted techniques. Samples from unexposed and exposed individuals were processed in parallel. Slides were scored blind and a large number of comparisons were made using a non-parametric test (Mann-Whitney “U” test). All laboratory controls responded as expected for chromosome-type aberrations and sister chromatid exchanges (SCE). There were no observed differences between sham and magnetic field exposed samples, and no synergy was observed between magnetic fields and X-rays, or magnetic fields and MMC. Two exceptions were noted, cells exposed to 504 μT magnetic fields had a statistically significant decrease in SCE frequency. Also, there was a significant increase in chromosome aberrations, compared to X-ray exposure alone, when cells were first exposed to X-rays and then cultivated in the presence of 88.4 μT magnetic fields. There is no plausible explanation why these two anomalous results should be statistically significant, except that a large number of comparisons were made during the study, and some spurious results could be expected.

Nordenson I, Mild KH, Jarventaus H, Hirvonen A, Sandstrom M, Wilen J, Blix N and Norppa H. Chromosomal aberrations in peripheral lymphocytes of train engine drivers. Bioelectromagnetics 2001;22(5):306-315.

The principal author is with the National Institute for Working Life, Umea, Sweden.

Abstract: Studies of Swedish railway employees have indicated that railroad engine drivers have an increased cancer morbidity and incidence of chronic lymphatic leukemia. The drivers are exposed to relatively high magnetic fields (MF), ranging from a few to over a hundred microtesla. Although the possible genotoxic potential of MF is unclear, some earlier studies have indicated that occupational exposure to MF

may increase chromosome aberrations in blood lymphocytes. Since an increased level of chromosomal aberrations has been suggested to predict elevated cancer risk, we performed a cytogenetic analysis on cultured (48 h) peripheral lymphocytes of Swedish train engine drivers. A pilot study of 18 engine drivers indicated a significant difference in the frequency of cells with chromosomal aberrations (gaps included or excluded) in comparison with seven concurrent referents (train dispatchers) and a control group of 16 office workers. The engine drivers had about four times higher frequency of cells with chromosome-type aberrations (excluding gaps) than the office workers ($P < 0.01$) and the dispatchers ($P < 0.05$). Seventy-eight percent of the engine drivers showed at least one cell per 100 with chromosome-type aberrations compared with 29% among the dispatchers and 31% among the office workers. In a follow-up study, another 30 engine drivers showed an increase ($P < 0.05$) in the frequency of cells with chromosome-type aberrations (gaps excluded) as compared with 30 referent policemen. Sixty percent of the engine drivers had one or more cells (per 100 cells) with chromosome-type aberrations compared with 30% among the policemen. In conclusion, the results of the two studies support the hypothesis that exposure to MF at mean intensities of 2-15 μT can induce chromosomal damage.

Comment: This paper describes separately a small pilot study and its follow-up, and then their combined results. The studies examined the effect of occupational magnetic fields on the rate of chromatid- and chromosome-type aberrations in the peripheral lymphocytes of engine drivers and a referent population. The magnetic field exposure of engine drivers was characterized by continuous 24 h measurements, and for referents, by spot measurements. The exposures for engine drivers were highly variable (averaging about 5 to 6 μT), with maximums ranging from 10 to 100 μT. The exposure to the referent population was on average about 0.2 μT. Both populations were subjected to the same erratic work schedules, including night shifts. The protocols used to collect and process blood samples were comparable for both cases and referents. Assay performance, however was not verified by use of a positive laboratory control. Slides were prepared using accepted cytogenetic techniques, and were assessed for aberrations in the follow-up study by two independent cytogenetic laboratories. Inter-laboratory comparisons indicated results could be pooled. Data was analyzed by non-parametric tests, without correction for multiple comparisons. The findings were inconclusive in that various combinations of chromosome-type aberrations and chromatid-type aberrations were mixed and matched for comparison. Based on chromosome-type aberrations, a difference that was “nearly” statistically significant was found. The authors then combined the pilot and the follow-up studies, using less than convincing arguments for pooling, and then found results that were statistically significant. However, the biological relevance of these differences is seriously open to question, as the effect of smoking was not considered, possibly because of the small study size. This study is highly contrived and reaches to find a statistical difference.

Skyberg K, Hansteen IL and Vistnes AI. Chromosomal aberrations in lymphocytes of employees in transformer and generator production exposed to electromagnetic fields and mineral oil. Bioelectromagnetics 2001;22(3):150-160.

The principal author is with the Department of Occupational Medicine, National Institute of Occupational Health, Oslo, Norway.

Abstract: The objective was to study the risk of cytogenetic damage among high voltage laboratory workers exposed to electromagnetic fields and mineral oil. This is a cross sectional study of 24 exposed and 24 matched controls in a Norwegian transformer factory. The exposure group included employees in the high voltage laboratory and in the generator soldering department. Electric and magnetic fields and oil mist and vapor were measured. Blood samples were analyzed for chromosomal aberrations in cultured lymphocytes. In addition to conventional cultures, the lymphocytes were also treated with hydroxyurea and caffeine. This procedure inhibits DNA synthesis and repair *in vitro*, revealing *in vivo* genotoxic lesions that are repaired during conventional culturing. In conventional cultures, the exposure group and the controls showed similar values for all cytogenetic parameters. In the DNA synthesis- and repair-inhibited cultures, generator welders showed no differences compared to controls. Among high voltage laboratory testers, compared to the controls, the median number of chromatid breaks was doubled (5 vs. 2.5 per 50 cells; $P<0.05$) the median number of chromosome breaks was 2 vs. 0.5 ($P>0.05$) and the median number of aberrant cells was 5 vs. 3.5 ($P<0.05$). Further analysis of the inhibited culture data from this and a previous study indicated that years of exposure and smoking increase the risk of aberrations. We conclude that there was no increase in cytogenetic damage among exposed workers compared to controls in the conventional lymphocyte assay. In inhibited cultures, however, there were indications that electromagnetic fields in combination with mineral oil exposure may produce chromosomal aberrations.

Comment: This was a cross-sectional study involving “testers” in two high voltage testing laboratories and welders in a generator production facility. The jobs in the testing laboratories involved exposures to high levels of power-frequency magnetic fields, and to high levels and uncommon pulses of electric and magnetic fields. The “generator” welders were exposed to high flux densities of high frequency (kHz) electric and magnetic fields and welding fumes. Both groups were exposed to frequent “releases” of a mineral oil mist into the workplace atmosphere. There was no separate study group that was exposed only to mineral oil vapors. The methods for determining exposures to power-frequency fields, kHz fields and mineral oil vapor were documented in detail. Controls were employees who neither worked in the testing or production facilities, and were matched to cases for sex, age and smoking status. The “tester” and welder groups each consisted of 12 exposed and 12 matched controls. Blood samples from an exposed individual and matched control were obtained and cultured at the same time under identical conditions. In addition to standard chromosome aberration assays, some of the samples were treated in the last 3 hours of cultivation (in addition to colcemid)

with caffeine and hydroxyurea to inhibit regular cell cycle DNA synthesis (cell-cycle progression) and repair (unscheduled DNA) synthesis. This treatment amplifies the number of detected chromosome and chromatid breaks, and increases the sensitivity of the assay to detect damage that otherwise would be masked in the conventional assay. About 200 cells were examined for each participating individual in the conventional chromosome aberration assay, and 50 cells per individual in the inhibited assay. The differences in exposure characteristics should have prevented data from the two exposed groups (testers and welders) from being pooled, but for completeness, the authors presented pooled data as well as data for the separate groups. When the two exposed groups were compared to the control, the differences in aberration frequencies were non-significant, regardless of whether the exposed groups were pooled or considered separately. Smoking status did not seem to make a difference. However, a significant difference was detected between the “testers” and matched controls when the repair inhibited cultures were examined. Smoking status appeared to be a cofactor. The health implications of the DNA strand breaks detected in the inhibited cultures is unknown. The biological relevance of the observed differences in the mean aberration rates per 50 cells examined is also unknown (5 aberrant cells in testers vs 3.5 in controls, 2 chromosome breaks in testers vs 0.5 in controls, and 5 chromatid breaks in testers vs 2.5 in controls), since this type of damage is rapidly repaired. The small sample size, 50 cells per individual in the inhibited assay, could have also been a factor. From standard tables, to detect a difference when the baseline aberration frequency is between 0.07 and 0.01, the sample size has to be between 200 and 435 cells (not 50 cells).

9.7.2 DNA Strand Break Damage

Singh N and Lai H. 60 Hz magnetic field exposure induces DNA crosslinks in rat brain cells. Mutat Res 1998;400(1-2):313-320.

The principal author is with the Bioelectromagnetics Research Laboratory, Department of Bioengineering, University of Washington, Seattle, WA, USA.

Abstract: In previous research, we found an increase in DNA strand breaks in brain cells of rats acutely exposed to a 60 Hz magnetic field (for 2 h at an intensity of 0.5 mT). DNA strand breaks were measured with a microgel electrophoresis assay using the length of DNA migration as an index. In the present experiment, we found that most of the magnetic field-induced increase in DNA migration was observed only after proteinase-K treatment, suggesting that the field caused DNA-protein crosslinks. In addition, when brain cells from control rats were exposed to X-rays, an increase in DNA migration was observed, the extent of which was independent of proteinase-K treatment. However, the X-ray-induced increase in DNA migration was retarded in cells from animals exposed to magnetic fields even after proteinase-K treatment, suggesting that DNA-DNA crosslinks were also induced by the magnetic field. The effects of magnetic fields were also compared with those of a known DNA crosslink-inducing agent

mitomycin C. The pattern of effects is similar between the two agents. These data suggest that both DNA-protein and DNA-DNA crosslinks are formed in brain cells of rats after acute exposure to a 60 Hz magnetic field.

Comment: The object of this study was to determine if exposure to power-frequency fields caused DNA-DNA and DNA-protein cross-links in the brain cells of rats. Rats were exposed, or sham exposed, to magnetic fields (0.5 mT, 60 Hz) for two hours and then killed after 4 hours. Their brains were isolated, crude single cell suspensions were prepared, mixed with agarose, and then cast, as thin films, onto a frosted glass slides. The first hypothesis was that if magnetic fields induced DNA-protein cross-links, then DNA migration would be evident on slides treated with proteinase-K (to remove proteins from the DNA) before electrophoresis, relative to cells with no proteinase-K treatment (DNA-protein cross-links on untreated slides would inhibit DNA migration). The second hypothesis was that if magnetic fields produced DNA-DNA cross-links, DNA migration on slides exposed to X-rays before electrophoresis would be inhibited, regardless of proteinase-K treatment (in the absence of magnetic field-induced DNA-DNA cross-links, X-ray damage would cause DNA to migrate further than the DNA on unexposed slides). The exposure system, consisting of Helmholtz coils, was adequately described and calibrated, and its performance during operation was capable of being monitored. Heat dissipation was not a problem. Exposure and sham exposures were carried out in a blinded manner, as were the preparation of cell suspensions and slides, and slide analysis. Mitomycin.C, a known DNA-DNA cross-linker, was used as positive control. The authors interpret the observed migration patterns of DNA as inferring the exposure to PFF produced both DNA-protein and DNA-DNA cross-links. A major weakness of the study is the subjective, operator-dependent nature of the analytical procedure, which makes replication difficult. The authors claim cells were analyzed at random, but rejected analyzing atypical cells. In fact, the selection process is subjective, which could result in selection bias, and, possibly, a systematic error of unknown direction and magnitude. In addition, the presence of DNA-DNA and DNA-protein lesions are inferred from the behavior of DNA migration patterns (rather than by chemical analysis), and over-interpretation is a possibility. The assay uses a positive control for DNA-DNA lesions but none for DNA-protein cross-links. To accept the study's conclusions as credible, a positive control for DNA-protein cross-links would be needed, or the nature of the lesions would have to be verified by qualitative chemical analysis.

Svedenstal BM, Johanson KJ, Mattsson MO and Paulsson LE. DNA damage, cell kinetics and ODC activities studied in CBA mice exposed to electromagnetic fields generated by transmission lines. In Vivo 1999(a);13(6):507-513.

The principal author is with the Department of Radioecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Abstract: CBA mice were exposed outdoors to 50 Hz electromagnetic fields (EMF), with a flux density of about 8 μT rms (root mean square), generated by a 220 kV

transmission line. Assays were performed in order to investigate the possible genotoxic effects after 11, 20 and 32 days of exposure, as well as the effects on body weight, leukocytes, erythrocytes, and the level of ornithine decarboxylase (ODC) activity in spleen and testis. DNA migration was studied on brain cells by single cell electrophoresis (comet assay). After 32 days of exposure a highly significant change of the tail/head ratio of the comets was observed ($p < 0.001$), showing DNA-damage. Further, a decreased number of mononuclear leukocytes ($0.02 < p < 0.05$) was observed in mice EMF-exposed for 20 days. In summary, our data indicate that transmission lines of this type may induce genotoxic effects in mice, seen as changes in the DNA migration. These results might have an important implication for health effects.

Comment: In this study young male CBA mice were exposed outdoors to magnetic fields of ~ 8 μT generated by a 220 kV (50 Hz) overhead transmission line. DNA damage, cell kinetics and ornithine decarboxylase activity (ODC) effects were assessed after 11, 20 and 32 days of exposure. Mice (74) were randomly assigned to exposure and sham categories. The neutral comet assay was used to assess DNA damage. No significant differences in DNA migration patterns were observed in cells isolated from brains of mice after 11 or 20 days of exposure. However, after 32 days, an increase in the tail/head ratio was observed in exposed mice relative to sham (130 comets were analyzed in total for exposed and sham). There was no indication from the author that the analyst was blind to the exposure status of the samples, no information was provided on the rates of false negatives or false positives associated with Kontron Imaging System, and a positive control was not used to establish the sensitivity of the entire analytical process. These shortcomings could have biased the results. It is possible that agents, other than magnetic fields, in the open field environment may have been responsible for the observed DNA damage at ~ 8 μT. There were no differences between exposed and controls in terms of ODC activity. Changes in leukocyte counts were inconsistent, with a difference being observed at 20 days, but not at 11 or 32 days. When results were pooled for the ratio of polychromatic to normochromatic erythrocytes, exposed mice were significantly lower than in sham (no justification for pooling was given).

Svedenstal BM, Johanson KJ and Mild KH. DNA damage induced in brain cells of CBA mice exposed to magnetic fields. In Vivo 1999(b);13(6):551-552.

The principal author is with the Department of Radioecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Abstract: DNA migration, using single cell gel electrophoresis (comet assay), was studied on brain cells of CBA mice exposed continuously to 50 Hz, 0.5 mT magnetic fields (MF) for 2 hrs, 5 days or 14 days. No differences were observed in the groups MF-exposed for 2 hrs and 5 days compared with controls. However, in the group

exposed to MF for 14 days, a significantly extended cell DNA migration was observed ($0.02 < p < 0.05$). These changes together with results from previous studies indicate that magnetic fields may have genotoxic effects in brain cells.

Comment: This study was an attempt to confirm in the laboratory, results previously reported for mice exposed under a high voltage overhead transmission line. In the previous study, DNA damage was detected, using the neutral comet assay, in the brain cells of mice after 32 d of continuous exposure under a high voltage transmission line. In the present study, CBA male mice were exposed to a 0.5 mT (50Hz) magnetic field for 2 h, 5 d and 14 d before brain cells were assessed for DNA damage. The exposure chamber has been described in a previous publication and was acceptable in all respects (Rannug, 1993). No significant differences were found between exposed and sham samples after 2 h and 5 d of exposure. However, at 14 days, there was a significant difference in the tail/head ratios of exposed samples relative to sham. There was no indication from the author that the analyst was blind to the exposure status of the samples, no information was provided on the rates of false negatives or false positives associated with Kontron Imaging System, and a positive control was not used to establish the sensitivity of the entire analytical process. These shortcomings could have biased the results.

Ivancsits S, Diem E, Pilger A, Rudiger H and Jahn O. Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts. Mutat Res 2002;519(1-2):1.

The principal author is with the Division of Occupational Medicine, University Hospital/AKH, Waehringer Guertel 18-20, A-1090, Vienna, Austria.

Abstract: Results of epidemiological research show low association of electromagnetic field (EMF) with increased risk of cancerous diseases and missing dose-effect relations. An important component in assessing potential cancer risk is knowledge concerning any genotoxic effects of extremely-low-frequency-EMF (ELF-EMF). Human diploid fibroblasts were exposed to continuous or intermittent ELF-EMF (50Hz, sinusoidal, 24 h, 1000 &mgr; T). For evaluation of genotoxic effects in form of DNA single- (SSB) and double-strand breaks (DSB), the alkaline and the neutral comet assay were used. In contrast to continuous ELF-EMF exposure, the application of intermittent fields reproducibly resulted in a significant increase of DNA strand break levels, mainly DSBs, as compared to non-exposed controls. The conditions of intermittence showed an impact on the induction of DNA strand breaks, producing the highest levels at 5 min field-on / 10 min field-off. We also found individual differences in response to ELF-EMF as well as an evident exposure-response relationship between magnetic flux density and DNA migration in the comet assay. Our data strongly indicate a genotoxic potential of intermittent EMF. This points to the need of further studies in vivo and consideration about environmental threshold values for ELF exposure.

Comment: This study examined the effect of intermittent, or continuous, exposure to power-frequency fields on DNA of human fibroblasts. The exposure system and cell culture incubator setup were adequately described. Exposures were carried out blind, and field and incubator performances could be monitored continuously throughout the treatments. Temperature control within the incubator was not an issue. The results show intermittent exposure to PFF caused a reproducible increase in DNA damage in cultured human diploid fibroblasts. The study is externally consistent in that cells exposed continuously to PFF did not show any DNA damage. The authors speculate that intermittent exposures could affect DNA repair differently than continuous exposures, with a continuous one allowing adaptation. The main weakness is the use of a subjective, operator-dependent analytical system, which means misclassification of the observed damage could be a source of error. However, it is not possible to judge the size of this possible error, since the analysis was done by a single individual. In addition, the study used two fibroblast cell lines established in the author's laboratory, instead of commercially available cell lines or lymphocytes from peripheral blood, which would have been readily available to those trying to replicate or confirm the study results. Taken together, these two factors makes independent replication difficult. Also, there were no positive controls, such bleomycin, to show what double strand breaks would look like, or low LET radiation, to show what single strand breaks would look like under their assay conditions. A minor shortcoming is the use of multiple comparisons, which would lead to some spurious results. The use of parametric tests with ordinal data is also objectionable (parametric tests are usually applied to interval scale measurements).

McNamee JP, Bellier PV, McLean JR, Marro L, Gajda GB, Thansandote A. DNA damage and apoptosis in the immature mouse cerebellum after acute exposure to a 1 mT, 60 Hz magnetic field. Mutat Res 2002;513(1-2):121-133.

The principal author is with the Consumer and Clinical Radiation Protection Bureau, Product Safety Programme, Health Canada, Ottawa, Ontario K1A 1C1, Canada.

Abstract: Several recent studies have reported that whole-body exposure of rodents to power frequency magnetic fields (MFs) can result in DNA single- and double-strand breaks in the brains of these animals. The current study was undertaken to investigate whether an acute 2 h exposure of a 1 mT, 60 Hz MF could elicit DNA damage, and subsequently apoptosis, in the brains of immature (10-day-old) mice. DNA damage was quantitated at 0, 2, 4, and 24 h after exposure using the alkaline comet assay. Apoptosis was quantitated in the external granule cell layer (EGCL) of the immature mouse cerebellum at 0 and 24 h after exposure to MF by the TdT-mediated dUTP nick-end labeling (TUNEL) assay. Four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. While increased DNA damage was detected by tail ratio at 2 h after MF exposure, no supporting evidence of increased DNA damage was detected by the other parameters. In addition, no similar differences were observed using these parameters at any of the other post-exposure

times. No increase in apoptosis was observed in the EGCL of MF-exposed mice, when compared to sham mice. Taken together, these results do not support the hypothesis that acute MF exposure causes DNA damage in the cerebellums of immature mice.

Comment: This is a confirmation-type study to determine whether evidence could be found to support the contention that exposure to magnetic fields could cause DNA strand break damage in the brains of rodents. The exposure system and assay procedures were adequately described and a positive control was used to demonstrate the sensitivity of the assay. Further comment can not be made because of an obvious conflict of interest.

9.7.3 Micronucleus Formation

Simko M, Kriehuber R, Weiss DG and Luben RA. Effects of 50 Hz EMF exposure on micronucleus formation and apoptosis in transformed and nontransformed human cell lines. Bioelectromagnetics 1998(a);19(2):85-91.

The principal author is with the Institute of Animal Physiology, Unit of Environmental Physiology, University of Rostock, Germany.

Abstract: Effects of applying extremely low-frequency electromagnetic fields (ELF-EMF) for different durations (24, 48, and 72 h) and different field intensities (0.1-1.0 mT) on micronucleus (MN) formation and induction of apoptosis were examined in a human squamous cell carcinoma cell line (SCL II) and in a human amniotic fluid cell line (AFC). A statistically significant increase of MN frequency and of
induction of apoptosis in SCL II cells after 48 h and 72 h continuous exposure to 50 Hz magnetic field (MF) (0.8 and 1.0 mT) was found. However, exposure of AFC cells to EMF of different intensities and for different exposure times showed no statistically significant differences when compared with controls. These results demonstrate that different human cell types respond differently to EMF. Dose-dependent induction of apoptosis and genotoxic effects, resulting in increased micronucleus formation, could be demonstrated in the transformed cell line, whereas the nontransformed cell line did not show statistically significant effects. These findings suggest that EMF could be a promotor but not an initiator of carcinogenic effects.

Comment: The object was to determine if magnetic fields were acting as a co-carcinogen or promoter in vitro, using a transformed and a non-transformed cell line. Presumably, if the magnetic field acted as a co-carcinogen or promoter, it would have likely effected the transformed cell line rather than the non-transformed one. Also, agents, such as phorbol myristate acetate (PMA), benzoyl peroxide and X-rays, have been shown to produce chromosome damage in vitro, mainly as single strand breaks, hence the rationale for micronuclei as the endpoint. However, there was no positive control (such as PMA, benzoyl peroxide, or X-rays), and therefore no way of knowing what a real response would look like. Under these circumstances, there is no way of

determining if the statistical differences observed were real or artifactual and, more importantly, if the differences were biologically relevant. The results of this study are therefore inconclusive.

Simko M, Kriehuber R, Lange S. Micronucleus formation in human amnion cells after exposure to 50 Hz MF applied horizontally and vertically. Mutat Res 1998(b);418(2-3):101-111.

The principal author is with the Institute of Animal Physiology, Division of Environmental Physiology, University of Rostock, Universitatsplatz 2, D-18055, Rostock, Germany.

Abstract: Micronucleus (MN) induction as a genotoxic effect of extremely-low-frequency electromagnetic fields (ELF-EMF, 50 Hz, 1 mT) was studied in human amniotic fluid cells (AFC) after continuous exposure to magnetic fields (MF), oriented horizontally and vertically with respect to the surface of the culture medium,
at different time points. To compare the effectiveness of different exposure systems, a Helmholtz-coil system and a so-called Merritt-coil system was used. A statistically significant increase in MN frequency could be detected in exposed cells compared to controls after 72 h continuous exposure to MF applied vertically in the Merritt-coil system, while no effect was found after exposure in the Helmholtz-coil system. Furthermore, a significant increase in MN induction occurred after 24, 48 and 72 h exposure to MF applied horizontally in the Helmholtz-coil system in comparison to controls, whereas horizontally MF generated in the Merritt-coil system induced no genotoxic effects. To exclude suppression of indirect EMF-induced DNA-lesions, we studied MN formation in the presence of N-Acetyl-p-aminophenol (APAP, Paracetamol®)), which is an inhibitor of DNA-repair mechanisms. We found a dose-dependent increase of MN formation in APAP-treated AFC cells, but no significant further increase in MN frequency after additional MF exposure. Therefore we conclude, that EMF-induced MN formation is not caused by directly or indirectly induced clastogenic mechanisms. The obtained results show that the orientation of MF with respect to the cell culture dish and the physical condition of the exposure system is of major importance for the induction of micronuclei in certain cell types. Therefore, the reason for inconsistent results published in the literature may be caused by the variability of exposure systems, the exposure conditions and the cell types used.

Comment: The two exposure systems were adequately described, as were the procedures for exposing and processing the cells. However, everything aside, this study had no positive control, and therefore no way of being able to determine if the observed changes, supposedly due to the magnetic fields, were credible. This is particularly true for the repair inhibition portion of the study. X-rays should have been used as a positive control so that a positive response, or inhibition of repair, could have been easily recognized. In this way, the reader could judge whether the observed changes in the frequency of micronuclei, attributed to the magnetic field exposure, were artifactual or real, or more importantly, biologically relevant.

Simko M, Richard D, Kriehuber R and Weiss DG. Micronucleus induction in Syrian hamster embryo cells following exposure to 50 Hz magnetic fields, benzo(a)pyrene, and TPA in vitro. Mutat Res 2001;495(1-2):43-50.

The principal author is with the Division of Environmental Physiology, Institute of Cell Biology and Biosystems Technology, University of Rostock, Universitatsplatz 2, D-18051 Rostock, Germany.

Abstract: Electromagnetic fields (EMFs) have been associated with increased incidence of cancer suggested by epidemiological studies. To test the carcinogenic potency of EMF, the in vitro micronucleus assay with SHE cells has been used as a screening method for genotoxicity. A 50 Hz magnetic field (MF) of 1 mT field strength was applied either alone or with the tumour initiator benzo(a)pyrene (BP) or the tumour promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). All three treatments were applied in single, double or triple treatment regimes. MF or TPA (1 nM) alone did not affect the number of micronuclei (MN) in initiated and non-initiated SHE cells. Changing the schedule of the typical initiation protocol, namely applying the initiator (BP) during exposure to MF, results in an 1.8-fold increased MN formation compared to BP treatment alone. Combined experiment with BP, TPA and MF did not cause further MN formation. Since initiation during MF exposure caused a significant increased MN formation, our findings suggest that MFs enhance the initiation process of BP. We think that this MF-enhanced co-carcinogenic effect is caused by an indirect "cell activation" process. The resulting genomic instability is proposed to be due to
free radicals and/or to the unscheduled "switching-on" of signal transduction pathways.

Comment: Syrian hamster embryo cell cultures were established from 13 day old embryos, which is an experimental system to detect cancer initiation or co-carcinogenic (co-initiation) activity. The exposure system was adequately described, as were the calibration and quality assurance protocols used to ensure reliable performance. The experimental design involved multiple treatments and a 3 day working protocol for all experiments. The micronucleus assay was performed without cytochalasin B. Benzoyl peroxide (BP) was used at a sub-threshold concentration (10^{-7}M) which had no effect on the frequency of micronuclei or on the rate of mitosis. Only MF for 72 hours and BP for the last 24 hours (48 to 72 hours) of incubation produced a statistically significant (x1.6-fold) increase in micronuclei. The authors conclude that MF acts as a co-carcinogen and enhances the action of the BP. However, it is not clear if a sub-threshold dose of BP would act as an initiator. It is also not clear whether a 1.6-fold increase in the frequency of micronuclei (from 22/1000 cells to 35/1000 cells) is biologically significant at these low frequencies, or is artifactual, or a spurious result, because of the large number of comparisons. The use of higher doses of BP, as a positive control, or a more efficient initiator may have increased the numbers so that readers would know what initiation and it’s enhancement, would look like in this system.

Abramsson-Zetterberg L and Grawe J. Extended exposure of adult and fetal mice to 50 Hz magnetic field does not increase the incidence of micronuclei in erythrocytes. Bioelectromagnetics 2001;22(5):351-357.

The principal author is with the Department of Environmental Toxicology, Uppsala University, Uppsala, Sweden.

Abstract: The flow cytometer-based micronucleus assay was used to study the effects on chromosomes in erythroid cells of CBA/Ca mice after extended exposure to 50 Hz magnetic field (MF), 14 μT, peak-to-peak (p-p). The study included two different experiments: (a) mice exposed in utero during 18 days of their prenatal stage, and (b) adult mice exposed for 18 days. In experiment (a) 35 days after exposure was terminated, peripheral blood was drawn from the mice exposed in utero to determine whether the exposure had a genotoxic effect on the pluripotent erythroid stem cells. About 200000 polychromatic erythrocytes (PCE) and 200000 normochromatic erythrocytes (NCE) were analysed from each of 20 exposed mice. The EMF exposure did not significantly change the frequency of micronucleated PCE or NCE in comparison with 20 sham-irradiated mice. There was no difference in the proportion of PCE between exposed and unexposed animals. Similarly, in experiment (b) no differences were seen between EMF exposed and unexposed adult mice when samples of peripheral blood were taken at the end of exposure and analyzed for micronuclei in PCE and NCE. The proportion of PCE was the same in both groups. The results indicate that exposure to EMF does not induce direct or indirect effects on chromosomes in erythroid cells expressed as increased levels of micronucleated erythrocytes of mice. No indications of delayed genetic effects were found.

Comment: The flow cytometric version of the erythrocyte micronucleus (MN) assay is capable of detecting, with acceptable sensitivity, MN in circulating red blood cells of mice chronically exposed to very low doses of ionizing radiation (Zetterberg, 1993). The MN are formed in erythrocyte precursors in bone marrow, and then released, together with their MN, into circulating blood, as part of the process of red blood cell renewal. The rationale was to apply the sensitive flow cytometry-based assay for quantifying erythroid micronuclei to study delayed effects in young mice and gender differences in adult mice. In the study on delayed effects, mice were exposed *in utero* to magnetic fields, and then examined for micronuclei 35 days after birth. Other studies using ionizing radiation found that the effects on chromosomes immediately after exposure were different from the so-called delayed effects which occurred after the passage of several cell cycles. However, ionizing radiation was not used as a positive control in the study of delayed effects, even though its involvement in the production of these effects has been well documented. Without a positive control, the negative result for delayed effects is inconclusive at best. In the other study, adult male and female mice were exposed to magnetic fields for 18 days to determine if any gender differences existed in incidence of erythroid micronucei, and ionizing radiation was used as a positive control. However, the positive control was an acute exposure, whereas for the gender study

mice were chronically exposed to the magnetic field. The sham exposure protocol was also not acceptable.

The description of the exposure system was acceptable. The procedure for collecting and processing blood was well documented and 400,000 cells were examined per mouse. Ionizing radiation was used as a positive control for direct damage, but not in the experiment involving the study of delayed or late events. Two experiments were conducted. In the first, pregnant mice were exposed for 18 days to 14 μT (50 Hz) magnetic fields, and then the offspring that had been exposed in utero were sacrificed 35 days after termination of exposure and their blood examined for MN, with the objective of looking for delayed effects of the magnetic field exposure. In a second experiment, adult mice (unmated female and male mice), were exposed for 18 days to the magnetic field and then sacrificed to determine if there were any gender differences in the frequency of MN formation. There was no statistical differences in the frequency of MN between any of the exposed and sham exposed groups. Weakness in this study are (i) an inadequate sham exposure, (ii) lack of description of how the analysts were blinded to sample status, and (iii) inadequate level of exposure (14 μT) which would have ensured a negative result.

9.7.4 Mutations

Walleczek J, Shiu EC and Hahn GM. Increase in radiation-induced HPRT gene mutation frequency after nonthermal exposure to nonionizing 60 Hz electromagnetic fields. Radiat Res 1999;151(4):489-497.

The principal author is with the Department of Radiation Oncology, Stanford University Medical School, California 94305-5403, USA.

Abstract: It is widely accepted that moderate levels of nonionizing electric or magnetic fields, for example 50/60 Hz magnetic fields of about 1 mT, are not mutagenic. However, it is not known whether such fields can enhance the action of known mutagens. To explore this question, a stringent experimental protocol, which included blinding and systematic negative controls, was implemented, minimizing the possibility of observer bias or experimental artifacts. As a model system, we chose to measure mutation frequencies induced by 2 Gy gamma rays in the redox-sensitive hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene in Chinese hamster ovary cells. We tested whether a 12 h exposure to a 60 Hz sinusoidally oscillating magnetic-flux density (Brms = 0.7 mT) could affect the mutagenic effects of ionizing radiation on the HPRT gene locus. We determined that the magnetic-field exposure induced an approximate 1.8-fold increase in HPRT mutation frequency. Additional experiments at Brms = 0.23 and 0.47 mT revealed that the effect was reduced at lower flux densities. The field exposure did not enhance radiation-induced cytotoxicity or mutation frequencies in cells not exposed to ionizing radiation. These results suggest that moderate-strength,

oscillating magnetic fields may act as an enhancer of mutagenesis in mammalian cells.

Comment: This study examined the ability of magnetic fields to enhance the mutagenic potential of ionizing radiation (gamma rays). The exposure system was acceptable, and the well known HPRT assay protocol for Chinese hamster ovary cells was described in detail. The study design was excellent. The HPRT assay is capable of detecting either DNA base pairs substitutions, large and small deletions, and DNA inversions. The strengths and weakness of the HPRT assay have been thoroughly documented in the open literature and it is considered to be a reliable, but somewhat insensitive, method for measuring mutation frequencies in mammalian cells. The results indicate that the mutation frequency of the gamma rays was increased x 1.8-fold when co-exposed to magnetic fields. While the differences were statistically significant, the biological relevance of such an increase, especially in an *in vitro* system, is unknown. These results were confirmed by a similar study, published in Japanese (Miyoshi, 1999).

Ansari RM and Hei TK. Effects of 60 Hz extremely low frequency magnetic fields (EMF) on radiation- and chemical-induced mutagenesis in mammalian cells. Carcinogenesis 2000;21(6):1221-1226.

The principal author is with the Center for Radiological Research, College of Physicians and Surgeons of Columbia University, VC 11-218, 630 West 168th Street, New York, NY 10032, USA.

Abstract: There is considerable uncertainty of the potential biological effects of extremely low frequency magnetic fields (ELF-MF or EMF) because of mixed results in epidemiological and laboratory studies. In the present study, exponentially growing human-hamster hybrid A(L) cells were treated with a 100 μT alternating EMF powered at 60 Hz for either 24 h or 7 days. Exposure to EMF was conducted either alone or in combination with graded doses of a physical or chemical carcinogen. gamma-radiation was chosen as a form of ionizing radiation while N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was chosen as a form of chemical contaminant. Exposure of A(L) cells to EMF alone for a period up to 7 days was non-cytotoxic and non-mutagenic. Concurrent EMF treatment did not increase either the cytotoxicity or induction of CD59- mutants by graded doses of gamma-rays or MNNG in A(L) cells. This study shows conclusively that short-term or long-term exposure to EMF alone neither affects the survival of A(L) cells nor increases the mutagenic potency of other environmental carcinogens.

Comment: In this study, the short- and long-term mutational effects of a 100 μT (60 Hz) magnetic field were examined in a human-hamster hybrid (A_L) cell line, alone and in combination with a mutagenic chemical or γ-irradiation. This assay for mutagenesis detects mutations at the gene and chromosomal levels, unlike the HPRT assay which detects mutations only at the chromosomal level. The human-hamster hybrid contains a

full set of Chinese hamster ovary genes, and a single copy of human chromosome 11, containing the CD59 gene, which encodes the CD59 cell surface antigen. When the cell surface marker is lost through a mutation in the gene on chromosome 11, specific antisera and complement cause cells with the surface marker to lyse, leaving the mutated cells to grow and form colonies. The magnetic field and sham exposure systems were carried out in the MC-2XC concentric coil system, a standardized system approved for use by the US National Institutes of Environmental Health Sciences. Gamma rays were provided by a Cesium-137 source, and irradiations were carried out at 1.5 and 3.0 Gy. After irradiation, cells were exposed to sham or magnetic fields continuously for 24 h or 7 days. Some cells were also treated with graded doses of a mutagenic chemical (MNNG) before exposure to sham or magnetic fields. Data was collected by counting colonies of the surviving mutants. Statistical analysis was adequate. Twelve experiments were carried out in total. There was no difference in mutation rates between sham and magnetic field exposed cells, or between cells co-exposed to graded doses of gamma rays and magnetic fields vs gamma rays alone, or between cells co-exposed to graded doses of MNNG and magnetic fields vs MNNG alone. The results for the positive controls were as expected.

Nakasono S, Ikehata M, Koana T and Saiki H. A 50 Hz, 14 mT magnetic field is not mutagenic or co-mutagenic in bacterial mutation assays. Mutat Res 2000;471(1-2):127-134.

The principal author is with the Bio-Science Department, Abiko Research Laboratory, Central Research Institute of Electric Power Industry, 1646 Abiko, Abiko-city, Chiba 270-1194, Japan.

Abstract: We used bacterial mutation assays to assess the mutagenic and co-mutagenic effects of power frequency magnetic fields (MF). For the former, we exposed four strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537) and two strains of Escherichia coli (WP2 uvrA, WP2 uvrA/pKM101) to 50 Hz, 14 mT circularly polarized MF for 48 h. All results were negative. For the latter, we treated S. typhimurium (TA98, TA100) and E. coli (WP2 uvrA, WP2 uvrA/pKM101) cells with eight model mutagens (N-ethyl-N'-nitro-N-nitrosoguanidine, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, 4-nitroquinoline-N-oxide, 2-aminoanthracene, N(4)-aminocytidine, t-butyl hydroperoxide, cumen hydroperoxide, and acridine orange) with and without the MF. The MF induced no significant, reproducible enhancement of mutagenicity. We also investigated the effect of MF on mutagenicity and co-mutagenicity of fluorescent light (ca. 900lx for 30 min) with and without acridine orange on the most sensitive tester strain, E. coli WP2 uvrA/pKM101. Again, we observed no significant difference between the mutation rates induced with and without MF. Thus, a 50 Hz, 14 mT circularly polarized MF had no detectable mutagenic or co-mutagenic potential in bacterial tester strains under our experimental conditions. Nevertheless, some evidence supporting a mutagenic effect for power frequency MFs does exist; we discuss the

potential mechanisms of such an effect in light of the present study and studies done by others.

Comment: The exposure system used in this study was adequately described and incorporated proper sham exposed controls and adequate temperature control. These bacterial tester strains detect point and frame shift mutagens. All exposures were to 14 mT (50 Hz) circularly polarized magnetic fields for 48 hours. The bacterial assays for detecting mutagens are well known and have been fully characterized and standardized over the years. This study involved the use of these standardized techniques to test the effect of magnetic fields alone, and in combination with a number of well known chemical mutagens and carcinogens, on the mutation frequencies of a battery of bacterial tester strains. The magnetic field alone did not increase mutation frequencies in any of the bacteria strains tested, nor did the combination of compounds and magnetic fields have any affect. This included mutagens that exert their action through free radical generating or photodynamic mechanisms.

Ding GR, Yaguchi H, Yoshida M and Miyakoshi J. Increase in X-ray-induced mutations by exposure to magnetic field (60 Hz, 5 mT in NF-kappaB-inhibited cells. Biochem Biophys Res Commun 2000;276(1):238-243.

The principal author is with the Department of Radiation Medicine, Fourth Military Medical University, Xi'an, 710032, China.

Abstract: It is established that extremely low frequency magnetic fields (ELFMF) at the flux densities, i.e., 5 mT and less, are not mutagenic. However, exposure to ELFMF enhances mutations induced by X-rays. In this study, we examined the effects of long-term exposure to 5 mT ELFMF on mutation induction and X-ray-induced mutations in human malignant glioma cells (MO54) with different mutant IkappaB-alpha (a critical inhibitor of NF-kappaB) genes. Cells were exposed or sham-exposed to 5 mT ELFMF for up to 8 days with or without initial X-rays (4 Gy), and the mutant frequency of hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene was analyzed. An obvious increase in X-ray-induced mutations was observed after treatment with ELFMF in combination with X-irradiation in MO54 cells with tyrosine mutant IkappaB-alpha gene other than with serine mutant IkappaB-alpha gene or vector alone. Exposure to ELFMF alone increased mutations significantly in MO54 cells with tyrosine mutant IkappaB-alpha gene. In addition, X-ray-induced apoptoic cells were increased in MO54-V cells after exposure to ELFMF, while an anti-apoptotic effect of magnetic field was found in MO54-SY4 cells. Our data suggest that exposure to 5 mT ELFMF may induce mutations and enhance X-ray-induced mutations, resulting from the inactivation of NF-kappaB through the inhibition of tyrosine phosphorylation.

Comment: This study is an extension of an earlier study (Miyoshi, 1999, published in Japanese, for abstract see Appendix - Genotoxicity) that found prolonged exposure to 5 mT (60 Hz) magnetic fields (1 - 6 weeks) significantly increased the rate of X-ray-

induced 6-thioguanine mutations in the HPRT assay. Walleczek (1999) reported the same effect from a 12 h exposure at 1 mT (60 Hz), in the HPRT CHO assay system. The present study focused on a transcription factor NF-kβ, which mediates the expression of genes. NF-kβ is sequestered in the cytoplasm as an inactive form complexed with the inhibitory factor Ikβ-α. NF-kβ, which is activated (i.e. released from the inhibitory complex) when a tyrosine or serine residue on the Ikβ-α, is phosphorylated in response to X-ray-induced DNA damage. The released NF-kβ translocates to the nucleus where it modulate genes involved in apoptosis and cell proliferation. To take advantage of this fact, MO54 cells (a malignant glioma) were transfected with an expression vector containing a gene encoding a form of Ikβ-α that could not be phosphorylated (and therefore would not allow NF-kβ to be released from the inactive NF-kβ-Ikβ-α complex). The HPRT assay was used with the MO54 parent cell and the MO54 cell containing the mutant Ikβ-α gene to monitor changes in mutation frequency. Prolonged exposure to magnetic fields alone at 5 mT (60 Hz) did not enhance mutation rates. However, magnetic fields decreased X-ray-induced mutations significantly in MO54 cells without the tyrosine/serine mutant Ikβ-α gene, but increased mutation frequency in cells that had the mutant gene. The differences in mutation rates were due to differences in the rate of apoptosis (the process for removing potentially mutated cells from an organism), with the rate being increased in the cells without mutated Ikβ-α genes, and decreased in cells with mutated Ikβ-α genes. Exposure to 5 mT (60 Hz) magnetic fields also appeared to enhance the spontaneous mutation rate. An obvious weakness of the study is the difficulty of independently replicating it, since it involved specialized expertise, and substantial resources and facilities to make several mutant cell constructs, and to verify their identity. The study is of interest because it explores the molecular mechanism of an established effect of magnetic/electric fields, albeit at high levels of exposure. The exposure system was acceptable, sham exposures were done properly, positive and negative controls were used throughout, the statistics were acceptable. Abstracts of three related papers, one originally published in Japanese (Miyakoshi, 2000) and two in English [(Miyakoshi, 1996) and (Yamagishi, 1997)] can be found in the Appendix - Genotoxicity.

Ding GR, Wake K, Taki M and Miyakoshi J. Increase in hypoxanthine-guanine phosphoribosyl transferase gene mutations by exposure to electric field. Life Sci 2001(a);68(9):1041-1046.

The principal author is with the Department of Radiation Medicine, the Fourth Military Medical University, Xi'an, China.

Abstract: Previously, we reported that exposure to extremely low frequency magnetic field (400 mT) increased in hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene mutations. However, it is unclear these mutations were induced by magnetic field (MF), electric field (EF), or both. To explore this question, a new exposure apparatus for EF was manufactured. We observed an increase in HPRT gene mutations in Chinese hamster ovary (CHO) cells after exposure to EF (10 V/m, 60 Hz) for 10 h. The mutant

frequency by EF-exposure was an approximate 2-fold of that by sham-exposure. Our data suggest that the mutations induced by exposure of cells to the variable magnetic field at 400 mT may be, in part, due to the induced EF.

Comment: This study indicates exposure to electric current can increase the mutation rate in CHO cells as measured by the HPRT assay. The results suggests DNA damage such as deletions may be the lesion responsible for the observed effect, but differential response via apoptosis can not be ruled out. The exposure system, sham exposure protocols, the use of positive and negative controls, and HPRT assay procedure appeared adequate (see Miyakoshi, 1996 in abstract for additional information). A two-fold statistically significant increase in the mean number of mutants was observed in exposed cultures. The biological relevance of this increase was not discussed. A similar observation was made by Walleczek (1999).

Ding GR, Nakahara T, Tian FR, Guo Y and Miyakoshi J. Transient suppression of X-ray-induced apoptosis by exposure to power frequency magnetic fields in MCF-7 cells. Biochem Biophys Res Commun 2001(b);286(5):953-957.

The principal author is with the Department of Radiation Medicine, Fourth Military Medical University, Xi'an, 710032, China.

Abstract: Epidemiological studies suggest that exposure to power frequency magnetic fields may be a risk factor for breast cancer in humans. To study the relationship between exposure to 60 Hz magnetic fields (MFs) and breast cancer, cell cycle distribution, apoptosis, and the expression of related proteins (p21, Bax, and Bcl-2) were determined in MCF-7 cells following exposure to magnetic fields (60 Hz, 5 mT) alone or in combination with X rays. It was found that exposure of MCF-7 cells to 60 Hz MFs for 4, 8, and 24 h had no effect on cell cycle distribution. Furthermore, 60 Hz MFs failed to affect cell growth arrest and p21 expression induced by X rays (4 Gy). Similarly, 60 Hz MFs did not induce apoptosis or the expression of Bax and Bcl-2, two proteins related to apoptosis. However, exposure of cells to 60 Hz MFs for 24 h after irradiation by X rays (12 Gy) significantly decreased apoptosis and Bax expression but increased Bcl-2 expression. The effects of exposure to 60 Hz MFs on X-ray-induced apoptosis and Bax and Bcl-2 expressions were not observed at 72 h. These data suggest that exposure to 60 Hz MFs has no effects on the growth of MCF-7 cells, but it might transiently suppress X-ray-induced apoptosis through increasing the Bcl-2/Bax
ratio.

Comment: This study examined the effect of magnetic fields on cell cycle progression, apoptosis, a protein expression in MCF-7 cells, a breast cancer derived cell line. Cell cycle progression and apoptosis are two important phenomena in the response of mammalian cells to DNA damage. Cell cycle arrest allows an extended time for repair, and cells that fail to repair can be removed by apoptosis. The exposure system used in these experiments has been adequately described in another paper (Miyakoshi, 1996 -

see Appendix for abstract). Well documented flow cytometry-based assays were used to examine cell cycle progression and apoptosis. The results show exposure of MCF7 cells to a 5 mT (60 Hz) magnetic field did not affect cell growth for up to 72 h of exposure, and the distributions of cells among G1, S , M and G2 phases were not different from that of sham-exposed controls. The magnetic field also failed to induce p21, Bax and Bcl-2 expression, similar to the results reported by Loberg (2000). When cells were pretreated with X-rays, prolonged exposure to magnetic fields did not affect X-ray-induced G1 or G2 phase arrest or p21 expression. However, the magnetic field transiently decreased X-ray-induced apoptosis after 24 h, but not 72 h after exposure. The Bcl-2 is a suppressor of apoptosis that forms heterodimers with the homologous protein BAX, a promoter of cell death. The over-expression of the Bcl-2 protein can block apoptosis and prolong cell survival, and thereby increase the likelihood of mutagenesis. In this study, magnetic fields inhibited BAX expression induced by the X-rays. The increase in the ratio of Bcl-2 to BAX, relative to cells exposed to X-rays alone, would transiently suppress apoptosis. This confirms a previous study by Sakakura (1998).

9.7.5 Cellular Repair and Sister Chromatid Exchanges

Yaguchi H, Yoshida M, Ejima Y and Miyakoshi J. Effect of high-density extremely low frequency magnetic field on sister chromatid exchanges in mouse m5S cells. Mutat Res 1999;440(2):189-194.

The principal author is with the Graduate School of Human and Environmental Studies, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan.

Abstract: The induction of sister chromatid exchanges (SCEs) was evaluated in the cultured mouse m5S cells after exposure to extremely low frequency magnetic field (ELFMF; 5, 50 and 400 mT). Exposure to 5 mT and 50 mT ELFMF led to a very small increase in the frequency of SCEs, but no significant difference was observed between exposed and unexposed control cells. The cells exposed to 400 mT ELFMF exhibited a significant elevation of the SCE frequencies. There was no significant difference between data from treatments with mitomycin-C (MMC) alone and from combined treatments of MMC plus ELFMF (400 mT) at any MMC concentrations from 4 to 40 nM. These results suggest that exposure to highest-density ELFMF of 400 mT may induce DNA damage, resulting in an elevation of the SCE frequencies. We suppose that there may be a threshold for the elevation of the SCE frequencies, that is at least over the magnetic density of 50 mT.

Comment: This study examined whether SCE were induced in cultured mouse cells by exposure to high flux density magnetic fields, and whether these fields amplified the action of mitomycin C, another inducer of SCE. The exposure system was adequately described and calibrated, with adequate quality assurance protocols. Temperature control was not an issue. Cell culture conditions were adequate, but the rodent cell line

is not widely available, making replication or confirmation studies difficult. Differences between exposed and sham were significant at only the 400 mT level but not at 5 and 50 mT, which indicates either an action, or an effective, threshold. There was no synergy between MMC and magnetic fields, suggesting the possibility that these agents produce SCE by different mechanisms. The results of this study are relevant to environmental issues, because it demonstrates that exposures to high flux density magnetic fields are capable of producing biological effects (which is analogous to early research on the biological effects of ionizing radiation), and it indicates the existence of some type of action threshold that is many times higher than levels found in the workplace or in homes.

Heredia-Rojas JA, Rodriguez-De La Fuente AO, del Roble Velazco-Campos M, Leal-Garza CH, Rodriguez-Flores LE and de La Fuente-Cortez B. Cytological effects of 60 Hz magnetic fields on human lymphocytes in vitro: sister-chromatid exchanges, cell kinetics and mitotic rate. Bioelectromagnetics 2001;22(3):145-149

The principal author is with the Subdireccion de Postgrado, Facultad de Ciencias Biologicas, Universidad Autonoma de Nuevo Leon, Mexico.

Abstract: Incubation for 72 h of human peripheral blood cultures in the presence of 60 Hz sinusoidal magnetic fields (MF) at magnetic flux densities of 1.0, 1.5, and 2.0 mT led to stimulation of lymphocyte proliferation but had no influence on the frequency of sister-chromatid exchanges (SCE). The cytotoxic potential of MF combined with the mutagen Mitomycin-C also was analyzed. An opposite effect between MF exposure and Mitomycin-C treatment in terms of cell kinetics and mitotic rate was found, whereas no variation in SCE frequency was observed for this coexposure condition.

Comment: Human blood was collected, and harvested for sister chromatid exchange analysis according to standard methods, as was the procedure for staining SCE. However, culture conditions consisted of a dry incubator and it is unclear how the lack of moisture (and the possibility of osmotic shock) would have affected results, nor is it clear how pH was maintained in the cultures. The set-up for sham exposures was only marginally acceptable. The slides were examined blind to exposure status, and 50 cells were scored per sample. It is also not clear how the blind status was maintained, given the obvious difference between the placement of exposed and control cultures in the incubator. Statistical analysis was only marginally acceptable, relying on non-parametric tests to examine the relationship between exposure and the proliferation and mitotic indices (PI and MI, respectively). Mitomycin C was used as a positive control for SCE. No statistically significant differences were found when the mean SCE frequency for exposed cells were compared with control cells, using acceptable ANOVA. The response to the positive control was acceptable. However, both the PI and MI were statistically different from controls. While these observed differences could have been due to the magnetic field, it is also possible that the small number of cells that were examined, the use of an unacceptable sham exposure, and the unacceptable incubation

conditions, combined to give a false positive signal. PI and MI were only analyzed at 72 h, and given the possibility of differences in temperature, pH, and growth rates between exposed and sham, it is possible that the control cultures reached their PI and MI peaks before the exposed cultures, which would also have resulted in a positive signal. The study design did not include a positive control for either PI or MI. In addition, the biological relevance of the observed differences for PI and MI are not known. Therefore, to be considered as a valid experimental design, both PI and MI should have been measured at early time points as well as at 72 hours.

Robison JG, Pendleton AR, Monson KO, Murray BK and O'Neill KL. Decreased DNA repair rates and protection from heat induced apoptosis mediated by electromagnetic field exposure. Bioelectromagnetics 2002;23(2):106-112.

The principal author is with the Department of Microbiology, Brigham Young University, Provo, Utah 84602, USA.

Abstract: In this study, we demonstrate that electromagnetic field (EMF) exposure results in protection from heat induced apoptosis in human cancer cell lines in a time dependent manner. Apoptosis protection was determined by growing HL-60, HL-60R, and Raji cell lines in a 0.15 mT 60 Hz sinusoidal EMF for time periods between 4 and 24 h. After induction of apoptosis, cells were analyzed by the neutral comet assay to determine the percentage of apoptotic cells. To discover the duration of this protection, cells were grown in the EMF for 24 h and then removed for 24 to 48 h before heat shock and neutral comet assays were performed. Our results demonstrate that EMF exposure offers significant protection from apoptosis (P<.0001 for HL-60 and HL-60R, P<.005 for Raji) after 12 h of exposure and that protection can last up to 48 h after removal from the EMF. In this study, we further demonstrate the effect of the EMF on DNA repair rates. DNA repair data were gathered by exposing the same cell lines to the EMF for 24 h before damaging the exposed cells and non-exposed cells with H2O2. Cells were allowed to repair for time periods between 0 and 15 min before analysis using the alkaline comet assay. Results showed that EMF exposure significantly decreased DNA repair rates in HL-60 and HL-60R cell lines (P<.001 and P<.01, respectively), but not in the Raji cell line. Importantly, our apoptosis results show that a minimal time exposure to an EMF is needed before observed effects. This may explain previous studies showing no change in apoptosis susceptibility and repair rates when treatments and EMF exposure were administered concurrently. More research is necessary, however, before data from this in vitro study can be applied to in vivo systems.

Comment: This is a complicated study involving multiple comparisons and multiple exposures. The study purports to show, that a 12 hour (or more) exposure of HL60 cells to magnetic fields before heat shock (followed by a further 13 h incubation before analysis for apoptosis by the neutral comet assay), inhibits apoptosis. Furthermore, this protection lasted up to 48 hours after termination of the magnetic field exposure. It is not clear from the article if all the necessary controls were incorporated into the

experimental design, for completeness two negative controls would be required, MF and no heat shock (to go with MF and heat shock), and no MF and no heat shock (to go with no MF and heat shock). DNA repair was also studied. A 24 h exposure to magnetic fields prior exposure of cells to hydrogen peroxide caused DNA repair rates to decrease in the immediate post treatment period, as measured by the alkaline comet assay. The presentation of repair as a line graph, rather than as a full table of results, makes it difficult to verify the conclusions. The use of transformed data, without justification, and a statistical package also prevent verification by alternative statistical tests. The exposure system was not acceptable, given the placement of the exposure apparatus (a Helmholtz coil) in one incubator and the sham culture in another incubator (not in an non-energized Helmholtz coil). This means that cultures were not processed blind nor is it possible to determine if the culture flasks were subjected to a temperature differential. A positive control was not used in the assay for apoptosis (neutral comet assay) and slides were apparently not scored blind, with the analysis being operator-dependent and subjective. The statistical analysis was done on transformed data using a statistical package that did not allow for easy verification. The study has both internal and external inconsistencies, meaning that it should be regarded as inconclusive.

Appendix - Genotoxicity

Miyakoshi J, Ohtsu S, Tatsumi-Miyajima J and Takebe H. A newly designed experimental system for exposure of mammalian cells to extremely low frequency magnetic fields. J Radiat Res (Tokyo) 1994;35(1):26-34.

The principal author is with the Department of Experimental Radiology, Faculty of Medicine, Kyoto University, Japan.

Abstract: To examine the biological effects of extremely low frequency magnetic field (ELFMF), we have designed and manufactured a new equipment for long-term and high-density exposure of cells to ELFMF. The ELFMF exposure system consists of a generator of magnets with a built-in CO_2 incubator, an alternating current (AC) power supply, a gas compressor and a thermocontroller for the incubator, and a cooling unit for the magnets. The CO_2 incubator made of acrylic resin is inserted into the inner-space of the silicon steel strip-cores. In this system, the temperature of the incubator is maintained at 37 ± 0.5 degrees C. The maximum magnetic flux density on the exposure area of the incubator is 500 mT (T; tesla) at a current of 556 A rms (rms; root mean square) at 50 Hz. The long-term (up to 120 hr) exposure of 400 mT ELFMF did not affect the growth of both HL60RG and CCRF-CEM cells originated from human leukemia. The post-X-irradiation exposure of 400 mT ELFMF for 2 h also did not affect the radiation sensitivity of GM0637 and TAT2SF cells originated from a normal human and an ataxia telangiectasia patient.

Miyakoshi J, Ohtsu S, Shibata T and Takebe H. Exposure to magnetic field (5 mT at 60 Hz) does not affect cell growth and c-myc gene expression. J Radiat Res (Tokyo) 1996;37(3):185-191.

The principal author is with the Department of Radiation Genetics, Faculty of Medicine, Kyoto University, Japan.

Abstract: We designed and manufactured equipment for long-term and low-density (0 to 9 mT) exposures of cultured cells to extremely low frequency magnetic fields (ELF-MF), and examined the effects of ELF-MF on cell growth and c-myc mRNA expression in Chinese hamster ovary (CHO) cells. The ELF-MF equipment consists of a CO_2 incubator with a built-in magnet generator using Helmholtz coils being 250 mm in inner diameter, 160 mm in distance and 128 turns, a slide regulator and a thermocontroller. No significant difference in the growth rate and the c-myc expression of CHO cells was observed with 5 mT ELF-MF exposure, sham-exposure and incubation in a conventional incubator.

Miyakoshi J, Yamagishi N, Ohtsu S, Mohri K and Takebe H. Increase in hypoxanthine-guanine phosphoribosyl transferase gene mutations by exposure to high-density 50-Hz magnetic fields. Mutat Res 1996;349(1):109-114.

The principal author is with the Department of Radiation Genetics, Faculty of Medicine, Kyoto University, Japan.

Abstract: Exposure to extremely low frequency magnetic field (ELFMF) of 50 Hz and 400 mT induced mutations in the hypoxanthine-guanine phosphoribosyl transferase gene of human melanoma MeWo cells. The mutant frequency was enhanced both by increasing the exposure period and the induced current intensity. Mutations induced by X-rays were enhanced by ELFMF exposure. No significant increase in mutant frequency occurred when DNA replication was inhibited during ELFMF exposure. DNA replication error is suspected of causing the mutations produced by ELFMF exposure.

Miyakoshi J and Yagi K. Inhibition of I kappaB-alpha phosphorylation at serine and tyrosine acts independently on sensitization to DNA damaging agents in human glioma cells. Br J Cancer 2000;82(1):28-33.

The principal author is with the Department of Radiation Genetics, Graduate School of Medicine, Kyoto University, Japan.

Abstract: Molecular mechanisms and/or intrinsic factors controlling cellular radiosensitivity are not fully understood in mammalian cells. The recent studies have suggested that nuclear factor kappaB (NF-kappaB) is one of such factors. The activation and regulation of NF-kappaB are tightly controlled by IkappaB-alpha, a cellular inhibitory protein of NF-kappaB. Most importantly, phosphorylation regulates

activity of the inhibitor IkappaB-alpha, which sequesters NF-kappaB in the cytosol. Two different pathways for the phosphorylation of IkappaB-alpha are demonstrated, such as serine (at residues 32 and 36) and tyrosine (at residue 42) phosphorylations. To assess a role of the transcription factor, NF-kappaB, on cellular sensitivity to DNA damaging agents, we constructed three different types of expression plasmids, i.e. S-IkappaB (mutations at residues 32 and 36), Y-IkappaB (mutation at residue 42) and SY-IkappaB (mutations at residues 32, 36 and 42). The cell clones expressing S-IkappaB and Y-IkappaB proteins became sensitive to X-rays as compared with the parental and vector-transfected cells. The cell clones expressing SY-IkappaB were further radiosensitive. By the treatment with herbimycin A, an inhibitor of phosphorylation, the X-ray sensitivity of cells expressing SY-IkappaB did not change, while that of the cells expressing S-IkappaB and Y-IkappaB and the parental cells was enhanced. Change in the sensitivity to adriamycin and UV in those clones was very similar to that in the X-ray sensitivity. The inhibition of IkappaB-alpha phosphorylation at serine and tyrosine acts independently on the sensitization to X-rays, adriamycin and UV. These findings suggest that the transcriptional activation induced by NF-kappaB may play a role in the DNA damage repair. The present study proposes a possibility that the inactivation of NF-kappaB by inhibition of both serine and tyrosine phosphorylations may be useful for the treatment of cancer in radio- and chemotherapies.

Yamagishi N, Miyakoshi J and Takebe H. Enhanced radiosensitivity by inhibition of nuclear factor kappa B activation in human malignant glioma cells. Int J Radiat Biol 1997;72(2):157-162.

The principal author is with the Department of Radiation Genetics, Faculty of Medicine, Kyoto University, Japan.

Abstract: To clarify the relationship between cellular radiosensitivity and nuclear factor kappa B (NF-kappa B) activation, an expression plasmid was constructed for I kappa B-alpha, a cellular inhibitory protein of NF-kappa B, and transfected it into two human malignant glioma cell lines. Cells overexpressing the I kappa B-alpha protein were more radiosensitive than the parental cells and one transfected clone with low expression. In the parental cell lines and one transfected clone with low expression, the sequence specific DNA-binding activity of NF-kappa B was considerably increased between 1 and 2 h after irradiation. In contrast, no increase in the DNA-binding activity was observed in the transfected clone overexpressing I kappa B-alpha protein. These results suggest that the activation of NF-kappa B may be one of the intrinsic responses determining cellular radiosensitivity.

Miyakoshi J, Yoshida M, Shibuya K and Hiraoka M. Exposure to strong magnetic fields at power frequency potentiates X-ray-induced DNA strand breaks. J Radiat Res (Tokyo) 2000;41(3):293-302.

The principal author is with the Department of Radiation Genetics, Graduate School of Medicine, Kyoto University, Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan.

Abstract: We examined the effect of an extremely low-frequency magnetic field (ELFMF) at 5, 50 and 400 mT on DNA strand breaks in human glioma MO54 cells. A DNA damage analysis was performed using the method of alkaline comet assay. The cells were exposed to X-rays alone (5 Gy), ELFMF alone, or X-rays followed by ELFMF at 4 degrees C or on ice. No significant difference in the tail moment was observed between control and ELFMF exposures up to 400 mT. X-ray irradiation increased DNA strand breaks. When cells were exposed to X-rays followed by ELFMF at 50 and 400 mT, the tail moment increased significantly compared with that for X-rays alone. When the exposure of cells was performed at 37 degrees C, no significant change was observed between X-rays alone and X-rays plus 400 mT. We previously observed that exposure to 400 mT ELFMF for 2 h increased X-ray-induced mutations (Miyakoshi et al, Mutat. Res., 349: 109-114, 1996). Additionally, an increase in the mutation by exposure to the ELFMF was observed in cells during DNA-synthesizing phase (Miyakoshi et al., Int. J. Radiat. Biol., 71: 75-79, 1997). From these results, it appears that exposure to the high density ELFMF at more than 50 mT may potentiate X-ray-induced DNA strand breaks.

Wan C, Fiebig T, Schiemann O, Barton JK and Zewail AH. Femtosecond direct observation of charge transfer between bases in DNA. Proc Natl Acad Sci U S A 2000;97(26):14052-14055.

The principal author is with the Laboratory for Molecular Sciences, Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, CA 91125, USA.

Abstract: Charge transfer in supramolecular assemblies of DNA is unique because of the notion that the pi-stacked bases within the duplex may mediate the transport, possibly leading to damage and/or repair. The phenomenon of transport through pi-stacked arrays over a long distance has an analogy to conduction in molecular electronics, but the mechanism still needs to be determined. To decipher the elementary steps and the mechanism, one has to directly measure the dynamics in real time and in suitably designed, structurally well characterized DNA assemblies. Here, we report our first observation of the femtosecond dynamics of charge transport processes occurring between bases within duplex DNA. By monitoring the population of an initially excited 2-aminopurine, an isomer of adenine, we can follow the charge transfer process and measure its rate. We then study the effect of different bases next to the donor (acceptor), the base sequence, and the distance dependence between the donor and acceptor. We find that the charge injection to a nearest neighbor base is crucial and the time scale is vastly different: 10 ps for guanine and up to 512 ps for inosine. Depending on the base sequence the transfer can be slowed down or inhibited,

and the distance dependence is dramatic over the range of 14 A. These observations provide the time scale, and the range and efficiency of the transfer. The results suggest the invalidity of an efficient wire-type behavior and indicate that long-range transport is a slow process of a different mechanism.

Bixon M, Giese B, Wessely S, Langenbacher T, Michel-Beyerle ME and Jortner J. Long-range charge hopping in DNA Proc Natl Acad Sci U S A 1999;96(21):11713-11716.

The principal author is with the School of Chemistry, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel.

Abstract: The fundamental mechanisms of charge migration in DNA are pertinent for current developments in molecular electronics and electrochemistry-based chip technology. The energetic control of hole (positive ion) multistep hopping transport in DNA proceeds via the guanine, the nucleobase with the lowest oxidation potential. Chemical yield data for the relative reactivity of the guanine cations and of charge trapping by a triple guanine unit in one of the strands quantify the hopping, trapping, and chemical kinetic parameters. The hole-hopping rate for superexchange-mediated interactions via two intervening AT base pairs is estimated to be 10(9) s(-1) at 300 K. We infer that the maximal distance for hole hopping in the duplex with the guanine separated by a single AT base pair is 300 +/- 70 A. Although we encounter constraints for hole transport in DNA emerging from the number of the mediating AT base pairs, electron transport is expected to be nearly sequence independent because of the similarity of the reduction potentials of the thymine and of the cytosine.

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10. SEARCH FOR EPIGENETIC MODES OF ACTION

10.1 Epigenetic (Non-genotoxic) Effects Summary

Few studies evaluated magnetic fields for epigenetic (non-genotoxic) effects in the period 1998 to 2002. Of particular interest were the results from studies by Yoshizawa (2002), Harris (2002) and Tian (2002) that found magnetic fields had no effect on cell proliferation or cell cycle progression in model transformed cell lines. The balance of the studies that examined these endpoints had weaknesses in their experimental designs or exposure systems, which made it difficult to interpret their findings. Studies with valid experimental designs and exposure systems also found magnetic fields did not effect cell viability (Loberg, 2000a&b) or cytoxicity [(Loberg, 2000a&b) and (Tian, 2002). Harris (2002) and Tian (2002) both found that exposure to magnetic fields, at high flux densities, inhibited gamma ray- or X-ray-induced cell cycle blockade in G2, which implies some cells with unrepaired DNA damage were able to progress through the cell cycle. Tian (2002) also found indication that X-ray induced apoptosis was inhibited by exposure to 5 mT (60 Hz) magnetic fields. These latter findings, while biologically interesting, are probably not relevant at environmental exposure levels.

Many studies, using a variety of cell lines, found that magnetic fields had no effect on the expression of MYC and other cancer-related early response genes [Owen (1998), Loberg (1999), Nakasono (2000), Morehouse (2000) and Yamori (2002)]. Rao also found magnetic fields did not effect the expression of APP695, a gene related to the development of Alzheimer's disease. While short-term exposure to magnetic fields may not effect gene expression, Zhou (2002) found evidence that longer term (72 h) exposures can alter cytokine production.

A few studies examined the effect of magnetic fields on intercellular communications. Gap junctional intercellular communication (GJIC) is an important process in the regulation of cellular differentiation and proliferation, and it has been postulated that magnetic fields can effect tumor promotion by inhibiting GJIC. There is also evidence that GJIC is inhibited by the activation of protein kinase C (PKC), an apparent obligatory step in tumor promotion. However, the methodology used to assess GJIC is still crude, based primarily on subjective, operator-dependent criteria. Under these analytical conditions, results run the risk of being biased.

The effect of magnetic fields on signal transduction demonstrates the difficulty of independent attempts to replicate results (also see abstract by Boorman in Appendix). The inability of two independent laboratories, whose main focus is signal transduction, to verify the findings of Uckun and colleagues, whose main function is not, weakens the hypothesis that magnetic fields effect biological systems by perturbation of signal transduction pathways. This is particularly relevant because the cells used in these studies were well characterized model systems.

Of the two studies that examined the effect of magnetic fields on cell differentiation, only Chen (2000) provides some interesting, but inconclusive, findings, which deserve further study.

Overall, the results from *in vitro* studies of epigenetic modes of action, find little evidence to support the hypothesis that magnetic fields can act as tumor promoters.

Table 20. Cell proliferation (CP), cell cycle progression (CCP), DNA synthesis (DNAS), cytotoxicity (CT), and viability (V) summary

Author	Year	Endpoint	Valid?	Effect?	Weaknesses
Cridland	1998	DNAS	No	NA	ED
Wei	2000	CP	inconclusive	NA	ED
Loberg	2000a	CP V CT	No Yes Yes	NA None None	ED None Apparent None Apparent
Loberg	2000b	V CT	No Yes	NA None	ED None Apparent
Van Den Heuvel	2001	CP	No	NA	ED
Yoshizawa	2002	CP	Yes	None	None Apparent
Harris	2002	CCP	Yes	Yes**	None Apparent
Tian	2002	CP CCP V CT	Yes Yes Yes No	None None None NR	None Apparent
Boland	2002	V; CT	No	Rat Brain	ED

Abbreviations:
ED = Experimental Design shortcomings
NA = limitations in study design prevents valid interpretation of results.
none = No statistical difference between exposed and sham treated cells.
NR = Not Relevant to environmental issues
** = magnetic fields inhibited X-ray induced G2/M blockade

10.2 Cell Proliferation, Cell Cycle Progression, DNA Synthesis, Cytotoxicity and Viability

Individual Study Summaries

Cridland NA, Haylock RGE and Saunders RD. 50 Hz magnetic field exposure alters onset of S-phase in normal human fibroblasts. Bioelectromagnetics 1999;20:446-452.

The principal author is with the National Radiological Protection Board, Chilton, Didcot, Oxfordshire, United Kingdom.

Abstract: This study was undertaken to investigate whether power frequency magnetic fields can affect the kinetics of cell cycle progression in exposed human cells. To achieve this, cultures of normal human fibroblasts were synchronised in the G0 phase of the cell cycle and exposed to 50 Hz magnetic fields at a range of flux densities. Progression through the cycle was monitored by examining the timing of entry into S phase, as characterized by the onset of DNA synthesis. Simultaneous positive controls were exposed to human recombinant fibroblast growth factor to demonstrate that the system was responsive to external stimuli. Exposure to magnetic fields at 20 and 200 μT induced a small but significant increase in the length of the G1 phase of the cell cycle. However, exposure at higher flux densities of 2 and 20 mT had no significant effect. These results are discussed in relation to weak magnetic field effects on free radical concentration.

Comment: The exposure system was crude, but well calibrated and characterized. However, the sham and exposure protocols were not equivalent. The exposure status of samples were not blind to the analyst. The study examined cell cycle progression from G0 into S-phase by measuring the incorporation of ^{3}H-deoxythymidine into DNA. The ^{3}H counts in exposed and sham samples should have been normalized to DNA content or cell numbers. While the author attributes the positive findings to the magnetic field, it is possible the results were due to a combination of differential treatment of exposed and control cells, differences between sham and exposed samples in terms of cell numbers, and possible analytical bias from processing non-blinded samples. The results are considered inconclusive, given these shortcomings.

Wei M, Guizzetti M, Yost M and Costa LG. Exposure to 60-Hz magnetic fields and proliferation of human astrocytoma cells in vitro. Toxicol Appl Pharmacol 2000;162(3):166-176.

The principal author is with the Department of Environmental Health, University of Washington, Seattle, Washington, 98105, USA.

Abstract: Epidemiological studies have suggested that exposure to electric and magnetic fields (EMF) may be associated with an increased incidence of brain tumors, most notably astrocytomas. However, potential cellular or molecular mechanisms involved in these effects of EMF are not known. In this study we investigated whether exposure to 60 Hz sinusoidal magnetic fields (0.3 -1.2 G for 3 -72 h) would cause proliferation of human astrocytoma cells. Sixty-hertz magnetic fields (MF) caused a time- and dose-dependent increase in proliferation of astrocytoma cells, measured by (3)H-thymidine incorporation and by flow cytometry, and strongly potentiated the effect of two agonists (the muscarinic agonist carbachol and the phorbol ester PMA). However, MF had no effect on DNA synthesis of rat cortical astrocytes, i.e., of similar, non-transformed cells. To determine the amount of heating induced by MF, temperatures were also recorded in the medium. Both 1.2 G MF and a sham exposure caused a 0.7 degrees C temperature increase in the medium; however, (3)H-thymidine incorporation induced by sham exposure was significantly less than that caused by MF. GF 109203X, a rather specific protein kinase C (PKC) inhibitor, and down-regulation of PKC inhibited the effect of MF on basal and on agonist-stimulated (3)H-thymidine incorporation. These data indicate that MF can increase the proliferation of human astrocytoma cells and strongly potentiate the effects of two agonists. These findings may provide a biological basis for the observed epidemiological associations between MF exposure and brain tumors.

Comment: The description of exposures system, its calibration, quality assurance protocols appear satisfactory. However, the analyst was not blind to the exposure status of the samples. The study purports to show that exposure to magnetic fields, alone or in combination with carbachol (a muscarinic agonist) or phorbol myristate acetate (PMA), increases cell proliferation. Cells were synchronized for 2 days in medium containing o.1% bovine serum albumin before being exposed to sham or magnetic fields. In the first assay involving ^{3}H-deoxythymidine uptake into DNA at S-phase, the radioactivity was not normalized to DNA content or cell numbers, and therefore this part of the experimental design is unacceptable. The second part of the study, flow cytometry was used to study the effect of magnetic fields on cell cycle progression in a transformed human astrocytoma cell and non-transformed rat cortical astrocytes. The paper does not supply sufficient information on flow cytometry protocols, or on the passage number of the astrocytoma cell line. For exposures at 72 h there is a possibility of osmotic shock due to water loss from the cultures, since temperature rose by 0.7^{o}C during sham or magnetic field exposure. The statistical strategy used to analyze the data was not justified. These shortcomings make the reliability of the data difficult to establish, and therefore the results must be considered inconclusive.

Loberg LI, Engdahl WR, Gauger JR and McCormick DL. Cell viability and growth in a battery of human breast cancer cell lines exposed to 60 Hz magnetic fields. Radiat Res 2000(a);153(5 Pt 2):725-728.

The principal author is with the Experimental Toxicology and Carcinogenesis Division, IIT Research Institute, Chicago, Illinois 60616, USA.

Abstract: Epidemiological data suggest that exposure to power-frequency (50/60 Hz) magnetic fields (MFs) may be a risk factor for breast cancer in humans. To determine whether MFs affect human breast cancer cells, we measured viability, growth and cytotoxicity in a battery of breast cancer cell lines after in vitro MF and sham exposure. Cells of three estrogen receptor-positive human breast cancer cell lines (MCF-7, ZR-75-1 and T-47D) and one estrogen receptor-negative human breast cancer cell line (MDA-MB-231) and normal (nontransformed) human breast epithelial cells were exposed to MFs (1 mT) or sham fields (<0.0001 mT) for 72 h. Cell viability was determined using the sulforhodamine B (SRB) assay at 0 and 72 h after the MF exposure period. Cell growth was measured as the change in SRB dye uptake over 72 h after MF exposure. MF exposure had no effect on cell viability or growth in any cell type examined. Similarly, MF exposure had no effect on cytotoxicity induced by exposure to the retinoid N-(4-hydroxyphenyl)retinamide. These data do not support the hypothesis that MF exposure stimulates growth of breast cancer cells.

Comment: Sulforhodamine B (SRB) binds to basic amino acids of cellular proteins and the solubilized stain is measured spectrophotometrically to determine relative cell growth or viability. It is a simple, widely used assay to screen for cytotoxicity, and is similar in sensitivity to the MTT assay. The exposure system was acceptable, as were the sham exposure protocols, and samples were processed and analyzed blind to exposure status. Experiment #1 examined the effect of a 1 mT magnetic field on the growth and viability of human beast cancer cell lines, including estrogen-receptor positive and negative lines and HME (normal human breast epithelial cells). Exposure to the magnetic field did not effect either cell proliferation or viability for any of the cell lines tested. The effect on proliferation was based on two measurements, at the beginning and end of a 72 hours. Measurements could also have been done at 24 and 48 hours to determine if any peaks in growth occurred at intermediate time points. The experiment on proliferation used no positive control, and cells were grown under optimal conditions so any stimulation of growth from exposure to the fields could have been missed. Also, there was no negative control to demonstrate what inhibition would have looked like. The statistical procedures used in data analysis were not properly justified. In experiment #2, the objective was to determine if the magnetic field could influence the cytotoxicity of 4-HPR (a retinoid compound) that is known to induce apoptosis in epithelial cells. No synergy was observed between magnetic fields and 4-HPR.

Loberg LI, Luther MJ, Gauger JR and McCormick DL. 60 Hz magnetic fields do not enhance cell killing by genotoxic chemicals in Ataxia telangiectasia and normal lymphoblastoid cells. Radiat Res 2000(b);153(5 Pt 2):685-689.

The principal author is with the Experimental Toxicology and Carcinogenesis Division, IIT Research Institute, Chicago, Illinois 60616, USA.

Abstract: Ataxia telangiectasia (AT) is an inherited autosomal recessive disease characterized by increased risk of cancer, immune deficiency, and neurodegeneration. Cells cultured from AT patients are highly sensitive to genotoxic agents and are deficient in cell cycle arrest after exposure to ionizing radiation. In consideration of their sensitivity to both ionizing and nonionizing radiation, AT cells may provide a sensitive model system to study the biological activity of other components of the electromagnetic spectrum. To characterize the effects of power-frequency (60 Hz) magnetic fields (MFs) in AT cells, we compared responses of AT and normal lymphoblast cells to sinusoidal MFs at 1.0 mT, either alone or in combination with the genotoxic agents mitomycin C or streptonigrin. The MF alone had no effect on cell growth or survival in a clonogenic assay in either AT or normal cells. The MF also had no effect on induction of cell death by mitomycin C or streptonigrin in either cell
type. AT cells do not demonstrate differential sensitivity to MF exposure. These results do not support the hypothesis that MFs interact with genotoxic agents to induce adverse biological effects in either normal or genetically susceptible human cells.

Comment: The exposure system was adequate as were calibration and quality assurance protocols. The analyst was blind to exposure status of samples. Cell culture was adequate. Statistical procedures for data analysis were adequate This study tests the hypothesis that magnetic fields influence carcinogenesis by modulating a cell's response to DNA damage. By inhibiting or perturbing the normal response to DNA damage, a magnetic field could increase the likelihood of 'fixing' a mutation or chromosome damage into the genetic make-up of the cell. This study used cells cultured from patients with the genetic disease ataxia telangiectasia (AT), and normal lympoblastoid cells as a model systems. AT cells are hypersensitive to cell killing and the damaging effects of ionizing radiation and radiomimetic drugs, such as streptonigrin. The sensitivity of AT cells to genetic damage should make them more susceptible than normal cells to the effects (if any) of magnetic fields. In the first experiment, AT and lymphoblastoid cells were exposed to 1 mT (60 Hz) magnetic field for 24 h, and then viability was assessed at the end of the exposure period, and at 24, 48, and 72 h later by Trypan blue exclusion and LDH assay. Lactate dehydrogenase release assay was performed on lysed cells, which provides a measure of cell number, and on non-lysed cells, which is a measure of membrane integrity (thereby confirming the trypan blue results). In a second experiment, the effect of magnetic fields on long-term viability was tested in normal and AT cells. Cells were exposed to a 1 mT (60Hz) field for 24 h, followed by two weeks of incubation, and a colony assay to measure survival. However, positive and negative controls were not used in this experiment. A third experiment was carried out to test a corollary hypothesis, that magnetic fields would increase the biological activity of DNA damaging agents. Lymphoid and AT cells were exposed simultaneously to mitomycin C or streptonigrin, and a 1.0 mT (60Hz) magnetic field for

24 h. Viability was measured by LDH assay at 0, 24, 48 and 72 hours after termination of the magnetic field exposure. There was no significant differences in survival between sham- and magnetic field exposed cells under any of the conditions examined. The only shortcoming was the lack of positive and negative controls in the first and second experiments. Exposure to magnetic fields did not alter the cell killing potential of streptonigrin or mitomycin.

Van Den Heuvel R, Leppens H, Nemethova G and Verschaeve L. Haemopoietic cell proliferation in murine bone marrow cells exposed to extreme low frequency (ELF) electromagnetic fields. Toxicol In Vitro 2001;15(4-5):351-355.

The principal author is with the Department of Environmental Toxicology, Boeretang 200, 2400 Mol, Belgium.

Abstract: As leukemia is one of the health hazards that is sometimes associated with exposure to extreme low frequency fields, we studied the in vitro effects of ELF fields on haemopoietic cell proliferation. First, the cytotoxic effect of 80 µT, 50 Hz magnetic fields on 3T3 cell proliferation was investigated using the neutral red test. Many chemicals are believed to cause damage because they interfere with basal or "housekeeping" cell functions. The basal cell functions are present in every cell. Non-specialized, actively dividing cells are suitable for measuring cytotoxic effects. Cytotoxic doses can be identified by exposing actively dividing cells in vitro and measuring growth inhibition caused by interference with these basal cell functions. 80 µT, 50 Hz magnetic fields caused no cytotoxicity: we were not able to demonstrate any interference with essential cell functions in the non-differentiated 3T3 cell line. Furthermore, the in vitro effects of ELF fields on murine haemopoietic and stromal stem cell proliferation were studied. Haemopoiesis is a continuous process, where mature blood cells are replaced by the proliferation and differentiation of more primitive progenitor and stem cells. Blood formation is tightly regulated by the stromal micro-environment. Exposure of murine bone marrow cells, from male and female mice, to 80 µT (50 Hz) magnetic fields showed a reduction in the proliferation and differentiation of the granulocyte-macrophage progenitor (CFU-GM) compared to non-exposed bone marrow cells. The results on the effect of the ELF-field on stromal stem cell proliferation (CFU-f) are somewhat equivocal at the moment. CFU-f from female mice showed a reduction while CFU-f from male mice were not decreased.

Comment: The exposure system was adequately described and calibrated, temperature control was not a problem. However, the sham exposure protocol was unacceptable and the exposure status of the samples were not blind to the analyst. Results were presented as percentages of control, instead of absolute numbers. The strategy used for statistical analysis was not justified. The results should be considered inconclusive.

Yoshizawa H, Tsuchiya T, Mizoe H, Ozeki H, Kanao S, Yomori H, Sakane C, Hasebe S, Motomura T, Yamakawa T, Mizuno F, Hirose H and Otaka Y. No effect of

extremely low-frequency magnetic field observed on cell growth or initial response of cell proliferation in human cancer cell lines. Bioelectromagnetics 2002;23(5):355-368.

The principal author is with the Power Engineering R & D Center, Tokyo Electric Power Co., Inc., Yokohama, Japan.

Abstract: An effect on the tumor promotion process, as represented by accelerated cell growth, has been indicated as one example of areas that demonstrate the possibility of biological effects of extremely-low frequency magnetic fields. We, therefore, exposed the five cell lines (HL-60, K-562, MCF-7, A-375, and H4) derived from human tumors to a magnetic field for 3 days to investigate the effects on cell growth. Prior to exposure or sham exposure, the cells were precultured for 2 days in low serum conditions. The number of growing cells was counted in a blind manner. To investigate the effect on the initial response of cell proliferation, two cell lines were synchronized in G1 phase by serum starvation and then exposed to a magnetic field for 18 h (H4 cells) or 24 h (MCF-7 cells), both with and without serum stimulation. The rate of DNA synthesis, taken as a measure of the cell proliferation, was determined by following the incorporation of [(3)H]-thymidine into the DNA. Three different magnetic field polarizations at both 50 and 60 Hz were used: linearly polarized (vertical); circularly polarized; and an elliptically polarized field. Magnetic field flux densities were set at 500, 100, 20 and 2 μT (rms) for the vertical field and at 500 μT (rms) for the rotating fields. No effect of magnetic field exposure was observed on either cell growth or the initial response of cell proliferation.

Comment: The exposure system (Yamazaki, 2000) and sham exposure protocol were adequate. Calibration of the exposure system, temperature control within the incubators, and quality assurance protocols were satisfactory. The exposures to magnetic fields could be linearly, circularly, and elliptically polarized to present a variety of exposure scenarios. Five cancer-derived cell lines were studied (including HL-60 and MCF-7), and all cells were used in the experiments within 10 cell passages. The investigators studied cell proliferation under conditions of serum deprivation, since at 10% fetal bovine serum, the proliferation rate would be at a maximum. A single lot of fetal bovine serum (FBS) was used throughout the experiments. Cells were synchronized by serum deprivation for two days prior to treatments. Cells were then split into a sham and magnetic field exposed groups. Cells were serum deprived during the three days of exposure, and an incubator control, which was give a growth stimulus by the addition of 10% fetal bovine serum, was used as a positive control for the experiment. Cells were exposed to 500, 20 and 2 μT magnetic fields for three days. Cell morphology was examined by scanning electron, and optical microscopy. The analyst was blinded to the exposure status of the culture dishes. Cell cycle progression was measured by propidium iodide staining and flow cytometry. The rate of DNA synthesis was determined by ^{3}H uptake, and the DNA concentration in each culture dish was measured by fluorometry using 3,5 diaminobenzoic acid fluorescence. Results were

expressed as ^{3}H uptake/µg DNA. The data was tested for homogeneity of variance by the F-test, if data variance was homogeneous, the Student's t-test was used to assess differences. If variances were heterogenous, Welch's t-test was used. An *a priori* strategy was determined if a significant difference was found: the experiment was to be repeated one more time and the results of all experiments were to be pooled and differences re-examined using Wilcoxon's rank sum test. The initial results for 2 cell lines warranted repetition and no differences were detected upon re-examination of all data. The spurious results (one an apparent false negative and the other an apparent false positive) were attributed to factors other than the magnetic field. The magnetic field did not effect cell proliferation, rate of DNA synthesis, or cell morphology at any of the exposure levels examined. This experiment was well planned and executed, and therefore the results stand without ambiguity.

Harris PA, Lamb J, Heaton B and Wheatley DN. Possible attenuation of the G2 DNA damage cell cycle checkpoint in HeLa cells by extremely low frequency (ELF) electromagnetic fields. Cancer Cell Int 2002;2(1):3.

The principal author is with the Section of Surgery, Division of Clinical Sciences (University of Sheffield),Clinical Sciences Centre, Northern General Hospital, Herries Road, Sheffield, S5 7AU, UK.

Abstract: *Background:* The issue remains unresolved as to whether low frequency magnetic fields can affect cell behaviour, with the possibility that they may be in part responsible for the increased incidence of leukaemia in parts of the population exposed to them. *Methods:* Combined treatment of HeLa cells with gamma-irradiation (1, 3 and 5 Grays) and extra low frequency magnetic fields of ~50 Hz was carried out under rigorously controlled conditions. *Results:* Synchronised cells progressing from S-phase arrived at mitosis on average marginally ahead of irradiation controls not exposed to ELF. In no instance out of a total of twenty separate experiments did this "double-insult" further delay entry of cells into mitosis, as had been anticipated. *Conclusion:* This apparently "non-genotoxic" agent (ELF) appears to be capable of affecting cells that would normally arrest for longer in G2, suggesting a weakening of the stringency of the late cycle (G2) checkpoint.

Comment: The exposure system was adequate as were calibration and quality assurance protocols. Temperature control was not a problem. The analyst was blind to the exposure status of samples. Culture conditions and protocols to manipulate cell cycle progression were adequate. The results implies that magnetic fields inhibit the radiation-induced G2 blockade, which would allow damaged cells to potentially transfer mutations through to subsequent cell cycles. Magnetic fields alone did not affect cell cycle progression or cell proliferation. While statistically significant, the results may not be biologically relevant when extrapolated to environmental levels of radiation exposure (but could be relevant at therapeutic doses). This study adds to the body of knowledge

that magnetic fields of high flux density may have subtle effects on radiation-induced G2 blockade.

Tian F, Nakahara T, Yoshida M, Honda N, Hirose H and Miyakoshi J. Exposure to power frequency magnetic fields suppresses X-ray-induced apoptosis transiently in Ku80-deficient xrs5 cells. Biochem Biophys Res Commun 2002;292(2):355-361.

The principal author is with the Department of Radiation Genetics, Graduate School of Medicine, Kyoto University, Yoshida-Konoe-cho, Sakyo-Ku, Kyoto, Japan.

Abstract: In an attempt to determine whether exposure to extremely low frequency (ELF) electromagnetic fields can affect cells, Ku80-deficient cells (xrs5) and Ku80-proficient cells (CHO-K1) were exposed to ELF electromagnetic fields. Cell survival, and the levels of the apoptosis-related genes p21, p53, phospho-p53 (Ser(15)), caspase-3 and the anti-apoptosis gene bcl-2 were determined in xrs5 and CHO-K1 cells following exposure to ELF electromagnetic fields and X-rays. It was found that exposure of xrs5 and CHO-K1 cells to 60 Hz ELF electromagnetic fields had no effect on cell survival, cell cycle distribution and protein expression. Exposure of xrs5 cells to 60 Hz ELF electromagnetic fields for 5 h after irradiation significantly inhibited G(1) cell cycle arrest induced by X-rays (1 Gy) and resulted in elevated bcl-2 expression. A significant decrease in the induction of p53, phospho-p53, caspase-3 and p21 proteins was observed in xrs5 cells when irradiation by X-rays (8 Gy) was followed by exposure to 5 mT ELF magnetic fields. Exposure of xrs5 cells to the ELF electromagnetic fields for 10 h following irradiation significantly decreased X-ray-induced apoptosis from about 1.7% to 0.7%. However, this effect was not found in CHO-K1 cells within 24 h of irradiation by X-rays alone and by X-rays combined with ELF electromagnetic fields. Exposure of xrs5 cells to 60 Hz ELF electromagnetic fields following irradiation can affect cell cycle distribution and transiently suppress apoptosis by decreasing the levels of caspase-3, p21, p53 and phospho-p53 and by increasing bcl-2 expression.

Comment: Ku80 is a heterodimer, each sub-unit of which is an ATP-dependent DNA helicase, capable of acting independently at sites of DNA double strand breaks to facilitate non-homologous end joining. Xrs5 cells are deficient in KU80 activity, whereas CHO-K1 cells are proficient in KU80 activity and can repair, within 24 h, 95% of all radiation-induced DNA double strand breaks. The magnetic field exposure system is the same one describe by Miyakoshi in Part I Genotoxicity, and is considered to be adequate in all respects, as was the experimental design. The relevant portion of the experiment found that exposure to 5 mT (60 Hz) magnetic field did not affect cell survival, cell cycle distribution or protein expression in xrs5 or CHO-K1 cells. In the second part of the experiment, exposure to a 5 mT(60 Hz) magnetic field for 5 hours inhibited X-ray-induced cell cycle arrest in xrs5 cells, but not in CHO-K1 cells. This implies magnetic fields allow some xrs5 cells with residual DNA damage to progress faster through the cell cycle than sham exposed controls or CHO-K1 cells. This is

similar to the findings of Harris (2002) above, who used HeLa cells. After 10 hours of exposure to magnetic field, X-ray induced apoptosis was inhibited in xrs5 cells, relative to sham, but not in CHO-K1 cells. This confirms previous findings of this laboratory in experiments discussed in Part I, Genotoxicity. However, very high doses of X-rays were required to demonstrate this effect, and the observed decrease in the rate of apoptosis (1.7% to 0.7%) would probably not be relevant at the lower exposures.

Boland A, Delapierre D, Mossay D, Dresse A and Seutin V. Effect of intermittent and continuous exposure to electromagnetic fields on cultured hippocampal cells. Bioelectromagnetics 2002;23(2):97-105.

The principal author is with the Laboratory of Pharmacology, Institute of Pathology B23a, University of Liege, Liege, Belgium.

Abstract: This study was designed to assess the effect of 50 Hz electromagnetic fields (EMFs) on hippocampal cell cultures in the presence or absence of either sodium nitroprusside (SNP, a NO donor) or Fe2+ induced oxidative stress. One week old cultured rat hippocampal cells were exposed to either intermittent EMFs (IEMFs, 50 Hz, 0-5 mT, 1 min ON/OFF cycles, repeated 10 times every 2 h, 6 times/day during 48 h) or continuous EMFs (CEMFs, 50 Hz, 0-5 mT for 48 h). In a second set of experiments, the effect on such EMFs applied in combination with oxidative stress induced by 0.5 microM Fe2+ or SNP was estimated. At the end of both sets of experiments, cell mortality was assessed by lactate dehydrogenase measurements (LDH). Neither type of exposure to EMFs was observed to modify the basal rate of cell mortality. The exposure to CEMFs in presence of either NO or Fe2+ did not induce any significant increase in cell death. However, when cells were exposed to EMFs in the presence of NO, we observed a significant increase in cell death of 11 and 23% ($P<0.001$) at 2.5 and 5 mT, respectively. This effect had some specificity because IEMFs did not modify the effect of Fe2+ on cell mortality. Although the effects of IEMFs reported in this study were only observed at very high intensities, our model may prove valuable in trying to identify one cellular target of EMFs.

Comment: The object of this study was to assess the effect of continuous or intermittent magnetic field exposure (cEMF, or iEMF, respectively) on, (i) the survival of cultured rat hippocampal cells, and (ii) the viability of these cells co-exposed to cEMF or iEMF and Fe^{+2} ions (which generate hydoxyl radicals) or sodium nitroprusside (SNP) (which generate nitric oxide radicals). A dose-response curve was not established for the chemical treatments. The sham and magnetic field exposure protocols were not equivalent, and the analyst was not blind to the exposure status of the samples. The intermittent exposure was generated by manually turning the exposure system ‘on’ or ‘off’ each minute for 20 minutes every 2 h for six times during the exposure period. This is unsatisfactory from a perspective of timing and the generation of transients and non-

uniform fields. Temperature changes in the culture dishes exposed to the magnetic fields were not assessed.

The hippocampi of embryonic rats were disaggregated according to a published protocol. Cells were allowed to remain in culture for 7 to 10 days before treatment. Proper dose-viability curves were not established for either SNP or Fe^{+2} alone (a preliminary experiment established SNP, but not Fe^{+2}, at 0.5 μM produced significant loss of cell viability). Cells were exposed to sham or to 0 -5 mT (50 Hz) magnetic fields for 48 h and then viability was measured by lactate dehydrogenase (LDH) release. However, this result was not normalized to cell number, which could have been obtained by measuring total LDH released after cell lysis. No effect on cell viability was observed in cells exposed to either iEMF or cEMF. A positive control was not used to establish assay performance. In a second experiment, cells were exposed for 24 h to iEMF, cEMF, or sham conditions, and then for a further 24 h in serum-free medium after addition of either SNP or Fe^{+2}. The shortcomings in experimental design make it difficult to draw valid conclusions.

10.3 Gene Expression

Table 21. Gene expression summary

Author	Date	Endpoint	Response	Relevance?	weakness
Tuinstra	1998	PKC	Yes	No	ED
Lagroye	1998	jun; fos	Yes	No	ED
Owen	1998	myc	No	Yes	None Apparent
Pipkin	1999	stress proteins	Yes	No	ED
Loberg	1999	myc	No	Yes	None Apparent
Loberg	2000c	multi-gene array	No	Yes	None Apparent
Nakasono	2000	expressed proteins	No	Yes	None Apparent (Qualitative)
Morehouse	2000	myc	No	Yes	None Apparent
Zhou	2002	cytokines	Mixed	Inconclusive	ED
Rao	2002	APP695	No	Yes	**
Yomori	2002	fos; jun; myc	No	Yes	None Apparent

Abbreviations:

APP695 = gene related to Alzheimer's disease
ED = Experimental Design shortcomings
fos = oncogene
jun = oncogene
myc = oncogene
PKC = Protein Kinase C
SP = Stress Proteins
** = pending communications from author.

Individual Study Summaries

Tuinstra R, Goodman E and Greenebaum B. Protein kinase C activity following exposure to magnetic field and phorbol ester. Bioelectromagnetics 1998;19:469-476.

The principal author is with the Biomedical Research Institute, University of Wisconsin-Parkside, Kenosha, Wisconsin.

Abstract: We examined the separate and combined effects of 60 Hz sinusoidal magnetic fields (MFs) and a phorbol ester on protein kinase C (PKC) activity in HL60 cells. No enhancement in PKC activity was observed when a cell culture was exposed to a 1.1 mT (rms) MF alone or to a combination of MF and 2 M phorbol 12-myristate 13-acetate (PMA) for 1 h. In a second set of experiments, cells were preexposed to a less than optimal concentration of PMA (50 nM) for 45 min, followed by a 15 min exposure to both PMA and MF. The data showed a greater decrease in cytosolic PKC activity and a larger increase in membrane activity than was induced by either 1 h PMA treatment alone or PMA and sham MF exposure. One logical conclusion from these data is that MFs may be acting in a synergistic manner on a pathway that has already been activated. Therefore, we suggest that MFs, rather than producing biological effects by a new pathway or mechanism of interaction, exert their effect(s) by interacting with already functioning reactions or pathways. If correct, the question of an MF's mechanism of interaction refocuses on how weak fields might enhance or depress a molecular reaction in progress, rather than on finding a new transduction pathway.

Comment: The object of this study was to determine the effect of a combined exposure to magnetic fields and PMA on protein kinase C activity (PKC) in HL60 cells. PMA crosses cell membranes and produces a prolonged activation of PKC by mimicking the action of diacylglycerol (DAG). During activation, PKC is transported from an inactive state in the cytosol to an active form in the cell membrane, where it triggers cellular changes, one of which can be the proliferation of initiated cells. The exposure system, its calibration and quality assurance protocols were adequate. Temperature control was not an issue. The analyst was blind to the exposure status of the samples. PMA was used as a positive control. Initial experiments used 2 μM PMA which, according to the

author maximally activated PKC, and a 1 h exposure to magnetic fields at 1.1 mT rms (60 Hz). Subsequent studies were done with a PMA concentration of 50 nM which apparently was sufficient to cause activation of PKC with some 'headroom' left over to detect any increase caused by exposure to the magnetic field for 15 minutes. However, the physiological concentration of PMA for most cells in culture is in the very low nM range (1 to 15 nM), whereas at higher concentrations it is cytotoxic. The assay to detect PKC activity in the cytosol and membranes has been described previously, and controls were used to correct for the radioactivity recovered in the samples. However, the assay was a complicated, multi-step procedure that incorporated at least one separation where the efficiency of recovery was assumed (not measured) and the possibility of differential recoveries for sham and exposed samples can not be ruled out, nor can the possibility of a systematic error. The statistical analysis of the data was not easy to verify from reading the paper. Data was log transformed before being analyzed using a 3-way ANOVA (simple statistical analysis should have sufficed). This suggests the author had to 'reach' for an answer. The author's interpretation of the data is not convincing, and the complexity of the experiment would make it difficult to confirm. Some of the results could also have been confounded by the toxicity of the PMA concentrations used. Results are inconclusive.

Lagroye I and Poncy JL. Influence of 50-Hz magnetic fields and ionizing radiation on c-jun and c-fos oncoproteins. Bioelectromagnetics 1998;19:112-116.

The principal author is with the CEA/DSV/SRCA/DRR-Laboratoire de RadioToxicologie, BP12-F-91680 Bruyores-le Chtel, France.

Abstract: The effect of magnetic fields (50 Hz, 100 μT rms sinusoidal magnetic field combined with a 55 μT geomagnetic-like field) and/or gamma rays of 60 Cobalt on the expression of the c-jun and c-fos proteins was investigated in primary rat tracheal epithelial cells and two related immortalized cell lines. Quite similar patterns and amplitudes of induction of these proteins were evidenced after either ionizing radiation or magnetic field exposure. No synergism after both treatments was observed. These findings suggest that magnetic fields explored in the present study may be considered as an insult at the cellular level.

Comment: One consequence of PKC activation is transcription of *c-jun* and *c-fos* oncogenes, which encode for protein products p39 and p62 respectively, members of the Activity Protein-1 (AP-1) transcription factor family. In normal cells, AP-1 is involved in growth factor-induced cellular proliferation, but its deregulation can lead to cell transformation. The cells used in this study were normal and immortalized rat tracheal epithelial, which have been widely used to study many environmental toxicants. The normal cells were used for only one passage while still in the diploid state, whereas the immortalized cells were aneuploid, and were used for a number of unspecified passages. It is not clear if the fetal bovine serum used in cell cultures was from multiple lots. This study investigated the effects of power frequency fields (104 μT rms (50 Hz)

combined with a geomagnetic-like static magnetic field of 55 μT) on the expression of p39 and p62 oncoproteins. Cells were used for the study on approaching confluence. Treatments included gamma radiation alone, magnetic fields alone, and then radiation followed by magnetic fields. Cells were exposed to magnetic fields for various times up to nine hours. The p39 and p62 oncoproteins were detected using rabbit anti-p39 and p62, visualized with ^{35}S-anti-rabbit-Ig, and quantified by scanning densitometry. Results were expressed as simple ratios of exposed to sham. Gamma rays, or magnetic fields alone, caused a significant increase in p39, as did a combined exposure to both gamma rays and magnetic fields. This study implies magnetic fields act as a tumor promoter in the multistage model of carcinogenesis. The efficiency of the protein extraction procedures was not verified during the assay, and therefore differential extraction of proteins from exposed and sham lysates is a possibility, which could result in a systematic error. This study requires confirmation.

Owen RD. MYC mRNA abundance is unchanged in subcultures of HL60 cells exposed to power-line frequency magnetic fields. Radiat Res 1998;150(1):23-30.

The principal author is with the FDA Center for Devices and Radiological Health, Rockville, Maryland 20850, USA.

Abstract: Epidemiological data have not demonstrated conclusively that there exists an association between exposure to power-line frequency electric and magnetic fields (EMFs) and cancer. Some laboratory studies performed to investigate possible mechanisms for such an association reported biological effects of EMF exposure, but attempts to confirm some such reports have had mixed success. The most publicized experiments in this regard were studies on the purported EMF-induced increase in MYC expression in HL60 cells. To address the accuracy and reproducibility of this effect, HL60 cells were exposed to 6 μT 60 Hz magnetic fields, and MYC expression was measured. Assay methods and exposure conditions were as close as practical to those of the investigators that originally reported a positive effect. A chemical agent was used to demonstrate that the cells were responsive to a known stimulus and that the experimental system was sufficiently sensitive to detect such a stimulus. The experimental system had sufficiently low basal variability to allow the detection of effects of the magnitude that had been reported previously. Using either cells from a commercial source or cells supplied by the original investigators, no evidence was obtained to support the hypothesis that EMF exposure could induce MYC expression.

Comment: This was a confirmation study that failed to find a significant association between exposure to magnetic fields and MYC expression, contrary to the original study.

Pipkin JL, Hinson WG, Young JF, Rowland KL, Shaddock JG, Tolleson WH, Duffy PH and Casciano DA. Induction of stress proteins by electromagnetic fields in cultured HL-60 cells. Bioelectromagnetics 1999;20:347-357.

The principal author is with the National Center for Toxicological Research, Jefferson, Arkansas

Abstract: HL-60 cells in culture were exposed for 2 h to a sinusoidal 0.1 or 1 mT (1 or 10 Gauss) magnetic field at 60 Hz and pulse labeled after exposure with radioactive isotopes by incubation by using either [35S]methionine, [3H]leucine, or [33P]phosphate. The radioactive labels were incorporated into cellular proteins through synthesis or phosphorylation. Proteins were extracted from electrostatically sorted nuclei, and the heat shock/stress proteins (sp) were analyzed for synthesis and phosphorylation by two-dimensional polyacrylamide gel electrophoresis. In the control cultures (no exposure to the magnetic field), sp 72c (cognate form) was faintly observed. A 0.1 mT exposure did not show sp metabolism to be different from that of the controls; however, after a 1 mT exposure of the HL-60 cells, sp 70i (inducible form) was synthesized ([35S]methionine incorporation). Sp 90 was not synthesized at either field level, but was phosphorylated ([33P]phosphate incorporation) in the 1 mT exposure. Sp 27 (isoforms a and b) was induced after a 1 mT exposure as reflected by labeling with [3H]leucine. These sps were not detected after a 0.1 mT exposure. After a 1 mT exposure and labeling with [33P], sp 27 isoforms b and c were phosphorylated whereas isoform a was not observed. Sps 70i, 72c, and 90 were identified by commercial sp antibodies. Likewise, polypeptides a, b, and c were verified as sp 27 isoforms by Western blotting. Statistical evaluation of sp areas and densities, determined from fluorographs by Western-blot analysis, revealed a significant increase in sps 90 and 27a after a 1 mT magnetic field exposure. The 1 mT magnetic field interacts at the cellular level to induce a variety of sp species.

Comment: The exposure system, calibration and quality assurance protocols were satisfactory. Sham and exposure protocols were adequate. Temperature control was not an issue. The analyst was blind to exposure status. The lab had extensive experience with heat shock biology. This study found that a brief exposure of HL60 cells to 1 mT magnetic field (but not 0.1 mT) induced heat shock proteins. The assay procedure was complex and multi-step involving a number of manipulations and separation steps that assumed the efficiencies of product recovery was equal for sham and exposed samples. Without proper quantitative recoveries, the possibility of systematic error exists. Some of the data processing routines were available only in the author's laboratory, making the results of this study difficult to confirm. This study suggests magnetic fields could be a general stressor, with an effective threshold at ~ 0.1 mT. Confirmation studies are required using simpler methodologies.

Loberg LI, Gauger JR, Buthod JL, Engdahl WR and McCormick DL. Gene expression in human breast epithelial cells exposed to 60 Hz magnetic fields. Carcinogenesis 1999;20(8):1633-1636.

The principal author is with the Experimental Toxicology and Carcinogenesis Division, Microbiology and Immunology Division and Electronics and Electromagnetics Section, IIT Research Institute, Chicago, Illinois 60616, USA.

Abstract: Epidemiology suggests a possible relationship between exposure to power frequency magnetic fields (EMF) and breast cancer. One mechanism through which EMF could stimulate breast cancer induction is via altered expression of oncogenes and/or tumor suppressor genes that regulate normal and neoplastic growth. To evaluate the hypothesis that EMF action in the breast is mediated by alterations in gene expression, transcript levels of c-myc and a battery of other cancer-associated genes were quantitated in human breast epithelial cells exposed to pure, linearly polarized 60 Hz EMF with low harmonic distortion. HBL-100 cells and normal (non-transformed) human mammary epithelial cells were exposed to EMF flux densities of 0.1, 1.0 and 10.0 Gauss (G) for periods ranging from 20 min to 24 h; concurrent sham controls were exposed to ambient fields (<0.001 G) only. Gene expression was quantitated using ribonuclease protection assays. EMF exposure had no statistically significant effect on basal levels of c-myc transcripts in either human breast cell model, and had no effect on alterations in c-myc expression induced by 12-O-tetradecanoylphorbol-13-acetate. Transcript levels of c-erbB-2, p53, p21, GADD45, bax, bcl-x, mcl-1, and c-foswere also unaffected by EMF exposure. These results suggest that EMF is unlikely to influence breast cancer induction through a mechanism involving altered expression of these genes.

Comment: The object was to evaluate the hypothesis that magnetic fields alter gene expression in breast epithelial cells. The hypothesis was tested by measuring transcript levels of a number of cancer-related genes in two human breast epithelial cell models (HBL-100, a transformed cell line and HME cells from normal breast epithelia) in response to magnetic fields (1 mT or 0.01 mT) or sham exposures. The exposure system, its calibration and quality assurance protocols were adequate. Cell culture conditions were satisfactory and the maximum cell passage number was specified for HME cells. Sham and exposures were done concurrently and the analyst was blind to exposure status of the samples. Total cellular RNA was isolated from samples and gene expression was determined by the ribonuclease protection assay, using available commercial kits. RNA-probe hybrids were separated by polyacrylamide gel electrophoresis, visualized by autoradiography and quantitated by scintillation counting of bands excised from the gel or by densitometric analysis of the autoradiographs. The ratio of expression of each gene was normalized to GAPDH (a house keeping gene used as an internal control). The magnetic field had no statistically significant effects on the basal expression of MYC in HBL-100 cells. However, there was no positive controls to demonstrate a positive or negative effect on expression. In a second experiment, the co-promoting effects of magnetic fields on the activation of MYC by phorbol myristate acetate (PMA) activity was examined. Within one hour, PMA alone induces a 3- to 6-fold increase in MYC expression in HBL-100 cells, with return to baseline at 24 h. Co-exposure of these cells to magnetic fields and PMA did not alter this pattern. In HME

(normal cells), exposure to PMA decreased MYC expression, possibly due to cytotoxicity. Exposure to magnetic fields did not affect transcript levels of other genes, but two exceptions were noted, at 1 mT; bax expression was increased 25% in HBL-100 cells and at 0.01 mT a 32% increase was seen in FOS expression, but not at 1 mT. With multiple comparisons, some false positives can be expected. The authors suggest the increase in bax expression was statistically significant but without biological relevance. This study confirms the findings of Owen, as reviewed above for MYC expression.

Loberg LI, Engdahl WR, Gauger JR, McCormick DL. Expression of cancer-related genes in human cells exposed to 60 Hz magnetic fields. Radiat Res 2000(c);153(5 Pt 2):679-684.

The principal author is with the Experimental Toxicology and Carcinogenesis Division, IIT Research Institute, Chicago, Illinois 60616, USA.

Abstract: Exposure to 60 Hz magnetic fields (MFs) may be a risk factor for human cancer. One mechanism through which MFs could influence neoplastic development is through alterations in the expression of cancer-related genes. Previous molecular studies of the action of MFs have measured effects on a limited number of genes. In the present studies, arrays containing cDNAs for 588 cancer-related genes were used to approach the hypothesis that the biological activity of MFsis mediated by alterations in gene expression. Cultures of normal (HME) and transformed (HBL-100) human mammary epithelial cells and human promyelocytic leukemia (HL60) cells were exposed to MFs at field strengths of 0, 0.01 or 1.0 mT for 24 h. Several genes were identified in MF-exposed cells whose expression was increased by at least twofold or decreased by 50% or more. However, no gene was found to be differentially expressed in each of three independent exposures for any cell type, and no relationship between exposure intensity and differential gene expression was found. These studies failed to identify a plausible genetic target for the action of MFs in human cells, and they provide no support for the hypothesis that MF exposure alters the expression of genes that are involved in cancer development.

Comment: To provide a more global evaluation of the hypothesis that exposure to magnetic fields alter gene expression, cDNA arrays were used to screen human cells for magnetic field-induced changes in the expression profiles of several hundred cancer-related genes. Gene expression profiles were generated using HL60, HBL-100 (transformed breast epithelial cells) and HME (normal human mammary epithelial cells).The exposure system was the same as that described in previous studies and the experimental design was adequate. For each cell type, three independent exposures to sham, 0.1 and 1.0 mT magnetic fields were made. Gene expression profiling was done using commercially available kits and analytical equipment. Two membranes were compared simultaneously, both visually and by densitometry (one membrane with RNA isolated from sham and the other with RNA isolated from MF-exposed cells). The genes included on the array were organized into 13 functional groups, housekeeping genes,

negative controls, blanks, and genomic DNA spots as orientation markers. Phorbol myristate acetate (PMA) was used as positive control (several genes are known to be up- or down-regulated by PMA). An *a priori* strategy was formulated to determine whether a change in gene expression had occurred (two-fold increase or a 50% decrease). Of the 588 cancer-related genes that were examined, magnetic field exposure did not result in any reproducible pattern of differential gene expression when compared with sham-exposed cells in three replications. This study was well done and it supports the expanding body of knowledge that indicates magnetic fields do not alter gene expression.

Nakasono S and Saiki H. Effect of ELF magnetic fields on protein synthesis in Escherichia coli K12. Radiat Res 2000;154(2):208-216.

The principal author is with the Bio-Science Department, Abiko Research Laboratory, Central Research Institute of Electric Power Industry, 1646 Abiko, Abiko-City, Chiba 270-1194, Japan.

Abstract: Escherichia coli K12 was used as a model system to determine whether ELF magnetic fields (MFs) are a general stress factor. The cells were exposed to ELF MFs (5-100 Hz) at a maximum intensity of 14 mT r.m.s. for circularly polarized MFs and 10 mT r.m.s. for vertically polarized MFs. The response of the cells to the MFs was estimated from the change in protein synthesis by using 2D PAGE. Approximately 1,000 proteins were separated on the 2D gels. The stress-responsive proteins such as CH10, DNAK, CH60, RECA, USPA, K6P1 and SODM were identified from the SWISS-2D PAGE database on the 2D gels. These proteins respond to most stress factors, including temperature change, chemical compounds, heavy metals, and nutrients. When the bacterial cells were exposed to each MF at 5 -100 Hz under aerobic conditions (6.5 h) or at 50 Hz under anaerobic conditions (16 h) at the maximum intensity (7.8 to 14 mT r.m.s.), no reproducible changes were observed in the 2D gels. Changes in protein synthesis were detected by 2D PAGE with exposure to heat shock (50 degrees C for 30 min) or under anaerobic conditions (no bubbling for 16 h). Increases in the levels of synthesis of the stress proteins were observed in heat-shocked cells (CH60, CH10, HTPG, DNAK, HSLV, IBPA and some unidentified proteins) and in cells grown under anaerobic conditions (DNAK, PFLB, RECA, USPA and many unidentified proteins). These results suggest that 2D PAGE is sufficient to detect cell responses to environmental stress. The high-intensity ELF MFs (14 mT at power frequency) did not act as a general stress factor.

Comment: This study examined the effect of a 14 mT (50 Hz) magnetic fields on mutation rates in E.Coli K12 cells. The exposure system was the same one described in another study by Nakasono on magnetic fields and mutation rates in bacterial tester strains. This is a well documented assay with acceptable sensitivity and specificity for identifying stress-related changes in gene expression. The protein end products of mutated gene expression was detected by 2-dimensional (qualitative) PAGE. Magnetic

fields did not produce any changes in gene expression under the experimental conditions. The magnetic field was not considered as a general stressor in this assay. This study adds support to the findings of Loberg (2000c).

Morehouse CA and Owen RD. Exposure of Daudi cells to low-frequency magnetic fields does not elevate MYC steady-state mRNA levels. Radiat Res 2000;153(5 Pt 2):663-669.

The principal author is with the FDA Center for Devices and Radiological Health, Rockville, Maryland 20850, USA.

Abstract: The effect of extremely low-frequency electromagnetic field (ELF EMF) exposures to human health has been widely debated. Epidemiological studies have found a possible correlation between increased cancer incidence and environmental ELF EMF exposures. Results from in vitro studies performed to examine the possible underlying bioeffects of ELF EMFs have varied greatly. Reported effects range from robust and reproducible effects to undetectable. In this study, Daudi cells were exposed to 60 Hz magnetic fields for 20, 40 or 60 min at flux densities of 12.5, 50, 100 or 500 microT. Exposures were performed in the Regional ELF-EMF Exposure Facility (Rockville, MD) to minimize variables that might contribute to a false positive effect. Exposures included sham/sham, exposed/sham or sham/exposed, and were performed with blinding with respect to type of exposure. 12-O-Tetradecanoylphorbol-13-acetate (TPA) treatment was used as a positive control. Total cellular RNA was isolated using a single-step technique. Human MYC expression was measured by northern blot hybridization as an indicator of the responsiveness of Daudi cells to experimental conditions. Beta-2-microglobulin (B2M) expression was measured simultaneously as an internal control. Exposure to a 60 Hz magnetic field did not significantly alter MYC expression in Daudi cells under any of the exposure conditions.

Comment: The exposure system, calibration, quality assurance, sham/magnetic field exposure, assay protocols were all satisfactory. This is a confirmation of a study by Saffer (1995) that found magnetic fields did not did not affect MYC expression in HL60 or Daudi cells. The study by Morehouse and Owen used northern blot hybridization to measure responsiveness in Daudi cells whereas Saffer used differential display PCR. The assay was well characterized and used both positive and negative controls. Morehouse and Owen confirmed the Saffer's results and found the magnetic field did not modulate the expression of MYC. This weakens the hypothesis that magnetic fields may affect cancer by modulating gene expression. However, failure to find an effect in HL60 or Daudi cells does not shed light on the capability of magnetic fields to contribute to neoplastic transformation, since these cells are already transformed. To overcome this shortcoming Saffer used JB6 cells which are initiated but not transformed. In a comprehensive study Saffer (1997) failed to find any affect of magnetic fields on MYC expression. This once again weakens the hypothesis that magnetic fields exert their

action by modulating gene expression. The argument however continues as other cancer-related genes could be involved.

Zhou J, Li C, Yao G, Chiang H and Chang Z. Gene expression of cytokine receptors in HL60 cells exposed to a 50 Hz magnetic field. Bioelectromagnetics 2002;23(5):339-346.

The principal author is with the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, People's Republic of China.

Abstract: The effects of a 50 Hz extremely low frequency (ELF) sinusoidal magnetic field (MF) on the expression of genes relating to cytokine receptors were studied in HL60 cells. Transcription levels of tumor necrosis factor receptor (TNFR) p55 and p75, interleukin-6 receptor-alpha (IL-6Ralpha) and transforming growth factor-beta receptor 1 (TGFbetaR1) were quantified in cells exposed to an intensity of 0.1 or 0.8 mT for periods ranging from 30 min to 72 h. Cells treated with 10 nM of phorbol 12-myristate 13-acetate (PMA) for 8 h served as a positive control. Gene expression values were assessed by the ribonuclease protection assay (RPA) and normalized to those of the noninducible gene GAPDH. The results showed that MF exposure at 0.1 and 0.8 mT for 72 h increased TNFR p75 and IL-6Ralpha mRNA expression in HL60 cells. No significant change in gene expression levels of TNFR p55 and TGFbetaR1 was observed under any of the exposure conditions. In addition, we report here for the first time that IL-6Ralpha mRNA expression can be suppressed by PMA in HL60 cells.

Comment: The exposure system was adequate, as were calibration and quality assurance protocols. However, the treatment of sham and exposed samples were not equivalent, and the number of sham exposed samples were inadequate. Temperature control was not a problem. The analyst was blind to exposure status, but the number of sham exposures used in the experiment were inadequate. Statistical analysis, and cell culture conditions were all acceptable. Phorbol myristate acetate (PMA) 10 nM was used as positive control. The study examined the effect of magnetic fields on the expression of genes relating to cytokine receptors in HL60 cells. Transcription levels of tumor necrosis factor receptor (TNFRp55 and TNFRp75), interleukin-6 receptor α (IL-6Rα) and transforming growth factor βR1 (TGFβR1) were quantified in cells exposed to 0.1 or 0.8 mT (60 Hz) magnetic fields for 0.5 to 72 h. Gene expression values were assessed by a commercially available ribonuclease protection assay (RPA) kit and values were normalized to those of the non-inducible gene GAPDH (used as an internal standard). All extractions and laboratory manipulations were done using a commercial kits, or widely available reagents of known quality. At 72 h, exposure to magnetic fields at 0.8 mT increased TNFRp75 and IL-6Rα mRNA expression by more than two-fold, but TNFRp55 and TGFβR1 were not increased. At times less than 72 h gene expression was not increased by the magnetic field exposure. At 0.1 mT, the expression of the two genes was increased less than two-fold. TNFRp75 are implicated in apoptosis and the

activation nuclear factor kappa B (NfκB). IL-6Rα is involved in regulation of cell differentiation and proliferation. The difference between the results found by Zhou, and those of Morehouse and Owens (2000) and Loberg (2000c), is in the length of time the cells were exposed to the magnetic fields (72 h for Zhou and only 24 h exposure in the other studies). However, the results of this study must be considered as inconclusive as sham exposures were inadequate. This experiment should be repeated after deficiencies in the sham have been addressed.

Rao R, Halperb J and Kisaalita WS. Effects of 60 Hz electromagnetic field exposure on APP695 transcription levels in differentiating human neuroblastoma cells. Bioelectrochemistry 2002;57(1):9-15.

The principal author is with the Cellular Bioengineering Laboratory, Biological and Agricultural Engineering Department, University of Georgia, Athens, GA 30602, USA.

Abstract: Epidemiological studies have suggested that workers with primary occupation that are likely to have resulted in the medium-to-high extremely low frequency (ELF) electromagnetic field (EMF) exposure are at increased risk of Alzheimer's disease (AD) pathogenesis. As a first step in investigating the possibility of an association between the ELF-EMF exposure and AD at the cellular level, we have used the differentiating IMR-32 neuroblastoma cells. In double-blind experiments, IMR-32 cells were exposed to the magnetic field intensities of 50, 100, and 200 μT at a frequency of 60 Hz for a period of 4 h at the three ages of differentiation (2, 10, and 16 days after incubation in differentiation medium). We used a custom-made Helmholtz coil setup driven by a 60 Hz sinusoidal signal from a function generator and an in-house built power amplifier. Total RNA extracted from the exposed cells was separated by the agarose gel electrophoresis and transferred to a nylon membrane for the northern hybridization. Digoxygenin-labeled APP695 RNA probes were used to detect changes in the APP695 mRNA levels in response to the ELF-EMF exposure. The results reported herein provided no support for any relationship between the APP695 gene transcription and IMR-32 differentiation age, as well as the magnetic field exposure. This study constitutes the first step towards investigating the possibility of an association between the ELF-EMF exposure and AD manifestations at the cellular level.

Comment: This study examined the effect of magnetic fields on the expression of the APP695 gene in a differentiating neuroblastoma cell line designated IMR-32. The gene APP695 is related to Alzheimer’s disease, and expresses amyloid precursor protein (or presenilin). The protein concentrates mainly in the brain, and has a receptor-like structure with extracellular, single transmembrane and cytoplasmic domains. The exposure system was acceptable, as were all the related protocols. The experimental design was also acceptable. The assay used to detect gene transcription was commercially available, and had an acceptable level of sensitivity. The magnetic field was applied to cells at various stages of differentiation, and no affect on APP695

transcription was observed. This study confirms those of Loberg, and of Morehouse and Owen that find short-term exposure (< 24 h) does not affect gene expression.

Yomori H, Yasunaga K, Takahashi C, Tanaka A, Takashima S and Sekijima M. Elliptically polarized magnetic fields do not alter immediate early response genes expression levels in human glioblastoma cells. Bioelectromagnetics 2002;23(2):89-96.

The principal author is with the Technical Research Center, the Kansai Electric Power Company, Inc., Hyogo, Japan.

Abstract: Expression of immediate early response genes such as c-fos, c-jun, and c-myc in response to 1-500 μT resultant ®) 60 Hz elliptically polarized (EP) magnetic fields (MFs), typical of environmental MFs polarization under overhead power lines, was analyzed in both at transcriptional and translational levels using human glioblastoma (T98G) cells. Pseudo synchronized T98G cells at G1 phase were exposed to EP-MFs (1, 20, 100, and 500 μT) for up to 3 h, but produced no statistical difference (P>0.05) in the levels of expression ratio at both the transcriptional and translational levels at 30 min for c-fos and c-jun and at 180 min for c-myc after serum stimulation. In addition, exposure of T98G cells to linearly (vertical and horizontal) and/or circularly polarized MFs (500 μT) produced no significant change (P>0.05) in the expression ratio at both transcriptional and post-transcriptional levels. Thus, there was no evidence that linearly or rotating polarized MFs enhanced early response gene expression in these studies. These results suggest that environmental MFs at 1-500 μT flux density are unlikely to induce carcinogenesis through a mechanism involving altered expression of the immediate early response genes.

Comment: The exposure system, calibration and quality assurance protocols were acceptable. The magnetic field was elliptically polarized to simulate an exposure in the vicinity of a 3-phase overhead power transmission line. This is in contrast to the studies of Loberg and Morehouse that used linearly polarized fields. Unlike most other studies on gene expression, Yomori examined the effect of magnetic fields at the levels of transcription and translation. Cells were exposed for periods of 0.5, 1,2 and 3 hours to magnetic fields ranging from 1 to 500 μT (60Hz). Preliminary studies were done to determine the time of maximum gene expression. The T98G cells were synchronized in G1 by serum deprivation, and were exposed to sham or magnetic fields after being stimulated by the addition of fetal bovine serum. Transcription was measured by Northern blots and translation by Western blots. All results were normalized to the 28S rRNA level as an internal standard. PMA was used as the positive control. The experimental design used commercially available kits and probes and all analyses were done using commercially available hardware and software. The statistical analysis of data was acceptable. Exposure to magnetic fields for up to three hours did not affect the expression of FOS, JUN or MYC at the level of transcription or translation.

10.4 Gap Junctions

Table 22. Gap junctions summary

Author	Date	End Point	Response?	Relevance?	Weakness
Li	1999	GJIC + MF GJIC+MF+PMA	No Yes	Inconclusive Inconclusive	SSS; SA SSS; SA
Griffin	2000	GJIC	No	Inconclusive	SSS; SA
Hu	2001	protein phos.	Inconclusive	Inconclusive	ED;SSS;SA

Abbreviations.
ED = (possible) Experimental Design shortcomings
GJIC = Gap Junctional Intercellular Communications.
+MF = in presence of Magnetic Field
+PMA = in the presence of Phorbol Myristate Acetate
Protein Phos = Protein Phosphorylation
SA = Subjective Assay
SSS = Small Sample Size

Individual Study Summaries

Li CM, Chiang H, Fu YD, Shao BJ, Shi JR and Yao GD. Effects of 50 Hz magnetic fields on gap junctional intercellular communication. Bioelectromagnetics 1999;(20):290-294.

The principal author is with the Microwave Institute, Zhejiang Medical University, Hangzhou, People's Republic of China.

Abstract: To explore whether the extremely low frequency (ELF) electromagnetic fields (EMFs) may act as cancer promoters or be synergistic with 12-O-tetradecanoylphorbol-13-acetate (TPA) in cancer promotion, an experiment was conducted on the effects of 50 Hz magnetic fields (MFs) on gap junctional intercellular communication (GJIC) of Chinese hamster lung (CHL) cells. Lucifer dye was loaded into CHL cells by iontophoretic injection, and the number of dye-coupled cells (DCC) 5 min after the injection was adopted as the index of GJIC. The effects of TPA at different concentrations and magnetic fields at different intensities, combined with 5 ng/ml TPA, were studied. The results showed that the suppression of TPA on GJIC was dependent on TPA concentration; the threshold concentration of TPA for CHL cells was between 1 and 5 ng/ml. After exposure to 0.8 mT magnetic field for 24 h, the number of DCC decreased to 6.08 ± 1.59, whereas the number of DCC in the control group was 9.84 ± 2.27 ($P < .05$). When the cells were exposed at 0.2, 0.4, and 0.8 mT for 24 h, combined with 5 ng/ml TPA treatment during the last 1 h, the number of DCC decreased to 5.52 ± 1.53, 5.00 ± 1.22, and 4.00 ± 1.29, respectively, which were significantly lower than the

values for the group treated with 5 ng/ml TPA alone (6.38 ± 1.39). It is suggested that certain intensities of 50 Hz magnetic field might act as cancer promoters, be additive with other promoters in cancer promotion, or both.

Comment: The exposure system was acceptable, capable of generating a uniform power frequency magnetic field of up to 1 mT. The system also incorporated a static field of 18.5 µT (14.1 µT horizontal and 12.0 µT vertical components). Calibration and quality assurance protocols were satisfactory. Temperature control was not an issue. Sham and exposure status of samples were blinded. Cell culture protocol was acceptable, but no mention was made of the number of cell passages used in the experiments. Assay for GJIC was by dye transfer. Dye was micro-injected into the cells iontophoretically by a negative pulse current (10 Hz 100 nA, 50 ms) for 2 - 4 s . The number of dye-coupled cells (DCC) was assessed by inverted fluorescent microscope for up to one hour after termination of exposures. The statistical protocol to assess differences between all treatments was acceptable. The DCC per injection was used as an index of GJC, and two to three cells per dish were microinjected. PMA was used as a positive control (5 ng/mL added to cultures one hour before the end of a 24 h exposure to sham or magnetic fields). Preliminary experiments were used to establish the cell's response to various doses of PMA. When cells were sham or exposed to magnetic fields alone, no effect was observed on GJC at 0.2, 0.4 mT, but at 0.8 mT, the number of DCC/injection was significantly reduced. When PMA was added at the last h of exposure, 0.2, 0.4 and 0.8 mT all acted synergistically with the PMA to reduce GJIC even more. The main weakness with this study is the reliance on subjective, analyst- dependent analyses for data collection, which makes replication difficult.

Griffin GD, Khalaf W, Hayden KE, Miller EJ, Dowray VR, Creekmore AL, Carruthers CW Jr, Williams MW and Gailey PC. Power frequency magnetic field exposure and gap junctional communication in Clone 9 cells. Bioelectrochemistry 2000;51(2):117-123.

The principal author is with the Department of Energy, Oak Ridge National Laboratory, Life Sciences (GDG, EGM, MWW) and Energy (PCG) Divisions, P.O. Box 2008, MS-6101 Oak Ridge, TN 37831-6101, USA.

Abstract: Exposure to a power-frequency magnetic field has been reported to produce a statistically significant inhibition of gap junctional communication (GJC) in Clone 9 cells that have been pre-stressed by treatment with low concentrations of chloral hydrate (CH) [C.F. Blackman, J.P. Blanchard, S.G. Benane, D.E. House, J.A. Elder, Double blind test of magnetic field effects on neurite outgrowth, Bioelectromagnetics, 19 (1998) 204-209]. This observation might provide mechanistic insight into the possible role of electromagnetic fields (EMFs) in the carcinogenic process, since cancer cells frequently show decreased or absent GJC, and tumor promoting chemicals have been observed to inhibit GJC. Magnetic field exposure conditions were 45 Hz, 23.8 µT rms+parallel DC 36.6 µT, for 30 min of exposure. The responses of Clone 9 cells to the

GJC-inhibiting effects of the tumor promoter 12-O-tetradecanoylphorbol 13-acetate and the chemical CH were evaluated and compared to reported results [S.G. Benane, C.F. Blackman, D.E. House, Effects of perchloroethylene and its metabolites on intercellular communication in Clone 9 rat liver cells, J. Toxicol. Environ. Health, 48 (1996) 427-437]. Before magnetic field exposure, cells were exposed for 24 h to either 3 (nine experiments) or 5 mM (11 experiments) CH to produce GJC of 67% or 50%, respectively, relative to unexposed controls. GJC was assessed microscopically using the scrape-loading technique and a blinded protocol. No statistically significant effect was observed due to magnetic field exposure with either CH concentration.

Comment: Gap junctions (GJ) are controlled pores, formed by specialized proteins called connexins located in the plasma membrane, which mediate the intercellular transfer of small molecules and ions. Experimental evidence implicate gap junctional intercellular communications (GJIC) in cellular differentiation and proliferation. The importance of gap junctions to cell proliferation has been implied by observations that (i) they are absence or reduced numbers in transformed cells, (ii) tumor promoters such as phorbol myristate acetate (PMA) reduce GJIC, and (iii) their functionality is impaired by the expression of certain oncogenes. In multistage carcinogenesis, the inhibition of GJIC is considered an important event in tumor promotion. The experiments by Griffin was to verify an abstract by Blackman that GJIC was inhibited in clone 9 cells (from rat liver) by exposure to magnetic fields. These cells are commercially available (Blackman, 1996). The exposure system used by Griffin was a duplicate of the one described by Blackman (Blackman, 1993). The exposure system consisted of Helmholtz coils. Construction and calibration were satisfactory, and quality assurance, sham exposure, and quality assurance protocols were all satisfactory. The magnetic field, like Blackman's, consisted of an AC field at 23.8 μT rms (45 Hz), and a parallel DC field of 36.6 μT. Temperature control was not a problem. All experiments were conducted blind to exposure status of the cells. Cell culture conditions were standard. Chloral hydrate (CH) and PMA which are known to modulate GJC, were used as controls (preliminary experiments had been conducted to characterize the clone 9's response to CH and PMA). Cells were treated with CH for 24 h and then exposed to sham or magnetic fields for 30 minutes. CH incubator controls were used throughout these experiments. The integrity of GJC was assessed microscopically by a subjective method that involved the migration distance of a fluorescent dye (Lucifer Yellow) through a monolayer of cells. The analyst was blinded to exposure status of the cells. The magnetic field did not modulate GJC under any of the assay conditions. Reasons, given by Griffin, for the failure to reproduce Blackman's results were (i) the lots of FBS were different, (ii) the dose response of clone 9 cells in the two labs were the same for PMA, but they were different for CH. This difference could be due to subtle differences in the two lots of cells (supplied by ATCC) or to the chemical purity of the CH, and (iii) subtle differences in seeding density and culture conditions. The effect of magnetic fields on gap junctional communications, under these laboratory conditions, has yet to be resolved.

Hu GL, Chiang H, Zeng QL and Fu YD. ELF magnetic field inhibits gap junctional intercellular communication and induces hyperphosphorylation of connexin43 in NIH3T3 cells. Bioelectromagnetics 2001;22(8):568-573.

The principal author is with the Microwave Laboratory, Zhejiang University School of Medicine, Hangzhou 310031, People's Republic of China.

Abstract: The effects of extremely low frequency (ELF) magnetic field on gap junctional intercellular communication (GJIC), protein levels, and phosphorylation of connexin43 (Cx43) were studied in NIH3T3 cells. The suppression of GJIC by 24 h, 50 Hz, 0.8 mT ELF magnetic field, 2 h, 3 ng/ml 12-O-tetradecanoylphorbol-13-acetate (TPA), or ELF combined with TPA treatment was confirmed by the fluorescence recovery after photobleaching (FRAP) analysis with a confocal microscope. The results showed that ELF or TPA exposure induced 50-60% inhibition of GJIC ($P < 0.01$). ELF combined with TPA enhanced the inhibition of GJIC. Western blot analysis using Cx43 specific antibodies showed obviously decreasing non phosphorylated Cx43 (P(0)) induced by ELF and/or TPA exposure. On the other hand, cells treated with ELF and/or TPA displayed a hyperphosphorylated Cx43 band (P(3)). However, there was no obvious changes in the level of Cx43 protein. The results implied that the P(3) band appeared to result from phosphorylation of P(0). But it remains possible that upon the ELF exposure P(0) is converted to P(1), P(2) or both and that P(3) is formed from P(1) or P(2) resulting in the observed hyperphosphorylation pattern. From the present study, we conclude that ELF magnetic field inhibits GJIC and the main mechanism is the hyperphosphorylation of Cx43.

Comment: This study examined the effect of power frequency magnetic fields on GJIC, protein levels, and the phosphorylation of connexin 43 (CX43), the main protein component of gap junctions in 3T3 cells. Phosphorylation of CX43 in 3T3 cells is the proposed mechanism by which GJC is suppressed. Preliminary studies demonstrated that magnetic field-induced suppression of GJC in CHL cells was inhibited by co-exposure with 10 nM of staurosporine (STS) or 10 µM of palmitoyl carnitine, two inhibitors of protein kinase C (PKC). Therefore, it was postulated that activation of PKC might mediate the phosphorylation of CX43, thereby shutting down GJC. The exposure system used in this study was the same one described by Li (1999) and was full acceptable, as were all protocols involved in its calibration and quality assurance. Cell culture procedures were satisfactory. The analyst was blinded to the exposure status of samples. Samples were exposed to sham or fields for 24 hours, and then to PMA during the last 2 hours of treatment. PMA was the positive control. All samples were assessed within one hour of terminating the exposure. GJC was measured manually by fluorescence recovery after photobleaching (FRAP), using a laser scanning confocal microscope. Less than 25 cells was scanned for each exposure category. This is a subjective, operator dependent assay, which together with the small sample size makes the reliability of the results questionable. The second part of the study examined the phosphorylation of CX43 in response to magnetic fields, in the absence and presence of

PMA. This was done using commercially available western blot technology, using commercially available monoclonal antibodies to detect unphosphorylated CX43. Commercially available secondary polyclonal chemiluminescence labelled antibodies were used to detect phosphorylated and phosphorylated CS43. The results indicate magnetic fields and PMA, or magnetic fields and PMA cause the concentration unphosphorylated CX43 to decrease. The secondary antibody recognizes both phosphorylated and unphosphorylated forms of CX43, results show (not convincingly) that the unphosphorylated CX43 decreased as the phosphorylated form increased. Data was acquired on commercially available chemiluminescence detector and analyzed by commercially available software. The assay was complicated and involved, requiring many separation steps, and the possibility of systematic error can not be entirely ruled out.

10.5 Signal Transduction

Table 23. Signal transduction summary

Author	Date	Endpoint	Response	Weakness
Dibirdik	Feb. 1998	ST	Yes	ED
Kristupaitis	May 1998	ST	Yes	ED
Miller	Dec. 1998	ST	No	None Apparent
Woods	Nov 2000	ST	No	None Apparent
Dibirdik	Dec 2000	ST	Yes	ED
Lindstrom	2001	ST	Yes	**

Abbreviations:
ED = (possible) Experimental Design shortcomings
NA = Not Available
ST = Signal Transduction
** pending communication from author

The next six studies will be discussed as a group, because they illustrate the difficulty of independent replication of positive findings allegedly produced by exposure to power-frequency fields (PFF). The original finding was published in 1995 and reported PFF could affect signal transduction in a biochemical pathway that was linked to proliferation and differentiation of B-lymphoid cells (Uckun 1995). Specifically Uckun and co-workers found that PFF stimulated a nine- and three-fold increases in Lyn and Syk kinases, a response that resulted in the marked increase in tyrosine phosphorylation of a number of protein substrates and a 2-fold increase in protein kinase C (PKC). By using mutant

cell constructs, the authors concluded activation of Lyn kinase was sufficient and necessary for PFF-induced cell signaling.

B-cell signaling pathways are mediated by the B-cell antigen receptor (BCR), which is expressed on the surface of immunocompetent B-cells. After engagement of BCR by an appropriate ligand, the first signal detected is the increased phosphorylation of tyrosine residues on multiple protein substrates. This involves recruitment of three families of non-receptor kinases: Src, including Lyn, the Syk family, and the Tec family such as Bruton's tyrosine kinase (BTK). Activation of the BCR culminates in B-cell proliferation.

The group in defense of these positive results consisted of the original investigator, Uckun, and his colleagues [Dibirdik (1998, 2000), Kristupaitis (1998) and Lindstrom (2001)]. The groups attempting replication were investigators from two independent laboratories, Miller (1998) in the USA and Woods (2000) in the UK. At issue was the claim that exposure of B-cells to PFF caused the phosphorylation of tyrosine residues of protein kinases LYN, SYK and BTK (Bruton's tyrosine kinase). This event triggered, in rapid sequence, the activation of phospholipase C-gamma 2 (PLCγ2), an increase in inositol triphosphate (IP3) turnover and the subsequent activation of protein kinase C (PKC), an enzyme linked to tumor promotion. Since phorbol myristate acetate (PMA), a well known tumor promoter, also activated PKC, then, by analogy, the link between magnetic fields and carcinogenesis was established.

In 1995, Uckun, reported that magnetic fields stimulated LYN protein, a protein tyrosine kinase of the Src family. Dibidik (1998) replicated and extended these findings in another B-cell system, the DT-40 chicken lymphoma B-cell model. In addition to wild-type DT-40 cells, a number of DT-40 cell constructs were used, including one deficient in PLCγ2, and a PLCγ2 deficient cell reconstituted with a Src domain that is essential for the PLCγ2-induced inositol triphosphate turnover. This study replicated the original observation and established the role of PLCγ2 as a key event in the activation of PKC. In the Dibirdik (1998) study, cells were exposed to 100 μT (60 Hz) in a Helmholtz coil for various times up to 5 minutes. The exposure system did not provide for true sham exposures. It is not clear from the manuscript whether the analysts were blinded to the exposure status of samples, or how the cell passage numbers differed between experiments. Analysis of IP3 was by a commercially available highly sensitive radioligand binding assay. In some experiments negative (genistein or herbimycin) and positive controls (anti-chicken IgM monoclonal antibody) were used. Following Dibirdik, Kristupaitis refined the mechanism by which magnetic fields exerted their biochemical effects. In this study, Bruton's tyrosine kinase (BTK) was identified as the end kinase that acted downstream from the LYN kinase and immediately upstream from PLCγ2. Thus, the sequence of events were purported to be, the magnetic field activation of LYN kinase, which in rapid succession activated SYK, BTK, PLCγ2 and IP3 turnover, and then PKC.

One month after the report by Kristupaitis, Miller (1998) published an attempted replication, using cells and cell constructs from Dibirdik's laboratory. Miller improved on the experimental design by using a duplicate exposure system so sham exposures could be carried out properly. In addition, the analysts were blind to the exposure status of samples. As far as possible procedures were duplicated and pervanadate was introduced as another positive control. In the end, Miller found that magnetic fields produced no change in any of the parameters studied.

Woods (2000) published another attempt to replicate the results of Uckun (1995), this time, in addition to DT-40 cells, a human pre-B cell line, designated as Nalm-6 was also used. The design of the exposure system was improved, calibration was thorough, and quality assurance protocols were improved. The exposure protocol included the capacity to do proper sham exposures, and the analysts were blind to the exposure status of samples. Protocols were also implemented to eliminate possible incubator bias. Temperature control during the exposures was not a problem. Experimental protocols were established with the cooperation of Uckum and colleagues, but also incorporated a number of improvements. All assays were carried out with commercially available kits or products. The statistical analysis was satisfactory and the p value was adjusted for multiple comparisons. The magnetic field did not induce activation of Btk or increase turnover of IP3 in DT-40 cells or Nalm-6 cells. In short, the study of Uckun could not be independently replicated a second time.

Lindstrom in Sweden also attempted to confirm (not replicate) Uckun's study using Jurkatt cells instead of DT40 cells (Lindstrom, 2001). Lindstrom laboratory is connected to Uckun's through Dr. Luben. Once again the study was conducted satisfactorily, this time with a minimum of potential flaws in the experimental design and once again magnetic fields were found to stimulate phosphoylation of a Src kinase, this time p56lck.

The fact that the original observations of Uckun can not be independently replicated by two laboratories that were actively engaged in signal transduction research means either: (i) the experimental condition(s) in Uckun's laboratory were so subtle that they could not be independently reproduced in other laboratories, (ii) systematic errors occurred in both laboratories trying to independently replicate Uckun's results, or (iii) operator bias present in Uckun's laboratory generated a false positive. The difficulty of independent replication is describe by Boorman (2000) (see Appendix-Epigenetic modes of action). Support for magnetic fields as a tumor promoter is weakened by the failure of two independent laboratories to replicate the studies of Uckun and colleagues.

Individual Study Summaries

Dibirdik I, Kristupaitis D, Kurosaki T, Tuel-Ahlgren L, Chu A, Pond D, Tuong D, Luben R and Uckun FM. Stimulation of Src family protein-tyrosine kinases as a proximal and mandatory step for SYK kinase-dependent phospholipase Cgamma2

activation in lymphoma B cells exposed to low energy electromagnetic fields. J Biol Chem 1998;273(7):4035-4039.

The principal author is with the Biotherapy Program, University of Minnesota, Minneapolis, Minnesota 55417, USA.

Abstract: Here, we present evidence that exposure of DT40 lymphoma B cells to low energy electromagnetic field (EMF) results in a tyrosine kinase-dependent activation of phospholipase Cgamma2 (PLC-gamma2) leading to increased inositol phospholipid turnover. B cells rendered PLC-gamma2-deficient by targeted disruption of the PLC-gamma2 gene as well as PLC-gamma2-deficient cells reconstituted with Src homology domain 2 (SH2) domain mutant PLC-gamma2 did not show any increase in inositol-1,4,5-trisphosphate levels after EMF exposure, providing direct evidence that PLC-gamma2 is responsible for EMF-induced stimulation of inositol phospholipid turnover, and its SH2 domains are essential for this function. B cells rendered SYK-deficient by targeted disruption of the syk gene did not show PLC-gamma2 activation in response to EMF exposure. The C-terminal SH2 domain of SYK kinase is essential for its ability to activate PLC-gamma2. SYK-deficient cells reconstituted with a C-terminal SH2 domain mutant syk gene failed to elicit increased inositol phospholipid turnover after EMF exposure, whereas SYK-deficient cells reconstituted with an N-terminal SH2 domain mutant syk gene showed a normal EMF response. LYN kinase is essential for the initiation of this biochemical signaling cascade. Lymphoma B cells rendered LYN-deficient through targeted disruption of the lyn gene did not elicit enhanced inositol phospholipid turnover after EMF exposure. Introduction of the wild-type (but not a kinase domain mutant) mouse fyn gene into LYN-deficient B cells restored their EMF responsiveness. B cells reconstituted with a SH2 domain mutant fyn gene showed a normal EMF response, whereas no increase in inositol phospholipid turnover in response to EMF was noticed in LYN-deficient cells reconstituted with a SH3 domain mutant fyn gene. Taken together, these results indicate that EMF-induced PLC-gamma2 activation is mediated by LYN-regulated stimulation of SYK, which acts downstream of LYN kinase and upstream of PLC-gamma2.

Kristupaitis D, Dibirdik I, Vassilev A, Mahajan S, Kurosaki T, Chu A,Tuel-Ahlgren L, Tuong D, Pond D, Luben R and Uckun FM. Electromagnetic field-induced stimulation of Bruton's tyrosine kinase. J Biol Chem 1998;273(20):12397-12401.

The principal author is with the Biotherapy Program, University of Minnesota, Minneapolis, Minnesota 55455, USA.

Abstract: Here we present evidence that exposure of DT40 lymphoma B-cells to low energy electromagnetic fields (EMF) results in activation of phospholipase C-gamma 2 (PLC-gamma2), leading to increased inositol phospholipid turnover. PLC-gamma2 activation in EMF-stimulated cells is mediated by stimulation of the Bruton's tyrosine kinase (BTK), a member of the Src-related TEC family of protein tyrosine kinases, which

acts downstream of LYN kinase and upstream of PLC-gamma2. B-cells rendered BTK-deficient by targeted disruption of the btk gene did not show enhanced PLC-gamma2 activation in response to EMF exposure. Introduction of the wild-type (but not a kinase domain mutant) human btk gene into BTK-deficient B-cells restored their EMF responsiveness. Thus, BTK exerts a pivotal and mandatory function in initiation of EMF-induced signaling cascades in B-cells.

Miller SC, Furniss MJ. Bruton's tyrosine kinase activity and inositol 1,4,5-trisphosphate production are not altered in DT40 lymphoma B cells exposed to power line frequency magnetic fields. J Biol Chem 1998;273(49):32618-32626.

The principal author is with the Signal Transduction Laboratory, Pharmaceutical Discovery Division, SRI International, Menlo Park, California 94025, USA.

Abstract: Exposure of wild-type DT40 lymphoma B cells or Bruton's tyrosine kinase (BTK)-deficient DT40 cells reconstituted with the human btk gene to a 1 gauss 60 Hz electromagnetic field (EMF) has been reported to rapidly increase inositol 1,4,5-trisphosphate (Ins 1,4, 5-P3) production (1,2). Here we have used BTK-deficient DT40 B cells reconstituted with the human btk gene to evaluate the reproducibility of these findings. An experimental design with blinded exposures and anti-IgM treatment to induce Ins 1,4,5-P3 production as a positive control, showed no significant effect of a 1-gauss 60 Hz EMF on Ins 1,4,5-P3 production. Because recent work has shown that the activation of BTK was required for EMF-responsiveness (2), we also evaluated the reproducibility of this finding in wild-type DT40 cells. BTK was activated in a dose- and time-dependent manner by treatment with the tyrosine phosphatase inhibitor pervanadate. However, the ability to detect BTK activation, as measured by increased autophosphorylation by immune complex kinase assay, was dependent on the kinase buffer. Using cells from the original investigators, no evidence was obtained to support the hypothesis that exposure to a 1 gauss 60 Hz EMF had a causal effect on protein-tyrosine kinase activities affecting Ins 1,4,5-P3 production.

Woods M, Bobanovic F, Brown D and Alexander DR. Lyn and syk tyrosine kinases are not activated in B-lineage lymphoid cells exposed to low-energy electromagnetic fields. FASEB J 2000;14(14):2284-2290.

The principal author is with the Laboratory of Lymphocyte Signalling, The Babraham Institute, Cambridge CB2 4AT, UK.

Abstract: Exposure of B-lineage lymphoid cells to a 100 μT 60 Hz AC magnetic field has been reported to stimulate the rapid activation of Lyn and Syk tyrosine kinases and the induction of protein tyrosine phosphorylation. These findings are significant because of the critical role played by these B cell signaling events in the control of growth and differentiation, and therefore the potential of electromagnetic field (EMF) exposure to induce cancer. We report the first study carried out with the aim of

reproducing the reported EMF effects on Lyn and Syk tyrosine kinases. The system used enabled EMF exposure conditions to be carefully controlled and also allowed experiments to be performed blind. The effects of a 100 µT 60 Hz AC magnetic field on protein tyrosine phosphorylation and on Lyn and Syk tyrosine kinase activities were investigated in Nalm-6 and DT40 B cells in the absence and presence of a 46 µT DC magnetic field. However, no significant effects of low-energy electromagnetic fields on tyrosine kinase activities or protein phosphorylation were observed.

Dibirdik I, Bofenkamp M, Skeben P and Uckun F. Stimulation of Bruton's tyrosine kinase (BTK) and inositol 1,4,5-trisphosphate production in leukemia and lymphoma cells exposed to low energy electromagnetic fields. Leuk Lymphoma 2000;40(1-2):149-156.

The principal author is with the Department of Biochemistry, Parker Hughes Institute, Roseville, Minnesota 55113, USA.

Abstract: We examined the effects of low energy electromagnetic field (EMF) exposure on the BTK kinase activity in B18-2 ([Btk-, rBTK(wt)] DT40) chicken lymphoma B cells and NALM-6 leukemic pre-B cells. Exposure of B 18-2 cells to EMF resulted in activation of BTK within 1 to 15 minutes in 8 of 8 independent experiments with stimulation indexes ranging from 1.2 to 13.3. While in some experiments the BTK stimulation was transient, in others the BTK activity continued to be significantly elevated for up to 4 hours. Similarly, exposure of NALM-6 cells to EMF resulted in activation of BTK within 30 minutes in 7 of 7 experiments with stimulation indexes ranging from 1.2 to 7.4. Stimulation of BTK activity in EMF exposed cells was associated with enhanced phosphoinositide turnover and increased inositol-1,4,5-trisphosphate (IP3) production in 7 of 13 experiments with DT40 cells and 7 of 13 experiments with NALM-6 cells. The likelihood and magnitude of an IP3 response after EMF exposure were similar to those after BCR ligation on DT40 cells and CD19 ligation on NALM-6 cells. These results confirm and extend our previous studies regarding EMF-induced biochemical signaling events in B-lineage lymphoid cells.

Lindstrom E[(1)], Still M[(2)], Mattsson MO[(3)], Mild KH[(1)] and Luben RA[(4)]. ELF magnetic fields initiate protein tyrosine phosphorylation of the T cell receptor complex. Bioelectrochemistry 2001;53(1):73-78.

The authors are with the [(1)]National Institute for Working Life, Umea, Sweden, [(2)]Department of Surgery, Umea University, Umea, Sweden, [(3)]Department of Biology, Orebro University, Orebro, Sweden, [(4)]Department of Biomedical Sciences, University of California, Riverside, CA , USA.

Abstract: The human T cell line Jurkat registers a sinusoidal extremely low frequency (ELF), 0.10 mT magnetic fields (MFs) at the level of the plasma membrane. In this study, the protein tyrosine phosphorylation (PY) of two membrane-associated proteins in

Jurkat cells were examined following a short-term MFs exposure, the chains and the Src kinases p56lck. These proteins are interesting to study since the earliest biochemical event upon T cell receptor (TcR) activation is PY of the chains. These signalling chains in the TcR complex was assessed using Western blotting and the activation of the p56lck kinase was analysed by in vitro kinase assay. The MFs exposure of Jurkat for 5 min activated p56lck and resulted in PY of . These findings are in line with earlier reports on how MFs exposure affects signal transduction in Jurkat.

10.6 Cell Differentiation

Table 24. Cell differentiation summary

Author	Date	Endpoint	Response?	Weakness
Verdugo-Diaz	1998	CA	yes	
Chen	2000	HB	yes	

Abbreviations:
CA = Catacholamine production as a marker for differentiation.
HB = Hemoglobin as a marker for differentiation

Individual Study Summaries

Verdugo-Diaz L[(1)], Palomero-Riverob M[(2)] and Drucker-Coln R[(2)]. Differentiation of chromaffin cells by extremely low frequency magnetic fields changes ratios of catecholamine type messenger. Bioelectrochemistry 1998;46(2):297-300.

The authors are with the [(1)]Departamento de Fisiologa, Facultad de Medicina, Universidad Nacional Autonoma de Mexico, Apdo. Postal 70-250, 04510, Mexico, D.F., Mexico, [(2)]Departamento de Neurociencias, Instituto de Fisiologia Celular, Universidad Nacional Autonoma de Mexico, Mexico, D.F., Mexico.

Abstract: Extremely low frequency magnetic fields (ELF MF) have a wide variety of effects in biological systems. Rat chromaffin cells in vitro show morphological and biochemical changes when exposed to ELF MF similar to those produced by nerve growth factor (NGF). To determine whether ELF MF alters catecholamine (CA) release, we used a culture of postnatal rat chromaffin cells which was differentiated by NGF or ELF MF for 7 days. Levels of catecholamine on media culture were detected by high pressure liquid chromatography with electrochemical detection (HPLC-ED) analysis. The results showed that differentiated cells released more dopamine than adrenaline, while chromaffin undifferentiated cells released more adrenaline than dopamine. In both cases noradrenaline release did not change. The results are

discussed in terms of the role of Ca2+ or some enzymes in the changes in messenger ratios.

Comment: Chromaffin cells were prepared from the adrenal glands of neonatal Wistar rats. Chromaffin cells synthesize, store, and release catecholamines (CA), serotonin, and other neuropeptides. This study compared the levels of adrenalin (A), dopamine (DA) and noradrenalin (NA) in non-differentiated chromaffin cells and in cells exposed to magnetic fields or nerve growth factor (NGF) which causes these cells to undergo differentiation. The catacholamines were assayed by high pressure liquid chromatography with electrochemical detection. The exposure system consisted of a pair of Helmholtz coils. Its performance characteristics were not describes and neither were any calibration or quality assurance protocols. It is unknown if temperature control was adequate. Sham exposures were not carried out and it is not clear if the analyst was blind to the exposure status of the samples. NGF was used as a positive control. Cell numbers were determined by measurement of cellular protein. However, the assay results were not normalized to protein concentration as a surrogate of cell numbers. Cells were exposed to 0.7 mT (60 Hz) for 7 days before assay. There are too many flaws in the experimental design, and results must be considered inconclusive.

Chen G, Upham BL, Sun W, Chang CC, Rothwell EJ, Chen KM, Yamasaki H and Trosko JE. Effect of electromagnetic field exposure on chemically induced differentiation of friend erythroleukemia cells. Environ Health Perspect 2000;108(10):967-972.

The principal author is with the Department of Pediatrics and Human Development, College of Human Medicine, Michigan State University, East Lansing, Michigan 48824, USA.

Abstract: Whether exposure of humans to extremely low frequency electromagnetic fields (ELF-EMF) can cause cancer is controversial and therefore needs further research. We used a Friend erythroleukemia cell line that can be chemically induced to differentiate to determine whether ELF-EMF could alter proliferation and differentiation in these cells in a manner similar to that of a chemical tumor promoter. Exposure of this cell line to 60 Hz ELF-EMF resulted in a dose dependent inhibition of differentiation, with maximal inhibition peaking at 40% and 40 mG (4 μT). ELF-EMF at 10 mG (1.0 μT) and 25 mG (2.5 μT) inhibited differentiation at 0 and 20%, respectively. ELF-EMF at 1.0 (100) and 10.0 G (1,000 μT) stimulated cell proliferation 50% above the sham-treated cells. The activity of telomerase, a marker of undifferentiated cells, decreased 100 [times] when the cells were induced to differentiate under sham conditions, but when the cells were exposed to 0.5 G (50 μT) there was only a 10 [times] decrease. In summary, ELF-EMF can partially block the differentiation of Friend erythroleukemia cells, and this results in a larger population of cells remaining in the undifferentiated, proliferative state, which is similar to the published results of Friend erythroleukemia cells treated with chemical-tumor promoters.

Comment: The objective of this experiment was to determine whether magnetic fields can alter proliferation and differentiation in Friend erythroleukemia cells (FELC) in a manner similar to that of a chemical tumor promoter. FELC can be chemically induced to differentiate into hemoglobin (Hb) producing cells by exposure to dimethyl sulfoxide (DMSO) or hexamethylene bis acetamide (HbMA). Tumor promoters, such as phorbol myristate acetate (PMA), inhibit differentiation, allowing cells to remain in the undifferentiated proliferative state. Epigenetic changes (key events) can alter gene expression at, (i) the transcription stagel (i.e. methylation of DNA, or acetylation of DNA binding proteins), (ii) the translation stage (i.e. altered stability of mRNA), or (iii) the post-translation stage (i.e protein phosphorylation). Inhibition of differentiation by magnetic fields in the FELC model would strengthen the possibility that magnetic fields act as a tumor promoter.

The exposure system, its calibration, characterization, and quality assurance protocols were adequate. Temperature control was not a problem. The analyst was blind to the exposure status of only some samples. Culture conditions were not ideal since the cells in the exposure chambers were incubated in an atmosphere of < 100% humidity. One lot of Fetal bovine serum was used throughout the study. However, the passage number varied with the procedure (cells were not thawed and used before a set number of passages). These are flaws in the experimental design since cells with higher passage numbers differentiate less than those with lower numbers. Cells were exposed to DMSO or HbMA immediately before exposure to the fields in exposure chambers that were only 98% saturated with water vapor. Cells were exposed to 100 μT magnetic fields for 3 or 4 days in screw cap flasks to minimize the possibility of osmotic shock due to evaporation (osmotic stress is know to induce cell signaling pathways). In the first experiment, differentiation was detected by the presence of hemoglobin (Hb), as detected by benzidine staining. Cells were examine on a hemocytometer (200-500 cells per chamber), and counts were visually assessed by two independent analysts. Cells were also prepared for flow cytometry using fluorescent secondary antibody to measure Hb-containing cells as a means of verifying the results from the microscopical method. Cell proliferation was measured at various times during exposure to magnetic fields by isolating DNA and measuring its concentration by absorbance at 260 nm. Telomerase, a marker for undifferentiated cells, was measured by polymerase chain reaction (PCR)-based TRAP (telomeric repeat amplification protocol) assay, available commercially. Data was collected on commercially available hardware and analyzed on commercially available software. Statistical methods were adequate.

Exposure to magnetic fields at 100 μT inhibited differentiation in cells treated with DMSO by ~35%, (ratio EMF/Sham = 0.64) as measured by benzidine staining of cells containing Hb. The flow method gave a ratio of 0.58. The percentage of differentiating cells appeared to be a function of the number of cell passages, with cells at higher passage numbers differentiating less. For cells exposed to 5 μT the passage number was < 5; at 100 μT the passage numbers were from 5 to 10, and higher for exposures of 1 mT. Telomerase activity decreased x 100 for sham exposed cells co-exposed to

DMSO but only x 10 for cells that were exposed to 100 μT magnetic fields. Cell numbers increased in cultures exposed to 100 μT fields for 4 days compared to sham exposed cells, indicating a higher number of undifferentiated cells in the exposed culture. Despite a number of flaws in the experimental design, this study deserves independent replication. Until then, the results should be considered interesting. The results found by Chen et al contrast to those of Revoltella (1993) that found magnetic fields did not affect differentiation (this paper however, had even more flaws than Chen's).

Appendix - Epigenetic Modes of Action

Boorman GA, Owen RD, Lotz WG and Galvin MJ Jr. Evaluation of in vitro effects of 50 and 60 Hz magnetic fields in regional EMF exposure facilities. Radiat Res 2000;153(5 Pt 2):648-657.

The principal author is with the National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709, USA.

Abstract: A weak association between magnetic-field exposure and increased incidences of cancer has been reported. While alterations in cellular processes after in vitro magnetic-field exposures have also been reported to provide plausibility for this association, other laboratories have been unable to repeat the findings. As part of an accelerated electric- and magnetic-field (EMF) research program, the National Institute of Environmental Health Sciences with the Department of Energy identified the replication of the published positive effects as a priority. Regional EMF exposure facilities were established to investigate major in vitro effects from the literature. These included effects on gene expression, intracellular calcium, colony growth in soft agar, and ornithine decarboxylase activity. The laboratories that first reported these effects provided experimental protocols, cell lines, and other relevant experiment details. Regional facility studies included sham/sham exposures (no applied field in either chamber) and were done in a blinded fashion to minimize investigator bias. In nearly all experiments, no effects of magnetic-field exposure were found. The effort provided insight into dealing with the difficulty of replication of subtle effects in complex biological systems. Experimental techniques provided some clues for the differences in experimental results between the regional facility and the original investigator. Studies of subtle effects require extraordinary efforts to confirm that the effect can be attributed to the applied exposure.

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Dibirdik I, Bofenkamp M, Skeben P and Uckun F. Stimulation of Bruton's tyrosine kinase (BTK) and inositol 1,4,5-trisphosphate production in leukemia and lymphoma cells exposed to low energy electromagnetic fields. Leuk Lymphoma 2000;40(1-2):149-156.

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11. ANIMAL STUDIES

11.1 Carcinogenesis and Cancer Progression

In this review, the validity of a study's results will be assessed by the appropriateness of the exposure system and the study design, according to the descriptions provided by the author. Consideration will be given to the following elements of the exposure system including, but not limited to, (i) the arrangement made for sham exposures, (ii) the characteristics of the field, and its uniformity within the exposure volume, (iii) the size of the exposure volume, (iv) the presence of stray fields, (v) the orientation of the exposure system relative to the Earth's magnetic field, (vi) the quality assurance steps taken to ensure system performance over the exposure period (including temperature control), (vii) whether the system operators were blind to the exposure status of the animals, and (viii) whether the ambient environmental conditions within the animal housing facility were characterized. The elements of the experimental design that will be considered important include (i) whether a full description was given of the animals used in the experiment, (ii) whether study objectives were clearly stated, (iii) the endpoints to be acquired were clearly identified, (iv) the assay used to acquire the data was appropriate and sufficiently well established that others could easily replicate the conditions under which the data was acquired, (v) the procedures used to insure assay performance and the quality of the acquired data (i.e. use of appropriate internal and external controls), (vi) whether the data was acquired blind to the exposure status of the samples, (vii) the quality assurance protocols to insure hardware used in the measurement process was properly calibrated, and (viii) the statistical procedures used to process data and test the hypothesis were appropriate. In lieu of a positive control, a well established background tumor incidence rate and variance for non-exposed control animals.

Whether the exposure system and the experimental design were acceptable depended largely on the description provided by the author. If a critical fact was omitted, or incompletely described, adopting a 'worst case scenario' was an option if, in the opinion of the reviewer, the omission had a high probability of resulting in compromised data. In this case, the study results were labeled as inconclusive. Once validity was established and the results were interpreted, the study was assigned to one of five categories, based, in part, on criteria adopted from the National Toxicology Program (NTP, USA), as summarized in NTP document TR488 (NIH report number 99-3978) titled "Toxicological and Carcinogenesis Studies of 60-Hz Magnetic Fields on F344/N Rats and B6C3F1 Mice". The categories are summarized below.

Category A. A study was assigned to category A if there was clear evidence of carcinogenic activity, such as, (i) a dose-related increase in malignant neoplasms, (ii) an increase in combined incidence of benign and malignant neoplasms, or (iii) a marked increase in benign neoplasms if, from prior knowledge, it had been established that such tumors progress to malignant neoplasms.

Category B. A study was assigned to category B if some evidence of carcinogenic activity had been demonstrated by a response that was intermediate between Categories A and C.

Category C. A study provided equivocal evidence of carcinogenic activity by demonstrating only a marginal increase in neoplasms.

Category D. A study assigned to category D demonstrated no evidence of carcinogenic activity (i.e. no increase in benign or malignant neoplasms).

Category E. A study is assigned to category E if, in the opinion of the reviewer, it had major qualitative or quantitative limitations that would not allow a valid interpretation of the data.

In assigning a study to a particular category, consideration was given to some key factors that were strongly associated with carcinogenesis:

(i) The occurrence of common vs uncommon tumors

(ii) Evidence there was progression from benign to malignant neoplasms, or from pre-neoplastic to neoplastic lesions. Benign lesions were assumed to progress to malignant neoplasms, unless there was established evidence to the contrary. Therefore, it was acceptable to estimate the incidence rate on the basis of the sum of benign and malignant neoplasms, since they represented different stages of tumor progression in the same organ or tissue.

(iii) Multiplicity of site-specific neoplasia.

(iv) The presence of a significant number of metastases.

(v) The presence of supporting information from other studies of site-specific hyperplasia or neoplasia in another species or sex.

(vi) The presence of a dose-response relationship.

11.2 Summary of Findings

Almost universally, these studies find no support for the hypothesis that magnetic fields initiate the development of neoplasms, under the experimental conditions of the studies. The only positive finding was for an increase in the incidence of thyroid neoplasms in male, but not female, Fisher 344 rats. There was no evidence of the development of neoplasms at sites where epidemiology suggests magnetic field related tumors should be found.

Table 25. Summary - general carcinogenesis

Author	Date	Species / Endpoint	Findings (Category)	Weakness
Zecca	1998	Sprague-Dawley rats, all cancers	Category D (exposed to magnetic and electric fields)	None Apparent
McCormick	1999	B6C3F1 mice, all cancers	Category D	None Apparent
Boorman	1999	Fisher F344 rats, all cancers	Category B - evidence of thyroid cancers in males only; no evidence of other cancers	None Apparent
Anderson	2001	Fisher 344 rats, leukemia	Category D	None Apparent
Otaka	2002	B6C3F1 mice, all cancers	Category D	None Apparent

Abbreviations:
NA = No Apparent shortcomings in experimental design.
ED = (possible) Experimental Design shortcomings.

Individual Study Summaries

McCormick DL, Boorman GA, Findlay JC, Hailey JR, Johnson TR, Gauger JR, Pletcher JM, Sills RC and Haseman JK. Chronic toxicity/oncogenicity evaluation of 60 Hz (power frequency) magnetic fields in B6C3F1 mice. Toxicol Pathol 1999;27(3):279-285.

The principal author is with the Illinois Institute of Toxicology Research Institute, Chicago 60616-3799, USA.

Abstract: A 2 year whole-body exposure study was conducted to evaluate the chronic toxicity and possible oncogenicity of 60 Hz (power frequency) magnetic fields in mice. Groups of 100 male and 100 female B6C3F1 mice were exposed to pure, linearly polarized, transient-free 60 Hz magnetic fields at flux densities of 0 Gauss (G) (sham control), 20 milligauss (mG), 2 G, and 10 G; an additional group of 100 male and 100 female B6C3F1 mice received intermittent (1 hr on/1 hr off) exposure to 10 G fields. A small but statistically significant increase in mortality was observed in male mice exposed continuously to 10 G fields; mortality patterns in all other groups of mice exposed to magnetic fields were comparable to those found in sex-matched sham controls. Body weight gains and the total incidence and number of malignant and benign

tumors were similar in all groups. Magnetic field exposure did not increase the incidence of neoplasia in any organ, including those sites (leukemia, breast cancer, and brain cancer) that have been identified in epidemiology studies as possible targets of magnetic field action. A statistically significant decrease in the incidence of malignant lymphoma was observed in female mice exposed continuously to 10 G fields, and statistically significant decreases in the incidence of lung tumors were seen in both sexes exposed continuously to 2 G fields. These data do not support the hypothesis that chronic exposure to pure, linearly polarized 60 Hz magnetic fields is a significant risk factor for neoplastic development in mice.

Comment: This is the peer reviewed summary of a portion of the National Toxicology Program report TR-488 (NIH Publications 99-3978) involving exposure of male and female B6C3F1 mice to 60 Hz magnetic fields. The exposure system was adequate, as were quality assurance protocols. The uniformity of the fields within the exposure chambers were adequately mapped. Sham exposures were conducted in non-energized exposure chambers, and technical staff were blind to the exposure status of samples. A positive control was not needed because the tumor incidence rates for control mice had been established by previous studies. Studies were conducted at the Illinois Institute of Technology (IIL) and quality assurance of the pathology was provided by an independent contractor. Standard toxicology studies and long-term carcinogenesis studies were conducted using a traditional B6C3F1 mouse model. Male and female mice were continuously exposed to pure, linearly polarized, transient-free 60 Hz magnetic fields at flux densities of 0 Gauss (G) (sham control), 0.02 G (20 μT), 2 G (200 μT) or 10 G (1 mT) 60 Hz magnetic fields for 18.5 h per day, 7 days per week for 106 weeks. An additional 100 male and 100 female mice were exposed to a 10 G (1 mT) intermittent magnetic field (1 h on and one h off) for 18.5 h per day, 7 days per week for 106 weeks. The study found no evidence to support the hypothesis that magnetic fields are carcinogenic in B6C3F1 mice, and was assigned to category D.

Boorman GA, McCormick DL, Findlay JC, Hailey JR, Gauger JR, Johnson TR, Kovatch RM, Sills RC and Haseman JK. Chronic toxicity/oncogenicity evaluation of 60 Hz (power frequency) magnetic fields in F344/N rats. Toxicol Pathol 1999(a);27(3):267-278.

The principal author is with the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA.

Abstract: A 2 year whole-body exposure study was conducted to evaluate the chronic toxicity and possible oncogenicity of 60 Hz (power frequency) magnetic fields in rats. Groups of 100 male and 100 female F344/N rats were exposed continuously to pure, linearly polarized, transient-free 60 Hz magnetic fields at flux densities of 0 Gauss (G) (sham control), 20 milligauss (mG), 2 G, and 10 G; an additional group of 100 male and 100 female F344/N rats received intermittent (1 h on/1 h off) exposure to 10 G fields. Mortality patterns, body weight gains throughout the study, and the total incidence and

number of malignant and benign tumors in all groups exposed to magnetic fields were similar to those found in sex-matched sham controls. Statistically significant increases in the combined incidence of C-cell adenomas and carcinomas of the thyroid were seen in male rats chronically exposed to 20 mG and 2 G magnetic fields. These increases were not seen in male rats exposed continuously or intermittently to 10 G fields or in female rats at any magnetic field exposure level. No increases in the incidence of neoplasms, which have been identified in epidemiology studies as possible targets of magnetic field action (leukemia, breast cancer, and brain cancer), were found in any group exposed to magnetic fields. There was a decrease in leukemia in male rats exposed to 10 G intermittent fields. The occurrence of C-cell tumors at the 2 lower field intensities in male rats is interpreted as equivocal evidence of carcinogenicity; data from female rats provides no evidence of carcinogenicity in that sex. These data, when considered as a whole, are interpreted as indicating that chronic exposure to pure linearly polarized 60 Hz magnetic fields has little or no effect on cancer development in the F344/N rat.

Comment: This is the peer reviewed summary of a portion of the National Toxicology Program report TR-488 (NIH Publications 99-3978) involving exposure of male and female F344/N rats to 60 Hz magnetic fields. The exposure system was adequate, as were quality assurance protocols. Sham exposures were conducted in non-energized exposure chambers, and technical staff were blind to the exposure status of samples. Studies were conducted at the Illinois Institute of Technology (IIT) and the quality assurance of the pathology was provided by an independent contractor. Standard toxicology studies and long-term carcinogenesis studies were conducted using a F344/N rat model. Male and female rats were continuously exposed to pure, linearly polarized, transient-free 60 Hz magnetic fields at flux densities of 0 (sham), 0.02 G (20 μT), 2 G (200 μT) or 10 G (1 mT) for 18.5 h per day, 7 days per week for 106 weeks. An additional 100 male and 100 female rats were exposed to a 10 G (1 mT) intermittent magnetic field (1 h on and one h off) for 18.5 h per day, 7 days per week for 106 weeks. Survival and mean body weights of exposed male and female rats were similar to sham exposed controls. The incidence of thyroid gland C-cell adenoma and carcinoma in male rats exposed to 0.02 G (60 Hz), adenoma in male rats exposed to 2 G fields, and combined adenoma and carcinoma in 0.02 G and 2 G male rats were significantly greater than in sham exposed controls. The incidence of mononuclear cell leukemia in male rats exposed to 10 G intermittent fields were significantly lower than controls. There was no evidence that exposure to magnetic fields increased tumor incidence in female rats. Also, there was no increased incidence of neoplasms at sites where epidemiology had suggested an association with magnetic fields. This study authors concluded that this study provides equivocal evidence that magnetic fields have carcinogenic potential in F344/N rats, and was assigned to category C.

Zecca L, Mantegazza C, Margonato V, Cerretelli P, Caniatti M, Piva F, Dondi D and Hagino N. Biological effects of prolonged exposure to ELF electromagnetic fields in rats:III. 50 Hz electromagnetic fields. Bioelectromagnetics 1998;19(1):57- 66.

The principal author is with the Institute of Advanced Biomedical Technologies, National Research Council, Milan, Italy.

Abstract: Groups of adult male Sprague Dawley rats (64 rats each) were exposed for 8 months to electromagnetic fields (EMF) of two different field strength combinations: 5 μT - 1kV/m and 100 μT - 5kV/m. A third group was sham exposed. Field exposure was 8 h/day for 5 days/week. Blood samples were collected for hematology determinations before the onset of exposure and at 12 week intervals. At sacrifice, liver, heart, mesenteric lymph nodes, bone marrow, and testes were collected for morphology and histology assessments, while the pineal gland and brain were collected for biochemical determinations. At both field strength combinations, no pathological changes were observed in animal growth rate, in morphology and histology of the collected tissue specimens (liver, heart, mesenteric lymph nodes, testes, bone marrow), and in serum chemistry. An increase in norepinephrine levels occurred in the pineal gland of rats exposed to the higher field strength. The major changes in the brain involved the opioid system in frontal cortex, parietal cortex, and hippocampus. From the present findings it may be hypothesized that EMF may cause alteration of some brain functions.

Comment: This study examined the effect, on adult male Sprague-Dawley rats, of exposure to combined electric and magnetic fields (5 μT - 1 kV/m and 100 μT - 5 kV/m) or sham exposures for 8 h per day, 5 d per week for 8 months (64 rats per group). Organs and tissues were examined histologically for lesions, blood chemistry was evaluated, and regions of the brain were assessed for neurochemical activity. Rats were communally housed and were exposed to light for the entire study period. The exposure system for generating the electric and magnetic fields were adequately described, as were the exposure conditions and housing facilities. Brain amines were analyzed by HPLC using isoproterenol as an internal standard. Dopamine and μ-opioid receptors were examined using previously published semi-quantitative receptor binding assays, and commercial software to analyze the binding data. At necropsy organs and tissues were examined macroscopically and microscopically for lesions. Blood chemistry was assessed by established protocols and serum chemistry was by commercial auto-analyzer. There was no significant differences observed between exposed and sham exposed rats in any of the assays except for some involving the μ-opioid receptor binding. The author concludes that this could indicate electric or magnetic field induced changes in brain chemistry. However, these changes could have been the result of the semi-quantitative nature of the receptor binding assays. This study was assigned to category D.

Anderson LE, Morris JE, Miller DL, Rafferty CN, Ebi KL and Sasser LB. Large granular lymphocytic (LGL) leukemia in rats exposed to intermittent 60 Hz magnetic fields. Bioelectromagnetics 2001;22(3):185-193.

The principal author is with the Battelle Memorial Institute, Richland, Washington, USA.

Abstract: An animal model for large granular lymphocytic (LGL) leukemia in male Fischer 344 rats was utilized to determine whether magnetic field exposure can be shown to influence the progression of leukemia. We previously reported that exposure to continuous 60 Hz, 1 mT magnetic fields did not significantly alter the clinical progression of LGL leukemia in young male rats following injection of spleen cells from donor leukemic rats. Results presented here extend those studies with the following objectives: (a) to replicate the previous study of continuous 60 Hz magnetic field exposures, but using fewer LGL cells in the inoculum, and (b) to determine if intermittent 60 Hz magnetic fields can alter the clinical progression of leukemia. Rats were randomly assigned to four treatment groups (18/group) as follows: (1) 1 mT (10 G) continuous field, (2) 1 mT intermittent field (off/on at 3 min intervals), (3) ambient controls (< 0.1 μT), and (4) positive control (5 Gy whole body irradiation from cobalt-60 four days prior to initiation of exposure). All rats were injected intraperitoneally with 2.2 x 10(6) fresh, viable LGL leukemic spleen cells at the beginning of the study. The fields were activated for 20 h per day, 7 days per week, and all exposure conditions were superimposed over the natural ambient magnetic field. The rats were weighed and palpated for splenomegaly weekly. Splenomegaly developed 9 -11 weeks after transplantation of the leukemia cells. Hematological evaluations were performed at 6, 8, 10, 12, 14, and 16 weeks of exposure. Peripheral blood hemoglobin concentration, red blood cells, and packed cell volume declined, and total white blood cells and LGL cells increased dramatically in all treatment groups after onset of leukemia. Although the positive control group showed different body weight curves and developed signs of leukemia earlier than other groups, differences were not detected between exposure groups and ambient controls. Furthermore, there were no overall effects of magnetic fields on splenomegaly or survival in exposed animals. In addition, no significant and/or consistent differences were detected in hematological parameters between the magnetic field exposed and the ambient control groups.

Comment: The objectives of this study were to: (i) replicate results of a study on continuous 60 Hz magnetic field exposures and leukemia progression, but using fewer LGL cells in the inoculum to detect any effect that might have been masked by the higher number of LGL cells used previously, and (ii) determine if an intermittent 60 Hz magnetic field could alter the clinical progression of leukemia. LGL leukemia in rats is an established experimental model in which F344/N rats spontaneously develop leukemia at increasing rates (20-40%) as they age. Spleen cells from leukemic rats are implanted into young rats which in turn develop leukemia within 7-12 weeks. The time course of the disease can be manipulated by altering the number of injected leukemia cells or by exposing the rats to Co-60 gamma rays. The study was conducted in accordance with the standards and requirements of Good Laboratory Practices (GLP) of the US Environmental Protection Agency 40 CFR 792. The exposure system, its calibration and characterization has been previously described (Rommereim, 1996). The experimental design included pre-screening of rats for the presence of viral antibodies and pathogenic organisms. A total of 72 rats were randomly distributed to four exposure groups (one each for intermittent or continuous magnetic field exposure,

one as a sham control and one as a Co-60 positive control). The study found neither continuous nor intermittent magnetic field exposures affected the progression of leukemia, or any of the hematological parameters examined. This confirms a previous study and extends the results to intermittent magnetic field exposures. This study was assigned to Category D.

Otaka Y, Chida T, Yamagishi Y and Kitamura S. Carcinogenicity test in B6C3F1 mice after parental and prenatal exposure to 50 Hz magnetic fields. Bioelectromagnetics 2002;23(3):206-213.

The principal author is with the Mitsubishi Chemical Safety Institute, Ibaraki, Japan.

Abstract: Some epidemiological studies suggest association of childhood cancer with occupational exposure of the parents to magnetic fields. To test this relationship, 50 each of C57BL/6J female and C3H/HeJ male mice were exposed for 2 and 9 weeks, respectively, to 50 Hz sham (group A), 0.5 (group B), and 5 mT (group C) sinusoidal alternating magnetic fields. They were mated under the exposure for up to 2 weeks, and the exposure was continued until parturition. All the B6C3F1 offspring, without adjusting numbers of animals, were clinically observed without exposure to magnetic field for a nominal 78 weeks from 6-8 weeks of age after weaning and then euthanized for pathological examination according to a routine carcinogenicity test. 540 pups entered the test, and the survival rate was 96.7%. No F1 mouse died of tumoral diseases before a male in A group died of stomach cancer at 43 weeks of age. The first animal death in the exposed groups due to tumor occurred at 71 weeks of age. Eighteen animals died before necropsy at 84 -86 weeks of age. No significant difference was detected in the final number of survivors and incidence of tumors between groups A and B, or A and C. Concerning reproduction total implants in group B were less than in group A and the difference was on the borderline of significance (P=.05). This difference was not reproduced in a later duplicate experiment.

Comment: The structure and performance characteristics of the exposure system used in this study has been described previously (Yasui, 1993). Pathogen-free C3H/HeJ male mice and C57BL/6J female mice were exposed to sham, 0.5 mT (50 Hz) or 5 mT (50 Hz) magnetic fields for 9 weeks, and then mated during exposure. Their offspring (B6C3F1 mice) were then examined at 84 -86 weeks of age for neoplasms in various tissues and organs. The incidence rate of neoplasms in sham exposed B6C3F1 mice has been established by historical studies. Blood samples were examined at intermediate time points. All protocols were adequately described and were acceptable. No significant difference was detected in the incidence of tumors in the offspring whose parents were exposed to magnetic fields when compared with offspring from sham exposed parents. There was no positive control, but historical rates of cancer incidence has been well established for this mouse model. This study is assigned to category D.

11.3 Tumor Promotion

Table 26. Summary - tumor promotion

Author	Date	Species/Endpoint	Result	Weakness
Kumlin	1998	mice, elevation of ODC	Inconclusive	Incomplete Description of Mice
Sasser	1998	SENCAR mice, elevation of ODC	No Effect	None Apparent
DiGiovanni	1999	SENCAR mice, various biomarkers of tumor promotion	No Effect	None Apparent
Mandeville	2000	Fisher 344 rats, promotion of neurogenic tumors	No Effect	None Apparent

Abbreviations:
MF = Magnetic Field exposure.
ODC = Ornithine Decarboxylase activity.

Kumlin T[1], Alhonen L[2], Junne J[2,3], Lang S[1], Kosma VM[4] and Juutilainen J[1]. Epidermal ornithine decarboxylase and polyamines in mice exposed to 50 Hz magnetic fields and UV radiation. Bioelectromagnetics 1998;19:388-391.

The authors are with the: [1]Department of Environmental Sciences, University of Kuopio, Kuopio, Finland, [2]Virtanen Institute, University of Kuopio, Kuopio, Finland, [3]Department of Biochemistry and Biotechnology, University of Kuopio, Kuopio, Finland and [4]Department of Pathology and Forensic Medicine, University of Kuopio, Kuopio, Finland.

Abstract: We studied the influence of magnetic fields (MFs) and simulated solar radiation (SSR) on ornithine decarboxylase (ODC) and polyamines in mouse epidermis. Chronic exposure to combined MF and SSR did not cause persistent effects on ODC activity or polyamines compared to the animals exposed only to UV, although the same MF treatment was previously found to accelerate skin tumor development. In an acute 24 h experiment, an elevation of putrescine and down-regulation of ODC activity was observed in the animals exposed to a 100 T MF. No effect was seen 24 h after a single 2-MED (minimal erythemal dose) exposure to SSR. The results indicate that acute exposure to 50 Hz MF does exert distinctive biological effects on epidermal polyamine synthesis.

Comment: The exposure system has been described previously (Kumlin, 1998) and was acceptable, as were calibration and quality assurance protocols. The mice were

described only as ODC K-2 transgenic and non-transgenic mice. The K-2 transgenic mice do not produce spontaneous skin tumors and their skin has elevated levels of ODC relative to non-transgenic mice. Polyamines are generally not elevated in the skin of these mice. In the chronic experiment, mice (half non-transgenic and half ODC transgenic) were exposed to continuous or intermittent magnetic fields or sham exposure for 10.5 months. Solar simulated radiation was used as a positive control. ODC was measured in skin samples by an established protocol and activity was expressed as pmol/mg of tissue. The polyamines were measured by an established HPLC method. Chronic exposure to magnetic fields alone did not affect ODC or polyamine levels in either transgenic or non-transgenic mice. A second, acute experiment was carried out in which mice were exposed to sham or magnetic fields for 24 h and then ODC and polyamines levels were measured. Only putrescine was elevated at 24 h. From this, the authors conclude ODC must have been elevated at an earlier time point. The results of this study are considered to be inconclusive.

Sasser LB, Anderson LE, Morris JE, Miller DL, Walborg EF Jr, Kavet R, Johnston DA and DiGiovanni J. Lack of a co-promoting effect of a 60 Hz magnetic field on skin tumorigenesis in SENCAR mice. Carcinogenesis 1998;19(9):1617-1621.

The principal author is with Battelle, Pacific Northwest Laboratories, Richland, WA 99352, USA.

Abstract: It has been proposed that extremely low frequency (ELF) magnetic fields may enhance tumorigenesis through a co-promotional mechanism. This hypothesis has been further tested using the two-stage model of mouse skin carcinogenesis, i.e. 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced promotion of skin tumors in mice initiated by a single subcarcinogenic dose of 7,12-dimethylbenz[a]anthracene. Experimentation described herein utilized the SENCAR mouse and examined the effect of a magnetic field on skin tumor promotion induced by three different doses of TPA within its dose-response range, i.e. 0.85, 1.70 or 3.40 nmol, administered twice per week. SENCAR mice (56/treatment group) were exposed to a 60 Hz magnetic field having a flux density of 2 mT for 6 h/day for 5 days/week and compared with mice exposed to the ambient magnetic field. Tumor incidence and multiplicity were monitored weekly for 23 weeks of TPA promotion. Statistical evaluation of the effects of the magnetic field on tumor incidence and multiplicity did not reveal any statistically significant effects; thus, within the sensitivity limits imposed by the animal model and the exposure parameters employed, no promotional or co-promotional effect of a 2 mT magnetic field on skin tumor development in SENCAR mice could be demonstrated.

Comment: The study protocol called for mice exposed to ambient fields as the control, in place of the traditional sham exposure in which mice are held in a non-energized or nulled exposure chamber. A related study (DiGiovanni, 1999) provided evidence to justify this procedural change. The SENCAR mouse model has been extensively characterized and evaluated as an indicator of tumorigenic potential for both chemicals

and physical agents. In previous experiments that evaluated the response of the SENCAR mouse model to 20 known carcinogens (Bull, 1986), the false negative rate was found to be 60%. Such poor sensitivity implies this assay is not likely able to detect weak tumor promoters. This study used an acceptable experimental design and, within the limits imposed by low sensitivity, confirms results of many authors that used this mouse model to demonstrate that a 2 mT magnetic field affects neither tumor incidence nor multiplicity.

DiGiovanni J, Johnston DA, Rupp T, Sasser LB, Anderson LE, Morris JE, Miller DL, Kavet R and Walborg EF Jr. Lack of effect of a 60 Hz magnetic field on biomarkers of tumor promotion in the skin of SENCAR mice. Carcinogenesis 1999;20(4):685-689.

The principal author is with the University of Texas M.D. Anderson Cancer Center, Science Park-Research Division, Smithville 78957, USA.

Abstract: It has been proposed that extremely low frequency magnetic fields may enhance tumorigenesis through a co-promotional mechanism. This hypothesis has been further tested using the two-stage model of mouse skin carcinogenesis, i.e. 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced promotion of skin carcinogenesis in mice initiated by a single subcarcinogenic dose of 7,12-dimethylbenz[a]anthracene. Experimentation utilized three different doses of TPA within its dose-response range (0.85, 1.70 or 3.40 nmol) and examined the following early biomarkers of tumor promotion after 1, 2 and 5 weeks of promotion: increases in epidermal thickness and the labeling index of epidermal cells, induction of epidermal ornithine decarboxylase activity and down-regulation of epidermal protein kinase C activity. Mice exposed to a 60 Hz magnetic field having a flux density of 2 mT for 6 h/day for 5 days/week were compared with mice exposed to an ambient magnetic field. Within the sensitivity limits of the biomarker methodology and the exposure parameters employed, no consistent, statistically significant effects indicative of promotion or co-promotion by the magnetic field were demonstrated.

Comment: This study describes the effect of a 2 mT (60Hz) magnetic field on early biomarkers of epidermal hyperplasia in SENCAR mice, complementing an earlier study by Sasser (1998), which described the effect of magnetic fields on tumor development in the same mouse model. A preliminary study was conducted to demonstrate the appropriateness of the dosing range of phorbol myristate acetate (PMA) to detect the effects on early biomarkers of tumor promotion (epidermal thickness, the labelling index of epidermal cells, the induction of epidermal ODC and the down-regulation of protein kinase C (PKC). In the primary experiment, three doses of PMA were selected for study (0.85, 1.70 and 3.40 nmol). In a separate preliminary experiment, mice that were exposed to a nulled magnetic field gave early biomarker results that were not significantly different from mice that were exposed to the ambient geomagnetic field (i.e. not held in a nulled exposure facility) in a room adjacent to the exposure facility. This

demonstrated that mice housed in a room separate from the activated exposure system could serve as an appropriate control for mice exposed to a 2 mT magnetic field. Such an exposure set up was used in this experiment. The results from the primary experiment demonstrate that exposure to a 2 mT (60Hz) magnetic field did not produce any consistent, statistically significant effects on DMBA/PMA-treated SENCAR mice, in terms of four early biomarkers of tumor promotion. The primary experiment also included groups of DMBA-initiated mice that were exposed to only the magnetic field (no PMA) or to the ambient fields to determine if there was any direct effect of magnetic fields alone on early biomarkers of tumor promotion. These results weaken the hypothesis that magnetic fields can act as tumor promoters or co promoters in the SENCAR mouse model system.

Mandeville R, Franco E, Sidrac-Ghali S, Paris-Nadon L, Rocheleau N, Mercier G, Desy M, Devaux C and Gaboury L. Evaluation of the potential promoting effect of 60 Hz magnetic fields on N-ethyl-N-nitrosourea induced neurogenic tumors in female F344 rats. Bioelectromagnetics 2000;21(2):84-93.

The principal author is with Biophage Incorporated, Montreal, Quebec, Canada.

Abstract: The present study investigated the possible effect of 60 Hz magnetic fields (MFs) as promoters of neurogenic tumors initiated transplacentally by a chemical carcinogen, N-ethyl-N-nitrosourea (ENU). In a preliminary study, 5 mg of ENU was shown to induce 30 to 40% neurogenic tumors in F344 rats offspring after 420 days of observation. In the present study, 400 female rats were divided into eight different groups (50 animals/group) and exposed in utero (on day 18 of gestation) to a single intravenous dose of either Saline (Group I), or ENU, 5 mg/kg (Group II to VIII). Dams in group II were given no further treatment while dams in Groups III to VII were exposed to 5 different intensities of MFs forty eight hours later. Animals in group III were sham exposed (<0.02 μT) while groups IV to VII were exposed to 2, 20, 200, and 2000 microT, respectively. Dams in Group VIII were injected intraperitoneally with 12-O-tetradecanoylphrobol-13-acetate (TPA; 10 micrograms/kg) from day 19 until delivery, and then their female offspring continued to be injected every 15 days, starting at day 14 after birth until sacrifice (positive controls). Accordingly, this study included three different types of controls: Internal controls (Groups II and III) and positive control (Group VIII). Body weight, mortality and clinical observations were evaluated in all groups of animals during in-life exposure. Necropsy was performed on all exposed and control animals that died, were found moribund or sacrificed at termination of the study. Histopathological evaluation was done for all brains, spinal cords, cranial nerves, major organs (lungs, liver, spleen, kidneys, pituitary, thyroid and adrenals) and all gross lesions observed during necropsy. All clinical observations and pathological evaluations were conducted under "blinded" conditions. The findings from this ENU/MFs promotion study clearly demonstrate that, under our defined experimental conditions, exposure to 60 Hz linear (single axis) sinusoidal, continuous wave MFs had no effect on the survival of female F344 rats or on the number of animals bearing neurogenic tumors. These

results suggest that MFs have no promoting effect on neurogenic tumors in the female F344 rats exposed transplacentally to ENU.

Comment: The objective of the study was to test the hypothesis that magnetic fields promote the growth of brain tumors in the offspring of F344 rats that had been initiated *in utero* to ethylnitrosourea (ENU), a transplacental carcinogen. A preliminary study established that 420 days after *in utero* exposure to ENU, 30-40% of the offspring would develop neurogenic tumors. The magnetic field exposure system, the facilities, and exposure protocols were all acceptable. The staff were blind to the exposure status of the samples. The offspring were exposed to 2, 20, 200 and 2000 μT (60Hz) magnetic fields for 20 h/d for 420 days. Phorbol myristate acetate (PMA) was used as a positive control. The central and peripheral nervous systems were evaluated histologically for the presence of neurogenic tumors. The magnetic fields did not promote the growth of neurogenic tumors under these experimental conditions. The results of this study do not support the hypothesis that magnetic fields are tumor promoters.

11.4 Breast Cancer Models

Table 27. Summary - breast cancer models

Author	Date	Species / Endpoint	Results	Weakness
Mevissen	1998(a)	SD rats; immune suppression	inconclusive	ED
Haussler	1999	SD rats; IL1, IL2 stimulation *ex vivo*	inconclusive; mitogen stimulation index not done	ED
Rosen	1999	SD rat pinealocytes	MF suppressed melatonin ex vivo	none apparent
John	1998	SD rats; melatonin suppression	no suppression	none apparent
Mevissen	1998(b)	SD rats; mammary tumors	MF-induced tumorigenesis	none apparent
Loscher	1998	SD rats; melatonin suppression	none found	none apparent

Thun-battersby	1999	SD rats; mammary tumors at lower DMBA dose, longer MF exposures	mammary tumorigenesis	none apparent
Anderson	1999	SD rats; mammary tumorigenesis (attempt to reproduce Loscher)	failed to reproduce Loscher's results	none apparent
Boorman	1999(b)	SD rats; mammary tumorigenesis (attempt to replicate Thun-Battersby)	inconclusive	ED
Mevissen	1999	SD rats; ODC enhancement	ODC increased at 2 w, but not a 8 and 13 w.	none apparent
Wilson	1999	dwarf Siberian Hamster; melatonin suppression	melatonin suppression (possible model for future studies)	none apparent
Bakos	2002	Wistar rats; melatonin suppression	none found	none apparent
Fedrowitz	2002	SD rats; tumor promotion; melatonin suppression	no melatonin suppression; MF-induced cell proliferation in mammary tissue	none apparent

Abbreviations:
ED = (possible) Experimental Design weakness.
MF = ELF magnetic fields.
SD = Sprague-Dawley.

Individual Study Summaries

Mevissen M, Haussler M, Szamel M, Emmendorffer A, Thun-Battersby S and Loscher W. Complex effects of long-term 50 Hz magnetic field exposure in vivo on immune functions in female Sprague- Dawley rats depend on duration of exposure. Bioelectromagnetics 1998(a);19(4):259-270.

The principal author is with the Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Hannover, Germany.

Abstract: In previous studies we have demonstrated that 50 Hz, 100 µT magnetic field (MF) exposure of female Sprague-Dawley rats for 13 weeks significantly enhances the development and growth of mammary tumors in a breast cancer model. The present study was designed to test the hypothesis that, at least in part, the tumor co-promoting effect of MF exposure is due to MF effects on the immune surveillance system, which is of critical importance in protecting an organism against the development and growth of tumors. For this purpose, female Sprague-Dawley rats of the same age as in the mammary tumor experiments were continuously exposed for different periods (2, 4, 8, and 13 weeks) to a 50 Hz, 100 µT MF. Control groups were sham-exposed simultaneously. Following the different exposure periods, splenic lymphocytes were cultured and the proliferative responses to the T-cell-selective mitogen concanavalin A (Con A) and the B-cell-selective pokeweed mitogen (PWM) were determined. Furthermore, the production of interleukin-1 (IL-1) was determined in the splenocyte cultures. The mitogenic responsiveness of T cells was markedly enhanced after 2 weeks of MF exposure, suggesting a co-mitogenic action of MF. A significant, but less marked increase in T-cell mitogenesis was seen after 4 weeks of MF exposure, whereas no difference from sham controls was determined after 8 weeks, indicating adaptation or tolerance to this effect of MF exposure. Following 13 weeks of MF exposure, a significant decrease in the mitogenic responsiveness of lymphocytes to Con A was obtained. This triphasic alteration in T-cell function (i.e., activation, tolerance, and suppression) during prolonged MF exposure resembles alterations observed during chronic administration of mild stressors, substantiating the hypothesis that cells respond to MF in the same way as they do to other environmental stresses. In contrast to T cells, the mitogenic responsiveness of B cells and IL-1 production of PWM-stimulated cells were not altered during MF exposure. The data demonstrate that MF in vivo exposure of female rats induces complex effects on the mitogenic responsiveness of T cells, which may lead to impaired immune surveillance after long-term exposure.

Comment: The object of this study was to determine if magnetic fields affect the growth of breast cancer by suppressing immune surveillance. The exposure system has been described in a previous publication (Baum, 1995) and is considered satisfactory. Rats (groups of nine) were exposed for 24h/d (except for a brief period for cage maintenance), 7d/w to 100 µT (50Hz) for 2, 4, 8 and 13 weeks. Sham exposed mice were treated in parallel in an identical, non-energized exposure system. Staff was blind to the exposure status of animals. There was no positive control. At the end of exposure period rats and the appropriate shams were killed and spleens were isolated and single cell suspensions prepared according to a standard protocol. Viability was satisfactory by trypan blue dye exclusion. ConconavalinA was used as a mitogen for T-cells in the spleen cell suspension and pokeweed mitogen was used to stimulate B-cells. Cell stimulation was assessed by ^{3}H-thymidine incorporation into DNA during S-phase. Cells were harvested by autosampler. Radioactivity was not normalized to DNA or protein

concentration. Samples that did not show a clear response to the mitogens were eliminated from the analysis. The extent of stimulation differed significantly between groups. However, at weeks 2 and 4, T-cells from magnetic field exposed rats were activated about 1.5-fold over sham. At week 8, there was no difference and at week 13,T-cells from the magnetic field exposed group showed significantly less stimulation than those from sham. The authors interpret this pattern as a typical response to a general stressor (a response in the short-term, adaptation in the intermediate term, followed by the possibility of toxicity in the long-term). However, the contribution of rejected results can not be predicted. Also, the variability in apparent T-cell activation, as defined by the authors, could as easily be due to incomplete recoveries during the cell collection phase of the assay to measure DNA synthesis, as results were not normalized to some surrogate of cell number. In addition, a x1.5 fold elevation in cell counts is not very convincing as an indication of T-cell stimulation. The magnetic field had no apparent effect on B-cell activation. Proper controls were not used to ensure assay sensitivity and performance, that is there were no positive control to demonstrate mitogen-induced T-cell activation, or it's inhibition. Interleukin-1 (IL-1) is an immunoregulatory molecule that is critical in the development of an immune response. In this study, IL-1 was measured semi-quantitatively by an in-house colorimetric assay utilizing MTT. The effect of melatonin on mitogen activation of T-cells is also not convincing. The authors conclude that inhibition of T-cell responsiveness after 13 weeks of exposure to magnetic fields may be evidence of long-term suppression of immune surveillance. However, the apparent increase in T-cell activation after 2 and 4 weeks of exposure stand as anomolous results, which could be due to age-related differences in spleen cell viability and mitogen responsiveness. The use of radioactive data that were not corrected for cell numbers, also could contribute to the variability of the data. These experimental shortcomings make the interpretation of results difficult, and the pattern of T-cell responsiveness, attributed to magnetic field acting like a general stressor, is therefore not convincing. As a result, these experiments provide little support for the hypothesis that magnetic fields exert their effect on mammary tumor development in this sub-line of Sprague-Dawley rats by inhibition of immune surveillance.

Haussler M, Thun- Battersby S, Mevissen M and Loscher W. Exposure of rats to a 50-Hz, 100 Tesla magnetic field does not affect the ex vivo production of interleukins by activated T or B lymphocytes. Bioelectromagnetics 20:295- 305, 1999.

The principal author is with Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Hannover, Germany

Abstract: Two separate, independent experiments were conducted to evaluate the effects of exposure of rats to a 50-Hz linearly polarized, 100 T magnetic field (MF) on the ex vivo production of interleukins (ILs) by mitogen-stimulated splenic lymphocytes. IL-1 and IL-2 were determined by proliferation assays, using IL-dependent murine T cell lines. In the first experiment, female Sprague-Dawley rats were treated with

7,12-dimethylbenz[a]anthracene (DMBA] at a dose of 20 mg per rat (four weekly gavage doses of 5 mg), and were either MF-exposed or sham-exposed for 14 weeks. This experimental protocol has previously been shown to result in a significant increase in breast cancer growth in response to MF exposure. Furthermore, MF exposure at 50-100 T for 3 months was recently found to induce a suppressed ex vivo proliferation of splenic T cells in response to mitogen stimulation, which could be a result of reduced IL production of spleen lymphocytes. However, the present experiments failed to demonstrate any significant difference between MF- and sham-exposed groups in production of IL-1 by mitogen-activated splenic B cells. In a second experiment, shorter MF exposure periods were studied with respect to IL production from mitogen-stimulated B and T cells. Groups of rats were MF- or sham-exposed for 1 day, 1 week, or 2 weeks, followed by preparation and activation of spleen lymphocytes. No significant difference in IL-1 or IL-2 production from stimulated B or T cells was seen. The data indicate that in vivo MF exposure of rats does not affect the ex vivo IL production of B or T lymphocytes, suggesting that the recently reported changes in T cell proliferation in response to MF exposure may not be mediated via alterations in B or T cell IL production.

Comment: The objective of this study was to extend the results of a previous study (Mevissen, 1998), that found magnetic fields could suppress mitogen-induced T-cell activation. The present study examined the effect of *in vivo* magnetic field exposure of female Sprague-Dawley rats on *ex vivo* production of IL-1 and IL-2 from mitogen activated B- and T-cells. The exposure system and animal handling protocols were the same as described in Mevissen (1998). The present experiment failed to demonstrate that magnetic fields had any effect on IL-1 or IL-2 production, based on the results from two independent assays (the bioassay used by Mevissen (1998) and ELISA kits).

Rosen LA, Barber I and Lyle DB. A 0.5 G, 60 Hz magnetic field suppresses melatonin production in pinealocytes. Bioelectromagnetics 1998;19(2):123-127.

The principal author is with the Division of Research Grants, National Institutes of Health, Bethesda, Maryland 20892-7854, USA.

Abstract: The objective of this study was to develop a model for testing various hypotheses concerning possible mechanisms whereby electromagnetic fields might induce suppression of nighttime melatonin production in rodents. A published method for digesting freshly obtained pineal glands to the single cell level was modified, yielding better than 95% viability. An in vitro exposure facility developed for the Food and Drug Administration was used for 12 h overnight exposures of primary pinealocyte cultures to 0.05 mT, 60 Hz, vertical AC and 0.06 μT, DC fields. After exposure, cells were separated from the supernatant by centrifugation. Supernatant melatonin was measured by ELISA assays. Data from 10 experiments demonstrated an average 46% reduction in norepinephrine-induced production of melatonin in the pinealocytes. The results support

the hypothesis that EM exposure can produce pineal gland melatonin suppression by affecting individual cells.

Comment: Cells were isolated from rat pineal glands using a previously described technique (Schaad, 1993). The exposure system was extensively evaluated and exposure and quality assurance protocols were acceptable. Staff were blind to the exposure status of samples. Temperature control and water loss from cultures were not an issue. Isolated pinealocytes were > 95% viable when isolated. The norepinephrine concentration used to stimulate melatonin production was properly titrated to give optimal response. Several different kits for measuring melatonin were examined. The exposure of norepinephrine-stimulated pinealocytes to magnetic fields was found to suppress melatonin production by about 46%.Some internally inconsistent results were attributed to the failure of norepinephrine to stimulate melatonin, rather than to failure of magnetic fields to suppress it production. Also, some inconsistency arose from the use of ELISA kits from different suppliers with different sensitivities and detection limits. Overall, the results support the hypothesis that magnetic fields might act through a mechanism that involves the suppression of melatonin production. However, this *in vitro* observation can not be readily extrapolated to whole animals.

John TM, Liu GY and Brown GM. 60 Hz magnetic field exposure and urinary 6-sulphatoxymelatonin levels in the rat. Bioelectromagnetics 1998;19(3):172-180.

The principal author is with the Clarke Institute of Psychiatry, Toronto, Ontario, Canada.

Abstract: Four separate experiments were carried out to investigate the effect of extremely low frequency magnetic field (MF) exposure (60 Hz, 1 mT rms) on urinary 6-sulphatoxymelatonin (aMT6s) levels in Sprague-Dawley rats. In the first experiment, immature male rats maintained under a regular 12 h daily photoperiod (white fluorescent light) were exposed to a 20 h daily MF exposure for 6 weeks. The second experiment was similar to the first, except that the MF exposure was limited to 10 days. In the third experiment, adult male rats acclimated to a combination of continuous dim red light and regular 12 h daily photoperiod (white fluorescent) were subjected to a single 1 h exposure to intermittent MF (1 min on and 1 min off cycles), 2 h before fluorescent lights went off. The fourth experiment was similar to the third, except that the animals received 2 consecutive days of 20 h daily exposure to intermittent MF, beginning 1 h before the fluorescent lights went off each day. In all four experiments, the circadian profile of urinary aMT6s was examined before, during, and after the MF exposure. No significant effect of 1 mT MF on indoleamine metabolism was observed in any of the above experiments. However, in one of the experiments (no. 4), both the control and the MF groups showed a lower aMT6s level during the exposure days, when compared with that of pre- and post-exposure days, suggesting that the existence of possible effects with lower field strengths at the range of stray field cannot be ruled out.

Comment: This study measured melatonin levels in Sprague-Dawley rats indirectly by an RIA kit for urinary 6-sulphatoxymelatonin (aMT6s). The intra- and inter-assay coefficient of variation were known at 8.6% and 12.5%, respectively The use of urine, rather than blood, allowed longitudinal studies to be conducted without the needed for multiple, potentially stressful, blood sampling. The exposure system was adequately described, calibration and quality assurance protocols were acceptable. The staff were blind to the exposure status of rats and to the samples during analysis. Melatonin levels were measured at 0100 h midway through the dark cycle, at the presumed peak of melatonin production in vivo. The light/dark cycle was lights on at 07:00 and off at 19:00 h. Rats were exposed to the magnetic field for 20 h per day, with 4 h off for cage maintenance from 11:00 - 15:00 h, at the presumed melatonin low. In experiment 1, rats were exposed to 1 mT rms (60 Hz) magnetic field for 20 h/day, as described, for 6 weeks. In experiment 2, rats were exposed to the magnetic field for 10 days, but melatonin was measured two days before exposure began and continued on a daily basis for three days after exposure ceased, and then on day 5, 7 and 14. In experiment 3, rats were exposed continuously to a red light in addition to their regular 12 h light/dark cycle, and an intermittent field was applied 1 h before the end of the light portion of the light/dark cycle. Experiment 4 was similar to 3, except the magnetic field exposure was for 20 h/day for two days. Both control and magnetic field-exposed rats exhibited robust circadian rhythms. None of the magnetic field exposures had any effect on melatonin levels. The observed differences in daytime melatonin levels between sham and exposed rats was attributed not to the magnetic field but to variations in melatonin levels of individual rats. It is possible that the sensitivity of the urine-based assay was too low to detect small changes in melatonin levels. However, no positive control was used to demonstrate what such a perturbation would look like. Therefore, the results of this study must be considered as inconclusive.

Mevissen M, Haussler M, Lerchl A and Loscher W. Acceleration of mammary tumorigenesis by exposure of 7,12-dimethylbenz[a]anthracene-treated female rats in a 50-Hz, 100-microT magnetic field: replication study. J Toxicol Environ Health 1998(b);53(5):401- 418.

The principal author is with the Department of Pharmacology, Toxicology, and Pharmacology, School of Veterinary Medicine, Hannover, Germany.

Abstract: In view of the methodological problems of epidemiological studies on associations between residential and occupational exposures to 50/60-Hz magnetic fields (MF) and increased incidence of cancers, laboratory studies are necessary to determine if 50/60-Hz MF can affect cancer development or growth. Recently, it was reported that alternating (50-Hz) MF of low flux density (100 μT) increase tumor growth and progression in a model of breast cancer in female rats in which mammary tumors were induced by the chemical carcinogen 7,12-dimethylbenz[a]anthracene (DMBA). The objective of the present study was to determine if a replicate experiment carried out in the same laboratory under the same experimental conditions yields a

significant increase in tumor development and growth of similar magnitude. For the MF experiment, a group of 99 female Sprague-Dawley rats was exposed to a homogeneous horizontally polarized MF for 24 h/d (minus time for weighing, tumor palpation, cage cleaning, cage rotation), 7 d/wk; another group of 99 rats was sham exposed. DMBA was administered intragastrically at a dose of 5 mg/rat at the first day of exposure and at weekly intervals thereafter up to a total dose of 20 mg/rat. Duration of MF or sham exposure was 91 d. In both MF-exposed and sham-exposed rats, the first tumors could be recorded 6 wk after the initial DMBA application. At 9 wk after DMBA application, the group of MF-exposed rats exhibited significantly more animals with tumors than the sham-exposed group. This significant difference in the rate of tumor development was observed throughout the subsequent period of exposure. After autopsy, the incidence of macroscopically visible mammary tumors was 62% in controls, but 83% in MF-exposed rats, with the 35% difference between groups being statistically significant. Data substantiate that long-term exposure of DMBA-treated female Sprague-Dawley rats in an alternating MF of low flux density promotes the development and growth of mammary tumors, thus indicating that MF exposure exerts tumor-promoting and/or copromoting effects. Furthermore, the data show that the effects of MF exposure in the DMBA breast cancer model are reproducible if the same experiment is repeated in the same laboratory.

Comment: This study reports the replication of previous results that demonstrate that exposure to magnetic fields increases the incidence of breast cancer in female Sprague-Dawley rats.

Loscher W, Mevissen M and Lerchl A. Exposure of female rats to a 100-microT 50 Hz magnetic field does not induce consistent changes in nocturnal levels of melatonin. Radiat Res 1998;150(5):557-567.

The principal author is with the Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Hannover, Germany.

Abstract: The hypothesis whereby alternating (50 or 60 Hz) magnetic fields such as those produced by electric power reduce the nocturnal production of melatonin in the pineal gland and thereby indirectly enhance development and growth of breast cancer has attracted a great deal of interest. In view of the potential importance of this hypothesis that there is a link between electric power and breast cancer, which is also known as the "melatonin hypothesis", we undertook various experiments in female Sprague-Dawley rats to evaluate whether 100 µT 50 Hz magnetic-field exposure, i.e. a flux density shown recently to exert a tumor (co)promoting effect in the 7,12-dimethylbenz[a]anthracene (DMBA) model of breast cancer in Sprague-Dawley rats, consistently reduces melatonin levels and, if not, which factors may be involved in the inconsistent effects of magnetic-field exposure on production of melatonin. Long-term exposure of female Sprague-Dawley rats to magnetic fields for 13 weeks did not alter the nocturnal levels of melatonin in the pineal gland or serum (determined 5 h

after the onset of darkness) significantly, irrespective of whether rats were treated with DMBA or not. In one experiment, when blood was sampled 3, 5 and 6 h after the onset of darkness after 2 weeks of magnetic-field or sham exposure, a significant decrease in melatonin was seen in magnetic-field-exposed rats at 6 h. However, the results could not be reproduced in two subsequent experiments in other groups of rats. Shorter (1 day, 1 week) or longer (4, 8, 13 weeks) exposure periods also did not result in any significant effects of the magnetic field on melatonin levels when blood sampling was performed either 5 or 6 h after onset of the dark phase. Various potential sources of variation in melatonin levels or in magnetic-field effects on melatonin levels were evaluated, but the reason(s) for the inconsistent effect of magnetic-field exposure remains unclear. Thus the present study failed to demonstrate a consistent effect of 100 μT 50 Hz magnetic-field exposure on melatonin levels in Sprague-Dawley rats.

Comment: This study is an attempt to replicate a previous study which found that exposure to 100 μT (50Hz) magnetic field for two weeks caused a decrease in melatonin levels 6 h after the onset of darkness. The experimental designs of the present and past experiments were the same, however, the previous results could not be replicated, and magnetic fields failed to affect melatonin levels in Sprague-Dawley rats. This inconsistency weakens the ‘melatonin’ hypothesis of breast cancer.

Thun-Battersby S, Mevissen M and Loscher W. Exposure of Sprague-Dawley rats to a 50-Hertz, 100-microTesla magnetic field for 27 weeks facilitates mammary tumorigenesis in the 7,12-dimethylbenz[a]-anthracene model of breast cancer. Cancer Res 1999;59(15):3627- 3633.

The principal author is with the Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine Hannover, Germany.

Abstract: We have shown previously (W. Loscher et al., Cancer Lett., 71: 75-81, 1993; M. Mevissen et al., Carcinogenesis (Lond.), 17: 903-910, 1996) that 50-Hz magnetic fields (MFs) of low (50 μT or 100 μT) flux density enhance mammary gland tumor development and growth in the 7,12-dimethylbenz[a]anthracene (DMBA) model of breast cancer in female Sprague Dawley rats. In these previous experiments, groups of rats were given 20 mg of DMBA (four weekly gavage doses of 5 mg each) and were MF- or sham-exposed for 13 weeks. The objective of the present study was to examine whether the use of a lower dose of DMBA (10 instead of 20 mg per rat), MF exposure of the rats before DMBA injection, and the increase of the MF exposure period after DMBA application to 26 weeks enhance the effect of MF on tumor development and growth. A group 99 rats was exposed to a homogeneous, horizontally polarized 100-microT MF of 50-Hz for 24 h/day for 7 days/week; another group of 99 rats was sham-exposed under the same environmental conditions as the MF-exposed rats. The exposure chambers were identical for MF-exposed and sham-exposed animals. The age of the rats was 45-49 days at the onset of exposure; duration of MF or sham exposure was 27 weeks. DMBA was administered p.o. at a dose of 10 mg/rat after 1 week of MF or sham

exposure. The animals were palpated once weekly from week 6 onwards to assess the development of mammary tumors. At the end of the exposure period, the animals were killed for the determination of number and volume and histological verification of mammary tumors. All of the recordings were done in a blinded fashion; i.e., the investigators were not aware which animals were MF- or sham-exposed. Mammary tumor development and growth was significantly enhanced by MF exposure, the most marked effect on tumor incidence (190% above sham control) being observed 13 weeks after DMBA administration. At the time of necropsy,
i.e., 26 weeks after DMBA administration, the incidence of histologically verified mammary tumors was 50.5% in controls and 64.7% in MF-exposed rats, the difference being statistically significant. More marked intergroup differences were recorded when tumor incidence was separately evaluated for each of the six mammary complexes, the most pronounced MF effect on tumor incidence being seen in the cranial thoracic complex. The data substantiate that, at least under the experimental conditions used in our laboratory, 50 Hz, 100 μT MF exposure significantly facilitates the development and growth of mammary tumors in the DMBA rat model of breast cancer.

Comment: This study confirms the results of previous studies by this laboratory group (Loscher, 1995) (Mevissen, 1996), (Mevissen, 1998) that magnetic fields significantly affect mammary tumor growth in DMBA-treated Sprague-Dawley rats. The exposure system, calibration and quality assurance protocols were acceptable, and temperature control was not an issue. The statistical procedures used to analyze the data were acceptable. Animal handling techniques were satisfactory, and the staff were blind to the exposure status of the samples. One interesting exposure variable was the use of a dim red light in the exposure room during the 12 h dark portion of the light/dark cycle. The authors state they believe this is a predisposing factor for the tumor promoting action of the magnetic fields. Rats were initiated by 10 mg DMBA by gavage, and then exposed to 100 μT magnetic fields for 24 h/d, 7 d/week for 27 w. There were 99 mice in each of the sham and magnetic field groups. Mammary tumors were visually identified, and then examined histologically. This study found magnetic fields produced a significant increase in the incidence of mammary tumors. A study by Anderson et al (1999) (see above) found the magnetic field produced no such effect under their experimental conditions. The differences in the experimental results were discussed by Anderson (2000). The differences between studies conducted by Anderson (1999) and colleagues in the USA, and Loscher and colleagues in Europe, was summarized by Anderson (2000) (see Appendix) and can be summarized as follows: (i) Differences in diet. (ii) The exposure period in Loscher's study, was shorter than in Anderson's study by ~ 500 h for the 13 week experiments. (iii) The US study group used different rooms for sham and magnetic field exposures. (iv) The Sprague-Dawley rat strain used by the US investigators were more sensitive to DMBA than the sub-line of Sprague-Dawley rats used by the European investigators (vanZwieten, 1984). The tumor promotion effects observed by Thun-Battersby can not be easily dismissed (replicated three times).

Anderson LE, Boorman GA, Morris JE, Sasser LB, Mann PC, Grumbein SL, Hailey JR, McNally A, Sills RC and Haseman JK. Effect of 13 week magnetic field exposures on DMBA-initiated mammary gland carcinomas in female Sprague-Dawley rats. Carcinogenesis 1999;20(8):1615-1620.

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Abstract: Several studies suggest that exposure to 50 Hz magnetic fields may promote chemically induced breast cancer in rats. Groups of 100 female Sprague-Dawley rats were initiated with four weekly 5 mg gavage doses of 7,12-dimethylbenz[a]anthracene (DMBA) starting at 50 days of age. After the first weekly DMBA administration, exposure to ambient fields (sham exposed), 50 Hz magnetic fields at either 1 or 5 G field intensity or 60 Hz fields at 1 G for 18.5 h/day, 7 days/week was initiated. Exposure continued for 13 weeks. A vehicle control group without DMBA was included. In a second study, using lower doses of DMBA, groups of 100 female Sprague-Dawley rats were initiated with four weekly doses of 2 mg of DMBA starting at 50 days of age followed, after the first weekly DMBA administration, by exposure to ambient fields (sham exposed) or 50 Hz magnetic fields at either 1 or 5 G field intensity for 18.5 h/day, 7 days/week for 13 weeks. Rats were weighed and palpated weekly for the presence of tumors. There was no effect of magnetic field exposure on body weight gains or on the time of appearance of mammary tumors in either study. At the end of 13 weeks, the animals were killed and the mammary tumors counted and measured. Mammary gland masses found grossly were examined histologically. In the first 13 week study, the mammary gland carcinoma incidences were 92, 86, 96 and 96% for the DMBA controls, 1 G, 50 Hz, 5 G, 50 Hz and 1 G, 60 Hz groups, respectively. The total numbers of carcinomas were 691, 528 ($P < 0.05$, decrease), 561 and 692 for the DMBA controls, 1 G, 50 Hz, 5 G, 50 Hz and 1 G, 60 Hz groups, respectively. In study 2, the mammary gland carcinoma incidences were 43, 48 and 38% for the DMBA controls, 1 G, 50 Hz and 5 G, 50 Hz groups, respectively. The total numbers of carcinomas were 102, 90 and 79 for the DMBA controls, 1 G, 50 Hz and 5 G, 50 Hz groups, respectively. There was no effect of magnetic field exposure on tumor size either by in-life palpation or by measurement at necropsy in either study. There was no evidence that 50 or 60 Hz magnetic fields promoted breast cancer in these studies in female rats. These studies do not support the hypothesis that magnetic field exposure promotes breast cancer in this DMBA rat model.

Comment: This is the peer reviewed publication of the National Toxicology Program (USA) report TR489 (NIH Publication 993979). The exposure system, its calibration and quality assurance protocols were acceptable. The study used female Sprague-Dawley rats and two different doses of DMBA (8 mg or 20 mg, administered by gavage) as the initiator, and, as the putative promoter, two magnetic flux densities at 50Hz (1 G and 5 G, or 0.1 and 0.5 mT respectively) and one at 60 Hz (1 G) for 18.5 h/d, 7 d/w for 13 weeks. The rats were exposed to a dim red light during the 12 h dark period of the

light/dark cycle (similar to Loscher's group). A vehicle control was also used in the study. Two studies were conducted. In the first, rats were initialed with 20 mg of DMBA, given in four divided doses, followed by 13 weeks of exposure to magnetic fields (DMBA alone was used as positive control). At 13 weeks, the cumulative proportion of DMBA-initiated, positive control rats (no EMF exposure) with palpable tumors approached 100% (Fig. 2), which was similar to the cumulative proportion of rats with mammary tumors that were initiated with DMBA but exposed to magnetic fields. Such an arrangement leaves no 'headroom' for a magnetic field-induced to be detected, if one were to occur. As a result, either the DMBA was more potent than anticipated, or, the rats were more sensitive to DMBA initiation than anticipated. In the second study, the DMBA was reduced to 8 mg, given in four divided doses followed by 13 weeks of exposure to magnetic fields (DMBA alone was the positive control). At the end of 13 weeks, the incidence of mammary tumors in the positive control was ~ 30-40%. Mammary tumors that were capable of being detected visually were removed and subjected to histopathologically evaluation. Under the conditions of the experiment, the magnetic field had no effect on the development or incidence of mammary tumors, or on tumor size, when compared with sham exposed controls. This study does not support the hypothesis that magnetic fields act as a tumor promoter. Consequently, the results are at odds with those of Loscher's group.

Boorman GA, Anderson LE, Morris JE, Sasser LB, Mann PC, Grumbein SL, Hailey JR, McNally A, Sills RC and Haseman JK. Effect of 26 week magnetic field exposures in a DMBA initiation-promotion mammary gland model in Sprague-Dawley rats. Carcinogenesis 1999(b);20(5):899-904.

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Abstract: Several studies have suggested that exposure to 50 Hz magnetic fields promote chemically induced breast cancer in rats. Groups of 100 female Sprague-Dawley rats were initiated with a single 10 mg gavage dose of 7,12-dimethylbenz[a]anthracene (DMBA) at 50 days of age followed by exposure to ambient fields (sham exposed), 50 Hz magnetic fields at either 1 or 5 Gauss (G) field intensity or 60 Hz fields at 1 G for 18.5 h/day, 7 days/week for 26 weeks. A vehicle control group without DMBA was included. Rats were palpated weekly for the presence of tumors. There was no effect of magnetic field exposure on body weight gains or the time of appearance of mammary tumors. At the end of 26 weeks, the animals were killed and the mammary tumors counted and measured. Mammary gland masses found grossly were examined histologically. The mammary gland carcinoma incidence was 96, 90, 95 and 85% ($P < 0.05$, decrease) for the DMBA controls, 1 G 50 Hz, 5 G 50 Hz and 1 G 60 Hz groups, respectively. The total numbers of carcinomas were 649, 494 ($P < 0.05$, decrease), 547 and 433 ($P < 0.05$, decrease) for the DMBA controls, 1 G 50 Hz, 5 G 50 Hz and 1 G 60 Hz groups, respectively. The number of fibroadenomas varied from 276 to 319, with the lowest number in the 1 G 60 Hz exposure group. Measurement of

the tumors revealed no difference in tumor size between groups. In this breast cancer initiation-promotion study in female Sprague-Dawley rats, there was no evidence that 50 or 60 Hz magnetic fields promoted breast cancer under the conditions of this assay. This study does not support the hypothesis that magnetic field
exposure can promote breast cancer in this rat model.

Comment: This study is an extension of the one by Anderson (1999). In the present study, Sprague-Dawley rats were initiated with a single 10 mg dose by gavage followed by 26 weeks of exposure to 1 G (50 Hz), 5 G (50 Hz) or 1 G (60 Hz) magnetic fields (18.5 h/d, 7d/w). DMBA alone was the positive control and a vehicle control was also used. The exposure system, calibration, quality assurance protocols were acceptable as were the animal handling protocols. A dim red light was used to illuminate the dark portion of the light/dark cycle. At 26 weeks, the cumulative proportion of DMBA-initiated, positive control rats (no EMF exposure) with palpable tumors approached 100%, which was similar to the cumulative proportion of rats with mammary tumors that were initiated with DMBA and exposed to magnetic fields. Such an arrangement leaves no 'headroom' for a magnetic field-induced effect to be detected, if one had occurred. As a result, the incidence of mammary tumors can not be evaluated by this study. There was no difference between positive control and magnetic field exposed mice in terms of the size of mammary tumors, nor was there any increase in tumor multiplicity in the magnetic field exposed mice over the positive control. The inability of the study to evaluate tumor incidence is a weakness, since this is the hallmark of carcinogenic potential.

Mevissen M, Haubler M and Loscher W. Alterations in ornithine decarboxylase activity in the rat mammary gland after different periods of 50 Hz magnetic field exposure. Bioelectromagnetics 1999;20:338-346.

The principal author is with the Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Hannover, Germany

Abstract: In a series of experiments with the chemical carcinogen DMBA (7,12-dimethyl[a]anthracene), we recently found that exposure of female Sprague-Dawley rats in 50 Hz magnetic fields (MF) in the microtesla range significantly facilitates the development and growth of mammary tumors. One possible explanation for this finding would be enhanced proliferation of breast epithelial stem cells by MF exposure, thereby increasing the sensitivity of these cells to chemical carcinogens. In line with this possibility, we previously determined that 50 Hz, 50 T MF exposure induces increases in ornithine decarboxylase (ODC), i.e., a key enzyme in cell proliferation, in the mammary gland of female Sprague-Dawley rats. In the present study, we examined the time course of this effect, by using different periods of exposure to a 50 Hz, 100 T MF. Furthermore, we determined ODC in different mammary complexes of the rat mammary gland to evaluate whether differences in response to MF exist over the anterior-posterior extension of this organ. Exposure of young female Sprague-Dawley rats induced marked increases in ODC in the mammary gland that

were similar to ODC increases seen in positive control experiments with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). However, this effect of MF critically depended on the duration of MF exposure, with no effect, or at least no consistent effect, for short (<1 week) or long (8 weeks and above) exposure periods, but a robust and reproducible enhancing effect on ODC activity after 2 weeks of exposure. Furthermore, we found that the effect of MF exposure depends on the part of the mammary complexes examined, the cranial thoracic (or cervical) complexes being particularly sensitive to ODC alterations in response to MF. This is in line with recent DMBA experiments of our group in which MF-induced increases in tumor development and growth were predominantly seen in this large cranial/cervical part of the mammary gland. The most likely explanation for the observed ODC changes after MF exposure is the melatonin hypothesis, although other cellular and molecular effects of MF might be involved as well.

Comment: This study measured the effect of 100 μT (50Hz) magnetic fields on ornithine decarboxylase (ODC) activity in the mammary complex of Sprague-Dawley rats. ODC is a key enzyme in the synthesis of polyamines (spermine, spermadine and putrescine) which are essential element for cell proliferation. ODC has also been postulated to play a role in tumor promotion. The objective of the present study was to confirm and extend the findings of a previous study (Mevissen, 1995) that reported magnetic fields alone enhanced ODC activity in mammary gland of rats. The exposure system, its calibration and quality assurance protocols were satisfactory, as described. Temperature control during exposure was not a problem. Animal handling and sham exposure protocols were adequate, and staff were blind to the exposure status of animals, and to sample status during the analysis of mammary tissue. Phorbol myristate acetate (PMA) was used as a positive control. The assay for ODC was based on ^{14}C release from ^{14}C-ornithine. Radioactivity was expressed per mg of protein. Preliminary studies were used to maximize the sensitivity of the assay by time series analysis and the use of ODC from E.Coli. The recoveries of analytes in separation steps were examined by quantitative analysis. Also different volumes of the sample were analyzed to confirm findings. The assay procedure for ODC was acceptable. The magnetic field increased the activity of ODC after two weeks of exposure, particularly in the cranial-thoracic mammary complex (less in other regions), the area where most mammary tumors had been found in previous studies by Loscher's group. These results were repeated a large number of times. No increase in ODC was detected after 8 or 13 weeks of exposure. There is no reason to reject this finding. To be accepted as valid, these findings would have to be independently repeated.

Wilson BW, Matt KS, Morris JE, Sasser LB, Miller DL and Anderson LE. Effects of 60 Hz magnetic field exposure on the pineal and hypothalamic-pituitary-gonadal axis in the Siberian hamster (Phodopus sungorus). Bioelectromagnetics 1999;20(4):224-232.

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Abstract: Experiments using the dwarf Siberian hamster Phodopus sungorus were carried out to determine possible neuroendocrine consequences of one-time and repeated exposures to 60 Hz magnetic fields (MF). Animals were maintained in either a short-light (SL, 8 h light: 16 h dark) or long-light (LL, 16 h light: 8 h dark) photoperiod. Acute (one-time, 15 min) exposure of male SL animals to a linearly polarized, horizontally oriented, 60 Hz MF (0.1 mT) gave rise to a statistically significant ($P < .005$) reduction in pineal melatonin content as determined 3 and 5 h after onset of darkness. In LL animals, acute exposure to 0.10 mT resulted in a significant decrease in pineal melatonin as measured 4 h after onset of darkness, whereas acute exposure to 50 microT showed no effect compared with sham exposure. In SL animals, an increase in norepinephrine was observed in the medial basal hypothalamus (including the suprachiasmatic nucleus) after acute exposure ($P < .01$). Daily MF exposure of SL animals to a combination of steady-state and on/off 60 Hz magnetic fields (intermittent exposure) at 0.1 mT for 1 h per day for 16 days was associated with a reduction in melatonin concentrations at 4 h after onset of darkness and an increase in blood prolactin concentrations ($P < .05$). Exposure of SL animals to a steady state 60 Hz MF for 3 h/day for 42 days resulted in a statistically significant reduction in body weight (ANOVA: $P > .05$), compared with sham-exposed SL animals. At 42 days, however, no significant changes in overnight melatonin or prolactin levels were detected. In both repeated exposure experiments, gonadal weights were lowest in the MF-exposed groups. This difference was statistically significant ($P < .05$) after 42 days of exposure. These data indicate that both one-time and repeated exposure to a 0.1 mT, 60 Hz MF can give rise to neuroendocrine responses in Phodopus.

Comment: This study characterizes the response of the dwarf Siberian hamster (DSH) to magnetic fields. The objective was to determine if the DSH could be used as a model system for the study of MF-induced bioeffects in humans. The DSH demonstrates magnetic orientation in the location of nests and is strongly photperiodic. Previous studies indicate magnetic fields can suppress melatonin production in the DSH. The neuroendocrine response to changes in photoperiod is rapid: within 35-40 days of the onset of a short photoperiod (8 h light, 18 h dark) there is testicular regression and changes in prolactin, testosterone and melatonin levels. The object of the present study was to determine the effects of one-time and repeated exposures to 60 Hz magnetic field on the pineal gland and the hypothalamic-pituitary-gonadal axis. Three hypotheses were tested, (i) whether acute (one-time) exposure to magnetic fields would suppress melatonin levels, (ii) whether magnetic fields would alter catecholamine (norepinephrine) levels in the hypothalamus, and (iii) whether repeated exposures to magnetic fields would alter circulating prolactin levels and gonadal status. The parameters of the photoperiod and magnetic field exposure systems and exposure facilities were all well characterized. Animal handling was acceptable. Staff was blind to the exposure status of the animals and the subsequent tissue and fluid samples. The

assays for all biochemicals were acceptable. Statistical analyses were acceptable. The study results demonstrate that acute (15 m) exposure to 0.1 mT magnetic field altered pineal gland and HPG function, as indicated by changes in melatonin and norepinephrine levels, respectively. Repeated daily exposures to magnetic fields increased levels of prolactin and reduced gonadal weight. The results indicate a pattern of neuroendocrine responses to magnetic fields in the photoperiod responsive DSH. Whether this animal model will ultimately be useful in the study of human breast cancer remains to be proven.

Bakos J, Nagy N, Thuroczy G, Szabo LD. One week of exposure to 50 Hz, vertical magnetic field does not reduce urinary 6-sulphatoxymelatonin excretion of male wistar rats. Bioelectromagnetics 2002;23(3):245-248.

The principal author is with the Department of Non-ionizing Radiation, National "Frederic Joliot -Curie" Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary.

Abstract: The effect of exposure to 100 or 50 μT, 50 Hz, vertical magnetic field on the excretion of 6-sulphatoxymelatonin (6SM) in the nocturnal urine of rats was studied. Twelve male Wistar rats were kept under 12:12 h light:dark conditions. The nocturnal urine of animals was collected in metabolic cages over 4 consecutive weeks. The concentration of 6SM in the rat urine was measured by 125I radioimmunoassay and normalized to creatinine concentration. After the first week of urine collection, 6 rats were exposed to 100 μT or 50 μT flux density magnetic fields (MF) for 8 h daily for 1 week. It was found that the excretion of the primary metabolite of melatonin in the urine, 6SM, did not show statistically significant changes during and after magnetic field exposure.

Comment: This study measured the effect of magnetic fields on the secretion of 6-sulphatoxymelatonin (6SM) in the urine of Wistar rats (bred in-house). The exposure facilities and calibration protocols were acceptable, as described. Animal handling protocols were adequate and rats were acclimatized before experimentation and the collection of urine. The assay for 6SM was by commercial RIA kit, and sensitivity and coefficients of variation parameters were given. Radioactive counts were normalized to creatinine concentration, which was determined by a clinical auto-analyzer. There is no indication that the analysts and staff were blind to the exposure status of animals or samples. Statistical analysis of the results was acceptable. The study found no significant differences in urinary 6SM levels between rats exposed to magnetic fields and sham-exposed controls. These results are similar to those of John (1998) and of Loscher et al (1998).

Fedrowitz M, Westermann J and Loscher W. Magnetic field exposure increases cell proliferation but does not affect melatonin levels in the mammary gland of female Sprague Dawley rats. Cancer Res 2002;62(5):1356-1363.

The principal author is with the Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, D-30559 Hannover, Germany.

Abstract: In line with the possible relationship between electric power and breast cancer risk as well as the underlying "melatonin hypothesis," we have shown previously (Thun-Battersby et al., Cancer Res., 59: 3627-3633, 1999) that 50 Hz magnetic fields (MFs) of low (100 µT) flux density enhance mammary gland tumor development and growth in the 7,12-dimethylbenz(a)anthracene model of breast cancer in female Sprague Dawley rats. On the basis of the melatonin hypothesis and previous observations of induction of ornithine decarboxylase in response to MF, we proposed that the effect of MF exposure on mammary carcinogenesis is related to enhanced proliferation of the mammary epithelium. The objective of the present study was to directly assess this proposal by the use of proliferation markers. Female Sprague Dawley rats were MF or sham exposed for 2 weeks at a flux density of 100 µT. Proliferation of epithelial cells in the mammary tissue and adjacent skin was examined by in vivo labeling of proliferating cells with bromodeoxyuridine (BrdUrd) and in situ labeling of the nuclear proliferation-associated Ki-67 protein by the antibody MIB-5. Furthermore, melatonin levels were determined after MF or sham exposure in the pineal gland and directly in the mammary tissue. In additional experiments, the tumor promoter 12-O-tetradecanoylphorbol-13-acetate was used for comparison with the effects of MF exposure. MF exposure significantly enhanced BrdUrd and Ki-67 labeling in the mammary epithelium, indicating a marked increase in cell proliferation. The most pronounced effect on proliferation was seen in the cranial thoracic (or cervical) mammary complexes, in which we previously had seen the most marked effects of MF exposure on mammary carcinogenesis. In contrast to the melatonin hypothesis, melatonin levels in pineal or mammary glands were not affected by MF exposure. Topical application of 12-O-tetradecanoylphorbol-13-acetate increased BrdUrd and Ki-67 labeling in epithelial cells of the skin, particularly in hair follicles, but not in the mammary tissue. The data demonstrate that MF exposure results in an increased proliferative activity of the mammary epithelium, which is a likely explanation for the cocarcinogenic or tumor promoting effects of MF exposure observed previously by us in the 7,12-dimethylbenz(a)anthracene model of breast cancer.

Comment: This is an extension of the studies by Loscher and colleagues. They present an alternative to the melatonin-breast cancer hypothesis, based on their previous observations. The alternative hypothesis basically says that magnetic fields act directly to stimulate proliferation of initiated cells in rat mammary epithelial tissue, and is based on previous observations that magnetic fields increased ornithine decarboxylase (ODC) activity in mammary epithelial tissue 2 to 8 weeks after start of exposure (Mevissen, 1999). ODC is considered a biomarker for tumor promotion, and its increase, induced by magnetic fields, preceded the appearance of a significant increase in the incidence of breast cancers, relative to sham exposed controls. Under these experimental conditions, melatonin levels remained unaffected by magnetic field exposures. Additional biomarkers of cell proliferation in mammary epithelial tissue was presented, including *in*

vivo labeling with bromodeoxyuridine, and *in situ* labeling of nuclear proliferation-associated Ki-67 protein. Labelling was particularly evident in the cervical mammary complexes, the site where most tumor developed in past studies by Loscher and colleagues. Phorbol myristate acetate (PMA) was also used as a positive control to demonstrate an enhancement of skin cell proliferation. However, PMA did not enhance cell proliferation in mammary epithelial tissue, which implies that magnetic fields alone, in this sub-line of Sprague -Dawley rat, acts as a breast cancer promoter.

11.5 Growth of Implanted Tumors

Table 28. Summary - growth of implanted tumors

Author	Date	Species / Endpoint	Results	Weakness
Galloni	2000	C3H/DBA 2J female mice; tumor growth	no affect of MF on growth or X_ray-induced killing	none apparent

Galloni P and Marino C. Effects of 50 Hz magnetic field exposure on tumor experimental models. Bioelectromagnetics 2000;21(8):608-614.

The principal author is with the Section of Toxicology and Biomedical Sciences, Environmental Department, ENEA, C.R. Casaccia, Rome, Italy.

Abstract: The aim of this study was to investigate the interaction between a 50 Hz, 2 mT magnetic field (MF) exposure and cell growth of mammary murine adenocarcinoma, injected into experimental mice. Six different experimental protocols were performed over 2 years; several different protocols of timing of exposure were tested. X-ray radiation was adopted as the positive control. Tumor incidence and the tumor development time were calculated. No effect was observed in any experiment, and there was no statistically significant difference related to time courses among the protocols used. Neither the time of tumor cell injection nor the time of exposure produced differences between unexposed, sham, and exposed mice. When X-ray radiation was applied, the cytotoxic effect of ionizing radiation was clear, but was not increased or modified by MF exposure. Finally, the study revealed how the host-tumor system has shown a distinctive variability, unmodified by MF exposure.

Comment: The tumor model used in this study was a moderately differentiated murine mammary adenocarcinoma (C3h tif) implanted into C3H/DBA 2J female mice. The use of this model in cancer studies has been previously described and characterized (Kallman, 1987). The exposure system was adequately described and appeared

satisfactory. Calibration of the coil assembly and the quality assurance protocols were acceptable. The sham mice were exposed to ambient magnetic fields and were placed in an area adjacent to the exposure chamber. There was no indication staff were blind to the exposure status of samples. Mice were inoculated with tumor cells and then either exposed to ambient or 2 mT (50 Hz) magnetic fields. X-rays were used as a positive control (the tumor-bearing area of the hind limb was irradiated with 10 Gy when the tumor reached ~ 200 mm^3). In untreated mice, tumors became palpable 10 to 15 days after injection. The magnetic field produced no significant effect either on tumor incidence and tumor development times, and there was no evidence that magnetic fields enhanced the cytotoxicity of X-rays.

Appendix - Animal Studies

Baldwin WS and Barrett JC. Melatonin: receptor-mediated events that may affect breast and other steroid hormone-dependent cancers. Mol Carcinog 1998;21(3):149-155.

The principal author is with the Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA.

Abstract: Epidemiological studies have suggested a possible link between extremely low frequency electromagnetic fields (EMFs) and increased rates of certain cancers. One cancer that has been postulated to be associated with EMF exposure is breast cancer, for which increased rates have been reported among electricians. These cancer associations are weak, and the link to EMF exposures remains tenuous. Understanding the mechanisms by which EMFs could have biological effects will help in elucidating the risk, if any, from EMFs. One hypothesis that has received considerable attention involves reduction of melatonin levels by EMFs. This hypothesis suggests that loss of melatonin affects a variety of hormonal processes such as estrogen homeostasis and thereby may increase breast cancer rates. Since this theory was first presented, putative melatonin receptors have been cloned, providing new tools with which to examine melatonin's mechanism of action and the melatonin hypothesis. These receptors are found in nuclear and membrane fractions of cells. The nuclear receptors (retinoid Z receptors) are found both in the brain and in non-neural tissues, whereas the membrane-bound receptors are found primarily in neural tissue and have a higher affinity for melatonin. These receptors may control a variety of hormonal and immunological functions, including the release of gonadotropins from the hypothalamus and pituitary and 5-lipoxygenase activity in B lymphocytes. This Working Hypothesis briefly reviews our current knowledge of melatonin receptors and then provides theories on how the inactivation of melatonin receptors may cause cancer and suggests areas of research for addressing this question.

Loscher W. Do cocarcinogenic effects of ELF electromagnetic fields require repeated long-term interaction with carcinogens? Characteristics of positive studies using the DMBA breast cancer model in rats. Bioelectromagnetics 2001;22(8):603-614.

The author is with the Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Bunteweg 17, D-30559 Hannover, Germany.

Abstract: The carcinogenic or cocarcinogenic potential of extremely low frequency (ELF; 50 or 60 Hz) magnetic fields (MFs) has been evaluated worldwide in diverse animal model systems. Though most results have been negative, weakly positive or equivocal results have been reported in several cancer models, including the rat DMBA (7,12-dimethylbenz[a]anthracene) model of mammary cancer. Based on the experimental conditions used in studies in which cocarcinogenic effects of ELF MF were found, it was recently proposed that MF exposure may potentiate the effects of known carcinogens only when the animals are exposed to both MF and carcinogen during an extended period of tumor development, i.e., when the carcinogen is given repeatedly during MF exposure. This review summarizes a series of experiments from our group, showing cocarcinogenic MF effects in the DMBA breast cancer model in rats, to test whether the above proposal is confirmed by existing data. Flux densities of 50 or 100 μT significantly increased the growth of mammary tumors, independent of whether DMBA was given in a single administration or repeatedly over a prolonged period. Thus, these data do not substantiate the hypothesis requiring repeated doses of DMBA during MF exposure. Instead, several other aspects of study design and experimental factors are identified that seem to be critical for the detection of cocarcinogenic effects of MF exposure in the rat DMBA mammary cancer model. These include the rat subline used, the dose of DMBA, the duration of MF exposure, the flux density, the background (sham control) tumor incidence, and the location of mammary tumors in the mammary gland complex. These and other experimental aspects may explain why some laboratories did not detect cocarcinogenic MF effects in the DMBA model. We hope that direct comparison of MF bioeffects in different rat sublines and further evaluation of other experimental differences between studies on MF exposure in the DMBA model will eventually determine which genetic and environmental factors are critical for potential carcinogenic or cocarcinogenic effects of ELF MF exposure.

Anderson LE[1], Morris JE[1], Sasser LB[1] and Loscher W[2]. Effects of 50- or 60-hertz, 100 microT magnetic field exposure in the DMBA mammary cancer model in Sprague-Dawley rats: possible explanations for different results from two laboratories. Environ Health Perspect 2000;108(9):797-802.

The authors are with [1]the Environmental and Health Sciences Division, Battelle, Richland, Washington, USA, and [2]the Department of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine, Bunteweg 17, D-30559 Hannover, Germany.

Abstract: In line with the possible relationship between electric power and breast cancer risk and the underlying melatonin hypothesis, 50-Hz magnetic field (MF) exposure at microtesla flux densities for either 13 or 27 weeks significantly increased the development and growth of mammary tumors in a series of experiments from Loscher's group in Germany. Loscher's group used the 7,12-dimethylbenz[a]anthracene (DMBA) model of breast cancer in Sprague-Dawley rats. The finding could not be replicated when a similar experimental protocol was used in a study conducted by Battelle in the United States. In the present paper, investigators from the two groups discuss differences between their studies that might explain the apparent discrepancies between the results. These differences include the use of different substrains of Sprague-Dawley rats (the U.S. rats were more susceptible to DMBA than the European rats), different sources for diet and DMBA, differences in environmental conditions, and differences in MF exposure metrics. Furthermore, the effects of MF exposure reported by Loscher's group, albeit significant, were weak. We also discuss the general problem of replicating such weak effects.

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12. EXPOSURE STANDARDS

12.1 Introduction

Studies of possible effects on human health from exposure to ELF electric and magnetic fields have led to the development of exposure guidelines and regulations, which are generally referred to as standards. A regulation is essentially a mandatory standard and is normally promulgated under an act of legislation. A guideline by itself has no legal force and is issued for guidance as a voluntary standard. However, guidelines become mandatory if they are referred to in a regulation.

Based on the currently available data and information in recent review reports [NRPB, 2001), ICNIRP (2001), (Health Council of the Netherlands, 2003) and NRPB, 2004a], there is no clear evidence of the carcinogenic effects of extremely low frequency (ELF) EMFs in adults, and no firm conclusions can be made whether these fields can cause cancer in children. Although the International Agency for Research on Cancer has classified power frequency magnetic fields as a possible carcinogen (IARC, 2002), there is insufficient data to determine whether or not a cause and effect (dose-response) relationship exists. In the absence of such a relationship, the data concerning childhood leukemia cannot be used to derive quantitative exposure guidance. As a consequence of inconclusive evidence from epidemiological studies, exposure standards are therefore derived from biological effects data and normally represent an exposure level below which no adverse effects are detected.

Standards for limiting EMF exposures usually provide occupational and general public exposure recommendations. Occupational limits are set at levels below thresholds for adverse health effects. An additional safety factor is then introduced to arrive at the limits for exposure of the general public. The lower limits take into account the fact that the age and health status of the general public may differ from those of workers.

The basic limits (restrictions) recommended in major ELF exposure standards are given in terms of the current densities or the electric fields produced in the body by induction. In practice, direct measurements of these quantities are feasible only under laboratory conditions. Recommended maximum exposure levels in terms of unperturbed electric and magnetic field strength, also known as reference levels, are therefore given in addition to the basic restrictions. These reference levels are obtained from the basic restrictions by mathematical modeling and by extrapolation from the findings of laboratory studies. Some exposure standards also provide recommendations for contact currents from energized metallic objects.

12.2 Existing Standards

In Canada, there are no national standards for occupational or residential exposure to ELF fields. Guidance for occupational exposure has been recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 2003). This guidance is designed for use by industrial hygienists in making decisions regarding safe levels of exposure to EMF found in the workplace. The ACGIH recommended limit values are based on an assessment of available data from laboratory research and human exposure studies, and on limiting induced internal currents to levels below those that are believed to produce adverse health effects. ACGIH cautions that these limit values should be used as guides in the control of exposure and should not be regarded as fine lines between safe and dangerous levels.

The most often cited exposure standards are the guidelines recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP), which were published in 1998 (ICNIRP, 1998). These guidelines, which cover the entire frequency range of time-varying EMF up to 300 GHz, have been adopted by a number of countries outside North America and by the European Union (CEU, 1999). In the ELF frequency range, the basic restrictions are provided in terms of current density to protect against acute exposure effects on central nervous system tissues in the head and the trunk of the body. A safety factor of 10 was incorporated in the design of restriction levels for occupational exposure to prevent these effects. For the frequency range from 4 Hz to 1 kHz, the basic restriction for occupational exposure is 10 mA/m^2. For the general public an additional factor of 5 is applied, resulting in a basic restriction of 2 mA/m^2. The reference levels for electric and magnetic fields were derived from these basic restrictions using mathematical modeling. A large circular loop was used for magnetic field induction modeling, while the exposure conditions and the size, shape and position of the exposed body in the field were taken into account in the electric field induction model.

Recommendations for preventing harmful effects from exposure to ELF fields have also been published by the Institute of Electrical and Electronics Engineers (IEEE, 2002). The recommendations, known as IEEE C95.6 Standard, are intended to apply to exposures of the general public as well as to individuals in the workplace (controlled environments). This standard introduces a different biological basis for designing exposure guidelines and distinguishes between different tissue types (brain, heart, hands, etc.) in setting basic restrictions. Probability factors obtained from the experimentally known distribution of thresholds for electrostimulation, augmented by appropriate safety factors, were used to derive numerical values for permissible exposure. Basic restrictions for particular regions of the body are given in terms of the *in situ* electric field, i.e. the electric field produced within the biological medium. For frequencies below 10 Hz, the *in situ* magnetic field is also recognized as a basic restriction. At higher ELF frequencies, only the *in situ* electric field determines the basic restriction. External magnetic field strength limits were derived from the basic

restrictions using an ellipsoidal induction model of the head and torso of a large individual. Exposure limits on environmental electric fields are included to avoid adversive or painful contact currents or spark discharges when a person touches energized metallic objects.

Besides ACGIH, ICNIRP and IEEE, a number of national organizations have also issued exposure guidelines for ELF fields. However, there are variations among the guidelines formulated by these organizations, especially dissimilarities between eastern European and western safety standards. These variations could be attributed to differences in the scientific data, philosophy and methodology used for standard development. Disparities in standards around the world have caused increasing public anxiety about EMF exposures. To address this anxiety, the Word Health Organization (WHO) has set up a process for harmonization of EMF standards worldwide. A number of international seminars and working group meetings organized by WHO for this process have been carried out under the International EMF Project. With inputs from scientists and government officials in different geographical regions around the world, WHO has produced a draft framework for harmonization of EMF standards. At the time of writing, the framework was under a final review. It is hoped that upon completion, the framework will be used as a basis for development of exposure limits and other control measures that will provide the same level of health protection to all people.

Of the ELF fields, those emitted from electrical installations that carry AC electricity are of most concern by the general public. Concerned individuals have frequently asked for information about safety standards with respect to human exposure to EMF from AC power lines, distribution substations, transformers and electrical appliances. In response to the need of such information, a number of standards for occupational exposure and exposure of the general public to EMF at power frequencies are summarized in Tables 25, 26 and 27. Additional information about EMF exposure standards is available on the WHO website (www.who.int/peh-emf/en).

Table 29. ICNIRP and selected IEEE basic restrictions for 60 Hz

	Maximum Permissible *In Situ* Electric Field in Brain, rms (V/m)		Maximum Permissible *In Situ* Electric Field in Other Tissue, rms (V/m)	
Standard	**Public**	**Occupational**	**Public**	**Occupational**
ICNIRP* (1998)	0.01	0.05	0.01	0.05
IEEE† (2002)	0.018	0.053	0.7	2.10

*ICNIRP – induced current density converted to induced electric field based on tissue conductivity = 0.2 S/m.

†IEEE – exceptions for heart, feet, wrists and ankles.

Table 30. ICNIRP and IEEE limits for touch/point contact currents from energized metallic objects for ELF frequencies

	Contact Current, rms (mA)	
Standard	**Public**	**Occupational**
ICNIRP (1998)	0.5	1
IEEE (2002)	0.5	1.5

Table 31. Reference levels for occupational exposure and exposure of the general public to electric and magnetic fields at power frequencies

	Electric Field Strength, rms (kV/m)		Magnetic Flux Density, rms (μT)	
Standard	**Public**	**Occupational**	**Public**	**Occupational**
ACGIH (2003) (60 Hz)		25		1000
ICNIRP (1998) (60 Hz)	4.17	8.33	83.33	416.67
IEEE (2002) (60 Hz)	5 10*	20	900	2710
Australia (1989)(50/60 Hz)	5	10	100	500
Bulgaria (1999) (50 Hz)		5		1200
Japan (2001) (50/60 Hz)	3	3		
Russia (1999) (50 Hz)	0.5 1†	5	10 50†	100

*Intermediate electric field exception within power transmission line rights-of-way
†Living areas outside buildings

At least three provinces in Canada and six states in the USA have set voluntary standards for electric fields at high-voltage transmission line corridors (rights-of-way). Of these US states, two also have standards for magnetic fields (NIEHS, 2002). The standards for transmission line electric fields in Canada have been set by electric utility companies and vary from province to province. For example, Hydro-Québec has established an internal design standard such that the electric field strength at the edge of the right-of-way is lower than 2 kV/m at 1 m above ground. The corresponding limits set up by Hydro One in Ontario and BC Hydro in British Columbia are 3 kV/m and 5 kV/m, respectively. The purpose of the transmission line electric field standards is to

ensure that an electric potential induced on large metal objects such as cars and trucks does not represent an electric shock hazard.

12.3 Precautionary Principle

There have been increasing requests from concerned citizens that the precautionary principle (PP) be used in a number of areas, including exposure to ELF fields. Concerns about the latter appear to arise from public anxiety over the safety of power transmission and distribution lines and transformers, which are installed in people's living environments without due consideration of people's feelings or input. While the scientific community acknowledges that research into the possible health effects of ELF fields has to date given no definitive conclusion, the public anxiety is increasing and has led concerned citizens to believe that a more cautious approach should be taken when managing EMF risks.

A number of countries have considered the need for PP, how to apply it for ELF exposure, and subsequent implications of its application. Some countries have incorporated PP into their standards. Switzerland, for example, has adopted ICNIRP reference levels for protection against adverse health effects, and has also introduced precautionary emission limitations (installation limit values) into their ordinance. These limit values must be respected for places of sensitive use, e.g. apartments, schools, hospitals, permanent workplaces, children’s playgrounds. At the power frequency of 50 Hz, the limit value for electric power lines, transformer stations and substations at maximum rated current is 1 μT. Similarly, the Italian regulation has introduced PP by incorporating attention levels and quality goals in addition to the exposure limits, which are based on the ICNIRP guidelines. For exposure to EMF generated by power lines, an attention value of 10 μT is adopted in children’s playgrounds, residential dwellings, school premises, and in areas where people stay for 4 h or more per day. A quality goal of 3 μT is adopted in the design of new power lines in the neighborhood of these sites, as well as in planning developments in the proximity of existing electrical installations.

However, other countries have different points of view. The opinion of the Health Council of the Netherlands is that the application of PP is not the same thing as taking measures to reduce exposures, but can include a number of actions (Health Council of the Netherlands, 2003). In fact, the extent of PP covers a multitude of measures ranging from moderate methods such as monitoring scientific developments and providing information, through active participation in the process of acquiring new knowledge by carrying out research, to stronger measures such as lowering exposure limits. Recently, the UK National Radiological Protection Board (NRPB) has recommended the adoption of the ICNIRP guidelines after conducting a comprehensive review of scientific evidence for limiting exposure to EMF (NRPB, 2004a and 2004b). NRPB’s view on PP is that it is important to consider the possible need for further precautionary measures in respect to exposure of children to power frequency magnetic fields. On this issue, WHO’s opinion is that if precautionary measures are introduced to reduce EMF exposure levels, it is

recommended that they be made voluntary and that health-based exposure limits be mandated to protect public health.

The above variation of actions and perspectives suggest that there is confusion about what PP means and how it should be applied. To assist member countries in the development of their public health policies, WHO has launched an initiative to clarify this important issue and organized a number of workshops to develop a common framework for application of PP to health issues. Subsequent to these workshops, WHO has produced a draft document titled “Framework to Develop Precautionary Measures in Areas of Scientific Uncertainty.” The draft document, in which case studies on ELF magnetic fields are included, was distributed to scientists, government officials and interested parties for review. At the time of writing, the draft document was being updated.

In Canada, the federal government has produced a document titled “A Framework for the Application of Precaution in Science-based Decision Making about Risk” (Government of Canada, 2003). The document outlines guiding principles for the application of precaution to science-based decision making in areas of federal regulatory activity for the protection of health and safety and the environment and the conservation of natural resources. The framework outlined in this document is related to a decision-making framework for identifying, assessing, and managing health risks, which has been adopted by Health Canada (Health Canada, 2000). In the area of EMF and health, Health Canada continually monitors the scientific literature, conducts research, provides information to interested parties and has actively participated in the International EMF Project. These activities are consistent with the Canadian government framework on precaution.

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13. CONCLUSIONS

The association between power-frequency fields (PFF) and human disease continues as a health issue. The objective of this review was to examine the scientific literature published between 1998 and 2002 inclusive and to determine if there is any new evidence to support the existence of a weak association between PFF and adverse health effects, including cancer and neurodegenerative disease. The overall conclusions for each section of the review are given below.

Brain Cancer

Studies of the association between the exposure of adults and children to residential PFF and brain tumors have produced inconsistent results. The meta-analysis of occupational studies indicates a slightly elevated risk of brain tumors for electrical workers without any discernible dose-response trend. Some well designed studies have also suggested a small increase in brain cancer risk while others have not. The inconsistency among the studies has been related to exposure misclassification.

Neurodegenerative Diseases

The studies that examined the association between PFF and the neurodegenerative diseases have many methodological shortcomings, which make it difficult to interpret their findings.

There is no convincing evidence to support an association between exposure to power-frequency magnetic fields and Amyotrophic Lateral Sclerosis (ALS). However, some studies have reported an association between the number of episodes of electric shock and ALS, but more studies will be needed to verify this finding.

The Alzheimer's studies do not provide convincing support for an association between PFF and Alzheimer's disease.

There is no evidence to support an association between occupational exposure to PFF and an increased risk of suicide.

The body of evidence to-date does not convincingly support the existence of an association between PFF and depression (depressive disorders). Once again, more studies will be needed to clarify this relationship.

Breast Cancer

To date, the epidemiological evidence in support of an association between PFF and breast cancer is weak to nonexistent. If recent reports identifying new risk factors for breast cancer can be verified by confirmation or replication, then the value of previous

epidemiological studies on PFF and breast cancer could be seriously questioned. Two sources of error have been identified in these studies: (i) inaccuracies in assessing long-term exposures, and (ii) failure to adjust for potential covariates and confounders.

Leukemia

Individual studies tend to show no association between exposure to PFF and childhood leukemia. However, when the results of these studies are combined into a 'meta-analysis,' there appears to be a small, but significant increased risk for young children exposed to very high levels of PFF. The risk factors for childhood leukemia are relatively unknown, and it is possible that some as-yet-to-be-identified confounder may be responsible for this observed association. More refined statistical and epidemiological methods will be needed to clarify this anomalous finding.

No significant association between a mother's exposure to EMF during pregnancy and childhood leukemia was detected.

There is no evidence that exposure to magnetic fields inside infant incubators is associated with an increased risk of childhood leukemia.

Overall, there is no evidence that electrical workers have an excess risk of leukemia as a consequence of occupational exposure to magnetic fields.

Miscarriage, Fertility and Reproductive Capacity

Measures of dose response (daily use, hours of use, or temperature setting) were not associated with increased risk. There is some indication that electric blanket use at the time of conception and in early pregnancy may be associated with a slight increase in risk of pregnancy loss.

Exposure of mice to magnetic fields has no adverse effect on fetal development.

Genotoxic and Epigenetic Modes of Action Laboratory Studies

Evidence from laboratory studies have found some evidence in support of the hypothesis that PFF can interact with biological systems. However, careful scrutiny of the literature indicates that many of the reported effects have never been verified by independent confirmation or replication studies.

There is no evidence from *in vitro* studies that PFF is genotoxic. Overall, the results from *in vitro* studies of epigenetic modes of action find little evidence to support the hypothesis that magnetic fields can act as tumor promoters.

Exposure Standards

In Canada, there are no national standards for occupational and general public exposure at frequencies below 3 kHz. However, a number of governmental and non-governmental organizations worldwide have issued exposure standards for ELF fields. These standards were derived from biological effects data since the epidemiological studies have not produced conclusive evidence. The majority of existing standards provide occupational and general public exposure recommendations. The most often cited and adopted exposure standards are the guidelines recommended by the International Commission on Non-Ionizing Radiation Protection. A similar standard has recently been published by the Institute of Electrical and Electronics Engineers. There are variations among the guidelines formulated by standard setting organizations, especially dissimilarities between eastern European and western safety standards. These variations could be attributed to differences in the scientific data, philosophy and methodology used for standard development. Disparities in standards around the world have caused increasing public anxiety about EMF exposures. To address this anxiety, the Word Health Organization (WHO) has set up a process for harmonization of EMF standards worldwide. Voluntary standards for transmission line electric fields in Canada have been set by electric utility companies to ensure that an electric potential induced on large metal objects does not represent an electric shock hazard.

There have been increasing requests from concerned citizens worldwide that the precautionary principle (PP) be used in a number of areas, including exposure to ELF fields. Some countries have incorporated PP into their exposure standards, while others have different points of view. WHO's opinion on this issue is that if precautionary measures are introduced to reduce EMF exposure levels, it is recommended that they be made voluntary and that health-based exposure limits be mandated to protect public health. The varied actions by different countries suggest that there is confusion about what PP means and how it should be applied. Due to this confusion, WHO has launched an initiative to develop a common framework for application of PP to health issues. A framework document on precaution has been produced by the Canadian government and is related to a decision-making framework for identifying, assessing, and managing health risks issued earlier by Health Canada.

14. POSITION STATEMENT FOR THE GENERAL PUBLIC ON THE HEALTH EFFECTS OF POWER-FREQUENCY (60 Hz) ELECTRIC AND MAGNETIC FIELDS

1. Electric and magnetic fields (EMFs) are produced by the generation, transmission, distribution and use of electrical energy at power frequencies (60 Hz in Canada). People are exposed to these fields while in close proximity to power lines and other electrical facilities, as well as electrical wiring, equipment and appliances in homes, schools and workplaces.

2. Studies to investigate the health effects of these fields have taken place around the world for more than thirty years. These studies include laboratory research into effects on cells and animals, as well as epidemiological (human health) studies looking at possible associations between exposures and diseases in the population. Short- and long-term scientific investigations have been conducted and are continuing.

3. Laboratory research has shown that power-frequency EMFs can interact with biological systems; however, results to date have not provided conclusive evidence that these fields cause adverse health effects, such as cancer. Epidemiological studies have not established an association between exposure to power-frequency EMFs and the development of cancer in adults. The evidence associating cancer in children with exposure to power-frequency EMFs remains inconclusive.

4. After a recent evaluation of the scientific data, the International Agency for Research on Cancer classified extremely-low-frequency (ELF) magnetic fields as "possibly carcinogenic to humans" based on studies of childhood cancer (http://monographs.iarc.fr). "Possibly carcinogenic to humans" is a classification used to denote an agent for which there is limited evidence of carcinogenicity in humans and less than sufficient evidence for carcinogenicity in experimental animals. In the case of ELF fields, the evidence is not strong enough to conclude that they definitely cause cancer in children. More studies are needed to draw firm conclusions.

5. Immediate biological effects can result from direct exposure but only at field strength levels well above those typically found in living environments. Peripheral nerve and muscle stimulation can be caused by intense magnetic fields and hair stimulation by intense electric fields. Minor shocks may be caused by touching poorly-grounded, conducting (metallic) objects located under some high voltage lines, as a result of electrical charge induced by high intensity electric or magnetic fields.

6. Based on the available scientific evidence to date, the Federal Provincial Territorial Radiation Protection Committee (FPTRPC) concludes that adverse health effects from exposure to power-frequency EMFs, at levels normally encountered in homes, schools and offices, have not been established. Protection of the public against acute effects such as minor shocks, that may occur from contact with conducting objects under high voltage power lines, can be achieved through awareness initiatives undertaken by the electrical power industry.

7. There have been increasing requests from concerned citizens that the precautionary principle (PP) be used in a number of areas, including exposure to EMFs. It should be noted that the extent of PP covers a variety of measures ranging from moderate methods such as monitoring scientific developments and providing information, through participation in the process of acquiring new knowledge by carrying out research, to stronger measures such as lowering exposure limits. Since there is no conclusive evidence that exposure to EMFs at levels normally found in Canadian living and working environments is harmful, FPTRPC is of the opinion that moderate measures and participation in the process of acquiring new knowledge are sufficient. These types of activity are consistent with the Canadian government framework on precaution.

8. The FPTRPC will continue to monitor scientific research relating to the health effects of power-frequency EMFs and will reassess its position periodically as new information becomes available.

Notes:

(a) This Position Statement replaces the previous Position Statement (first released by the FPTRPC in November 1998 and updated in October 2002).

(b) This Position Statement is not intended to provide direction on health and safety aspects of electromagnetic interference by EMFs with medical electronic devices, including cardiac pacemakers. Electromagnetic interference with such devices requires different considerations from those used in the evaluation of human health effects.

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