—— LAW OFFICES ——

DeCOTIIS, FITZPATRICK, COLE & WISLER, LLP

NEW YORK, NY
TRENTON, NJ

GLENPOINTE CENTRE WEST
500 FRANK W. BURR BOULEVARD, SUITE 31
TEANECK, NEW JERSEY 07666
TELEPHONE: (201) 928-1100
TELEFAX: (201) 928-0588
www.decotiislaw.com

Catherine E. Tamasik, Esq.
Direct Dial: 201-907-5265
Email:ctamasik@decotiislaw.com

July 9, 2009

VIA OVERNIGHT MAIL
Ken Sheehan, DAG
Division of Law
Dept. of Law and Public Safety
124 Halsey Street
Newark, New Jersey 07102

Kristi Izzo, Secretary of the Board
New Jersey Board of Public Utilities
2 Gateway Center
Newark, New Jersey 07102

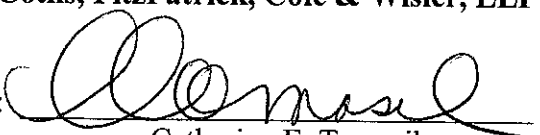
Re: I/M/O THE PETITION OF PUBLIC SERVICE ELECTRIC AND GAS COMPANY FOR A DETERMINATION PURSUANT TO THE PROVISIONS OF N.J.S.A. 40:55D-19 (SUSQUEHANNA-ROSELAND) BPU DOCKET NO. EM 09010035

Dear Mr. Sheehan, and Ms. Izzo:

On behalf of our clients, the Fredon Township School District and the Willow Lake Day Camp, and in accordance with the Schedule as amended by the Board of Public Utilities on May 13, 2009, we submit herewith the Testimony of Martin Blank, Ph.D. which is in reply to Public Service Electric & Gas Company's Petition and supporting testimony.

Thank you for your attention.

DeCotiis, FitzPatrick, Cole & Wisler, LLP

By: 
Catherine E. Tamasik

cc: Tamara L. Linde, Esq., V.P. Regulatory, PSEG Services Corporation
All Parties Designated on the Attached Service List
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STATE OF NEW JERSEY
BOARD OF PUBLIC UTILITIES

IN THE MATTER OF THE PETITION OF PUBLIC SERVICE ELECTRIC AND GAS COMPANY FOR A DETERMINATION PURSUANT TO THE PROVISIONS OF N.J.S.A. 40:55D-19 (SUSQUEHANNA-ROSELAND)

BPU DOCKET No. : EM09010035

TESTIMONY OF MARTIN BLANK, Ph.D.
ON BEHALF OF MUNCIPAL INTERVENORS IN OPPOSITION TO THE SUSQUEHANNA-ROSELAND TRANSMISSION LINE PROJECT

I. Summary of Testimony

Along with the growing need for electric power in modern society, there are growing concerns about the health risks arising from exposure to the electromagnetic fields (EMF) associated with transmission and use of electric power. Scientific research has continued the study of biological processes affected by EMF and the consequences to human health. The present level of understanding of biological mechanisms underscores the need for greater protection of populations exposed to EMF, especially young children. Recent research has even provided a plausible biological explanation for the link between EMF and leukemia at the 3-4 milligauss (mG) level found in epidemiology studies. shall present evidence regarding EMF and leukemia showing that:

- EMF affects many fundamental biological processes at field strengths in the range of observed epidemiology thresholds
- Low levels of EMF stimulate stress protein synthesis (**the stress response**), a protective cellular mechanism in reaction to such harmful stimuli as high temperature and acidity.

Activation of the stress response indicates that cells react to low levels of EMF as potentially harmful.

- It is clear that EMF leads to DNA chain separation, since initiation of stress protein synthesis requires the two strands of DNA to come apart. EMF interacts with DNA in the stress response at very low intensities, and can cause damage to DNA at higher intensities. EMF stimulation of DNA is important, because cancer is associated with changes in DNA called mutations.
- The relevance of DNA damage is seen in a study where children missing DNA repair genes have a much greater incidence of leukemia.
- EMF has also been associated with harmful biological effects in adults. EMF inhibits the secretion of melatonin which normally inhibits the growth of breast cancer cells, and breast cancer cells have been shown to grow faster in EMF. EMF also increases the incidence of Alzheimer's disease and senile dementia, the incidence increasing with the duration of EMF exposure.
- There are plausible molecular mechanisms to account for the observed biological effects of EMF. Biochemical studies have shown that EMF can accelerate electron transfer reactions and that electrons can be displaced in DNA. The physical properties (electron affinity, fluorescence depolarization) of the CTCT groups in DNA activated by EMF could contribute to these interactions.

In the past, EMF safety issues only dealt with the need to protect against acute large EMF effects, such as electric shock or the firing of nerves, and not against chronic low level effects. Recent research, showing potentially harmful biological effects associated with chronic low level EMF, indicates the need for greater protection. We now know that even weak magnetic fields

are biologically active. They easily pass into cells and are unlike electric fields that are attenuated at the cell membrane by a factor of over a million. For this reason, even weak EMF constitute a significant risk, especially to children who spend many hours a day in schools and summer camps located near power lines. The magnetic fields exceed biologically active levels at the edge of the right of way (ROW), and for some distance beyond ROW.

II. Testimony of Martin Blank, Ph.D.

Q.1. Please state your name and address.

A.1. My name is Martin Blank. I reside at 157 Columbus Drive, Tenafly, NJ 07670.

Q.2. Please state your profession and describe your academic responsibilities.

A.2. I am Associate Professor of Physiology and Cellular Biophysics at the College of Physicians and Surgeons, Columbia University in New York City. My primary responsibility has been to conduct research, and I have specialized in the study of electromagnetic fields (EMF) and their effects on cell biochemistry and cell membrane function. I have recently specialized in the study of stress proteins and charge transport enzymes (protein catalysts). I have also taught Medical Physiology to first year medical, dental and graduate students, including a year as Course Director in charge of 250 students. My Curriculum Vitae is provided as **Exhibit A**.

Q.3. Please describe your formal education.

A.3. I have a Ph.D. degree from Columbia University (1957) in physical chemistry, as well as a Ph.D. from Cambridge University (1959), England, in colloid science. Colloid science was an interdisciplinary department that included biology, physics and chemistry that would be called biophysics in the United States.

Q.4. Please state related academic and industrial research experience.

A.4. In addition to Columbia University and Cambridge University, I have worked in other academic settings: Polymer Department, Weizmann Institute (Israel); Bioengineering Department, University of California-Berkeley; Pharmacology Department, Hebrew University (Israel); Biochemistry Department, Monash University (Australia); Frumkin Institute of Electrochemistry (Moscow, USSR); Biophysics Department, University of Warsaw (Poland); Chemical Physics Department, Tata Institute for Fundamental Research (India); Chemistry Department, University of the Negev (Israel); Biology Department, University of Victoria (Canada); Department of Theoretical Physics, Kyoto University (Japan).

I have also done research in industrial laboratories: California Research Corp, Richmond, CA; Esso Research and Engineering Corp, Linden, NJ; 3 Unilever Research Labs (Port Sunlight and Welwyn in England, and Vlaardingen in the Netherlands).

Q.5. Please describe your scientific publications

A.5. I am author of over 200 peer-reviewed papers and reviews, and 12 edited books on electrical properties of biological systems. The books include Proceedings of the First World Congress on "Electricity and Magnetism in Biology and Medicine"; "Biomembrane Electrochemistry"; "Nerve-Muscle Function", based on the 4th Erice (Italy) course; "Electromagnetic Fields: Biological Interactions and Mechanisms" for the American Chemical Society series, *Advances in Chemistry.*

Q.6. Please state other relevant professional experience

A.6. I worked for the United States Office of Naval Research (ONR) as a Liaison Scientist in London (UK) and as a Program Officer in Arlington (US), where I developed and managed a research program in biomembrane electrochemistry. I have also consulted for other research agencies, including the American Institute of Biological Sciences (AIBS) and Electric Power

Research Institute (EPRI), as well as private corporations, helping to evaluate proposed and ongoing research programs. In 2008, I was invited to address the Brazil Chamber of Deputies on EMF safety.

I have served as an officer of various scientific societies, editor of scientific journals, reviewer of scientific papers for publication and proposals for funding, as well as an expert advisor in the evaluation of the performance of research laboratories for various government agencies.

Q.7. Do you have a particular perspective on research?

A.7. The various research and teaching roles I have assumed in my career have generally involved an interdisciplinary approach to complex problems. This has given me a broad perspective on scientific research and specifically in bioelectromagnetics.

I reported on interdisciplinary research in biology at ONR-London, and organized interdisciplinary symposia as Chairman of the Biology Division of the Electrochemical Soc., as Bioelectrochemical Soc. President, and as Bioelectromagnetics Soc. President. This was also true of other scientific meetings I organized, such as the 4th International Symposium on Bioelectrochemistry (1976), the first Gordon Research Conference on Bioelectrochemistry (1980), and four interdisciplinary courses at the Majorana Center, Erice (Italy). The Gordon Conference enabled our group to organize the First (1992) and Second (1997) World Congresses on Electricity and Magnetism in Biology and Medicine. My editorial work for the Journal of the Electrochemical Society, and American Editor of Bioelectrochemistry and Bioenergetics was also interdisciplinary. My most recent activities have been more directly related to research and safety issues in Bioelectromagnetics. I was one of the organizers of the Bioinitiative working group that published the online Bioinitiative report (2007) evaluating electromagnetic safety

standards. I am also Editor of the special issue of Pathophysiology devoted to Electromagnetic Fields that will be published in August 2009.

Q.8. How do you see the role of science in determining EMF safety?

A.8. The need to base EMF safety standards on science is widely recognized. The IEEE guideline for revision of C95.1-1991 safety standards begins with that principle (*Bioelectromagnetics, Supplement 6*). Science is both a body of information and a method to obtain new information. Scientific research is designed to answer questions, and 'scientific proof' is best understood in terms of the old meaning of 'proof' as 'test'. Scientific proof does not rely on 'weight of the evidence', where one keeps a scoreboard of positive versus negative results and merely tallies the numbers. In scientific proof, number and weight do not count. It is the continuing accumulation of relevant data from all sources and the testing of scientific hypotheses regarding the biological effects of EMF that has provided useful information. It is in this way that scientific data from both epidemiology and laboratory studies continue to contribute to our understanding of the health risks associated with exposure to EMF. What started as an epidemiological link between EMF and childhood leukemia is now better understood in terms of EMF interactions with DNA. This current understanding of the scientific data underscores the need for greater protection from EMF exposure, especially for children.

Q.9. Please provide a brief history of biological effects of EMF as it relates to the proposed PSEG powerline project?

A.9. Years ago when EMF safety standards were first considered, they were meant to protect only against large and acute effects, such as electric shock or the firing of nerves, and not against chronic effects at low level EMF exposures. Recent research has shown potentially harmful biological effects at low level EMF exposures for extended periods of time, so there is a great

need to reconsider EMF safety. This is especially true for magnetic fields. Unlike electric fields that are attenuated at cell membranes by a factor of over a million, magnetic fields pass easily into cells and are biologically active. For this reason, even weak magnetic fields constitute a significant risk to living cells, especially to the rapidly growing cells in children.

Recent awareness of EMF risk started in 1979, when an epidemiology study by Wertheimer and Leeper suggested an association between magnetic field exposure and an increased risk of leukemia. The evidence from subsequent epidemiology and laboratory research led to the May 1999 National Institute of Environmental Health Sciences ("NIEHS") Report to the Congress (see Executive Summary, **Exhibit B**) to recommend "*that the power industry continue its current practice of siting power lines to reduce exposures and continue to explore ways to reduce the creation of magnetic fields around transmission and distribution lines without creating new hazards.*" The NIEHS-EMF review panel announced in June 1998 that magnetic fields should be considered a "*possible human carcinogen*", and two pooled analyses (*Greenland et al, Epidem 2000; Ahlbom et al, Brit J Cancer 2000*), the first analyzing 15 major studies and the second 9 major studies, subsequently showed a statistically significant doubling of the risk of childhood leukemia at EMFs exceeding 3-4mG. (The analyses are **Exhibits C and D.**) The epidemiological evidence was strong enough to serve as a basis for practical recommendations, and laboratory research has provided data on plausible mechanisms.

Q.10. Is the 3-4mG level similar to EMF thresholds for other biological effects?

A.10. Low EMF thresholds in several biological systems have been published in peer reviewed journals. The first five values in the following Table are from our laboratory at Columbia University, and the measured thresholds for changes in enzyme activity and in biosynthesis of stress proteins are in the range of the epidemiology threshold.

Biological EMF Thresholds (60Hz range)

Reactions:	Na,K-ATPase	2-3mG
	Cytochrome C Oxidase	5-6mG
	Malonic acid oxidation	1-2mG
Stress response:	HL60 cells	<8mG
	Sciara larva cells	<8mG
Cancer cells:	Block inhibition by melatonin	
	(Breast cancer cells)	2-12mG
Epidemiology:		3-4mG

The biochemical reactions are central to biological function. Electron transfer to cytochrome C oxidase is a critical step in converting foodstuff into ATP, the fuel used to power living cells. The Na,K-ATPase utilizes the ATP to drive the biological 'pump' that maintains the ionic composition of living cells. The '**stress response**' is a reaction to potentially harmful agents, such as high temperature, toxic metals, alcohol, etc, that leads to stimulation of DNA and the synthesis of stress proteins. Stimulation of the stress response by EMF shows that cells react to relatively low EMF levels as potentially harmful. EMF blockage of the inhibition of breast cancer cell growth by melatonin will be discussed in Q.14.

Q.11. How extensive is the health risk due to EMF levels near power lines?

A.11. All of the biological systems listed above, as well as many inter-connected ones, would be stimulated within the ROW of existing powerlines. In addition to the risk of leukemia, there would be changes in the fundamental cellular processes of energy production and utilization, as well as activation of DNA that contains genetic information. It is clear that the 3-4 mG level set in the NIEHS report to Congress would be exceeded along the proposed powerline route that

includes areas near the school and summer camp where children would be exposed to an increased health risk. It is also clear that the added 500 kV line would raise the EMF above the present level.

Actually, a 3-4 mG field does not indicate safety below that level. It is important to realize that doubling the risk at 3-4mG implies a lesser but still existing non-zero risk at levels just below that. Even when the PSE&G quoted fields are below 3-4 mG, the values quoted are **median values** with fields being higher half the time and lower half the time. EMF is bound to vary according to the power demands, and the median values are bound to be exceeded. Since there is no indication how high the values can go, they could exceed the thresholds of many additional biochemical reactions.

There are other issues that are difficult to evaluate, but, undoubtedly contribute to adverse biological effects.

- There is evidence that the radiofrequency RF 'noise' that accompanies a 60Hz signal may cause harmful effects (e.g., cancer, diabetes).
- The ground currents that account for a percentage of the AC return current are an indeterminate additional source of magnetic field exposure and depend on grounding, local circuitry, usage, etc.

A desirable optimal design would generate peak magnetic fields that are as small as possible, and as 'clean' (pure 60 Hz sine wave) as possible.

Q.12. How long must one be exposed to EMF to result in a biological change?

A.12. There is relatively little information about the effect of exposure duration on adverse health effects. Recent evidence from the Alzheimer's disease and dementia study discussed in Q.14. shows an increased incidence with exposure duration over a period of 15 years. Studies of

radio frequency EMF and cell phone use show an increased risk of brain tumors at 10 years. The peak incidence of leukemia in children at 3-4 years of age indicates that it can occur in a much shorter time. Current ideas of mechanism suggest that EMF initiates the change in DNA, which then follows a different course.

At the cellular level, there are indications that in addition to very low thresholds, some biological changes occur after very short exposure durations. Studies on EMF stimulation of the enzyme ornithine decarboxylase (*Litovitz et al. Biochem Biophys Res Comm 178: 862-865, 1991; Bioelectromagnetics 14: 395-403, 1993*) showed that a full response could be obtained when cells were exposed for only 10sec. The studies used either pure low frequency sine waves or modulated radiofrequency (RF) sine waves. In both cases, the EMF signal had to be continuous, since gaps in the sine wave resulted in a reduced response. The very short time to be effective would be expected if EMF acts as an initiator of DNA strand separation, as in the mechanism discussed below in Q.13.

Q.13. Please show how EMF interaction with DNA helps in understanding the stress response and the possible link to cancer.

A.13. The DNA molecule in a cell nucleus is a long, tightly coiled, double helix. The two strands of the helix are connected by four interacting chemicals called bases given the symbols C, G, A, and T. In human DNA there are about 3 billion bases that interact as pairs, C with G and A with T, one base from each strand. The sequence of the bases along the DNA is in a code needed to make the proteins essential for life, and that has been deciphered in the "Human Genome" project. Each protein is encoded in a separate segment called a gene, and individual genes are activated by specific chemicals in regions of the gene called promoters.

The integrity of the DNA is essential for life, since changes in the information for making proteins generally damage the cell. An accumulation of changes (mutations) in the DNA is associated with the development of cancers, diseases that are believed to arise from a multi-step process: initiation (damage to DNA in at least two places), promotion (effect on cellular processes that causes loss of control over protein synthesis) and progression (tumor growth). Cancer mechanisms are not well understood, and different mechanisms may be operating in each tissue. However, there is agreement that interaction with DNA and damage to DNA is a key factor.

Protein synthesis in a cell is regulated by a system that activates DNA to supply particular proteins when more are needed. When there is a potentially harmful change in a cell's environment (a stress), stress proteins are synthesized. The stress response, first identified in reaction to elevated temperatures, is used by all species in response to harmful environmental stimuli (e.g., high temperature, low oxygen, toxic metal ions).

Research has shown that cells react to EMF as a stress and make stress proteins. Stimulation of the stress response indicates that cells react to EMF as a potentially harmful stimulus at field strengths slightly above normal background levels. The response to **EMF requires remarkably low energy input** - over a trillion times lower than the thermal energy needed to evoke a response, as shown in the following Table:

ENERGY to STIMULATE STRESS RESPONSE

Form of Energy	Stimulus	Energy Density
Magnetic	8mG	2.6×10^{-7} joules/m^3
Thermal	+ 5.5°C	$2.3 \times 10^{+7}$ joules/m^3

Our laboratory was the first to report EMF stimulation of the stress response, and we identified EMF responsive regions with -CTCT- sequences in the promoter of the stress protein (hsp70). Inactivating these sequences by removal or mutation, eliminates the response to EMF. Inserting these sequences into an artificial construct containing a gene, causes the gene to be activated by EMF. (See **Exhibit E**) Linkage of EMF responses with specific regions of DNA provides a non-invasive, precise technique for gene activation. Columbia University has a patent for this process based on our research.

Recent studies show that DNA conducts electrons along the bases within the double helix, and we have shown that EMF accelerate electron transfer reaction rates. The velocity of charge movement calculated from experiments with the enzyme, Na,K-ATPase, 1000 m/s, is similar to ultrafast electron transfer in DNA of 400 m/s. The forces at low field strengths that affect enzyme reactions are large enough to move electrons in DNA and could lead to repulsive forces that cause chain separation. The physical properties (electron affinity, fluorescence depolarization) of the chemical groups (CTCT) activated by EMF could contribute to interactions with DNA. From estimates of the balance of forces (repulsion-attraction) at the DNA bases, sites rich in C and T, as in the identified -CTCT- sequences, appear to be more likely to come apart when repulsive forces are generated by EMF. These calculations (*Blank and Goodman, J Cell Physiol, 2004*) suggest a plausible mechanism for stimulation of DNA by EMF, and provide a rationale for EMF specific sequences that are effective in this process. A more general discussion of plausible EMF mechanisms can be found in *Blank, Electromagnetic Biology and Medicine, 2008.* (The two papers are attached as **Exhibits F and G**.)

Q.14. Has EMF been linked to diseases other than leukemia?

A.14. As mentioned in the reply to Q.10., EMF exposures have been shown to modify

the tumor suppressing action of melatonin secreted by the pineal gland in the brain. (*Liburdy et al. J Pineal Res 14:89-97, 1993*). Studies replicated in four labs show that 2mG blocks the growth-inhibiting action of melatonin on human estrogen receptor-positive, breast cancer cells, as well as the near-complete blockage of the anticancer (chemotherapeutic) drug Tamoxifen. A field strength of 2mG has no effect, indicating that the threshold for this effect lies between 2mG and 12mG.

There are currently many studies of tumors in the head (gliomas, acoustic neuroma, parotid gland tumors) correlated with the use of cellphones. These are generally discussed in terms of the radio frequency EMF that carries the cellphone signals, although there are low frequency components (12Hz, 217Hz) associated with the transmission that could be involved.

A recent study from Switzerland (*Hus et al., American Journal of Epidemiology, January 2009*) (**Exhibit H**) found that exposure to relatively low EMF from 220-380 kV power lines is correlated with an increase in the incidence of Alzheimer's disease and senile dementia. The risk for those living within 50m compared to over 600m, increased with duration of exposure over 5, 10 and 15 years, with a doubling of the risk at 15 years. The fields were not measured, but it is possible to estimate that the fields at 50m were in the range of 8-10mG, based on data published by the Bonneville Power Administration.

EMF interaction with DNA is important, because cancer is associated with changes in DNA (mutations) and a probable explanation of epidemiology results. We know that EMF activates DNA in the stress response at very low intensities, and can cause damage to DNA at higher intensities (*Lai and Singh, Bioelectromagnetics 18:156-165, 1997*). The relevance of DNA damage induced by EMF is reinforced in a recent epidemiology study where children missing the DNA repair genes were found to have a 4 fold greater incidence of leukemia from

exposure to EMF as low as 1.4-1.8mG (*Yang et al, Leukemia and Lymphoma 49: 2344-2350, 2008*) (**Exhibit I**).

Q.15. Please summarize the evidence linking EMF and cancer

A.15. From what we have learned about EMF interactions with cells, the plausibility of a link between low frequency EMF and childhood leukemia, and the possibility of other diseases, can be stated with growing confidence:

- Epidemiology studies show a doubling of the risk of childhood leukemia associated with EMF exposures in excess of 3-4mG. EMF is also associated with greater growth of breast cancer cells and an increase in the incidence of Alzheimer's disease and senile dementia.
- At the cellular level, stimulation of the cellular stress response (a cellular protective mechanism) by EMF indicates that cells react to EMF as harmful. The same low level of EMF can also affect fundamental cellular processes.
- A plausible molecular mechanism has been proposed for stimulation of protein synthesis by EMF, based on interaction with electrons in DNA and identification of a specific DNA sequence that has been shown to respond to EMF.
- Children missing the genes needed to repair DNA have a four fold greater incidence of leukemia from exposure to EMF as low as 1.4mG. This recent result links DNA damage by EMF to leukemia.

Q.16. Please assess the cancer risk from EMF of the proposed PSEG powerline.

A.16. The proposed addition of the 500kV PSEG line to the existing 230kV line is designed to minimize the EMF produced by distributing the current in three cables that carry current out of phase. However, there can be no doubt that the added 500 kV line would add to the level of EMF from the existing 230kV line and thereby create an additional potential hazard. This would

be contrary to the recommendations of the May 1999 NIEHS Report to the Congress. The studies cited here indicate that even the weak magnetic fields that extend beyond the ROW of the proposed power lines have the ability to cause significant changes in living cells by affecting fundamental biological processes, and predisposing them to the development of cancer and other diseases. It is therefore essential to minimize exposure to EMF, where errors in DNA that can occur during cell division are most likely in rapidly growing children.

Because of the wide range of biological systems affected, the low response thresholds, the possibility of cumulative effects by repetitive stimulation and the inadequacy of exposure standards, it is urgent that the proposed powerline be moved to a distance where the anticipated magnetic fields will not pose a hazard to the community. At the very least, **peak EMF levels should not exceed 3-4mG**. The recent study linking the absence of DNA repair genes to EMF induced leukemia (**Exhibit I**) suggests that half that value, **1.4-1.8mG, would be a more prudent peak limit** to aim for.

EXHIBITS

Exhibit A Curriculum Vitae - Martin Blank

Exhibit B Executive Summary, National Institute Environmental Health Sciences ("NIEHS") Report to the Congress, May 1999.

Exhibit C Greenland, Sheppard, Kaune, Poole, Kelsh. 2000. A Pooled Analysis of Magnetic Fields, Wire Codes, and Childhood Leukemia. Epidemiology 11:624-634.

Exhibit D Ahlbom, Day, Feychting, Roman, Skinner, Dockerty, Linet, McBride, Michaelis, Olsen, Tynes, Verkasalo. 2000. A pooled analysis of magnetic fields and childhood leukemia," Brit J Cancer 83:692-698.

Exhibit E Lin, Blank, Rossol-Haseroth, Goodman. 2001. Regulating genes with electromagnetic response elements. J Cellular Biochemistry 81:143-148.

Exhibit F Blank, Goodman. 2004. A mechanism for stimulation of biosynthesis by electromagnetic fields: charge transfer in DNA and base pair separation. Published online J Cellular Physiology, 9 July 2007.

Exhibit G Blank. 2008. Protein and DNA reactions stimulated by electromagnetic fields. Electromagnetic Biology and Medicine, 27: 3-23.

Exhibit H Hus, Spoerri, Egger, Roosli. 2009. Residents near power lines and Mortality from neurodegenerative diseases: longitudinal study of the Swiss population. American Journal of Epidemiology 169:167–175.

Exhibit I Yang, Jin, Yan, Tian, Tang, Shen. 2008. Case-only study of interactions between DNA repair genes and low frequency electromagnetic fields in childhood leukemia. Leukemia and Lymphoma 49: 2344-2350.

EXHIBIT A

Columbia University, College of Physicians & Surgeons

Department of Physiology and Cellular Biophysics
630 West 168 Street
New York, NY 10032
mb32@columbia.ed

Telephone: (212) 305-3644
Telefax: (212) 305-5775
EMAIL:

January 10, 2006

Mr. Robert J. Pellatt,

Commission Secretary

British Columbia Utilities Commission

900 Howe Street, Box 250

Vancouver, BC V6Z 2N3 sent via Email: Commission.Secretary@bcuc.com

Dear Mr. Pellatt,

Re: **FortisBC Inc. Order No. G-114-05 / Project No. 3698407CPCN Application for Nk'Mip Substation and Transmission Line**

Mr. Hans Karow, Coalition to Reduce Electropollution (CORE), has asked me to provide testimony addressing the electromagnetic pollution issue associated with the proposed project cited above. As indicated in my CV (attached), I have spent many years studying the effects of low frequency electromagnetic fields (EMF) at both the cellular and molecular levels, and I have published extensively in peer reviewed journals.

Before addressing the main points, let me state that EMF from a 63kV power line will exceed the 3-4mG level within about 70-80 feet of the line, at typical power levels, based on the Bonneville Power Administration data. This level field will extend over an even wider range at peak power levels. Many biological systems are perturbed at relatively low EMF, but it has been shown that the risk of leukemia in children is doubled at the 3-4mG level. This field level is well within current safety limits, but the scientific basis of these limits is open to serious questions (Blank and Goodman, 2004) that challenge the capacity of these limits to be protective.

The main points I wish to emphasize are the following:

- recent epidemiological studies in the power frequency range suggest increased risk of leukemia associated with exposure to EMF

- current safety guidelines are not based on biological thresholds, and are many times above the levels that epidemiological studies have correlated with elevated risk of leukemia in children

- EMF thresholds of biological reactions are very low. Very low field strengths stimulate the stress response, the protective cellular reaction to potentially harmful stimuli

- the mechanism by which EMF cause changes in several well-documented biochemical systems involves interaction with electrons. Such a mechanism would affect many biological reactions, and possibly lead to cancer on interaction with DNA

Recent epidemiological studies indicate need for caution

Since the Wertheimer, Leeper paper of 1979, there have been many epidemiological studies of the effects of EMF in the power frequency range. Two recent meta analyses by groups of experts (Greenland et al, Epidem 2000; Ahlbom et al, Brit J Cancer 2000), of 15 and 9 major studies respectively, have shown a statistically significant doubling of the risk of childhood leukemia when exposures to low frequency EMF exceed 3-4mG. While the small number of cases of high exposure has resulted in a lack of statistical significance, the doubling of the risk of leukemia has persisted in many studies near the "significant" level. By pooling cases, it has been possible to demonstrate statistical significance. There is now quite general agreement that the epidemiological evidence indicates an association of EMF with childhood leukemia when exposures exceed 3-4mG.

Current safety guidelines are not based on biological mechanisms

In assessing the potential biological impact and risk of exposure, one would generally turn to the safety standards set by professional agencies such as ICNIRP and IEEE. However, the standards set by these agencies are unrelated to biological thresholds. They are based solely on the heating of tissue that results from the energy deposited by EMF. The energy deposition rate, the SAR, does not take into account many biological properties that change long before a change in the SAR can be detected. This fundamental flaw in the current standards makes them unreliable as a basis for safety:

- they assume that there are no biological reactions unless heating of cells occurs. The EMF thresholds discussed below, show that significant biological reactions occur in cells at very low EMF, in the absence of heating. These 'non-thermal' reactions raise an alarm regarding questions of safety.

- the standards were derived assuming that in EMF, the magnetic fields do not act directly, but only through the relatively weak electric fields they induce. This is not so. We have shown that both electric and magnetic fields can affect cells. In fact, magnetic fields penetrate cells far more effectively than electric fields at low frequency.

The SAR is a valid measure of energy deposition rate, but not of safety. It was derived at a time when all one could measure was temperature increase. Because of scientific advances, it is now possible to show many biological changes due to EMF that occur within the current safety guidelines. The current guidelines have been challenged by scientists, e.g., by an international commission that met in Catania, Italy in September 2002.

EM fields stimulate the cellular stress response

Regarding the question of safety, the most important observation is activation of stress protein synthesis in cells by EMF at both power and radio frequencies. The stress response occurs in reaction to a variety of potentially harmful influences in the environment, such as high temperature, toxic metal ions, alcohol, deviations of pH from neutrality, etc. For this reason, stimulation of the stress response by EMF can be seen as a direct answer by cells to the safety question. Cells react to EMF as a significant departure from a normal environment and as potentially harmful.

The stress proteins are the same whether stimulated by fields or by an increase in temperature, but the response to EMF requires much lower energy input. In Sciara salivary gland cells, the threshold energies of the EMF and thermal stimuli needed to evoke a stress response differ by 14 orders of magnitude, as shown in the Table below.

ENERGY to STIMULATE STRESS RESPONSE

Form of Energy	Stimulus	Energy Density (joules/m^3)
Magnetic	**8mG**	**2.6×10^{-7}**
Thermal	**5.5°C**	**$2.3 \times 10^{+7}$**

In addition to the stress response, many biological reactions, such as enzyme systems and electron transfer reactions, are affected by weak EMF. Low thresholds have been measured in several systems, and the values have been published in peer review journals. The Table below shows that the measured thresholds for changes in reaction rates of enzymes, the BZ reaction (oxidation of malonic acid), and reactions in DNA leading to biosynthesis of stress proteins, are in the range of cut-off thresholds in epidemiological studies. The table also has an entry for EMF needed to block the inhibition of breast cancer cell growth by melatonin. That study has been replicated in six labs, and it shows that a low EMF of 12mG blocks the growth-inhibiting action of melatonin on human estrogen receptor-positive, breast cancer cells, as well as the near complete blockage of the anticancer (chemotherapeutic) drug Tamoxifen. An EMF of 2mG has no effect, indicating that the threshold for an effect on these cancer cells lies between 2mG and 12mG.

Biological EM Field Thresholds (power frequency range)

Reactions:	**Na,K-ATPase**	**2-3mG**
	Cytochrome C Oxidase	**5-6mG**
	BZ (redox) reaction	**1-2mG**
DNA:	**Stress proteins (HL60 Cells)**	**<8mG**
	Stress proteins (Sciara Cells)	**<8mG**
Cells:	**Block inhibition by melatonin (Breast cancer cells)**	**2-12mG**
	Epidemiology threshold (leukemia)	**3-4mG**

Stimulation of the stress response by EMF shows that they activate DNA as the first step in protein synthesis. Several labs have shown that DNA can conduct electrons within its structure. Therefore, it appears possible for EMF to activate DNA by generating repulsive forces when interacting with electrons in DNA. We have shown that specific regions of DNA are associated with the response to EMF, and inactivating these sequences by removal or mutation eliminates the response to EMF. Inserting these DNA sequences into an artificial construct containing a gene makes the gene EMF-responsive. In brief, our understanding of mechanism has reached the point where we have identified an EMF sensitive DNA sequence, have transplanted it and have reactivated it with EMF. (We have obtained a patent for this process.) That experiment, together with the breast cancer cell study indicate that EMF can enter into health related mechanisms at very low field strengths.

Recommendation

Based on recent research on biological changes induced by EMF, it is wise and prudent to recommend minimizing exposure by all reasonable methods, especially of school age children, with the aim of being below 3-4mG at peak power levels. **ALARA** (As Low As Reasonably Achievable) has been a policy with regard to radiation safety, and the European Union has adopted a related measure, the **Precautionary Principle**, as a general approach to environmental issues. Italy and Austria have applied this approach to EMF, and I have organized a symposium on the Precautionary Principle for the next meeting of the Bioelectromagnetics Society.

On request, I am prepared to provide additional information and clarification. Please feel free to contact me via e-mail mb32@columbia.edu , or telephone provided above.

Martin Blank, PhD
Associate Professor of Physiology and Cellular Biophysics
Columbia University

Enclosure: Curriculum Vitae

CC: Mr. George Isherwood, Director Reg. Affairs, FortisBC George.Isherwood@fortisbc.com
Mr. Hans Karow, CORE hkarow@shaw.ca

MARTIN BLANK

CURRICULUM VITAE

Address Home 157 Columbus Drive, Tenafly, N.J. 07670
(Tel: 201-266-4076; FAX: 201-266-4076; email: mbphd32@yahoo.com)
Office Dept. of Physiology, College of Physicians and Surgeons,
Columbia University, 630 West 168th Street, New York, NY 10032
(Tel: 212-305-3644; FAX: 212-305-5775; email: mb32@columbia.edu)

Personal

Born	February 28, 1933	New York, New York
Married	Marion Sue Hersch	July 3, 1955 (3 children)

Education

1950-1954	City College of New York, BS Magna Cum Laude (Chemistry)
1954-1957	Columbia University, PhD (Physical Chemistry)
1957-1959	Cambridge University, England, PhD (Colloid Science)

Academic Appointments

1954-1955	Assistant in Chemistry, Columbia University
1955-1957	Research Fellow (Chemistry), Columbia University
1957-1959	Postdoctoral Research Fellow, Cambridge University, England
1959-1964	Instructor in Physiology, Columbia University
1964-1968	Assistant Professor of Physiology, Columbia University
1968-present	Associate Professor of Physiology and Cellular Biophysics, Columbia University

Other Appointments

Summer 1956	Chemist, California Research Corp, Richmond, CA.
Summer 1957	Chemist, Esso Research and Engineering Co, Linden, NJ.
Fall 1961	Research Fellow, Cambridge University, England
Summer 1964	Chemist, Unilever Research Lab, Cheshire, England
Summer 1966	Visiting Scientist, Polymer Dept, Weizmann Institute, Israel
Summer 1967	Chemist, Unilever Research Lab, Hertfordshire, England
Summer 1968	Visiting Scholar, Bioengineering Dept, University of California, Berkeley
Summer 1969	Research Chemist, Unilever Research Lab, Vlaardingen, Netherlands
1970	Visiting Professor, Pharmacology Dept, Hebrew University, Israel
1974-1975	Physiologist, Office of Naval Research, London, England
1982 (6 mo.)	Visiting Lecturer, Biochemistry Dept, Monash University, Australia
1984-1985	Biologist, Office of Naval Research, Arlington, VA
1986-1988	Part-time IPA Biologist, Office of Naval Research, Arlington, VA
1989 (May)	Visiting Professor, Acad Sci USSR, Inst Electrochemistry, Moscow, and Dept of Biophysics, Univ of Warsaw, Poland
1992 (Nov)	Visiting Professor, Tata Institute, Bombay, India
1995 (spring)	Visiting Professor, Dept of Chemistry, University of the Negev, Beersheba, Israel Visiting Scientist, Dept of Biology, University of Victoria, BC, Canada
2005 (July)	Visiting Professor, Dept of Theoretical Physics, Kyoto University, Japan

Honors

1953	Elected to Phi Beta Kappa, City College
1956	Elected to Sigma Xi, Columbia University
1955-1957	Consumers Union Research Fellowship, Columbia University
1957-1959	Postdoctoral Research Fellowship, National Heart Institute, Cambridge University
1960-1970	Research Career Development Award (USPHS), Columbia University
1975	Certificate of Appreciation, Office of Naval Research, London
1982 (June)	Distinguished Visiting Professor, Univ Western Australia
1984	Distinguished Lecturer in Physiology, Wayne State University
1985	Certificate of Commendation, Office Naval Research, Arlington
1987	Invited Lecturer, International Biophysics Congress, Jerusalem
1988	Invited Lecturer, Univ of Bologna, 900th Anniversary Symposium
1989 (May)	Visiting Professor, Acad Sci USSR, Institute of Electrochemistry, Moscow and Dept of Biophysics, University of Warsaw, Poland
1990	Certificate of Appreciation, The Electrochemical Society
	Yasuda Award, Bioelectrical Repair and Growth Society
1992	Invited Opening Speaker, First Congress of European Bioelectromagnetics Association, Brussels, Belgium
	(Nov) Visiting Professor, Tata Institute, Bombay, India
1992-1993	Editor-in Chief, Proceedings, First World Congress on "Electricity and Magnetism in Biology and Medicine"
1993-1999	American Editor, "Bioelectrochemistry and Bioenergetics"
	Certificate of Appreciation, American Chemical Society, Environment Division
1995 (spring)	Visiting Professor, Dept of Chemistry, University of Beersheba, Israel
	Visiting Scientist, Dept of Biology, University of Victoria, BC, Canada
1997	Plenary Lecturer, Second World Congress on "Electricity and Magnetism in Biology and Medicine", Bologna, Italy
2002	Plenary Lecturer, Bioelectromagnetics Society, Quebec, Canada.
2005 (July)	Visiting Professor, Dept of Theoretical Physics, Kyoto University, Japan
2005	Plenary Lecturer, Conference 'Biological Effects of Electromagnetic Fields', Kyoto, Japan
2007	Invited Lecturer, House of Deputies of Brazil on Biological Effects of EM Fields, Brazil

Areas of Research

General Experimental and Theoretical Areas:

Electromagnetic field effects on cells (stress response, enzyme reactions, DNA)
Membrane biophysics and transport mechanisms (active, passive, excitation mechanisms)
Biopolymers (surface and electrical properties of proteins, DNA)

Theoretical Models of Processes in Membranes and Biopolymers:

Electric and magnetic field effects on electron transfer reactions, enzymes, DNA
Ion fluxes in excitable membranes and ion gating in channels
Cooperative reactions in membranes, hemoglobin

Specific Biological Systems:

Electron transfer reactions: Belousov-Zhabotinski (oxidation of malonic acid), cytochrome oxidase
Enzymes: Na,K-ATPase, cytochrome oxidase, F_0F_1ATPase (effects of ions and EM fields)
Proteins: hemoglobin, red cell membrane, lung surfactant, Sciara salivary gland proteomics
Cells: red blood cells, sperm cells, HL60, Sciara salivary gland, E. coli
Membranes: red blood cells, sperm cells, membrane enzymes

Interfaces, Monolayers (proteins, lipids, ions), Bilayers:

Permeability (to water, gases, ions) and Rheology (elasticity, yield stress, flow)
Electrical effects: Adsorption, Electrode Noise, Surface Potential

Teaching

Faculty of Medicine - College of Physicians and Surgeons, Columbia University
- Medical Physiology - from 1961 to 1991
  - Lectures- physical biochemistry, membranes, transport.
  - Demonstrations- membrane properties, lung surfactant, analog computer.
  - Laboratory teaching including mammalian experiments.
- Course Director, 1989-1990
  - Computerized syllabus and administration (30 faculty, 310 students)
  - Introduced lab reports and new lab exercise
- Summer Science Teachers Program, 1995, 2000, 2004

Faculty of Pure Science - Graduate School of Arts and Sciences, Columbia University
- Basic Principles in Membrane Biophysics - Physical biochemistry (1970 - present)
  - membranes, electrical properties, ion transport
- Membrane Biophysics - Surfaces, membranes, channels, model systems.
- Graduate Seminar - Basic papers on membranes and transport.
- Control Mechanisms in Physiology - Lectures and lab on analog computer.
- Principles of Physiology - Lectures on biophysics (membranes, biopolymers)

Ettore Majorana Center, Erice, Italy-International School of Biophysics (Co-Director of 4 courses)
- 1981 Bioelectrochemistry I: Redox Processes
- 1984 Bioelectrochemistry II: Membrane Phenomena
- 1988 Bioelectrochemistry III: Charge Separation Across Biomembranes
- 1991 Bioelectrochemistry IV: Nerve-Muscle Function

National Medical School Review
- Lectures on Membranes, Nerve, Muscle

City University of New York (Graduate School)
- Surface Chemistry - Lectures on Surface Chemistry in Biology

Tata Institute, Bombay, India
- Course in Bioelectrochemistry

University of Beersheba (Department of Chemistry), Israel
- Course in Biophysics

Faculty Committees

Admissions, Faculty Council (and Executive Committee of the Faculty Council), By-Laws (Formulation of Stated Rules), First Year Faculty, Divisional Elections Commission, ad hoc tenure and department review committees.

Department of Physiology: Director of Seminar Program 1973-1984, Graduate Committee, Undergraduate Committee

Society Memberships

American Association for the Advancement of Science
Bioelectromagnetics Society
Bioelectrochemical Society
American Chemical Society (Colloid and Surface Chemistry Division)
Biophysical Society
Electrochemical Society (Organic and Biological Division)

Professional Activities

Editorial Boards

Bioelectrochemistry and Bioenergetics - Editorial Board, 1978 -1998;
Co-Editor, 1981 - 1987; North American Editor, 1993 - 1998
Journal of Electrochemical Society - Divisional Editor (Biology), 1978 -1991
Journal of Colloid and Interface Science - Advisory Board, 1978 -1981
Colloids and Surfaces (founded 1979) - Editorial Board, 1979 -1986

Guest Editor, Pathophysiology (2008-2009)

Special Issue of on EMF

Bioelectrochemical (BES) Society

Founding Member, March 1979; Vice President, 1979 - 1988; President, 1988 - 1992.
Co-organizer, 4th International Symposium, Woods Hole, MA, 1977.
Plenary Lecturer, Weimar, DDR, 1979.
Organizing Committee, Topical Lecturer, Jerusalem, 1981.
Scientific Committee, Invited Lecturer, Stuttgart, Germany, 1983; Bologna, Italy, 1985.
Liaison to Bioelectromagnetics Society Board, 1984-1996.
Organizing Committee, Invited Lecturer, Szeged, Hungary, 1987.
Honorary Committee, Invited Lecturer, Pont-a-Mousson, France, 1989; Bielefeld, Germany, 1992; Seville, Spain, 1994; Israel, 1996.
Organizer, Symposium on Biological Effects of Environmental EM Fields, Israel, 1996.
International Scientific Committee, Invited Lecturer, Denmark, 1998; Bratislava, Slovakia, 2001; Florence, Italy, 2003.

Bioelectromagnetics (BEMS) Society

Invited Lecturer, BEMS meetings, San Francisco, CA, 1985; Madison, WI, 1986; Stamford, CT, 1988; Quebec, Canada, 2002
Invited Speaker, BEMS Workshop on Cooperative Phenomena, Bethesda MD, 1988
Invited Speaker, BEMS Gene Workshop, Los Angeles, CA, 1993
Board of Directors, 1989-1992; liaison from BES 1985-1996.
President Elect, 1996; President, 1997-1998; Past President, 1998-1999 (Nominating Comm, Journal Comm, Public Affairs Comm)
Plenary Lecturer, Quebec, Canada, 2002
Symposium Organizer, Speaker (Bioelectromagnetic Mechanisms), Washington, DC, 2004
Symposium Organizer, Speaker (Precautionary Principle), Cancun, Mexico, 2006
Symposium Co-Organizer, (Detecting Risk and Societal Responses), San Diego, 2008

BioInitiative Working Group (2005 - 2007)

An international group of scientists focused on EMF issues (including science, public policy and public health). **The BioInitiative Report,** entitled 'A Scientific Perspective on Health Risk of Electromagnetic Fields' was published on line on August 31, 2007. http://www.bioinitiative.org/report/index.htm
Author of Section 7, pp. 1-40. Evidence for Stress Response (Stress Proteins)

World Congress on Electricity and Magnetism in Biology and Medicine

1992-3 Executive Committee, Site Selection Committee, Program Committee.
1992-3 Editor-in-Chief of Proceedings Volume, First World Congress
1994-7 Vice President, Executive Committee for Second World Congress
Chairman, Technical Program Committee, Second World Congress

International School of Biophysics, Erice, Italy; Co-Director and Lecturer in following:
Bioelectrochemistry I: Biological Redox Reactions and Energetics, 1981.
Bioelectrochemistry II: Membrane Phenomena, 1984.
Bioelectrochemistry III: Charge Separation Across Biomembranes, 1988.
Bioelectrochemistry IV: Nerve-Muscle Function, 1991.

Division of Colloid and Surface Chemistry, American Chemical Society
Symposium Chairman, "Surface Chemistry of Biological Systems", 1966; 1969.
VK LaMer Award Committee, 1971-1976, Chairman 1975-1976
Symposium Chairman, "Bioelectrochemistry", Miami, 1978; Cleveland, 1981; Washington, 1983; Denver, 1987.
Program Committee, Biology and Medicine, Chairman, 1979-1983.
Invited Lecturer, Colloid and Surface Science Symposium, Ann Arbor, 1987.
Invited Lecturer, Biological Interfacial Reactions Symposium, Atlanta, 1991.

Division of Organic and Biological Electrochemistry (Electrochemical Society)
Symposium Chairman, "Electrochemical Processes at Biological Membranes", Seattle, 1978
Officer: Secy-Treas 1979-1981; Vice Chair 1981-1983; Chair 1983-1985.
Board of Directors, Electrochemical Society, 1983-1985.
Symposium Chairman, "Electrical Double Layers in Biology", Toronto, 1985.
Invited Speaker, "Ion Transfer Across Interfaces", Boston, 1986.
Member, Interdivisional Committee on Chemical Sensors, 1984-1987.
Invited Speaker, "Redox and Interfacial Properties", Washington, 1991.

Gordon Research Conferences
Speaker, "Chemistry at Interfaces", 1963.
Speaker, "Sensory Transduction in Microorganisms", 1978.
Day Chairman and speaker, "Chemistry at Interfaces", 1974.
Organizing Chairman, First Conference "Bioelectrochemistry", 1980.
Day Chairman and speaker, "Bioelectrochemistry", 1982.
Speaker, "Bioelectrochemistry", 1984, 1986, 1988.
Speaker, "Protons and Membrane Reactions", 1985.
Speaker, "Physicochemical Aspects, Transport in Microvasculature", 1985.
Discussion Leader, "Bioelectrochemistry", 1990, 1992, 1994, 1996, 1998, 2000 (Oxford), 2002.
Speaker, "Bioelectrochemistry", 2004.

Invitations to Miscellaneous Meetings, Workshops, Panels (Departmental Seminars not listed)
Chairman and Lecturer, "Physical Chemistry of Interfacial Transport: Biological Interfaces - Flows and Exchanges" NY Heart Assoc, 1968
Chairman and Lecturer, "Transport and Rheology of Interfacial Layers", Internat Conf on Surface and Colloid Science, Jerusalem, Israel, 1981
Lecturer, "Structure and Function in Excitable Cells", Biophysical Congress Satellite Conf, Woods Hole, MA 1981
Lecturer, "Biophysics of Cell Surface", Arendsee, DDR, 1981
Guest Speaker, CIBA Foundation, Biological Effects of Electromagnetic Fields, London, 1984
Lecturer, "Electrochemical Growth Stimulation", International Society of Electrochemistry, Berkeley, CA, 1984
Lecturer, "Biophysics of Cell Surface", Heringsdorf, DDR, 1985
Lecturer, Bioelectrical Repair & Growth Soc, Utrecht, Netherlands, 1986
Lecturer, IEEE/Engineering in Biology and Medicine Soc, Fort Worth, TX, 1986

Lecturer, International Biophysics Congress, Jerusalem, Israel, 1987
Session Organizer, IEEE/Engineering in Biology and Medicine Soc, Boston, MA, 1987
Lecturer, Bioelectrical Repair & Growth Soc, Washington, DC, 1988
Lecturer, "Chemistry Physics of Electrified Interfaces", Bologna, Italy, 1988 Symposium
Organizer, "Bioelectrochemistry", AIChE, Washington, DC, 1988
Speaker, BEMS Workshop on Cooperative Phenomena, Bethesda MD,1988
Speaker, National Research Council, "Health Effects of EM Fields", Washington, DC, 1989
Lecturer, "Electrobiology Today", Bologna, Italy, 1989
Speaker, California Department of Health Service Workshop on "ELF Field Exposure and Possible Health Effects", Berkeley, CA 1991
Speaker, FASEB Symposium on "Cancer, EM Fields and Biological Systems", Atlanta, GA 1991
Panelist, EPA- NYC Dept of Health Panel on Health Effects of EM Fields, New York, NY, 1991
Panelist, BEMS Workshop, Research Agenda, Health Effects of EM Fields, Milwaukee, WI, 1991
Opening Speaker, First Congress of European Bioelectromagnetics Association, Brussels, 1992
Speaker, EPRI Workshop on Neurobiology, Asilomar, CA, 1992
Speaker, FASEB Symposium, Biological Effects of Electromagnetic Fields, Anaheim, CA, 1992
Panelist, Molecular Electronics Symposium, First World Congress on Electricity and Magnetism in Biology & Medicine, Orlando, FL, 1992
Lectures (4) on Bioelectrochemistry of Proteins and Membranes, Tata Inst, Bombay, India, 1992
Plenary Lecture, Bioelectrochemical Society of India, Bombay, 1992
Speaker, Biophysical Society Public Policy Symposium on Biological Effects of Electromagnetic Fields, Washington, DC, 1993
Organizer, ACS Symp, Biological Effects of Environmental EM Fields, Denver, CO, 1993
Speaker, Helen Hayes Hospital, Haverstraw, NY, 1993
Speaker, Bell Labs (Series on EMF), Murray Hill, NJ 1993
Speaker, International Society of Molecular Electronics & Biocomputers, Gaithersberg, MD, 1993
Speaker, International Society of Toxicology, New Orleans, 1993
Speaker, ACS Conference on Chemical Health and Safety, Garden City, 1993
Panelist, Deadline Club, "Tension over High Tension", New York, 1993
Organizer and Speaker, Biophysical Society Workshop on Biological Effects of Environmental Electromagnetic Fields, New Orleans, LA, 1994
Speaker, ACS Conference on Environment, Hofstra University, NY, 1994
Lecturer, Hackensack Meadowlands Environment Center, Lyndhurst, NJ, 1994
Plenary Lecture, International Society of Electrochemistry, Portugal, 1994
Seminar Lecturer, Weizmann Institute, Rehovoth, Israel, 1995
Seminar Lecturer, Hebrew University-Hadassah Medical School, Jerusalem, Israel, 1995
Distinguished Lecturer, Wayne State University Medical School, Detroit, MI, 1995
Lecturer, Centre for Environmental Health, Victoria, BC, 1995
Lecturer, Victoria Cancer Clinic, Royal Jubilee Hospital, Victoria, BC, 1995
Speaker, First World Congress in Magnetotherapy, London, UK, 1996
Speaker, Applied Physics Division, CSIRO, Sydney, Australia, 1996
Speaker, Complementary Healing Conference, Baltimore, MD, 1996
Speaker, Vermont Law School Conference "Unplugged", Killington, VT, 1996
Speaker, 9th International Congress on Stress, Montreux, Switzerland, 1997
Speaker, Internat'l Comm Non-Ionizing Radiation Protection/ World Health Org (ICNIRP/WHO) Seminar, Bologna, Italy, 1997
Plenary Lecturer, Second World Congress on "Electricity and Magnetism in Biology and Medicine", Bologna, Italy, 1997
Speaker, EMF - Scientific and Legal Issues , Catania, Italy, 2002
Speaker, Chemistry and Biochemistry Departments, CUNY. 1998

Speaker, 10th International Congress on Stress, Montreux, Switzerland, 1999
Speaker, Electromed99, Norfolk, VA, 1999
Speaker, Tutorial on Magnetic Fields, Procter & Gamble, Cincinnati, 1999
Speaker, Potential Therapeutic Applications of Magnetic Fields, Vanderbilt Univ, 1999
Speaker, North American Academy of Magnetic Therapy, Los Angeles, 2000
Speaker, 3rd International Conference on Bioelectromagnetism, Slovenia, 2000
Speaker, Electromed2001, Portsmouth, VA, 2001
Speaker, EBEA Conference on EMF. Helsinki, 2001
Plenary Lecturer, Bioelectromagnetics Society, Quebec, Canada, 2002
Speaker, XXVII URSI General Assembly, Maastricht, Netherlands, 2002
Speaker, EMF - Scientific and Legal Issues , Catania, Italy, 2002
Speaker, Physics Colloquium, University of South Florida, 2003
Speaker, RIFE Conference. Topic: Electromagnetic Fields and Living Cells. Seattle, 2004
Plenary Lecturer, Conference 'Biological Effects of Electromagnetic Fields', Kyoto, Japan, 2005
Speaker, Conference on EMF and the Precautionary Principle, Benevento, Italy, 2006
Keynote Speaker, Conference on Cell Towers and Wireless Technologies, Everett, MA, 2007
Invited lecturer on Biological Effects of EM Fields, Chamber of Deputies, Brasilia, Brazil, 2007
Speaker, Conference on Responsible Cell Tower Siting. Cornwall, CT, 2008

Grant Review Consultant

Office of Naval Research, Department of Defense
IPA Biologist, Manager of Membrane Electrochemistry ARI, 1986-1988
Chairman, Panel on Biological Sciences Div, August 1986
Member, Panel on Interdisciplinary Research, April 1979
Electric Power Research Institute, Palo Alto, CA
Member, Basic Sciences Advisory Committee, 1987-1991
National Institutes of Health
Radiation Study Section, 1991
(several ad hoc Study Sections and site visit committees)
National Science Foundation
US Army Research Office
US-Israel Binational Science Foundation
Petroleum Research Fund
Medical Research Council - Canada
Australian Research Grants Committee
Research Corporation (Providence, Rhode Island)
University and Polytechnic Grants Committee, Hong Kong
International Science Foundation (for Former Soviet Union), Washington, DC
Breast Cancer Research Program, University of California
US Army Medical Research and Materiel Command, Neurotoxin Exposure Program, AIBS
US Army Radiofrequency Radiation Research Program, AIBS

Consultant to various environmental groups on biological effects of electromagnetic fields (power frequency and radiofrequency)

PUBLICATIONS - Books, Reviews, Chapters

1. Blank M (1957) The Transfer of Monolayers through Surface Channels. **PhD Dissertation,** Chemistry Department, Columbia University, 54pp.
2. Blank M (1959) The Permeability of Monolayers to Carbon Dioxide and Oxygen. **PhD Dissertation,** Department of Colloid Science, Cambridge University, England, 105pp.
3. Blank M (1967) Editor, Symposium "Surface Chemistry of Biological Systems". **Journal of Colloid and Interface Science** 24:1-127.
4. Blank M and Britten JS (1970) Physical Principles in Monolayer and Membrane Permeation. in **"Physical Principles of Biological Membranes"**, edited by F Snell et al; Gordon & Breach, New York, pp 143-163.
5. Blank M (1970) Editor, **"Surface Chemistry of Biological Systems"**. Volume 7, "Advances in Experimental Medicine and Biology", Plenum Press, New York, 340pp.
6. Blank M (1972) The Measurement of Monolayer Permeability, in **"Techniques of Surface Chemistry and Physics"**, Volume I. Good, Stromberg, Patrick (eds); Marcel Dekker Inc., New York, pp 41-88
7. Blank M (1979) Monolayer Permeability. **Progress in Surface & Membrane Science** 13:87-139.
8. Blank M (1979) Surface Pharmacology: Drug Binding Equilibria and Ion Transport in Membrane Structures. **Pharmacology and Therapeutics** 7:313-328.
9. Blank M (1980) Editor, **"Bioelectrochemistry: Ions, Surfaces and Membranes"**, Advances in Chemistry, Volume 188, American Chem Soc, Washington, DC, 527pp.
10. Blank M (1981) Surface Pharmacology: Drug Binding Equilibria and Ion Transport in Membrane Structures, in **International Encyclopedia of Pharmacology and Therapeutics**, Inhibitors of Mitochondrial Functions, M Erecinska, DF Wilson (eds). Pergamon, New York, pp 19-34.
11. Milazzo G and Blank M (1983) Editors, **"Bioelectrochemistry I: Biological Redox Reactions"**, School of Biophysics, Erice, Italy. Plenum, New York, 348pp.
12. Blank M (1983) Transmembrane Potentials and Redox Reactions from the Physiological Point of View. in **"Bioelectrochemistry I: Biological Redox Reactions"**, G Milazzo, M Blank (eds), Plenum, New York, pp 227-247.
13. Blank M (1983) The Effects of Surface Compartments of Ion Transport Across Membranes. in **"Structure and Function in Excitable Cells"**, DC Chang, I Tasaki, WJ Adelman, HR Leuchtag (eds); Plenum, New York, pp. 435-449.
14. Blank M (1986) Editor, **"Electrical Double Layers in Biology"**, Plenum, NewYork, 319pp
15. Blank M (1987) The Surface Compartment Model: A Theory of Ion Transport Focused on Ionic Processes in the Electrical Double Layers at Membrane Protein Surfaces. **Biochimica et Biophysica Acta - Reviews on Biomembranes** 906:277-294.
16. Blank M and Findl E (1987) Editors, **"Mechanistic Approaches to the Interaction of Electric and Electromagnetic Fields with Living Systems"**, Plenum, New York, 439pp.
17. Milazzo G and Blank M (1987) Editors, **"Bioelectrochemistry II: Membrane Phenomena"**, International School of Biophysics, Erice, Italy. Plenum, New York, 543pp.
18. Blank M (1987) An Electrochemical Perspective on Excitable Membranes, Channels and Gating. in **"Bioelectrochemistry II: Membrane Phenomena"**, G Milazzo, M Blank (eds); Plenum, New York, pp. 431-456.
19. Blank M (1988) Recent Developments in the Theory of Ion Flow Across Membranes Under Imposed Electric Fields. In **"Modern Bioelectricity"**, AA Marino (ed); Dekker, New York, pp 345-364.
20. Markov M and Blank M (1988) Editors, **"Electromagnetic Fields and Biomembranes"**, Plenum, New York, 309pp.

Blank M (1990) Editor, **Syllabus for Human Physiology Course**, 13th Edition, Physiology Department, Columbia University, New York, 704pp.

22. Milazzo G and Blank M (1990) Editors, **"Bioelectrochemistry III: Charge Separation across**

Membranes", Plenum, New York, 337pp.

23. Blank M (1991) Membrane Transport: Insight from Colloid Science. in **"Interfacial Phenomena in Biological Systems"**, M Bender (ed). Dekker, New York, pp 337-366.
24. Blank M (1993) Electrochemistry of Nerve Excitation, **"Modern Aspects of Electrochemistry"** Number 24, edited by RE White et al, Plenum, New York, pp1-37.
25. Blank M (1993) Editor-in-Chief, Proceedings of First World Congress on **"Electricity and Magnetism in Biology and Medicine"**, San Francisco Press, 952pp.
26. Blank M and Vodyanoy I (1994) Editors, **"Biomembrane Electrochemistry"**, Advances in Chemistry Series of the American Chemical Society Press, 605pp.
27. Blank M (1994) An Electrochemical Model of Voltage Gated Channels. **Advances in Chemistry** 235:429-446.
28. Melandri BA, Milazzo G and Blank M (1994) Editors, **"Bioelectrochemistry IV: Nerve-Muscle Function"**. Life Sciences Volume 267, Plenum, New York, 376pp.
29. Blank M (1995) Editor, **"Electromagnetic Fields: Biological Interactions and Mechanisms"**, **Advances in Chemistry**, Volume 250, American Chemical Society Press, 512pp.
30. Blank M (1995) Biological Effects of Electromagnetic Fields: An Overview. **Advances in Chemistry** 250:3-12.
31. Blank M (1995) Electric Stimulation of Protein Synthesis in Muscle. **Advances in Chemistry** 250:143-153.
32. Blank M (1995) Electric and Magnetic Field Signal Transduction in the Membrane Na,K-ATPase. **Advances in Chemistry** 250:339-348.
33. Goodman R and Blank M (1995) The Biosynthetic Stress Response in Cells Exposed to Electromagnetic Fields. **Advances in Chemistry** 250:423-436.
34. Blank M (1997) Effects of Electromagnetic Fields on Cells as a Basis for Therapy. in **Proceedings of the First World Congress in Magnetotherapy**, pp. 151-156, London, May 1996.
35. Blank M (1997) Studies on the Mechanism of Electromagnetic Field Interactions with Cells: I-The Cellular Stress Response in Electromagnetic Fields; II-Electric and Magnetic Signal Transduction in a Membrane Protein. **Electric Power Research Institute Report TR-108947,** 99 pp.

Goodman R and Blank M (1998) Magnetic Field Induces Expression of hsp70. **Cell Stress and Chaperones** 3:79-88.

37. Goodman R and Blank M (2002) Insights into Electromagnetic Interaction Mechanisms. **Journal of Cellular Physiology** 192:16-22.
38. Blank M and Goodman R (2004) Initial interactions in electromagnetic field-induced biosynthesis. **Journal of Cellular Physiology** 199:359-363.
39. Blank M (2008) Protein and DNA Reaction Stimulated by Electromagnetic Fields. **Bioelectromagnetic Biology and Medicine** 27: 3-23.

PUBLICATIONS - Papers

1. LaMer VK and Blank M (1956) The Transfer of Surface Films through Surface Channels- Geometrical Factors. **Journal of Colloid Science** 11:608-616. 1956.
2. Blank M and LaMer VK (1957) The Mechanism of Transfer of Surface Films. Proceedings of the **Second International Congress on Surface Activity**, Vol II, pp 102-108.
3. Blank M and LaMer VK (1957) The Transfer of Monolayers through Surface Channels - II. Mechanism. **Journal of Physical Chemistry** 61:1611-1614.
4. Blank M and Roughton FJW (1960) The Permeability of Monolayers to Carbon Dioxide. **Transactions of the Faraday Society** 56:1832-1841.
5. Blank M (1961) The Effect of Vapors on Monolayer Permeability to Carbon Dioxide. **Journal of Physical Chemistry** 65:1698-1703.
6. Blank M and LaMer VK (1962) The Energy Barrier for Monolayer Penetration, in **"Retardation of Evaporation by Monolayers"**, edited by VK LaMer. Academic Press, New York, pp. 59-66.
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Book Reviews

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EXHIBIT B

NIEHS REPORT on

Health Effects from Exposure to Power-Line Frequency Electric and Magnetic Fields

Prepared in Response to the 1992 Energy Policy Act (PL 102-486, Section 2118)

NIEHS

National Institute of Environmental Health Sciences
National Institutes of Health

Supported by the NIEHS/DOE

EMF RAPID
Electric and Magnetic Fields Research and Public Information Dissemination Program

NIH Publication No. 99-4493

NIEHS REPORT on

Health Effects from Exposure to Power-Line Frequency Electric and Magnetic Fields

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National Institute of Environmental Health Sciences
National Institutes of Health

Dr. Kenneth Olden, Director

Prepared by the
NIEHS EMF-RAPID Program Staff

NIH Publication No. 99-4493

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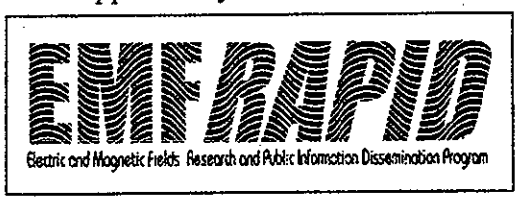

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

May 4, 1999

Dear Reader:

In 1992, the U.S. Congress authorized the Electric and Magnetic Fields Research and Public Information Dissemination Program (EMF-RAPID Program) in the Energy Policy Act. The Congress instructed the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health and the U.S. Department of Energy (DOE) to direct and manage a program of research and analysis aimed at providing scientific evidence to clarify the potential for health risks from exposure to extremely low frequency electric and magnetic fields (ELF-EMF). The EMF-RAPID Program had three basic components: 1) a research program focusing on health effects research, 2) information compilation and public outreach and 3) a health assessment for evaluation of any potential hazards arising from exposure to ELF-EMF. The NIEHS was directed to oversee the health effects research and evaluation, and the DOE was given the responsibility for overall administration of funding and engineering research aimed at characterizing and mitigating these fields. The Director of the NIEHS was mandated upon completion of the Program to provide this report outlining the possible human health risks associated with exposure to ELF-EMF. The scientific evidence used in preparation of this report has undergone extensive scientific and public review. The entire process was open and transparent. Anyone who wanted "to have a say" was provided the opportunity.

The scientific evidence suggesting that ELF-EMF exposures pose any health risk is weak. The strongest evidence for health effects comes from associations observed in human populations with two forms of cancer: childhood leukemia and chronic lymphocytic leukemia in occupationally exposed adults. While the support from individual studies is weak, the epidemiological studies demonstrate, for some methods of measuring exposure, a fairly consistent pattern of a small, increased risk with increasing exposure that is somewhat weaker for chronic lymphocytic leukemia than for childhood leukemia. In contrast, the mechanistic studies and the animal toxicology literature fail to demonstrate any consistent pattern across studies although sporadic findings of biological effects have been reported. No indication of increased leukemias in experimental animals has been observed.

The lack of connection between the human data and the experimental data (animal and mechanistic) severely complicates the interpretation of these results. The human data are in the "right" species, are tied to "real life" exposures and show some consistency that is difficult to ignore. This assessment is tempered by the observation that given the weak magnitude of these increased risks, some other factor or common source of error could explain these findings. However, no consistent explanation other than exposure to ELF-EMF has been identified.

Epidemiological studies have serious limitations in their ability to demonstrate a cause and effect relationship whereas laboratory studies, by design, can clearly show that cause and effect are possible. Virtually all of the laboratory evidence in animals and humans and most of the mechanistic work done in cells fail to support a causal relationship between exposure to ELF-EMF at environmental levels and changes in biological function or disease status. The lack of consistent, positive findings in animal or mechanistic studies weakens the belief that this association is actually due to ELF-EMF, but it cannot completely discount the epidemiological findings.

The NIEHS concludes that ELF-EMF exposure cannot be recognized at this time as entirely safe because of weak scientific evidence that exposure may pose a leukemia hazard. In my opinion, the conclusion of this report is insufficient to warrant aggressive regulatory concern. However, because virtually everyone in the United States uses electricity and therefore is routinely exposed to ELF-EMF, passive regulatory action is warranted such as a continued emphasis on educating both the public and the regulated community on means aimed at reducing exposures. The NIEHS does not believe that other cancers or non-cancer health outcomes provide sufficient evidence of a risk to currently warrant concern.

The interaction of humans with ELF-EMF is complicated and will undoubtedly continue to be an area of public concern. The EMF-RAPID Program successfully contributed to the scientific knowledge on ELF-EMF through its support of high quality, hypothesis-based research. While some questions were answered, others remain. Building upon the knowledge base developed under the EMF-RAPID Program, meritorious research on ELF-EMF through carefully designed, hypothesis-driven studies should continue for areas warranting fundamental study including leukemia. Recent research in two areas, neurodegenerative diseases and cardiac diseases associated with heart rate variability, have identified some interesting and novel findings for which further study is ongoing.

Advocacy groups have opposing views concerning the health effects of ELF-EMF. Some advocacy groups want complete exoneration and others want a more serious indictment. Our conclusions are prudent and consistent with the scientific data. I am satisfied with the report and believe it provides a pragmatic, scientifically-driven basis for any further regulatory review.

I am pleased to transmit this report to the U.S. Congress.

Sincerely,

Kenneth Olden, Ph.D.
Director

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EXECUTIVE SUMMARY

Introduction

Electrical energy has been used to great advantage for over 100 years. Associated with the generation, transmission, and use of electrical energy is the production of weak electric and magnetic fields (EMF). In the United States, electricity is usually delivered as alternating current that oscillates at 60 cycles per second (Hertz, Hz) putting fields generated by this electrical energy in the extremely low frequency (ELF) range.

Prior to 1979 there was limited awareness of any potential adverse effects from the use of electricity aside from possible electrocution associated with direct contact or fire from faulty wiring. Interest in this area was catalyzed with the report of a possible association between childhood cancer mortality and proximity of homes to power distribution lines. Over the next dozen years, the U.S. Department of Energy (DOE) and others conducted numerous studies on the effects of ELF-EMF on biological systems that helped to clarify the risks and provide increased understanding. Despite much study in this area, considerable debate remained over what, if any, health effects could be attributed to ELF-EMF exposure.

In 1992, the U.S. Congress authorized the Electric and Magnetic Fields Research and Public Information Dissemination Program (EMF-RAPID Program) in the Energy Policy Act (PL 102-486, Section 2118). The Congress instructed the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health and the DOE to direct and manage a program of research and analysis aimed at providing scientific evidence to clarify the potential for health risks from exposure to ELF-EMF. The EMF-RAPID Program had three basic components: 1) a research program focusing on health effects research, 2) information compilation and public outreach and 3) a health assessment for evaluation of any potential hazards arising from exposure to ELF-EMF. The NIEHS was directed to oversee the health effects research and evaluation and the DOE was given the responsibility for overall administration of funding and engineering research aimed at characterizing and mitigating these fields. The Director of the NIEHS was mandated upon completion of the Program to provide a report outlining the

possible human health risks associated with exposure to ELF-EMF. This document responds to this requirement of the law.

This five-year effort was signed into law in October 1992 and provisions of this Act were extended for one year in 1997. The Program ended December 31, 1998. The EMF-RAPID Program was funded jointly by Federal and matching private funds and has been an extremely successful Federal/private partnership with substantial financial support from the utility industry. The NIEHS received $30.1 million from this program for research, public outreach, administration and the health assessment evaluation of ELF-EMF. In addition to EMF-RAPID Program funds from the DOE, the NIEHS contributed $14.5 million for support of extramural and intramural research including long-term toxicity studies conducted by the National Toxicology Program.

NIEHS Conclusion

The scientific evidence suggesting that ELF-EMF exposures pose any health risk is weak. The strongest evidence for health effects comes from associations observed in human populations with two forms of cancer: childhood leukemia and chronic lymphocytic leukemia in occupationally exposed adults. While the support from individual studies is weak, the epidemiological studies demonstrate, for some methods of measuring exposure, a fairly consistent pattern of a small, increased risk with increasing exposure that is somewhat weaker for chronic lymphocytic leukemia than for childhood leukemia. In contrast, the mechanistic studies and the animal toxicology literature fail to demonstrate any consistent pattern across studies although sporadic findings of biological effects (including increased cancers in animals) have been reported. No indication of increased leukemias in experimental animals has been observed.

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association is actually due to ELF-EMF, but it cannot completely discount the epidemiological findings.

The NIEHS concludes that ELF-EMF exposure cannot be recognized as entirely safe because of weak scientific evidence that exposure may pose a leukemia hazard. In our opinion, this finding is insufficient to warrant aggressive regulatory concern. However, because virtually everyone in the United States uses electricity and therefore is routinely exposed to ELF-EMF, passive regulatory action is warranted such as a continued emphasis on educating both the public and the regulated community on means aimed at reducing exposures. The NIEHS does not believe that other cancers or non-cancer health outcomes provide sufficient evidence of a risk to currently warrant concern.

The interaction of humans with ELF-EMF is complicated and will undoubtedly continue to be an area of public concern. The EMF-RAPID Program successfully contributed to the scientific knowledge on ELF-EMF through its support of high quality, hypothesis-based research. While some questions were answered, others remain. Building upon the knowledge base developed under the EMF-RAPID Program, meritorious research on ELF-EMF through carefully designed, hypothesis-driven studies should continue for areas warranting fundamental study including leukemia. Recent research in two areas, neurodegenerative diseases and cardiac diseases associated with heart rate variability, have identified some interesting and novel findings for which further study is ongoing.

Background

Program Oversight and Management

The 1992 Energy Policy Act created two committees to provide guidance and direction to this program. The first, the Interagency Committee (IAC), was established by the President of the United States and composed of representatives from the NIEHS, the DOE and seven other Federal agencies with responsibilities related to ELF-EMF. This group receives the report from the NIEHS Director and must prepare its own report for Congress. The IAC had responsibility for developing a strategic research agenda for the EMF-RAPID Program, facilitating interagency coordination of Federal research activities and communication to the public and monitoring and evaluating the Program.

The second committee, the National EMF Advisory Committee (NEMFAC), consisted of representatives from public interest groups, organized labor, state governments and industry. This group was involved in all aspects of the EMF-RAPID Program providing advice and critical review to the DOE and the NIEHS on the design and implementation of the EMF-RAPID Program's activities.

ELF-EMF Health Effects Research

The EMF-RAPID Program's health effects research initiative relied upon accepted principles of hazard identification and risk assessment to establish priorities. All studies supported by the NIEHS and the DOE under this program were selected for their potential to provide solid, scientific data on whether ELF-EMF exposure represents a human health hazard, and if so, whether risks are increased under exposure conditions in the general population. Research efforts did not focus on epidemiological studies (i.e. those in the human population) because of time constraints and the number of ongoing, well-conducted studies. The NIEHS health effects research program focused on mechanistic, cellular and laboratory studies in the areas of neurophysiology, behavior, reproduction, development, cellular research, genetic research, cancer and melatonin. Mechanistic, cellular and laboratory studies are part of the overall criteria used to determine causality in interpreting epidemiological studies. In this situation, the most cost-effective and efficient use of the EMF-RAPID Program's research funds was clearly for trying to clarify existing associations identified from population studies. The DOE research initiatives focused on assessment of exposure and techniques of mitigation.

The EMF-RAPID Program through the combined efforts of the NIEHS and the DOE radically changed and markedly improved the quality of ELF-EMF research. This was accomplished by providing biological and engineering expertise to investigators and emphasizing hypothesis-driven, peer-reviewed research. Four regional facilities were also set-up where state-of-the-art magnetic field exposure systems were available for in-house and outside investigators to conduct mechanistic research. The EMF-RAPID Program through rigorous review and use of multi-disciplinary research teams greatly enhanced the understanding of the interaction of biological systems with ELF-EMF.

Information Dissemination and Public Outreach

The EMF-RAPID Program provided the public, regulated industry and scientists with useful, targeted information that addressed the issue of uncertainty regarding ELF-EMF health effects. Two booklets, a question and answer booklet on ELF-EMF and a layman's booklet addressing ELF-EMF in the workplace, were published. A telephone information line for ELF-EMF was available where callers could request copies of ELF-EMF documents and receive answers to standard questions from operators. The NIEHS also developed a web-site for the EMF-RAPID Program where all of the Program's documents are on-line and links are available to other useful sites on ELF-EMF. Efforts were made to include the public in EMF-RAPID Program activities through sponsorship of scholarships to meetings; holding open, scientific workshops; and setting aside a two-month period for public comment and review on ELF-EMF and the workshop reports. In addition, the NIEHS sponsored attendance of NEMFAC

members at relevant scientific meetings and at each of the public comment meetings.

Health Risk Assessment of ELF-EMF Exposure

In preparation of the NIEHS Director's Report, the NIEHS developed a process to evaluate the potential health hazards of ELF-EMF exposure that was designed to be open, transparent, objective, scholarly and timely under the mandate of the 1992 Energy Policy Act. The NIEHS used a three-tiered strategy for collection and evaluation of the scientific information on ELF-EMF that included: 1) three science review symposia for targeted ELF-EMF research areas, 2) a working group meeting and 3) a period of public review and comment. Each of the three symposia focused on a different, broad area of ELF-EMF research: mechanistic and cellular research (24-27 March 1997, Durham, NC), human population studies (12-14 January 1998, San Antonio, TX) and laboratory human and clinical work (6-9 April 1998, Phoenix, AZ). These meetings were aimed at including a broad spectrum of the research community and the public in the evaluation of ELF-EMF health hazards, identifying key research findings and providing opinion on the quality of this research. Discussion reports from small discussion groups held for specific topics were prepared for each meeting.

Following the symposia, a working group meeting (16-24 June 1998, Brooklyn Park, MN) was held where a scientific panel reviewed historical and novel evidence on ELF-EMF and determined the strength of the evidence for human health and biological effects. Stakeholders and the public attended this meeting and were given the opportunity to comment during the process. The Working Group conducted a formal, comprehensive review of the literature for research areas identified from the symposia as being important to the assessment of ELF-EMF-related biological or health effects. Separate draft documents covering areas of animal carcinogenicity, animal non-cancer findings, physiological effects, cellular effects, theories and human population studies (epidemiology studies) in children and adults for both occupational and residential ELF-EMF exposures were rewritten into a single book. The Working Group characterized the strength of the evidence for a causative link between ELF-EMF exposure and disease in each category of research using the criteria developed by the International Agency for Research on Cancer (IARC).

The IARC criteria fall into four basic categories: sufficient, limited, inadequate and evidence suggesting the lack of an effect. After critical review and discussion, members of the Working Group were asked to determine the categorization for each research area; the range of responses reflected the scientific uncertainty in each area. A majority of the Working Group members concluded that childhood leukemia and adult chronic lymphocytic leukemia from occupational exposure were areas of concern. For other cancers and for non-cancer health endpoints, the Working Group categorized the experimental data as

providing much weaker evidence or no support for effects from exposure to ELF-EMF.

Following the Working Group Meeting, the NIEHS established a formal review period for solicitation of comments on the symposia and Working Group reports. The NIEHS hosted four public meetings (14-15 September 1998, Tucson, AZ; 28 September, Washington, DC; 1 October 1998, San Francisco, CA; and 5 October 1998, Chicago, IL) where individuals and groups could voice their opinions; the meetings were recorded and transcripts prepared. In addition, the NIEHS received 178 written comments that were also reviewed in preparation of this report. The remarks that NIEHS received covered many areas related to ELF-EMF and provided insight about areas of concern on behalf of the public, researchers, regulatory agencies and industry.

EXHIBIT C

British Journal of Cancer (2000) 83(5), 692–698

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A pooled analysis of magnetic fields and childhood leukaemia

A Ahlbom[1], N Day[2], M Feychting[1], E Roman[3], J Skinner[2], J Dockerty[4], M Linet[5], M McBride[6], J Michaelis[7], JH Olsen[8], T Tynes[9] and PK Verkasalo[10,11,12]

[1]Division of Epidemiology, National Institute of Environmental Medicine, Karolinska Institute, Sweden; [2]Strangeways Research Laboratory, University of Cambridge, UK; [3]Leukaemia Research Fund Centre for Clinical Epidemiology, University of Leeds, UK; [4]Childhood Cancer Research Group, University of Oxford, UK; [5]Division of Cancer Epidemiology and Genetics, National Cancer Institute, USA; [6]Cancer Control Research Programme, British Columbia Cancer Agency, Canada; [7]Institute of Medical Statistics and Documentation, University of Mainz, Germany; [8]Institute of Cancer Epidemiology, Danish Cancer Society, Denmark; [9]Institute of Epidemiological Cancer Research, Norway; [10]Department of Public Health, University of Helsinki, Finland; [11]Finnish Cancer Registry; [12]Department of Public Health, University of Turku, Finland

Summary Previous studies have suggested an association between exposure to 50–60 Hz magnetic fields (EMF) and childhood leukaemia. We conducted a pooled analysis based on individual records from nine studies, including the most recent ones. Studies with 24/48-hour 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 3203 children with leukaemia 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 leukaemia 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. © 2000 Cancer Research Campaign

Keywords: EMF; cancer; childhood leukaemia; meta-analysis; pooled analysis; epidemiology

It is now twenty years since Wertheimer and Leeper (1979) published the first study suggesting an association between residential exposure to extremely low frequency magnetic fields (EMF) and childhood cancer. Ever since, this has been a controversial issue with the findings from several, but not all, subsequent epidemiological studies being consistent with an association, particularly with respect to residential exposure and childhood leukaemia (Portier and Wolfe, 1998). However, many of the reports have been based on small numbers of exposed cases, and despite intense experimental research no known biophysical mechanism to explain an effect has been established.

We conducted a pooled analysis based on primary data from nine studies on EMF and childhood leukaemia, addressing three specific questions:

1. Do the combined results of these studies indicate that there is an association between EMF exposure and childhood leukaemia risk, which is larger than one would expect from random variability?
2. Does adjustment for confounding from socioeconomic class, mobility, level of urbanization, detached/not detached dwelling, and level of traffic exhaust change the results?
3. Do the combined data support the existence of the so-called wire code paradox, that is, a stronger association between proxy measures of EMF and cancer than between direct measurements and cancer?

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Correspondence to: A Ahlbom

METHODS

The original plan for this project was to include all European studies that addressed the question of an association between EMF and childhood leukaemia and were based on either 24 or 48 hour magnetic field measurements or calculated fields. At the time five such studies were reported (Feychting and Ahlbom, 1993; Olsen et al, 1993; Verkasalo et al, 1993; Tynes and Haldorsen, 1997; Michaelis et al, 1998). In addition, a nationwide childhood cancer study was in progress and near completion in the UK (UKCCS, 1999). Since we were not aware of any other European study to be published in the near future, the inclusion of the UK study would give us a complete set of European studies. We felt that if we could also incorporate new studies from non-European countries this pooled analysis would be up to date and presumably stay current for several years. We were aware of three more studies in other parts of the world with compatible information that were all nearly

Table 1 Relevant characteristics for studies included in the pooled analysis

	Subjects			Exposure measures			Matching variables			Potential confounders: Common		Potential confounders: Study specific (no. of groups)					
	Cases	Controls	Year of diagnosis	Long measurements	Calculated fields	Wire codes	Sex	Year of birth	Area of diagnosis	Detached or other house	Mobility quintile	Social group	Measure of social status: Mother's education	Income	Urbanisation	Car exhaust	Other
Canada	272	304	1990–94	✓		✓	✓	✓	✓	✓	✓		3		2		
Denmark	833	4746	1968–86		✓		✓	✓			✓	5			4		
Finland[a]	29	1027	1974–93		✓		✓	✓							2		
Germany	175	409	1992–95	✓			✓	✓	✓	✓	✓	2			3	2	2[c]
New Zealand	86	80	1990–93	✓			✓	✓			✓		5		2		
Norway	148	572	1965–89		✓		✓	✓	✓	✓	✓	6			2		
Sweden	36	508	1960–85		✓		✓	✓	✓	✓	✓	4			2	3	
USA[b]	595	530	1989–94	✓		✓	✓	✓	✓	✓	✓			6	4		
UK	1073	2224	1992–96	✓			✓	✓	✓			7					

Specification of exposure information selected for the pooled analysis

Canada	Latest home inhabited before diagnosis for which a 24-hour bedroom measurement was available (may not be same home for long measurement & wire code)
Denmark	Latest home inhabited before diagnosis for which a calculated field was available
Finland	Calculated field for 12 months prior to diagnosis was provided especially for this exercise (may be average of values for more than one home)
Germany	Latest home inhabited before diagnosis (was home at diagnosis for almost all individuals)
New Zealand	Home inhabited at diagnosis
Norway	Latest home inhabited before diagnosis in which child lived in the power line corridor, field calculated for entire period
Sweden	Latest home inhabited before diagnosis in which child lived in the power line corridor, field calculated for entire period
USA	Latest home inhabited before diagnosis for which a record was available (may not be same home for long measurement & wire code)
UK	Home inhabited at diagnosis (UKCCS selection meant that the child must have lived there for previous 12 months)

[a]Case control data generated from the original cohort; [b]acute lymphoblastic leukaemia only; [c]East/West Germany.

completed or recently completed, so we could include those too (Linet et al, 1997; Dockerty et al, 1998, 1999; McBride et al, 1999). Table 1 lists the studies and their relevant characteristics. A fourth study was also near completion in Ontario, Canada, but it was decided that since this study did not provide 24-hour indoor measurements, or anything similar to it, the exposure information in this study was not similar enough to justify inclusion (Green et al, 1999a,b). In effect, all large-scale published studies with extended indoor measurements or calculated fields were included in the pooled analysis with the exception of a few studies that were not population based.

The primary analyses reported here were all discussed and agreed upon prior to the commencement of the work. This included diagnostic categories, exposure definitions, time period for evaluation, cut points, confounders, and statistical methods. In addition certain analyses were done to confirm that the findings from these primary analyses were not dependent on these specifications and yet other analyses were done with an exploratory purpose.

This pooled analysis focused on childhood leukaemia, even though several of the studies also included other cancer diagnoses. The US study included only acute lymphocytic leukaemia (ALL). We did analyses both for total leukaemia and for ALL, but for brevity the more detailed results are given for total leukaemia. There was some variation with respect to age groups in the studies, and we decided to use the age interval 0–14 years.

Since we wanted the data to be as consistent as possible across studies, the data that we used from a particular study were sometimes different from those that formed the basis for the original publication from that study. This was particularly the case with the exposure variables (Table 1). In effect, the study-specific results that we report in this article differ to various degrees from the results as reported in the original publications. These differences are biggest for the US study. Compared with the published results of the US study, the pooled analysis included fewer cases and controls (34 cases and 90 controls were excluded because 24/48-hour measurements were missing), limited the study period to the year prior to diagnosis rather than the five years immediately prior to diagnosis, restricted the number of residences for which measurements were utilized to one per subject rather than all homes resided in during the five years immediately prior to diagnosis, and used geometric means rather than arithmetic means.

In studies with long magnetic field measurements (24/48-hour), these were chosen as the primary exposure measure. The publication from the Canadian study uses personal measurements, but to achieve consistency with the other studies we chose to use the in-home measurements instead. In the UK, a two-phase measurement strategy was used, according to which 48-hour measurements were conducted when either a shorter measurement (108 minutes) or a characteristic of the residency indicated that EMF exposure was elevated. These measurements were all treated as long measurements because almost all elevated readings would come from 48-hour measurements. None of the adjustments to the measured exposure that were presented in the UKCCS analysis were used in the pooled analysis. (It should be noted that these adjustments had negligible effect.)

As a summation of all measurements for one subject, over the 24/48 hours, most of the centres used arithmetic means. We decided, however, to use geometric means from all studies, because they are less affected by outliers. For comparison we also analysed the data using arithmetic means. Therefore, each centre provided the geometric means as well as the arithmetic means, regardless of what they used in their original publication.

All centres without long measurements had calculated fields, i.e., calculations of magnetic fields based upon distance between the subject's home and the nearby power line, line characteristics, and load on the line. For these centres calculated fields were evaluated as the primary measure.

We also analysed wire-codes (i.e., a proxy measure of residential magnetic field level, based on the distance and configuration of nearby power lines) for all North American studies. These were classified and analysed according to the original Wertheimer–Leeper scheme (Wertheimer and Leeper, 1982). We also developed a European version of the wire-code, but eventually decided that the differences between the North American and the European distribution systems were too large to make this meaningful. The wire-code analyses, therefore, only included the North American studies.

With respect to the reference time for exposure characterization, there was considerable variation across studies. Residential measurement data were available for various periods from birth to diagnosis. We decided to aim for the average exposure during the last year prior to diagnosis for the cases and the corresponding age for the controls. We achieved this by using the exposure information for the home at the time of diagnosis for the cases and the home lived in by the matched control at the same age; when this information was unavailable we used instead the latest time period prior to diagnosis (Table 1). The reasons were that all studies could provide exposure data specified in this way and that exposure close to date of diagnosis is relevant to the hypothesis that EMF, if anything, would act as a promoter.

All studies utilized a matched case-control design, although the matching variables were not the same in all studies (Table 1). In Finland the original publication reported findings from a cohort study, but in preparation for this pooled analysis a control group was selected and the data were evaluated using a matched case-control design with 3 additional years of follow-up. Because we wanted to use as many as possible of the cases and controls to increase the flexibility of the analysis, we decided to ignore the matching. Instead we included adjustment for age and sex in all analyses, with age classified into one-year groups up to five years of age and then into five year groups. In all analyses, the measurement studies were also adjusted for socio-economic status, according to centre-specific definitions (Table 1). In addition, we adjusted for residence in the eastern or western part of the country in Germany.

One of the aims of this study was to test whether adjustment for any available covariate would have an effect on the summary relative risk estimates. In addition to the covariates included in the basic model, the following factors were available: socioeconomic status, mobility, level of urbanization, detached/not detached dwelling, and level of traffic exhaust. All of these variables were not available in all studies (Table 1). For socioeconomic class, level of urbanization, residential mobility, and traffic exhaust, the basic information and the definitions varied between centres as described in Table 1.

To estimate a summary relative risk across centres, a logistic regression model was applied to the raw data, with centres represented by dummy variables. We did this for measurement studies and calculated field studies separately but also across all studies. In the primary analyses, exposure was categorized in the four levels: < 0.1 μT; 0.1–<0.2 μT; 0.2–<0.4 μT; ≥ 0.4 μT and entered into the model with the use of dummy variables. The wire-code analyses were treated correspondingly. In addition, a similar analysis but with continuous exposure was conducted, the results of which are reported as relative risks per 0.2 μT intervals. This continuous analysis was also the basis for a likelihood ratio test of homogeneity of effects across studies.

RESULTS

Table 2 gives the absolute numbers of subjects by case/control status, study, and exposure level. In total there are 3247 cases and 10 400 controls. The UK provided by far the largest number of cases, while Denmark had the largest number of controls. In the highest exposure category (≥ 0.4 μT) there were 44 cases and 62 controls, with the largest number of cases from the USA and the largest number of controls from Sweden. Out of the 3247 cases, 2704 (83%) are ALL cases. The US study was restricted to ALL, which explains why the US numbers are the same in the left and right panels of Table 2.

In Table 3 we summarize the primary results for total leukaemia. For each centre the relative risks are estimated by exposure level and with adjustment for the basic potential confounders. Some of the studies are based on small numbers, particularly the highest exposure categories, and in some instances there are zero cases or controls. Although some of the centre-specific relative risk estimates are of little interest in themselves, particularly in the higher categories, all studies still provide information for the summary measures. The last column of the table gives the results of the logistic regression analysis with continuous exposure. The homogeneity test based on the continuous analysis across all nine centres resulted in a χ^2 with eight degrees of freedom of 10.7 corresponding to a P value of 0.22. The interpretation is that the variation in point estimates between the studies, is not larger than one would expect from random variability. We compared results for matched versus unmatched analyses to confirm that ignoring the matching did not introduce a bias. Because the results were similar, we only report the unmatched results.

Across the measurement studies, the summary relative risk is estimated at 1.87 (95% CI.: 1.10–3.18) in the highest exposure category, with a corresponding P value of 0.01. The two lower categories have estimates close to unity. For the calculated fields studies the summary measure for the top exposure category is 2.13 (0.93–4.88), with a P value of 0.04.

In the very last line of Table 3, we give the summary relative risk estimate across all studies, regardless of whether the study is a measurement study or a calculated field study. We consider this an analysis based on the exposure measure that is closest to the specified magnetic field measurement and time period of study defined for the pooled analysis. The relative risk estimates in the two intermediate exposure categories are near the no effect value, while in the top category (≥ 0.4 μT) the relative risk estimate is 2.00 (95% CIs: 1.27–3.13), with a P value of 0.002. The continuous analysis gives a relative risk estimate per 0.2 μT of 1.15 (1.04–1.27) with a test for trend P value of 0.004.

Table 2 Absolute numbers of childhood leukaemia cases and controls by study and exposure level

Measurement studies										
Leukaemia cases	< 0.1	0.1–0.2	0.2–0.4	≥ 0.4	Total	ALL cases < 0.1	0.1–0.2	0.2–0.4	≥ 0.4	Total
Canada	174	56	29	13	272	151	50	26	12	239
Germany	156	12	5	2	175	130	10	5	2	147
New Zealand	76	6	4	0	86	64	5	3	0	72
UK	1018	38	13	4	1073	859	34	10	3	906
USA	418	111	49	17	595	418	111	49	17	595
Total	1842	223	100	36	2201	1622	210	93	34	1959
Controls	< 0.1	0.1–0.2	0.2–0.4	≥ 0.4	Total					
Canada	215	53	26	10	304					
Germany	380	21	6	2	409					
New Zealand	72	8	0	0	80					
UK	2099	91	26	8	2224					
USA	386	95	44	5	530					
Total	3152	268	102	25	3547					
Calculated fields studies										
Leukaemia cases	< 0.1	0.1–0.2	0.2–0.4	≥ 0.4	Total	ALL cases < 0.1	0.1–0.2	0.2–0.4	≥ 0.4	Total
Denmark	830	1	0	2	833	596	0	0	2	598
Finland	27	0	1	1	29	25	0	1	1	27
Norway	140	6	2	0	148	92	5	2	0	99
Sweden	27	3	1	5	36	17	1	0	3	21
Total	1024	10	4	8	1046	730	6	3	6	745
Controls	< 0.1	0.1–0.2	0.2–0.4	≥ 0.4	Total					
Denmark	4736	2	8	0	4746					
Finland	991	19	10	7	1027					
Norway	542	13	7	10	572					
Sweden	438	30	20	20	508					
Total	6707	64	45	37	6853					

Table 3 Total leukaemia. Relative risks (95% CI) by exposure level and with exposure as continuous variable (RR per 0.2 μT) with adjustment for age, sex, and SES (measurement studies) and East/West in Germany. Reference level: < 0.1 μT. Observed (O) and expected (E) case numbers ≥ 0.4 μT, with expected nos. given by modelling probability of membership of each exposure category based on distribution of controls including covariates.

Type of study	0.1–< 0.2 μT	0.2–<0.4 μT	≥ 0.4 μT	O	E	Continuous analysis
Measurement studies						
Canada	1.29 (0.84–1.99)	1.39 (0.78–2.48)	1.55 (0.65–3.68)	13	10.3	1.21 (0.96–1.52)
Germany	1.24 (0.58–2.64)	1.67 (0.48–5.83)	2.00 (0.26–15.17)	2	0.9	1.31 (0.76–2.26)
New Zealand	0.67 (0.20–2.20)	4 cases/0 ctrls	0 cases/0 ctrls	0	0	1.36 (0.40–4.61)
UK	0.84 (0.57–1.24)	0.98 (0.50–1.93)	1.00 (0.30–3.37)	4	4.4	0.93 (0.69–1.25)
USA	1.11 (0.81–1.53)	1.01 (0.65–1.57)	3.44 (1.24–9.54)	17	4.7	1.30 (1.01–1.67)
Calculated fields studies						
Denmark	2.68 (0.24–30.45)	0 cases/8 ctrls	2 cases/0 ctrls	2	0	1.50 (0.85–2.65)
Finland	0 cases/19 ctrls	4.11 (0.48–35.1)	6.21 (0.68–56.9)	1	0.2	1.15 (0.79–1.66)
Norway	1.75 (0.65–4.72)	1.06 (0.21–5.22)	0 cases/10 ctrls	0	2.7	0.78 (0.50–1.23)
Sweden	1.75 (0.48–6.37)	0.57 (0.07–4.65)	3.74 (1.23–11.37)	5	1.5	1.31 (0.98–1.73)
Summary						
Measurement studies	1.05 (0.86–1.28)	1.15 (0.85–1.54)	1.87 (1.10–3.18)	36	20.1	1.17 (1.02–1.34)
Calculated fields studies	1.58 (0.77–3.25)	0.79 (0.27–2.28)	2.13 (0.93–4.88)	8	4.4	1.11 (0.94–1.30)
All studies	1.08 (0.89–1.31)	1.11 (0.84–1.47)	2.00 (1.27–3.13)	44	24.2	1.15 (1.04–1.27)

In the measurement studies, because several of the relative risk estimates were higher when geometric rather than arithmetic means were employed the data were reanalysed using arithmetic means. Although the summary relative risk for all measurement studies was still elevated 1.59 (1.04–2.45), it was lower than that obtained when the analysis was based on geometric means.

While the primary categorical analyses were based on the predetermined cut off points, we evaluated the robustness of the results by also using other cut off points. With 0.3–<0.4, 0.4–<0.5 and ≥ 0.5 μT as the three highest categories we found, across all studies and for total leukaemia, relative risks of 1.60, 2.54 and 1.75, respectively.

The largest studies and therefore the studies that carry most of the weight in the summations are those from the US, Canada, and the UK. If the US study were to be excluded, the summary estimate for the highest exposure category would be reduced from 2.00 to 1.68 (1.00–2.83; $P = 0.03$). The exclusion of Canada would increase the summary estimates to 2.14 (1.27–3.61), while exclusion of the UK study would increase it to 2.29 (1.41–3.74). Table 3 also gives the expected number of cases in the highest category under the null

Table 4 Acute lymphocytic leukaemia. Relative risks (95% CI) by exposure level with adjustment for age, sex, and SES (measurement studies) and East/West in Germany. Reference level: < 0.1 μT.

Measurement studies	0.1–<0.2 μT	0.2–<0.4 μT	≥0.4 μT
Canada	1.33 (0.85–2.07)	1.44 (0.79–2.60)	1.65 (0.68–4.01)
Germany	1.29 (0.58–2.89)	2.19 (0.62–7.71)	2.21 (0.29–16.7)
New Zealand	0.71 (0.21–2.44)	3 cases/0 ctrls	0 cases/0 ctrls
UK	0.89 (0.59–1.34)	0.87 (0.42–1.84)	0.88 (0.23–3.39)
USA	1.11 (0.81–1.53)	1.01 (0.65–1.57)	3.44 (1.24–9.54)
Calculated fields studies			
Denmark	0 cases/2 ctrls	0 cases/8 ctrls	2 cases/0 ctrls
Finland	0 cases/19 ctrls	4.31 (0.50–37.2)	6.79 (0.74–62.6)
Norway	2.25 (0.78–6.55)	1.49 (0.30–7.45)	0 cases/10 ctrls
Sweden	0.88 (0.11–7.19)	0 cases/20 ctrls	3.46 (0.84–14.3)
Summary			
Measurement studies	1.07 (0.87–1.31)	1.15 (0.84–1.56)	1.95 (1.14–3.35)
Calculated fields studies	1.42 (0.58–3.45)	0.84 (0.25–2.81)	2.23 (0.88–5.65)
All studies	1.08 (0.88–1.32)	1.12 (0.84–1.51)	2.08 (1.30–3.33)

Table 5 Summary relative risks. (95% CI) for total leukaemia by exposure level based on best available measure with adjustment for potential confounders. Germany also includes East/West adjustment.

	0.1–<0.2 μT	0.2–<0.4 μT	≥ 0.4 μT
All studies but Finland			
Age, sex	1.07 (0.88–1.29)	1.11 (0.84–1.47)	1.91 (1.21–2.99)
Age, sex, SES	1.08 (0.89–1.31)	1.10 (0.82–1.46)	1.92 (1.22–3.02)
All studies but UK			
Age, sex, SES	1.18 (0.94–1.48)	1.15 (0.84–1.58)	2.28 (1.40–3.71)
Age, sex, SES, Urban	1.13 (0.90–1.42)	1.09 (0.79–1.50)	2.24 (1.37–3.67)
All studies but UK, Denmark, Finland, and NZ			
Age, sex, SES	1.20 (0.96–1.52)	1.15 (0.83–1.58)	1.97 (1.19–3.25)
Age, sex, SES, type of dwelling	1.21 (0.96–1.52)	1.15 (0.83–1.59)	1.97 (1.19–3.26)
All studies but UK and Finland			
Age, sex, SES	1.19 (0.95–1.49)	1.13 (0.83–1.55)	2.20 (1.34–3.61)
Age, sex, SES, mobility	1.18 (0.94–1.48)	1.14 (0.83–1.56)	2.20 (1.34–3.61)
Sweden and Germany			
Age, sex, SES	1.37 (0.71–2.64)	1.28 (0.47–3.51)	3.30 (1.24–8.81)
Age, sex, SES, car exhaust	1.36 (0.70–2.63)	1.27 (0.46–3.49)	3.24 (1.22–8.63)

Reference level: < 0.1 μT.

hypothesis. The total number of excess cases across all studies is 20, the largest number being contributed by the US study.

We then restricted these analyses to ALL. Since the ALL cases make up as much as 83% of all cases and since the controls are the same, the ALL results must be similar to the total leukaemia results. The results in Table 4 show that this is indeed the case, but in the highest exposure category the ALL relative risks are somewhat higher than for total leukaemia.

We also looked separately at other leukaemia to see whether the observed excess risk was restricted to the ALL group. The summary relative risk for other leukaemia was 1.42 in the highest exposure category, but based on only 4 exposed cases.

Next we addressed the issue of a possible effect of adjustment for more covariates. The results of this analysis are given in Table 5. In addition to the centres using different definitions of potential confounders we also faced the problem that all centres did not have data on all potential confounders. When we adjusted for a particular confounder we therefore included only those studies that have data on that confounder. Because of the centre specific differences in relative risks we could not compare the adjusted results calculated from only a subset of the studies to the basic model results calculated from all the studies. Therefore, in Table 5 we present results with and without adjustment for a potential confounder for the group of studies that the estimates are based upon. As can be seen in Table 5, for none of the potential confounders does the adjustment result in anything but minor changes in any of the relative risk estimates.

The final issue is the so-called wire-code paradox. Table 6 has the results according to wire-code categories including a summary estimate for the two North American studies. In the table we also give magnetic field levels for each wire code category. The relative risk for the highest wire-code category is 1.24 (0.82–1.87) so these analyses do not provide evidence for the existence of such a paradox.

DISCUSSION

We did not find any evidence of an increased risk of childhood leukaemia at residential magnetic field levels < 0.4 μT. We did, however, find a statistically significant relative risk estimate of two for childhood leukaemia in children with residential exposure to EMF ≥ 0.4 μT during the year prior to diagnosis. Less than 1%

Table 6 Total leukaemia. Relative risks (95% CI) by wire-code with adjustment for age, sex, SES (local definitions) and mobility, number of subjects, and EMF levels based on subset of subjects with measurement on home used in wire code analysis.

North American studies	UG/VLCC[1]	OLCC[2]	OHCC[3]	VHCC[4]
Canada	1	0.98 (0.66–1.46)	0.75 (0.52–1.10)	1.59 (0.90–2.82)
Case/control	151/154	77/77	83/105	39/23
USA	1	1.03 (0.73–1.44)	1.04 (0.71–1.51)	0.87 (0.47–1.61)
Case/control	177/173	119/115	88/87	24/26
All North American studies	1	1.01 (0.78–1.30)	0.89 (0.68–1.16)	1.24 (0.82–1.87)
EMF level, median in controls	0.04	0.05	0.08	0.11

[1]Under ground/very low current configuration; [2]Ordinary low current configuration; [3]Ordinary high current configuration; [4]Very high current configuration.

of subjects were in this highest exposure category. The results did not change following adjustment for the potential confounders. In addition, the existence of the so-called wire-code paradox could not be confirmed.

Earlier analyses of the hypothesis of an association between EMF and cancer have sometimes been criticized on the grounds that the findings might be a consequence of so-called data dredging. In order to avoid this and because this work has been a collaborative effort of a rather large group of investigators we specified which primary analyses we planned to do and how to do them before we commenced the analysis; this was before the results of several of the individual studies were known.

The fact that we had access to the raw data from each study gave us two substantial advantages. First, it allowed us to make the data from the various centres as compatible as possible, which was particularly important for the exposure variables. For example, it made it possible to use the same cut-off points in all studies, to use geometric means of the measurements, and to focus on exposure during the year preceding diagnosis. Second, we could arrange data in ways that were of little interest in themselves for some of the individual centres because of small numbers, but still of considerable interest for the total material. In particular this made it possible to analyse, in a consistent way, higher cut-off points than the commonly used 0.2 μT.

For the measurement studies, the findings may have reflected effects of selection bias due to non-participation. Differences were observed in several measures of socioeconomic status between cases and controls, particularly in the US study, with controls generally characterized by higher socioeconomic status than cases. In a recent analysis, Hatch et al found that exclusion of partial or non-cooperative participants from analyses of either in-home magnetic field measurements or wire-codes tended to increase the risk estimates for childhood leukaemia in the US study (Hatch et al, 2000). This was confirmed in the UK study in which there was a moderate association between a deprivation index and measured magnetic fields (UKCCS, 1999). This suggests that at least some of the elevation of risk estimates arose from differential participation of cases and controls.

Exposure measurements from both calculated and measured field studies are subject to error. Time-weighted average in a single 24- or 48-hour period immediately prior to diagnosis may not represent typical levels or the proper metric at the time period that is relevant for assessing risk of leukaemia, if any, and may not reflect the exposure of a child living in the home. Calculated fields are also averages over time and do not take individual characteristics of homes into consideration. Since elevated risk appears to be confined to only the small fraction of children who are highly exposed and since we have no basis for determining the pattern of measurement errors in each study, we cannot reliably infer the underlying risk function that would be consistent with the observed risk pattern.

One feature of our results is the high degree of consistency between the group of studies with measured fields and the group of studies with calculated fields. This may be of significance when considering potential confounders because in the calculated fields studies, the dominant source of exposure is high voltage power lines, while in the measured fields studies internal sources (such as ground currents, household wiring, and exposures from electrical appliances) may predominate. In effect one would not expect the same confounders to be operating in these two types of studies. This may also be of significance when considering selection bias problems, because the calculated fields studies are using population registries in a way that makes selection bias a small issue. In this comparison between the measurement studies and the calculated fields studies, one must keep in mind, however that the calculated fields studies are small and based only on a total of 8 cases with exposure in the highest exposure category.

One of our goals was to see whether controlling for as many putative confounders as possible would change the results, but none of the covariates that we had access to changed the results in any substantial way when included in the models. On the other hand, none of these is an established risk factor for childhood leukaemia. Indeed, knowledge about risk factors for childhood leukaemia is very limited so one cannot exclude the possibility that adjustment for some other variable would have an effect. For the moment we can only conclude that mobility, traffic exhaust, type of dwelling, and urban/rural residency are not important confounders when studying EMF and childhood leukaemia.

An interesting finding in our analysis relates to the so-called wire-code paradox. In an earlier review, an expert committee noted on the basis of the earlier studies that there is a stronger association between markers for EMF exposure and leukaemia risk than between direct measurements and leukaemia risk (National Research Council, 1996). Our data based on subsequent studies do not support this. In fact, the two North American studies show no evidence of increased risk associated with residing in homes in high wire-code categories. It is also worth noting that the measured magnetic fields are low in all the wire-code categories. The reasons for the elevated risk estimates for high wire-code categories in the earlier North American studies are unclear, although considerable potential for bias has been noted for both studies carried out in Denver (Portier and Wolfe, 1998).

The results of numerous animal experiments and laboratory studies examining biological effects of magnetic fields have

produced no evidence to support an aetiologic role of magnetic fields in leukaemogenesis (Portier and Wolfe, 1998). Four lifetime exposure experiments have produced no evidence that magnetic fields, even at exposure levels as high as 2000 μT, are involved in the development of lymphopoietic malignancies. Several rodent experiments designed to detect promotional effects of magnetic fields on the incidence of leukaemia or lymphoma have also been uniformly negative. There are no reproducible laboratory findings demonstrating biological effects of magnetic fields below 100 μT.

Our results have clear implications for future studies. The level of significance that we see for the excess risk at high exposure makes chance an unlikely explanation. Future studies will be of use only if the operation of selection bias and confounding can be adequately addressed, and if there are sufficient numbers with exposure over 0.4 μT.

In summary, for exposure up to 0.4 μT our data demonstrate relative risks near the no-effect level. For the very small proportion (0.8%) of subjects with exposure above 0.4 μT, the data show a two-fold increase, which is unlikely to be due to random variability. The explanation for the elevated risk estimate is unknown, but selection bias may have accounted for some of the increase.

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EXHIBIT D

A Pooled Analysis of Magnetic Fields, Wire Codes, and Childhood Leukemia

Sander Greenland,[1] Asher R. Sheppard,[2] William T. Kaune,[3] Charles Poole,[4] and Michael A. Kelsh,[5] for the Childhood Leukemia-EMF Study Group

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 and 0.2–0.3 microtesla (μT) categories with the 0–0.1 μT category, but the Mantel-Haenszel summary odds ratio comparing $\geq$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. (Epidemiology 2000;11:624–634)

Keywords: childhood neoplasms, electromagnetic fields, environmental exposure, leukemia, magnetic fields, wire codes.

The question of health effects of extremely low-frequency electromagnetic fields (EMFs) remains an unsettled topic.[1] The National Institute of Environmental Health Sciences funded our research team to conduct a pooled analysis of those studies of EMF and childhood leukemia for which original data could be obtained. We felt that a direct analysis of individual study data would allow a more reliable evaluation of interstudy differences in results (heterogeneity). It also could allow more reliable evaluation of dose-response relations and effects on public health than could a combination of summaries from studies, especially in light of the very different analyses presented in the published reports. The present paper reports our analyses.

From the [1]Department of Epidemiology, UCLA School of Public Health, Los Angeles; [2]Asher Sheppard Consulting, Redlands, and Department of Physiology, Loma Linda University, CA; [3]EM Factors, Richland, WA; [4]Department of Epidemiology, University of North Carolina School of Public Health, Chapel Hill, NC; and [5]Exponent Health Group, Menlo Park, CA.

Address correspondence to: Asher Sheppard Consulting, 108 Orange Street, Suite 8, Redlands, CA 92373-4719.

The Childhood Leukemia-EMF Study Group consists of the above authors; data contributors: A. Ahlbom and M. Feychting (Karolinska Institute), R. Coghill (Coghill Research Laboratories), Electric Power Research Institute (EPRI) (magnetic field survey data), J. Dockerty (University of Otago), A. Fajardo-Gutiérrez (Centro Médico Nacional Siglo XXI), J. Fulton (Rhode Island Department of Health), M. Koskenvuo (University of Turku), M. Linet (National Cancer Institute), S. London (National Institute of Environmental Health Sciences), M. McBride (British Columbia Cancer Agency), J. Michaelis (Johannes Gutenberg-University of Mainz), J. Olsen (Danish Cancer Society), J. Peters (University of Southern California), E. Pukkala (Finnish Cancer Registry), D. Savitz (University of North Carolina), J. Schüz (Johannes Gutenberg-University of Mainz), L. Tomenius, T. Tynes (Norwegian Radiation Protection Authority), P. Verkasalo (University of Helsinki), and N. Wertheimer (University of Colorado); and the Pooled Database Assembly Team, which included R. Mrad, B. Smith, and K. Zhao (Exponent Health Group), and M. Atherton (EcoAnalysis, Inc.).

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Subjects and Methods

Studies

From literature searches, we identified 24 studies[2–25] that presented data on household EMF or power-supply wiring information and childhood leukemia. To be eligible for inclusion in our pooled analysis, the study had to have obtained quantitative magnetic field measures for individual subjects or enough information to approximate Wertheimer-Leeper wire codes.[1] Nineteen studies[2–16,22–25] had eligible data. Five articles reporting four studies[22–26] appeared after our initial search in 1998; investigators in two of those studies[22,23] supplied data in time for inclusion here. One study group[16] refused our data request. Two studies[8,15] were conducted using identical methods within the same

TABLE 1. Description of Studies in Pooled Analyses [All Are Case-Control Studies (Verkasalo Nested in Cohort)]

First Author	Location	Measurements*	Matching Factors†
Coghill[2]	England	Direct	Age, sex
Dockerty[23]	New Zealand	Direct	Birth quarter, sex
Fajardo-Gutiérrez[3]	Mexico	WC	Age, sex
Feychting[4]	Sweden	Calc; some direct	Birth year, sex, diagnosis year, parish, transmission-line corridor
Fulton[5]	Rhode Island	WC	Birth year
Green[24]	Ontario	WC‡	Birth year, sex
Linet[6]	Eastern U.S.	Direct; some WC	Age, race, RDD
London[7]	Los Angeles	Direct; WC	Age, sex, race; some friend, RDD
McBride[22]	Canada	Direct; WC	Age, sex, area
Michaelis[8]	Germany	Direct	Birth date, sex; some by locale
Olsen[9]	Denmark	Calc	Birth year, sex, diagnosis date
Savitz[10]	Denver	WC; some direct	Age, sex, RDD
Tomenius[11]	Sweden	Direct	Age, sex, birth district
Tynes[12]	Norway	Calc	Birth year, sex, municipality
Verkasalo[13]	Finland	Calc	Age, sex
Wertheimer[14]	Denver	WC	Birth date; some by county

* Calc = magnetic field exposure calculated from configuration and electric load data; direct = direct magnetic field measurements; WC = wire code.
† RDD = random-digit dialing.
‡ Only wire code data from published report used here. Green *et al*[24,25] also obtained magnetic field data.

country and treated as one study.[8] Fulton *et al*[5] and Tomenius[11] published analyses that used residence as the analysis unit, but we used individual-level exposures from their data. We thus had anonymous records on individual subjects from 15 distinct studies.

Table 1 summarizes the studies included here. All are case-control studies. Verkasalo *et al*[13] initially conducted and reported a person-time cohort study. They supplied data from an unpublished case-control study nested within their cohort, based on all cases observed in the cohort plus ten controls for each case, and which obtained additional covariate data; the controls were age-sex matched but otherwise randomly sampled from the cohort. The two Swedish studies[4,11] had a small overlap in source populations and so share a few cases, but this overlap could not be identified from the available data. Most studies had geographic restrictions on their source populations beyond those shown in Table 1; some had restrictions to areas near or crossed by high-voltage lines.[4,12,13]

Primary Measures

Twelve studies supplied magnetic field exposure estimates for some or all individuals. For four Nordic studies,[4,9,12,13] we used estimates calculated by the original investigators from measured proximity to power lines and historical current-supply data. For eight studies,[2,6–8,10,11,22,23] we used estimates based on direct measurements (measured magnetic fields at the front door of the residence,[11] measured fields in the child's bedroom,[2,7,8,23] averaged fields in several rooms,[6,10] and averaged personal and house measures[22]).

Some studies[4,6–8,10,22] supplied more than one type of magnetic field measurement. For example, there were normal- or low-power measurements, spot and 24-hour measurements, mean and median values, data from the residence at diagnosis, and data from other residences. There is as yet no measure of magnetic field exposure that is known to be biologically the most relevant. In the absence of such knowledge, it would be best to examine a number of different measures. This was indeed done in several studies, but it raises multiplicity problems that are difficult to deal with statistically in even a single study. For a pooled analysis of the studies here, there would be more than 100 combinations of measures (although we did not have all measures for all of the studies).

To avoid multiplicity issues and to keep our task manageable, we defined our target measure to be a child's time-weighted average exposure up to 3 months before diagnosis. When we had several measures from a study, we used a measure that, based on earlier work,[27–29] seemed likely to provide the best approximation to this target. In particular, we preferred calculated historical fields or averages of multiple measurements rather than spot measurements when there was a choice. Table 2 summarizes the measurements used from each study. We also conducted analyses of each supplied measure and a

TABLE 2. Magnetic-Field Measures Used in Primary Analyses

First Author	Summary Measure Description*
Coghill[2]	Nighttime (8:00 pm to 8:00 am) recordings in child's bedroom
Dockerty[23]	Arithmetic mean of 24-hour recordings in child's bedroom
Feychting[4]	Average of calculations based on distances, phases, and loads of above-ground lines
Linet[6]	Time-weighted household mean based on typical child activity patterns and 24-hour child bedroom measurements and spot measurements in kitchen and family room; front door measurement when these data were not available; includes multiple homes covering 70% or more of the reference period (up to 5 years before diagnosis date)
London[7]	Arithmetic mean of 24-hour recordings in child's bedroom
McBride[22]	Time-weighted mean based on 48-hour personal monitoring plus predictions from perimeter measurements
Michaelis[8]	Arithmetic mean of 24-hour recordings in child's bedroom
Olsen[9]	Average of calculations based on distances; phases; and loads of 50–400-kV transmission lines, cables, and substations within areas calculated as potentially having ≥0.1 μT exposure
Savitz[10]	Arithmetic mean of low-power spot measurement in three or more locations (child's bedroom, parent's bedroom, other room occupied by child ≥1 hour/day, front door)
Tomenius[11]	Maximum uniaxial value outside front door of single-family homes and apartments
Tynes[12]	Average of calculations based on distances, phases, and loads of above-ground lines ≥11 kV
Verkasalo[13]	Average of calculations based on distances, typical line configuration, and loads of overhead 110–400-kV lines

* For details see original reports.

limited sensitivity analysis of summaries based on revisions of initial choices.

All North American studies[3,5–7,10,14,22,24] obtained wire code data. Wire codes from two studies[5,14] were recalculated from original data on distances to type of distribution line. Wire codes from one study[3] were in a unique three-level form.

Other Information

Studies varied considerably in the covariates available for control and in their completeness of exposure and covariate information. One study[11] supplied no covariate data and so was excluded from covariate-adjusted analyses. Several studies[4–9,12,22,23] supplied at least one socioeconomic variable on some or all subjects. One important ecologic covariate available for all studies was location; studies in North America involved 60-Hz fields with 110–125-V home supply, whereas all other studies involved 50-Hz fields with 220–240-V power. Thus, all comparisons of 60-Hz *vs* 50-Hz fields are also comparisons of 110–125-V *vs* 220–240-V systems and of North America *vs* other locations.

There are several discrepancies between the data we report and those in some published reports.[6,7,10,14,22,23] Some differences arose because we did not impose exclusion criteria used by certain authors. For example, we included ten Down-syndrome subjects excluded by Linet *et al*[6] because we could not identify such subjects in other studies and we could not identify any bias that would justify such an exclusion. Other differences arose from postpublication corrections or additions to the study data by the original investigators and from our use of exposure measures and cutpoints different from those used in the original publications; these differences led to especially large upward changes for the Tomenius[11] and McBride *et al*[22] estimates. A few small discrepancies were unresolved; no such discrepancy appeared capable of producing more than negligible differences in summary results.

Coghill *et al*[2] and Linet *et al*[6] restricted their cases to acute lymphoblastic leukemia (ALL). Because about 80% of childhood leukemias are ALL, and because not all datasets distinguished leukemia subtypes, we conducted no analysis restricted to ALL.

Statistical Methods

Data were analyzed using inverse-variance weighted (Woolf), Mantel-Haenszel, and maximum-likelihood (ML) tabular methods, and using ML logistic regression.[30,31] (Inverse variance methods were included because they are common in meta-analysis.) All *P*-values were derived from score statistics or deviance (log likelihood-ratio) statistics.[30] For magnetic field exposures, dose response was examined using category indicators and splines in logistic models.[31–33] All results were adjusted for study: tabular analyses were always stratified on study, and all regressions included indicators for study.

All magnetic field measurements were converted into units of microtesla (μT). Only two studies[6,7] had more than four cases above 0.4 μT; therefore, for categorical magnetic field analyses, values above 0.3 μT were combined in a single category to ensure cell counts large enough for all statistical procedures. To avoid the trend distortions and power loss associated with percentile-category boundaries,[33,34] we used equally spaced boundaries below the 0.3-μT cutpoint. We combined low-exposure wire codes (UG = underground, VLCC = very low current code, and OLCC = ordinary low current code) into a single "LCC" low-current reference category for comparison with the two high-exposure wire codes (OHCC = ordinary high current, and VHCC = very high current). Previous results indicate that the three low-current categories do not correspond to meaningful differences in EMF exposure or childhood leukemia risk.[3,5–7,10,22,24,35] Furthermore, in three studies,[5,6,14] the proportions of subjects with a UG or VLCC code were too small to yield efficient estimates using those codes as reference category; in another,[6] UG and VLCC were combined in the supplied data; and in another,[3] low-current codes had been combined in data collection.

Complications arose in accounting for the variety of matching protocols used. Most studies matched on certain covariates (typically sex, age or birth date, and some sort of geographic unit). Many studies experienced some failures to match, leading to fewer subjects available for matched analyses than unmatched analyses. Several considerations led us to focus on unmatched analyses with analytic control for matched covariates. First, this choice provided the most subjects for analysis. Second, this choice avoided further efficiency loss due to the type of analysis overmatching documented by Brookmeyer *et al*.[36] Third, this choice also helped avoid small-sample bias away from the null due to sparse matched-set counts in study-specific analyses[37]; although we would expect the unmatched analyses to suffer some small bias toward the null, we thought this possibility preferable to a potentially large bias away from the null due to sparse data. Fourth, results from matched analyses were less stable but exhibited the same patterns seen in the unmatched analyses.

Results for Magnetic Fields

Categorical Analyses

Table 3 displays the distribution of magnetic field measurements among the studies supplying such measurements. There are extensive differences among the studies, ranging from Olsen *et al*[9] (which has only 0.5% of cases and controls above 0.1 μT) to Linet *et al*[6] (which has more than one-third of measured subjects above 0.1 μT). Values above 0.3 μT are relatively infrequent in all studies. The differences appear associated chiefly with location rather than with measurement method (direct *vs* calculated). Distributions in North American studies tend to be much higher than those in European studies, probably reflecting differences in power systems (for example, more overhead wires and lower household

TABLE 3. Study-Specific Distributions of Magnetic-Field Data

First Author	Magnetic-Field Category (μT)							
	≤0.1	>0.1–≤0.2	>0.2–≤0.3	>0.3–≤0.4	>0.4–≤0.5	>0.5	Total	No Measure*
Cases								
Coghill[2]	48	5	2	0	1	0	56	0
Dockerty[23]	72	9	3	1	1	1	87	34
Feychting[4]	30	1	1	2	0	4	38	0
Linet[6]	403	152	41	20	13	9	638	46
London[7]	110	30	5	9	4	4	162	68
McBride[22]	174	77	32	11	1	2	297	102
Michaelis[8]	150	17	3	3	3	0	176	0
Olsen[9]	829	1	0	0	0	3	833	0
Savitz[10]	24	7	2	3	0	0	36	62
Tomenius[11]	129	16	5	0	0	3	153	0
Tynes[12]	146	2	0	0	0	0	148	0
Verkasalo[13]	30	1	0	0	1	0	32	3
Controls								
Coghill[2]	47	9	0	0	0	0	56	0
Dockerty[23]	68	13	1	0	0	0	82	39
Feychting[4]	488	26	18	10	2	10	554	0
Linet[6]	407	144	41	17	5	6	620	69
London[7]	99	28	6	2	2	6	143	89
McBride[22]	194	96	28	5	3	3	329	70
Michaelis[8]	372	29	7	4	0	2	414	0
Olsen[9]	1,658	3	2	2	0	1	1,666	0
Savitz[10]	155	28	10	3	2	0	198	67
Tomenius[11]	546	119	24	4	2	3	698	21
Tynes[12]	1,941	25	7	5	4	22	2,004	0
Verkasalo[13]	300	9	6	4	0	1	320	30

* No measure for a residence at or before time of diagnosis (cases) or corresponding index date (for controls).

voltage in North America), per capita electricity consumption,[38] and grounding practices. The higher distribution in Feychting and Ahlbom[4] compared with the other Nordic studies reflects the fact that the source population was restricted to children dwelling within 300 meters of high-voltage lines[4] (although Verkasalo *et al*[13] imposed a 500-meter limit and Tomenius[11] restricted subjects to census wards with transmission lines).

Table 4 displays odds ratio estimates computed directly from the raw counts underlying Table 3 and summary estimates assuming common odds ratios for each analysis category. The study-specific and summary esti-

TABLE 4. Study-Specific Odds Ratio Estimates and Study-Adjusted Summary Estimates, Magnetic Field Data (Reference Category, ≤0.1 μT)

First Author	Magnetic Field Category (μT)					
	>0.1–≤0.2		>0.2–≤0.3		>0.3	
	Estimate	95% CL	Estimate	95% CL	Estimate	95% CL
Coghill[2]	0.54	0.17, 1.74	No controls		No controls	
Dockerty[23]	0.65	0.26, 1.63	2.83	0.29, 27.9	No controls	
Feychting[4]	0.63	0.08, 4.77	0.90	0.12, 7.00	4.44	1.67, 11.7
Linet[6]	1.07	0.82, 1.39	1.01	0.64, 1.59	1.51	0.92, 2.49
London[7]	0.96	0.54, 1.73	0.75	0.22, 2.53	1.53	0.67, 3.50
McBride[22]	0.89	0.62, 1.29	1.27	0.74, 2.20	1.42	0.63, 3.21
Michaelis[8]	1.45	0.78, 2.72	1.06	0.27, 4.16	2.48	0.79, 7.81
Olsen[9]	0.67	0.07, 6.42	No cases		2.00	0.40, 9.93
Savitz[10]	1.61	0.64, 4.11	1.29	0.27, 6.26	3.87	0.87, 17.3
Tomenius[11]	0.57	0.33, 0.99	0.88	0.33, 2.36	1.41	0.38, 5.29
Tynes[12]	1.06	0.25, 4.53	No cases		No cases	
Verkasalo[13]	1.11	0.14, 9.07	No cases		2.00	0.23, 17.7
Study-adjusted summaries*						
Woolf	0.96	0.81, 1.14	1.08	0.80, 1.45	1.83	1.34, 2.49
MH	0.95	0.80, 1.12	1.06	0.79, 1.42	1.69	1.25, 2.29
Study + age + sex adjusted†						
MH	1.01	0.84, 1.21	1.06	0.78, 1.44	1.68	1.23, 2.31
Spline‡	1.00	0.81, 1.22	1.13	0.92, 1.39	1.65	1.15, 2.36

95% CL = 95% confidence limits.

* MH = Mantel-Haenszel; maximum-likelihood summaries differed by less than 1% from these summaries; based on 2,656 cases and 7,084 controls. Summary tests: 3-degree-of-freedom (df) MH categorical $P = 0.01$; 1 df Mantel trend $P = 0.06$ (from continuous data).

† Excludes Tomenius *et al*[11] (no covariate data); based on 2,484 cases and 6,335 controls with age and sex data; 3-df MH categorical $P = 0.01$; 1 df Mantel trend $P = 0.04$ (from continuous data).

‡ Estimates comparing odds at category means (0.14, 0.25, and 0.58 *vs* 0.02 μT) from a quadratic logistic spline with one knot at 0.2 μT, plus age and sex terms.

mates tend to show little or no association of fields below 0.3 μT with leukemia, but all studies with cases and controls in the >0.3 μT category exhibit positive associations for that category. The differences across studies were within chance variation (deviance $P = 0.42$ using exposure categories in Table 4), as were differences between studies with different measures [ML odds ratios for >0.3 μT = 1.70 from studies with calculated fields and 1.68 from studies with direct measurement; 95% confidence limits (95% CL) for ratio of odds ratios = 0.46, 2.22] or different field frequencies (ML odds ratios for >0.3 μT = 1.97 from studies with 50-Hz fields and 1.58 from studies with 60-Hz fields; 95% CL for ratio of odds ratios = 0.66, 2.36).

The Tomenius data[11] included no covariate and so were excluded from covariate-adjusted analyses. The penultimate line of Table 4 shows the age-sex-study-adjusted Mantel-Haenszel estimates. The exclusions and adjustments had negligible effect, and odds ratio differences across age and sex categories (not shown) were within chance variation. Table 5 summarizes categorical analyses upon restriction to subjects with no missing data. Neither restriction nor adjustment for available covariates changed the qualitative result that there was little or no association evident below 0.2 μT, but some positive association was evident above 0.3 μT.

Trend Analysis

The final line of Table 4 displays estimated odds ratios from a logistic model fit to individual-level magnetic field data using a quadratic spline for field along with age, squared age, and sex terms. The spline has a single knot at 0.2 μT (the middle category boundary) and so has one linear and two quadratic magnetic field terms; the model thus uses 3 degrees of freedom for field, the same number of degrees of freedom as in the four-category analysis. The spline estimate under each category is the leukemia odds ratio comparing the mean field measure in that category with the mean field measure in the ≤0.1 μT category and is thus a continuous-data analogue of the categorical summary estimate. Unlike the categorical analysis, the spline analysis imposes a smooth dose-response relation between field level and leukemia. Nonetheless, the spline results are similar to the categorical results: there appears to be little or no association below 0.2 μT but some association comparing high with low exposures; furthermore, differences among covariate-specific curves (not shown) were within chance variation.

Figure 1 displays a graph of the "floated" case-control ratios[39] fit by the spline model, along with pointwise confidence limits. This figure is a plot of the fitted odds of being a case *vs* being a control in our studies. Assuming these odds are proportional to the underlying childhood leukemia rates, this plot is an estimate of the *shape* of the curve relating leukemia rates to magnetic fields under the spline model.[39] The vertical axis corresponds to geometric mean case-control ratios rather than to odds ratios, but ratios of different points on the curve equal the model-fitted odds ratios[39]; for example, the ratio of the curve heights at 0.58 and 0.02 μT (the means of the >0.3 and ≤0.1 μT categories) is 1.65, equal to the final odds ratio in Table 4. We caution against focusing on the central curve, however, because the data are compatible with a wide range of trends, including nonmonotonic, linear, and exponentially increasing shapes. For example, the strictly increasing trend above 0.1 μT is *not* a statistically stable feature, in that curves that plateau or even decline above 0.6 μT also fit the data well.

Influence and Sensitivity Analyses

As with covariate adjustment, neither single-study deletions nor alternative choices for the exposure measure altered results qualitatively, nor did deletion of large field values (for example, the five subjects above 2.0 μT, all controls from Tynes and Haldorsen[12]). Although the highest-category estimates and the fitted curve varied considerably with category-boundary and model choices, these choices also did not alter the basic qualitative results.

Use of alternatives among the supplied exposure measures produced only small differences in the summaries; we did not have all measures from all studies, however. Missing data varied with choice of measure, and this variation sometimes had more influence on estimates than the choice of measure. Two studies[4,13] supplied calculated yearly exposure of children; we used these data to construct alternative-exposure measures that might arguably approximate more closely our target than the measures used in the original reports and in our analysis above. Use of these alternatives had little effect on the study-specific odds ratios below 0.3 μT but raised the >0.3-*vs*-≤0.1 odds ratio to 5.9 (95% CL = 2.0, 17) for Feychting and Ahlbom[4] and to 10 (95% CL = 1.4, 74) for Verkasalo *et al*.[13] Some of this increase may only be increased small-sample bias[37] due to reduction in numbers above 0.3 μT. In any event, use of these alternatives changed the summaries by only a few percent.

The calculated-field measures from the Nordic studies were based on high-voltage lines and did not include contributions from sources such as in-home wiring and appliances.[4,9,12,13] The effect of the latter omissions is not straightforward to assess, because fields are vector additive and so may even destructively interfere with one another, depending on the relative orientation and phase of the contributions from different sources. One study[4] supplied spot measurements as well as calculated fields on 24 of 38 cases and 344 of 554 controls. These dual measurements permitted instrumental-variable corrections[40] for estimates from the calculated fields in the Nordic studies. Because these corrections involve strict assumptions and require extensive technical description,[40] they were not used in Tables 3 and 5, and we omit details. The main result was that odds ratio estimates from the Nordic studies[4,9,12,13] were corrected toward the null. Nonetheless, because these studies contributed so few cases at the higher exposure levels, the corrections had only a small effect on the overall summary estimates.

TABLE 5. Study-Specific Odds Ratio Estimates and Study-Adjusted Summary Magnetic Field Estimates from Data Restricted to the 2,078 Cases and 5,516 Controls with Complete Covariate Data, without and with Covariate Adjustment* (Reference Category, ≤0.1 μT)

	Magnetic Field Category (μT)					
	>0.1–≤0.2		>0.2–≤0.3		>0.3	
First Author	Estimate	95% CL	Estimate	95% CL	Estimate	95% CL
Restricted, no covariate adjustment						
Coghill[2]	0.30	0.06, 1.52	No controls		No controls	
Dockerty[23]	0.65	0.24, 1.78	3.05	0.31, 30.1	No controls	
Feychting[4]	0.63	0.08, 4.77	0.90	0.12, 7.00	4.44	1.67, 11.7
Linet[6]	1.06	0.81, 1.40	0.99	0.63, 1.58	1.70	1.01, 2.87
London[7]	1.08	0.58, 2.01	1.07	0.28, 4.12	1.82	0.75, 4.43
McBride[22]	0.88	0.61, 1.28	1.30	0.75, 2.25	1.45	0.64, 3.27
Michaelis[8]	1.45	0.78, 2.72	1.06	0.27, 4.16	2.48	0.79, 7.81
Olsen[9]	1.03	0.09, 11.4	No cases		4.13	0.37, 45.7
Savitz[10]	1.68	0.66, 4.30	1.30	0.27, 6.29	3.89	0.87, 17.4
Tynes[12]	1.11	0.26, 4.74	No cases		No cases	
Verkasalo[13]	1.13	0.14, 9.25	No cases		2.04	0.23, 18.0
MH*	1.02	0.85, 1.23	1.10	0.81, 1.51	1.87	1.35, 2.60
Restricted and covariate adjusted						
Coghill[2]	0.28	0.06, 1.44	No controls		No controls	
Dockerty[23]	0.66	0.24, 1.81	2.83	0.29, 27.9	No controls	
Feychting[4]	0.60	0.08, 4.54	0.80	0.10, 6.22	4.57	1.72, 12.1
Linet[6]	1.07	0.81, 1.42	0.96	0.61, 1.52	1.67	0.99, 2.82
London[7]	1.02	0.55, 1.89	0.98	0.25, 3.75	1.82	0.75, 4.44
McBride[22]	0.85	0.59, 1.23	1.24	0.72, 2.14	1.40	0.62, 3.18
Michaelis[8]	1.24	0.66, 2.33	0.93	0.24, 3.64	2.02	0.64, 6.37
Olsen[9]	1.03	0.09, 11.4	No cases		3.74	0.34, 41.4
Savitz[10]	1.78	0.70, 4.57	1.27	0.26, 6.17	4.08	0.91, 18.2
Tynes[12]	1.12	0.26, 4.78	No cases		No cases	
Verkasalo[13]	1.13	0.14, 9.25	No cases		2.05	0.23, 18.1
MH*	1.01	0.82, 1.25	0.94	0.65, 1.37	2.06	1.40, 3.01

95% CL = 95% confidence limits; MH = Mantel-Haenszel.
* Excludes Tomenius *et al*[11] (no covariate data). Covariate adjustment is for age and sex, plus social and economic variables in nine studies.[4,6–9,12,13,22,23] Covariate-adjusted summary: 3-degrees-of-freedom Mantel-Haenszel categorical $P = 0.01$.

The dip in the curve in Figure 1 below 0.1 μT is mostly attributable to the Danish data,[9] in which exposures below 0.1 μT were effectively set to 0 when calculating averages, and which contributed about one-quarter of the subjects in the ≤0.1 μT category. When this study was deleted, the dip disappeared, but the curve remained mildly sigmoidal.

Noncontributing Studies

Myers *et al*[16] reported only one case and two controls for "non-solid tumors" above 0.1 μT; exclusion of this study could not have influenced our results to an important degree. Most of the data from the much larger study by Green *et al*[24] were neither presented in categories that could be combined directly with our categories nor broken into analysis categories above 0.15 μT; the estimates in this study varied considerably with the measure and adjustment used, but all had wide confidence intervals and were statistically compatible with our results. Crude data from a personal-monitoring substudy by Green *et al*[25] produced odds ratios of 1.20 (95% CL = 0.59, 2.41), 1.76 (95% CL = 0.82, 3.80), and 0.71 (95% CL = 0.18, 2.88) comparing 0.1–0.2, 0.2–0.3, and >0.3 μT with ≤0.1 μT, reflecting the small numbers in this substudy. The U.K. Childhood Cancer Study group[26] reported birthdate-sex-socioeconomic status-adjusted odds ratios for total leukemia of 0.78 (95% CL = 0.55, 1.12), 0.78 (95% CL = 0.40, 1.52), and 1.68 (95% CL = 0.40, 7.10) comparing categories of 0.1–0.2, 0.2–0.4, and >0.4 μT with ≤0.1 μT; our pooled data yielded age-sex-study-adjusted ML estimates of 1.01 (95% CL =

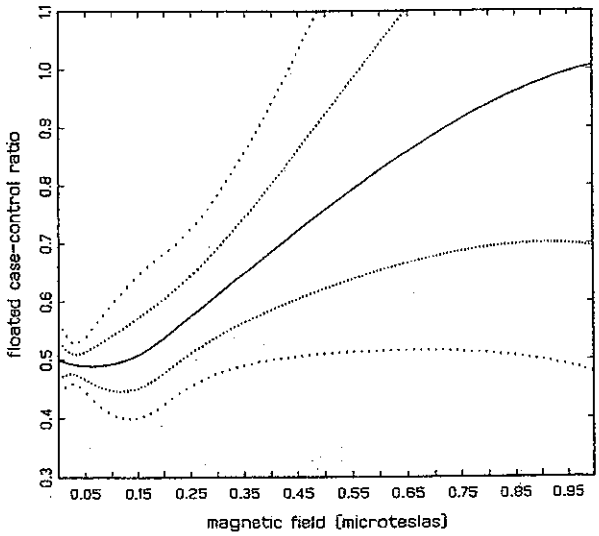

FIGURE 1. Floated case-control ratios[39] from 3-degree-of-freedom quadratic-logistic spline model fit to pooled magnetic field data, with adjustment for study, age, and sex. Inner dotted lines are pointwise 80% confidence limits; outer dotted lines are pointwise 99% confidence limits.

TABLE 6. Distribution of Residential Magnetic Field Measurements in Electric Power Research Institute Survey of U.S. Homes[41] (N = 987) (Categories Exclude Lower Boundary)

Category (μT)	No. of Homes in Category	%
≤0.05	437	44.2
0.05–0.1	277	28.1
0.1–0.2	173	17.5
0.2–0.3	55	5.6
0.3–0.4	20	2.0
0.4–0.5	8	0.8
0.5–0.6	7	0.7
0.6–0.75	6	0.6
over 0.75	4	0.4

Median = 0.06 μT, mean = 0.09 μT, and maximum = 1.01 μT.

0.84, 1.21), 1.25 (95% CL = 0.96, 1.61), and 1.60 (95% CL = 1.03, 2.48) using the same categories.

Attributable-Fraction Analysis

We estimated the excess fraction of U.S. childhood leukemia incidence that would be attributable to magnetic field exposures above 0.05 μT, under the assumption that the dose-response estimate in Figure 1 represents the causal effects of fields. To estimate the U.S. population distribution of field exposure, we used data from a utility-based cluster-sampled survey conducted by the Electric Power Research Institute (EPRI).[41] The data we obtained (Table 6) comprised spot field measurements averaged across rooms within each of 987 homes sampled from residences served by 301 EPRI utilities, which together served about 67% of U.S. homes.[41]

When these data were combined with the spline function in Figure 1 using a model-based attributable-fraction formula,[42] we obtained a population attributable-fraction estimate of 3% for the effect of magnetic fields greater than 0.05 μT (95% CL = −2%, 8%). The estimate is nearly the same if one uses any reference level up to 0.15 μT (rather than 0.05 μT), reflecting the fact that 90% of surveyed homes are in the 0–0.2 μT range, in which the fitted ratios exhibit little variation. The wide confidence interval reflects the uncertainty about the distribution of exposure, as well as the considerable uncertainty about dose response. We further caution that our estimate refers only to effects of ambient residential fields and excludes effects of unmeasured personal field sources such as electric blankets.

We did not have survey data for Europe, but given the low Northern European exposures seen in Table 3, we would expect a correspondingly lower attributable-fraction estimate for Northern Europe.

Results for Wire Codes

Table 7 displays the distribution of wire codes among the studies supplying such codes, as well as data from Table V of Green *et al.*[24] As with fields, there are extensive differences among the studies, ranging within the U.S. from 15% with OHCC or VHCC codes in Linet *et al*[6] to nearly 50% with those codes in London *et al.*[7] These differences reflect well-documented differences in power-grid architecture within the United States.[41]

Table 8 displays odds ratio estimates computed directly from the raw counts underlying Table 7, and the corresponding covariate-adjusted estimates. Summary estimates are omitted because of the extensive unexplained heterogeneity among the study-specific results; for example, the VHCC odds ratios are less than 1 in three studies and more than 2 in three others (homogeneity $P = 0.005$). We found no covariate that accounted for the large variation in results, but deletion of Wertheimer and Leeper[14] increased the homogeneity P-value to 0.11; no other single-study deletion increased the homogeneity P-value above 0.04. Eliminating Wertheimer and Leeper[14] and Fulton *et al*[5] (the two earliest studies) yielded summary ML odds ratios of 1.02 (95% CL = 0.87, 1.22) for OHCC and 1.50 (95% CL = 1.17, 1.92) for VHCC based on 1,457 cases and 1,962 controls from six studies[3,6,7,10,22,24] (deviance $P = 0.005$ for wire code; homogeneity $P = 0.15$).

As with fields, confounder adjustment had little effect on the wire code results beyond reducing the number of subjects, resulting in less stable estimates and more pronounced heterogeneity. For example, adjustment changed the Savitz *et al*[10] estimate of the VHCC odds ratio from 2.6 (95% CL = 0.92, 7.5) to 3.8 (95% CL = 1.2, 12); this change was entirely due to the deletion of

TABLE 7. Study-Specific Distributions of Wire-Code Data

First Author	Wire Code				
	VLCC*	OLCC	OHCC	VHCC	No Measure
Cases					
Fajardo-Gutiérrez[3]	13†		92	82	0
Fulton[5]	7	67	33	10	0
Green[24]§	82	41	26	6	46
Linet[6]	180	120	91	25	268‡
London[7]	34	66	71	43	16
McBride[22]	152	77	83	39	48
Savitz[10]	32	38	21	7	0
Wertheimer[14]	4	86	53	13	7
Controls					
Fajardo-Gutiérrez[3]	20†		102	65	0
Fulton[5]	8	126	65	26	0
Green[24]§	172	81	65	14	74
Linet[6]	179	117	93	27	273‡
London[7]	37	87	54	24	30
McBride[22]	157	77	105	23	37
Savitz[10]	108	103	46	8	0
Wertheimer[14]	17	107	26	6	7

VLCC = very low current code; OLCC = ordinary low current code; OHCC = ordinary high current code; VHCC = very high current code.

* VLCC includes underground (UG).

† Low-current categories not distinguished; translated as "baja" = LCC (low current code), "mediana" = OHCC, "alta" = VHCC.

‡ Subjects in Linet *et al*[6] had to meet a "residential stability" criterion to be wire coded.

§ Taken from Table V of Green *et al.*[24]

TABLE 8. Study-Specific Odds Ratio Estimates and Study-Adjusted Summary Estimates without and with Restriction and Covariate Adjustment, Wire-Code Data [Reference Category: LCC (OLCC + VLCC + UG)]

	Wire Code			
	OHCC		VHCC	
First Author	Estimate	95% CL	Estimate	95% CL
Without restriction or adjustment				
Fajardo-Gutiérrez[3]	1.39	0.65, 2.95	1.94	0.90, 4.19
Fulton[5]	0.92	0.55, 1.52	0.70	0.32, 1.52
Green[24]*	0.82	0.50, 1.36	0.88	0.33, 2.35
Linet[6]	0.97	0.69, 1.34	0.91	0.52, 1.61
London[7]	1.63	1.05, 2.53	2.22	1.26, 3.91
McBride[22]	0.81	0.57, 1.14	1.73	1.00, 2.99
Savitz[10]	1.38	0.77, 2.46	2.64	0.92, 7.54
Wertheimer[14]	2.81	1.63, 4.83	2.99	1.09, 8.15
With restriction and adjustment†				
Fajardo-Gutiérrez[3]	1.41	0.66, 2.99	2.05	0.95, 4.43
Fulton[5]	0.79	0.40, 1.53	0.54	0.21, 1.41
Linet[6]	0.99	0.70, 1.41	0.92	0.51, 1.66
London[7]	1.46	0.91, 2.35	2.25	1.21, 4.20
McBride[22]	0.79	0.56, 1.12	1.55	0.89, 2.68
Savitz[10]	1.52	0.82, 2.83	3.77	1.22, 11.7
Wertheimer[14]	2.84	1.65, 4.89	3.10	1.14, 8.47

OLCC = ordinary low current code; VLCC = very low current code; UG = underground (LCC combines these three categories); OHCC = ordinary high current code; VHCC = very high current code.
* Computed from Table V of Green *et al.*[24]
† Excludes Green *et al*[24] (which was not in our database); restricted to subjects with covariate data; covariate adjustment is for age and sex, plus social or economic variables in four studies.[5–7,22]

15 cases and 23 controls without covariate data. Adjusted results in our three-level format could not be computed from Green *et al*,[24] but their own adjustment produced little change in their estimates.[24,Table V] Fajardo-Gutiérrez supplied additional data on wiring configurations that allowed one of us (W. T. K.) to construct an alternative approximation to the Wertheimer-Leeper wire code in this study.[3] This alternative coding produced OHCC and VHCC (*vs* LCC) odds ratios of 1.5 (95% CL = 0.80, 2.9) and 1.2 (95% CL = 0.80, 1.9), which appear less consistent with other studies than the odds ratios from the original coding (Table 8).

Four studies[6,7,10,22] recorded both magnetic fields and wire codes, allowing us to examine these exposures together (Table 9). Because these analyses involve only a fraction of all subjects and because fields and codes are strongly associated (mean fields of 0.09 for LCC, 0.13 for OHCC, and 0.19 for VHCC), the results are even more unstable. Nonetheless, the associations seen with fields and codes entered into the same model were similar to the associations seen with separate models for the measures.

Discussion

For brevity and on scientific grounds, we restricted this report to analyses specified as *a priori* relevant to the main study question: Are magnetic fields or wire codes consistently associated with childhood leukemia? Our prior restrictions were meant to avoid analyses that "capitalize on chance" (small numbers and unstable estimates) either to reinforce or refute a particular hypothesis. Such restrictions are especially important in dose-response analyses of magnetic fields because of suggestions that the entire topic of EMF research is a product of unconstrained data dredging.[43]

Purely categorical dose-response analyses (that is, those conducted without regard to ordering, spacing, or smoothness constraints) can almost always be made to yield nonmonotone patterns by using categories small enough so that category-specific estimates become unstable. To avoid such problems, we supplemented our initial categorical analyses with smooth regression analyses (splines) rather than with smaller categories. We believe that dose-response modeling is important in the present context because, even upon pooling, there are still too few data to reject any plausible dose-response shape, especially above 0.2 μT. In particular, the data appear to be statistically consistent with anything from

TABLE 9. Summary Odds Ratio Estimates Based on 850 Cases and 1,004 Controls from Four Studies with Both Magnetic Field Measurements and Wire Codes[6,7,10,22] (Reference Categories: ≤0.1 μT and LCC)

	Estimates from Logistic Regression* with					
	Magnetic Field Alone		Wire Code Alone		Field and Wire Code	
	Estimate	95% CL	Estimate	95% CL	Estimate	95% CL
Field (μT)						
0.1–0.2	1.08	0.86, 1.35			1.02	0.81, 1.29
0.2–0.3	1.10	0.76, 1.60			1.01	0.69, 1.48
>0.3	1.52	0.99, 2.33			1.38	0.89, 2.13
P value†	0.27				0.55	
Wire code						
OHCC			1.15	0.92, 1.44	1.13	0.90, 1.42
VHCC			1.65	1.15, 2.35	1.58	1.18, 2.28
P value†			0.02		0.04	

LCC = low current code; OHCC = ordinary high current code; VHCC = very high current code.
* Includes study indicators.
† From deviance tests of all categories.

curves that are nearly flat to curves that rise and then fall at high exposures to curves that rise faster than exponentially.

We had planned to use available information to impute magnetic field values for subjects having only wire codes, on the basis of information relating codes to field measurements.[10,35] Nonetheless, because of the heterogeneity among wire code results and doubts about the accuracy of the imputation, we decided to forego those analyses.

One interesting result from our analysis is resolution of an apparent "wire code paradox." It has been remarked that wire codes show more consistent associations with childhood cancers across studies than do magnetic fields. The paradoxical element arose in part from the presumption that wire codes were a proxy for fields and thus should show less consistent associations if fields have an effect. An examination of our tables suggests that, after allowing for statistical variability, wire codes in fact show less consistent associations with childhood leukemia than do magnetic fields. Nonetheless, adjustment for measured fields does not reduce the association of wire codes with childhood leukemia (Table 9). Perhaps only fields are biologically relevant, but errors in the field measures are so large that wire codes pick up much of the field effect; another possibility is that both measures only reflect effects of some biologically relevant exposure that is missing from our data.

One can of course raise many criticisms of the individual studies, which would increase the already large uncertainty in our results. For example, confounding effects of socioeconomic status, residential mobility, residence type, viral contacts, and traffic density have been raised as possible explanations for the observed associations.[44–51] These confounding hypotheses are themselves problematic. First, a confounding explanation requires the confounder to have an effect considerably larger than the observed association, as well as a strong association with exposure.[30,Ch. 2] These attributes have not yet been demonstrated for the hypothesized confounders across the different populations that display positive associations. Adjustment for recorded socioeconomic and housing factors produced only small changes in the field-leukemia association, but our data on such factors are incomplete and we have only limited data on other potential confounders. Some results suggest that traffic-density effects may be large enough to partly explain the associations seen here.[44–47] We thus recommend that future studies obtain data on traffic density and ambient pollution levels, as well as details of socioeconomic status and residence history.

Biases due to measurement errors are undoubtedly present in and vary across all of the studies, but their assessment is not wholly straightforward. One problem is that there is no agreed-upon definition of the target exposure, although it is often thought of as some sort of average or cumulative exposure during some biologically relevant time before leukemia diagnosis. Only under fairly restrictive conditions[40,52] can one be certain that the net bias due to such error will be toward the null. Unfortunately, there is little or no evidence to establish such detailed attributes of the errors, and there is no basis for assuming such attributes are the same across studies and measures. For example, although some U.S. studies have found clear associations between fields measured at the front door, average magnetic fields in the home, and personal exposure to children[27,53] and another U.S. study found some repeatability of spot measures over extended time periods,[54] these associations are not large enough to ensure that the measures would tend to exhibit similar associations with childhood leukemia. Furthermore, the associations are imperfect enough to indicate that probably all of the measures suffer considerable error as proxies for any biologically relevant exposure measure (if one exists). One study suggested that electric rather than magnetic fields may be the relevant exposure.[2] Other studies conflict with this suggestion, however, insofar as the electric-field associations with childhood leukemia reported in those studies tended to be null or smaller than the reported magnetic-field associations.[7,10,23,25]

Selection biases may be present in the studies, but for most there is little evidence that would establish their magnitude or even their direction with any certainty. Some studies reported low response rates (for example, field measurements were obtained on only half the identified potential controls in McBride *et al*[22]), and accurate response rates cannot be determined for all studies. Whether such problems have led to serious bias remains a matter of speculation; the limited evidence from U.S. studies appears conflicting (for example, contrast Savitz *et al*[10,p.35] with Hatch *et al*[51] and Savitz and Kaune[55]).

Given the preceding considerations, it seems reasonable to suppose that measurement and validity differences are responsible for some of the variation in study-specific results. Those considerations also raise a serious criticism of our analysis, in that we pooled different magnetic field measures without demonstrating that all of the measures are comparable or combinable. Indeed, it is highly implausible that the measures we used (or any other choices among available measures) reflect common underlying exposure and error distributions. Furthermore, our criteria for choosing measures when we had a choice are not compelling (for example, minimize missing data), and one could reasonably argue in favor of other choices[56] (although not without dispute[57,58]). We expected that measure heterogeneity would lead to extra variation among the study-specific results, so we are all the more surprised that the observed variation was limited. We caution, however, that other choices could lead to very different degrees of variation; our results may not even be typical of what would be seen upon trying all defensible choices (although exploring the full range of choices would not indicate which choice is most valid). These problems should further expand the considerable uncertainty apparent in our results.

Another meta-analytic issue is that of publication bias. Because of the publicity surrounding the topic, we speculate that the data in small unpublished studies (if any exist) would have little influence on the results, and

that all large studies of this topic get published. Unfortunately, there are as yet too few published studies of fields or wire codes and childhood leukemia to support a reliable analysis of this bias,[59,60] and current methods for analyzing the bias are not well suited for relations that require several degrees of freedom to summarize.

Our attributable-fraction estimate is subject to further criticism through its dependence on the EPRI survey.[41] The survey measurements are of residential fields and therefore exclude sources such as school exposures and electric blankets; this exclusion error probably increases with age, especially upon school entry. Furthermore, selection bias could have been introduced because the survey homes were not limited to homes with children. Nonetheless, we think our estimate shows that any population effect of fields is probably much too small to detect via ecologic or time-trend studies; large ecologic variation or trends in leukemia rates would more likely be due to ecologic or temporal confounding than to real EMF effects.

In light of the above problems, the inconclusiveness of our results seems inescapable; resolution will have to await considerably more data on high electric and magnetic-field exposures, childhood leukemia, and possible bias sources. It also appears to us that, if an effect exists below 0.2 μT, it is probably too small to reach consensus about it via epidemiologic investigation alone. In contrast, both our categorical and trend analyses indicate that there is some association comparing fields above 0.3 μT to lower exposures, although there are as yet insufficient data to provide more than a vague sense of its form and its possible sources. We believe individual-level studies that focus on highly exposed populations would be needed to clarify this association. Such populations might be found in densely settled areas of some industrialized countries, such as Japan.[61] Even in these countries, efficiency might be improved by restricting the source population to locales containing transmission lines, as was done in some Scandinavian studies.

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EXHIBIT E

Journal of Cellular Biochemistry 81:143–148 (2001)

Regulating Genes with Electromagnetic Response Elements

Hana Lin,[1] Martin Blank,[2] Karin Rossol-Haseroth,[3] and Reba Goodman[1]*

[1]Department of Pathology, Columbia University Health Sciences, New York, NY 10032
[2]Department of Physiology, Columbia University Health Sciences, New York, NY 10032
[3]Department of Medicine, Columbia University Health Sciences, New York, NY 10032

Abstract A 900 base pair segment of the c-*myc* promoter, containing eight nCTCTn sequences, is required for the induction of c-*myc* expression by electromagnetic (EM) fields. Similarly, a 70 bp region of the HSP70 promoter, containing three nCTCTn sequences, is required for the induction of HSP70 expression by EM fields. Removal of the 900 base pair segment of the c-*myc* promoter eliminates the ability of EM fields to induce c-*myc* expression. Similarly, removal of the 70 bp region of the HSP70 promoter, with its three nCTCTn sequences, eliminates the response to EM fields. The nCTCTn sequences apparently act as electromagnetic field response elements (EMRE). To test if introducing EMREs imparts the ability to respond to applied EM fields, the 900 bp segment of the c-*myc* promoter (containing eight EMREs) was placed upstream of CAT or luciferase reporter constructs that were otherwise unresponsive to EM fields. EMREs-reporter constructs were transfected into HeLa cells and exposed to 8 μT 60 Hz fields. Protein extracts from EM field-exposed transfectants had significant increases in activity of both CAT and luciferase, compared with identical transfectants that were sham-exposed. Transfectants with CAT or luciferase constructs *lacking* EMREs remained unresponsive to EM fields, i.e., there was no increase in either CAT or luciferase activity. These data support the idea that EMREs can be used as switches to regulate exogenously introduced genes in gene therapy. J. Cell. Biochem. 81:143–148, 2001.

Key words: electromagnetic field response elements; gene therapy

Low frequency electromagnetic (EM) fields induce increased expression of the stress response gene HSP70 [Lin et al., 1997; Goodman and Blank, 1998]. There are several parallels in the biochemical pathways induced by EM fields and heat shock, but there are striking differences as well. Both pathways involve the binding of heat shock factor 1 (HSF1) to a heat shock element (HSE), but regulation of HSP70 gene expression by EM fields requires three nCTCTn binding sites in the HSP70 promoter that lie between −230 and −160, upstream from the transcription initiation site. These three nCTCTn sequences appear to act as electromagnetic field response elements (EMREs), since the ability of an EM field to induce stress proteins gradually disappears as the EMREs are mutated one by one [Lin et al., 1998a, 1999]. Removal of EMREs by mutation does not affect the response to heat shock, since the heat shock domain is downstream from the EM field domain in the HSP70 promoter (between −106 and −67) [Lin et al., 1997, 1998b, 1999].

We previously showed that a 900 bp region in the c-*myc* promoter (−1257 to −353) was responsive to EM fields [Lin et al., 1994]. Recent reanalysis of this 900 bp region revealed eight nCTCTn sequences within this DNA fragment. These eight EMREs in the c-*myc* promoter could account for the EM field sensitivity of the c-*myc* gene, and the resultant increased c-*myc* transcript levels in cells exposed to EM fields [Jin et al., 1997].

To determine whether EMREs can serve as switches to regulate exogenously introduced genes, the 900 bp fragment of the c-*myc* promoter was placed upstream of CAT or luciferase reporter constructs that were otherwise unresponsive to EM fields. EMRE-reporter constructs were transfected into HeLa cells and

Abbreviations used: CAT, chloramphenicol transferase; EM, electromagnetic; Hz, hertz (cycles/s); EMRE, electromagnetic field response element.

*Correspondence to: R. Goodman, Columbia University Health Sciences, Department of Pathology, 630 W. 168th Street, New York, NY 10032. E-mail: rmg5@columbia.edu

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transfectants exposed to EM fields. Protein extracted from EM field-exposed transfectants showed increased CAT and luciferase activities, whereas no increase in CAT or luciferase was measureable in the controls. Three kinds of controls were used: transfectants that were sham-exposed; transfectants lacking EMREs; and non-specific protein. These data support the theory that EMREs can be inserted into the promoters of exogenously introduced genes to serve as switches that respond to EM fields. This would provide a new and powerful non-invasive technique for regulating gene expression *during* gene therapy.

MATERIALS AND METHODS

Cell culture and transfections. As previously described, HeLa cells were used for transient transfections and the lipofectin method (Gibco/BRL, Cat. No. 18292-011) was used for transfection as described [Lin et al., 1997, 1998a,b].

900 bp segment from the c-myc promoter. The 900 bp region of the c-*myc* promoter containing eight copies of nCTCTn extends from −353 (*Pvu*II site) to −1257 (*Cla*I site).

***p*ΔH-11-CAT HSP70 deletion construct.** (This plasmid was kindly provided by Dr. R. Kingston, Department of Genetics, Harvard University). A diagrammatic representation of this construct is presented by Lin et al. [1999]. This construct contains the first 111 base pairs upstream from the transcription initiation site and includes the heat shock domain (−106 to −67). There are no nCTCTn binding sites in this construct and it is unresponsive to EM fields [Lin et al., 1999].

Construction of EMRE-CAT expression vector. Plasmid pΔ11-CAT was digested with *Hind*III and *Pvu*II, harvested from gel (Fig. 1A). Two oligonucleotides were used for PCR which allowed us to create two enzyme sites and amplify the 900 bp region from c-*myc* promoter.

1. CCTGAGCTCTTCTTTGATCAGAATCGATA
2. TCTAAGCTTCTTTGATCAGAATCGATG

One microliter of plasmid (digested with *Hind*III and *Pvu*II) was mixed with 3 μl PCR product, placed at 12°C overnight for ligation and transformed using DH52 bacteria. Clone hybridization verified insert.

Construction of EMRE-luciferase expression vector. A luciferase expression vector PGL3 (Promega) was digested with Sac1 and Sma1 and harvested from a gel (Fig. 1B). Two oligonucleotides (see above) were used for PCR which allowed us to create two enzyme sites and amplify the 900 bp region from c-*myc* promoter. One microliter of digested plasmid was mixed with 3 μl PCR product, placed at 12°C overnight for ligation and transformed using DH52 bacteria. Clone hybridization verified insert.

Protein. Protein was extracted and concentrations determined as previously described [Lin et al., 1997, 1998a,b, 1999].

CAT assay. CAT assays were performed as previously described [Lin et al., 1997, 1998a]. Results were quantified using a PhosphorImager and ImageQuant software.

Luciferase assay. Luciferase activity was determined (Luciferase Assay Kit) (Promega #E1501) and results quantified as suggested by Promega.

Magnetic field exposures of transfectants. Transfectants were exposed and sham-exposed as previously described [Lin et al., 1998a,b, 1999].

Heat shock. Samples from cells that had been heat shocked (43°C) served as positive controls for CAT assay. Petri dishes containing transfectants were wrapped in Parafilm, placed in a Mu metal box (to shield them from exposure to the magnetic fields generated by the water bath heating motor) and immersed in the water bath at 43°C for 30 min. Petri dishes were removed from the water bath and, following an additional 30 min at 37°C, protein was extracted [Lin et al., 1997].

Sinusoidal electromagnetic field exposure system. Two fully functional exposure units provided simultaneous sham and experimental exposures. Exposures used Helmholtz coils (Electric Research and Management, Pittsburgh, PA) that consisted of 19-gauge wire bundles wound 164 times around a rectangular form 13 cm long and 14 cm wide with 8 cm spacing. The coils were energized by a function generator (11 MHz Wavetek Stabilized Function Generator; model 21). A digital multimeter was used to measure the field intensity and verify the systems operation (Fluke 87 digital multimeter). Field parameters were monitored with a Hitachi V-1065 100 MHz oscilloscope and calibrated inductive search coil (25X; Electro-Biology Inc., Parsippany, NJ). Detailed description of the exposure system, including

background magnetic fields in the incubater, harmonic distortion, DC magnetic fields, and mean static magnetic fields in the incubator, both vertical and horizontal components, can be found in Jin et al. [1997]. Cells were placed on a Plexiglas stand in a horizontal orientation, i.e., the entire area of the dish was exposed to the field. The bottom of the dish was 2 cm below the axis level. The height from dish bottom to top surface of liquid was ~1.1 cm; the height of the liquid was 0.6 cm. The calculated electric field was ~11 μV/m for an 8 μT 60 Hz exposure.

Mu metal shielding. Helmholtz coils were enclosed within Mu metal containers to minimize stray fields during EM field exposures. Both active (experimental) and sham-exposed (controls) coils were enclosed in a 30 cm high, 15 cm diameter cylindrical Mu metal container (0.040 inch thickness) (Amuneal Corp. Philadelphia, PA). The 60 Hz shielding factor is (min) 90.1 (39.08 dB). Sham-exposed controls and experimental exposures were performed simultaneously in identical Mu metal containers.

Statistical analyses. A minimum of five experiments were performed to assure statistical significance. Statistical significance is determined by multifactor analysis of variance program (INSTAT).

RESULTS

EMREs Increase Luciferase Activity in Transfectants Exposed to EM Fields

To determine whether the nCTCTn sequences (EMREs) that are EM field responsive would confer EM field responsiveness to a reporter construct lacking these sequences, a 900 bp region from the c-*myc* promoter containing eight copies of nCTCTn, was ligated to a PGL3 plasmid containing a portion of the SV40 promoter and carrying the luciferase gene (Fig. 1B). This plasmid construct was transfected into HeLa cells and the transfectants exposed to 8 μT 60 Hz fields for 30 min, followed by an additional 30 min out of the field prior to protein extraction for the luciferase assay. Luciferase activity increased at an average of 61%. Three sets of controls were used (Fig. 2A): (1) sham-exposed transfectants that served as controls for EM field exposure, and showed no significant luciferase activity; (2) transfectants containing the luciferase reporter construct *without* the 900 bp insert served as controls for background and showed no measurable luciferase activity; and (3) non-specific protein served as negative controls with no measurable activity. These transfectants were not responsive to

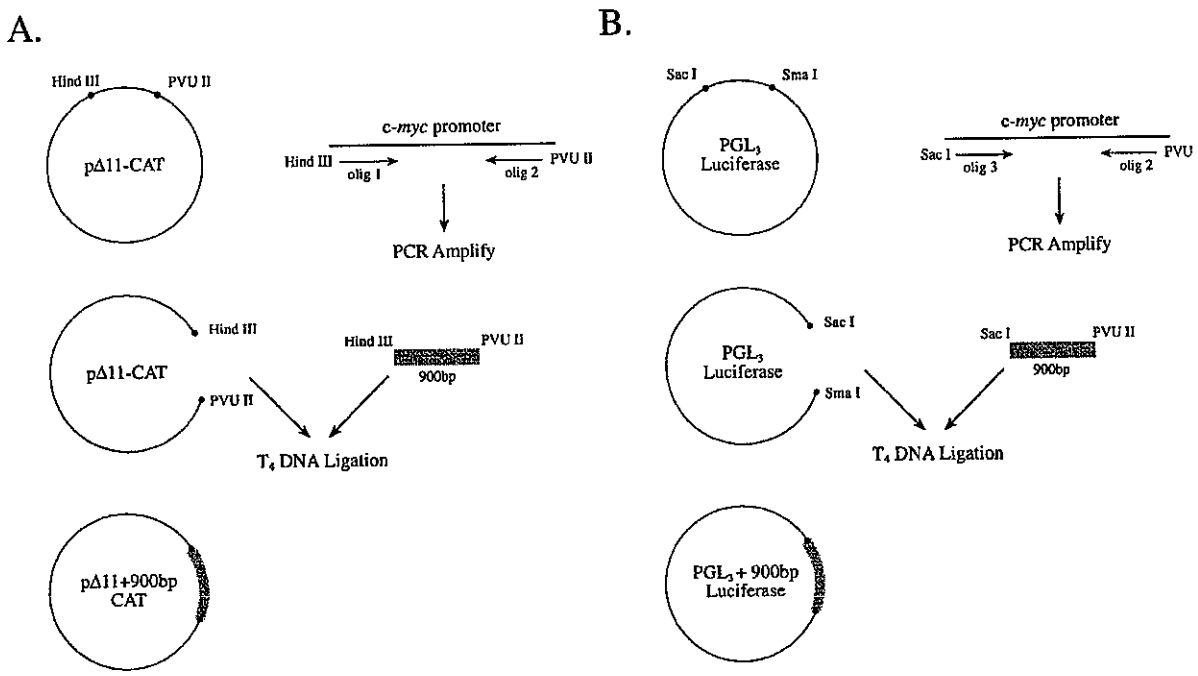

Fig. 1. Construction of EMRE-expression vectors. **A:** pΔ11 + 900 bp + CAT; **B:** PGL_3 + 900 bp + luciferase.

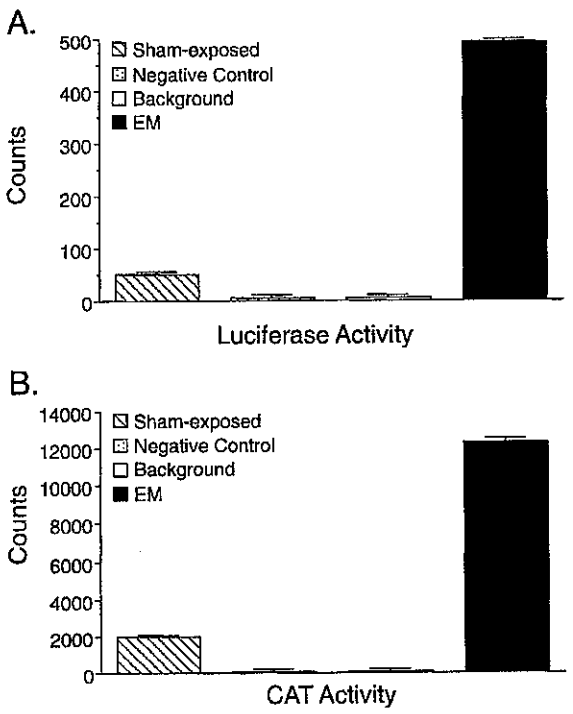

Fig. 2. CAT and luciferase activities. Samples in lane 1 were sham-exposed (30 min); samples in lanes 2, 3, and 4 were exposed to 8 μT 60 Hz EM fields (30 min). **A:** (1) luciferase activity in protein extracted from transfectants containing luciferase construct *plus* the 900 bp insert (sham-exposed); (2) luciferase activity using non-specific protein (negative control) (EM field-exposed); (3) luciferase activity in protein extracted from transfectants containing luciferase construct *minus* the 900 bp insert (EM field-exposed); (4) luciferase activity in protein extracted from transfectants containing luciferase construct *plus* the 900 bp insert (EM field-exposed). **B:** (1) CAT activity in protein from transfectants containing CAT construct *plus* the 900 bp insert (sham-exposed); (2) CAT activity using non-specific protein (negative control) (EM field-exposed); (3) CAT activity in protein from transfectants containing CAT construct *minus* the 900 bp insert (EM field-exposed); (4) CAT activity in protein from transfectants containing CAT construct *plus* the 900 bp insert (EM field-exposed).

heat shock, as expected from the absence of heat shock consensus sequences (nGAAn) in this plasmid construct.

EMREs Increase CAT Activity in Constructs Exposed to EM Fields

In similar experiments with a CAT reporter construct, the 900 bp region from the c-*myc* promoter containing eight nCTCTn was ligated to pΔ11- CAT (Fig. 1A), transfected into HeLa cells, and the transfectants exposed to an 8 μT 60 Hz field for 30 min, followed by an additional 30 min out of the field prior to protein extraction for the CAT assay. There was an average 60% increase in CAT activity. The same three sets of controls described above were employed in these experiments (Fig. 2B): (1) sham-exposed transfectants, served as controls for EM field exposure, and showed no significant CAT activity; (2) transfectants containing the CAT reporter construct *without* the 900 bp insert (pΔ11-CAT) served as controls for background; protein extracts from these transfectants showed no measurable CAT activity; and (3) non-specific protein served as negative controls. Transfectants with and without the 900 bp insert were heat shocked for 30 min at 43°C followed by protein extraction after an additional 30 min out of the heat. There was an average 45% increase in CAT activity in heat shocked transfectants. The pΔ11 plasmid contains the heat shock domain, −106 to −67, therefore response to heat shock served as an additional control.

DISCUSSION

Since EM fields penetrate tissues without attenuation, they must penetrate to the cell nucleus with its DNA and interact with moving charges there [Blank and Goodman, 1999]. That there are conducting electrons in DNA has been shown by Porath et al. [2000], who have made direct measurements of electrical transport through DNA, and by Wan et al. [1999], who have measured the dynamics of DNA-mediated electron transfer at the femtosecond level. Conduction in DNA appears to depend on specific structure, since different DNA sequences have different conductivities [Meggers et al., 1998]. Therefore, EM fields could theoretically interact preferentially with specific DNA sequences, and the nCTCTn sequences (EMREs) in the HSP70 and c-*myc* promoters used in these studies may be such sequences.

Of course, at this stage, it is possible for some unidentified indirect mechanism to be at play, but we have shown that these sequences are critical for EM field responsiveness in our experiments, and other data appear to support this. Verdugo-Diaz et al. [2000], in totally unrelated investigations, showed that low frequency EM field stimulation in nigro-striatal lesioned rats with chromaffin transplants in-

duced changes in the subventricular zones and led to significant motor improvements in a rat Parkinson model. A second report from the same laboratory [Olivares-Banuelos et al., 2000] has used differential display to analyze possible alterations in DNA of EM field-exposed chromaffin cells. Differential bands observed in the EM field-exposed group show changes in gene expression induced by EM fields. One specific differential band in the EM field-exposed samples, containing 349 bp, was sequenced. In an independent analysis of this DNA fragment we have identified three copies of the EM field response element (nCTCTn) that we describe in this report. A computer search is currently underway to determine whether this 349 bp DNA fragment is contained in the promoters of any known genes, possibly a specific gene related to the differentiation process of chromaffin cells.

Advantages of Using EMREs for Gene Therapy

Gene therapy was proposed about 20 years ago as a way to ameliorate genetic defects by providing a source for missing essential genetic components. The injection of *copies of the gene* responsible for the production of a specific protein directly into the targeted area by means of a viral vector was considered a mode of insuring that the protein required would be synthesized at the site where it was needed. This approach offered a distinct advantage over prior conventional treatment of metabolic diseases, which required continuous injection of *gene product* from exogenous sources.

The principle behind gene therapy is simple, but practical application has been difficult. Failure of early gene therapy was mainly due to three problems:

1) difficulties in efficiently transducing primary quiescent human cells in vivo;
2) strong immune responses to the gene therapy vectors, as well as to the foreign therapeutic transgenes that rapidly eliminated transgene expressing cells in humans;
3) the ability of many cell types to shut off the viral promoters that controlled transgene expression in humans.

One positive outcome of these early efforts at gene therapy was the demonstration that introducing cloned genes into humans could be safe, with little or no morbidity. More recently, new vectors have been engineered, including adenoviruses and even naked DNA, enhancing the efficiency of in vivo gene delivery and reducing the immunogenicity of vectors and transgenes.

We have demonstrated that EM fields induce gene expression [Goodman and Blank, 1998; Lin et al., 1999] and that activation of the gene by EM fields requires specific EMREs, which control genes when placed upstream of reporter constructs. Their ability to confer EM field responsiveness suggests the use of EMREs in the control and regulation of gene therapy. The characterization of a cellular promoter system that can be regulated, such as described here, provides a novel, noninvasive technique for the regulation of transgene expression in humans without interfering with normal physiologic function. The applied EM field can be directed to the region where the gene product is needed and, since the EM field intensities needed to affect EMREs are well below the human perception threshold, their introduction and presence would not be felt by the patient. An example of such application would be the introduction of an exogenous insulin gene containing one or more EMREs placed upstream of the gene. Regulation would be provided by the simple and safe application of EM fields. The whole operation would be made automatic by having the EM field generating circuit activated by an implanted glucose sensor responsive to pre-set blood glucose levels.

How Many EMREs are Required for EM Field Responsiveness?

Our results show that the eight nCTCTn sequences (EMREs) in the 900 bp DNA fragment from the c-*myc* promoter are effective in regulating CAT or luciferase activity. However, not all eight EMREs may be needed for a response [Lin et al., 1999]. We previously demonstrated that the EM-induced expression of HSP70 is mediated through *three* EMREs in the human HSP70 promotor. EM field exposure of HSP70 promoter constructs, linked to a CAT reporter gene and containing all three sites, showed more than a threefold increase in CAT activity. Yet, the presence of even one site was sufficient for a 1.5-fold increased CAT response. These data show that even a single EMRE can promote interaction with EM fields. The data also suggest that the level of interaction appears to be roughly proportional to the number of EMREs.

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EXHIBIT F

Journal of Cellular Physiology

A Mechanism for Stimulation of Biosynthesis by Electromagnetic Fields: Charge Transfer in DNA and Base Pair Separation

MARTIN BLANK,[1]* AND REBA GOODMAN[2]

[1]Department of Physiology Columbia University, New York, New York

[2]Department of Pathology Columbia University, New York, New York

Electrons have been shown to move in DNA, and a specific DNA sequence is associated with the response to EM fields. In addition, there is evidence from biochemical reactions that EM fields can accelerate electron transfer. Interaction with electrons could displace electrons in H-bonds that hold DNA together leading to chain separation and initiating transcription. The effect of charging due to electron displacement on the energetics of DNA aggregation shows that electron transfer would favor separation of base pairs, and that DNA geometry is optimized for disaggregation under such conditions. Electrons in the H-bonds of both DNA and the surrounding water molecules fluctuate at frequencies that are much higher than the frequencies of the EM fields studied. The characteristics of the fluctuations suggest that the applied EM fields are effectively DC pulses and that interactions extend to microwave frequencies.
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How weak electromagnetic (EM) fields interact with DNA to stimulate protein synthesis is currently not well understood. An important clue, however, is the identification of a specific DNA sequence on the gene promoter that is associated with the response to EM fields. When this sequence is transfected into the promoter of a reporter gene, previously unresponsive to EM fields, this gene is now EM field-responsive. Previous research showed that EM field induction of the HSP70 gene involved signaling pathways that could respond to feedback information from the DNA interaction mechanism. An EM field sensitive DNA sequence suggests that EM fields may interact both directly and indirectly with DNA. The initial interaction could involve the displacement of electrons in the H-bonds that hold DNA together, thereby causing chain separation and initiating transcription and translation. Electrons have been shown to move in DNA and data from biochemical reactions indicate that EM fields can accelerate electron transfer. Interaction with electrons could account for activation of DNA by weak low frequency EM fields as well as the more energetic high frequencies. It has also been shown using multi-subunit proteins that charging leads to disaggregation. A simple model of the effect of charging due to electron displacement on the energetics of DNA aggregation shows that electron transfer would favor separation of base pairs, and that DNA geometry is optimized for disaggregation under such conditions. Electric fields exert comparable forces on electrons and also stimulate biosynthesis, as expected. The proposed mechanism suggests that there could be a maximum frequency for the EM field response, and that modifications of the charge on DNA affect the response.

EM Field Stimulation of Transcription

The interplay between experiment and theory usually catalyzes scientific development, but thus far, studies of EM field interactions with biological systems have not led to a generally accepted explanation of established biological effects. Theoretical approaches have been proposed, based on cyclotron resonance of ions (Liboff, 1985) and related approaches (e.g., Lednev, 1991; Blanchard and Blackman, 1994), the forced vibration of ions (Panagopoulos et al., 2002), and effects on electron transfer (Blank and Goodman, 2002, 2004; Blank, 2005). The very low energy of the fields that are reported to be effective have even led to theoretical papers that question the validity of the experiments themselves (Valberg et al., 1997; Weaver et al., 1998). The lack of success in defining a mechanism may be due to trying to find a single over-arching principle that would describe a variety of experimental observations. For example, cyclotron resonance undoubtedly applies to charges in DC and AC fields in a vacuum, but would not be expected to apply to hydrated ions in membrane channels. In all cases, the low energies that are effective need to be explained, especially in activating the signaling pathways in the stress response.

Theoretical approaches to EM field mechanisms would probably do better to focus on a single well characterized biological effect and the low energy processes that could be involved. Two such attempts consider effects on electron transfer in a mechanism for EM field-DNA interactions that initiate transcription (Blank and Goodman, 2004), as well as for EM field acceleration of the Na,K-ATPase that leads to ion pumping (Blank, 2005). Elson (2006) has also considered the possibility that charge transfer in DNA may be important in affecting the rate of development of living systems. These approaches incorporate experimentally observed processes as links in a causal chain. This paper proposes the following processes in DNA activation of transcription:

EM fields displace electrons in DNA. This causes transient charging of small groups of base pairs. At the charged sites, disaggregation forces overcome H-bonds. Disaggregation of the two chains at those sites enables transcription.

Abbreviations: EM, electromagnetic; Hz, hertz; ELF, extremely low frequency; RF, radio frequency.

*Correspondence to: Martin Blank, Department of Physiology, Columbia University, 630 West 168 Street, New York, NY 10032.
E-mail: mb32@columbia.edu

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We know that power frequency (ELF) fields alter RNA transription patterns (Goodman et al., 1983), induce upregulation of the early response genes, c-*fos* (Rao and Henderson, 1996) and c-*myc* (Lin et al., 1994, 1996), and the stress response gene HSP70 (Goodman et al., 1994; Lin et al., 1997; Goodman and Blank, 1998). Radio frequency (RF) fields have also been shown to induce stress response genes (Kwee et al., 2001; Leszczynski et al., 2002; Shallom et al., 2002; Weisbrot et al., 2003). Additional studies that support EM field interaction with DNA are electron conduction in DNA (Wan et al., 1999, 2000) and EM field-induced DNA single and double strand breaks (Lai and Singh, 1997, 2004; Diem et al., 2005; Ivancsits et al., 2005; REFLEX Project Report, 2004).

However, not all cell types respond to EM fields. A series of recent studies using both ELF and RF fields found, in addition to DNA strand breaks, cell type specific genotoxic effects from exposures to ELF fields (Sarimov et al., 2004; Winker et al., 2005; REFLEX Project Report, 2004). One effect of ELF fields on DNA that has been repeated many times in different laboratories is that 12 mG fields interfere with the ability of Tamoxifen to inhibit the growth of MCF7 breast cancer cells at low thresholds (2–12 mG; Liburdy, 2003).

The 'dilemma' of the cell line that does not respond to EM fields and the inability to 'replicate' positive reports have plagued this area of research for many years, and has led to mistrust of data. One such controversy concerned two cell lines of HL60 cells obtained from different sources. The discrepancy was resolved when it was shown that the two cell lines in question had dramatically different growth rates as well as differences in response to EM field exposure (Jin et al., 1997). These results show that cell lines that have been maintained for a long period of time in different laboratories must be characterized before using them in EM field experiments: for example, number of passages; whether they are transformed; and their genomic and proteomic composition. Effects of ELF and RF have been shown to differ depending upon a number of factors including different human donors and how long a cell line has been maintained in a specific laboratory. Natural selection takes place at each cell passage and eventually the genome of the cell line is permanently altered. Inability to replicate published data can be due to any number of factors. One such factor could be the presence or absence of the EM-field sensitive DNA sequence on the promoters of some of their genes, as described below.

Biochemical Signaling Pathways

In the absence of EM fields, an important series of cellular signaling events normally occurs prior to upregulation of gene expression. These events are controlled by members of the mitogen activating phosphokinase (MAPK) family. Transcription factors in the p38 MAPK pathway are involved during both ELF and RF exposures (Leszczynski et al., 2002, 2004). Increased phosphorylation of specific transcription factors has also been shown when cells and tissues are exposed to EM fields (Jin et al., 2000; Leszczynski et al., 2002; Weisbrot et al., 2003). In considering how EM fields affect DNA and the regulation and control of gene expression, it is important to take into account that the chain of events coming into the cell from outside is comprised of a large number of transcription factors that are regulatory proteins. Some of these enter the nucleus and bind to specific recognition sites on the DNA of the promoter.

How these ongoing events may be affected by EM field stimulated processes in the DNA is currently unknown, but the biochemical signaling pathways are inter-connected much like the intermediary metabolism charts, and they connect with the products of DNA transcription (Lin et al., 1996). The EM field can initiate DNA transcription by itself once the DNA sequences in the promoter transduce the field energy, and this sets in motion the inter-connected processes that are activated in the stress response. Figure 1 shows a diagram linking activation of DNA with activation of the biochemical pathways. By this mechanism DNA stimulation can occur directly via the DNA molecule itself, as well as indirectly via the biochemical pathways, without necessarily involving interaction with the cell membrane.

The stress response, characterized by synthesis of stress proteins (e.g., hsp70), can be induced by elevated temperatures ('heat shock') as well as EM fields, but the stimuli act on distinctly different parts of the promoter. See Figure 2. Upregulation of the HSP70 gene by EM fields occurs in the absence of elevated temperature. The promoters of both HSP70 and another EM field-sensitive gene, c-*myc*, have multiple copies of a specific nucleotide sequence that responds to EM field exposures. This consensus sequence, nCTCTn (shown as the MYC binding sites in Fig. 2), is upstream on the promoter relative to the transcription initiation site from a different nucleotide sequence that is associated with the heat shock response (Lin et al., 1994, 1999, 2001). EM field exposure of HSP70 deletion constructs, linked to a CAT or Luciferase reporter genes and containing all three nCTCTn binding sites, showed more than a three-fold increase in CAT and Luciferase activity (Lin et al., 1998, 1999, 2001). The presence of even one nCTCTn binding site is sufficient for a 1.5-fold increase. To demonstrate EM field specificity and sensitivity, nCTCTn sequences were mutated one by one. The CAT and Luciferase assays showed that the ability of an EM field to induce hsp70 protein disappears as the sequences are mutated (Lin et al., 1998, 1999). Since the nCTCTn sequences have low electron affinities and electrons are easily displaced, these data support the idea that EM fields could interact with electrons in the promoter of the gene.

EM Fields Interact With Electrons in Biochemical Reactions

One expects electric (E) and magnetic (B) fields to interact most strongly with electrons, because of their unusually high charge to mass ratio. In quantum theory, this basic assumption, known as the Born–Oppenheimer Approximation, applies to sub-atomic reactions. Electrons are assumed to respond instantaneously compared to protons and heavier atomic nuclei

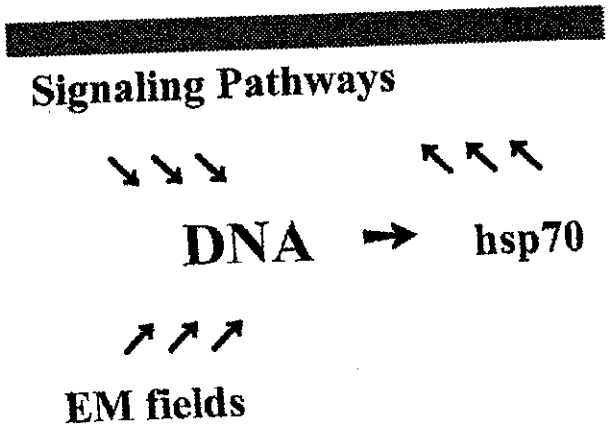

Fig. 1. A diagram showing interaction of EM fields and the biochemical signaling pathways with DNA leading to synthesis of the stress protein hsp70. The stress protein hsp70 acts as a negative feedback agent in controlling its own synthesis. We assume this to be characteristic of feedback mechanisms in the signaling pathways of EM field activated mechanisms.

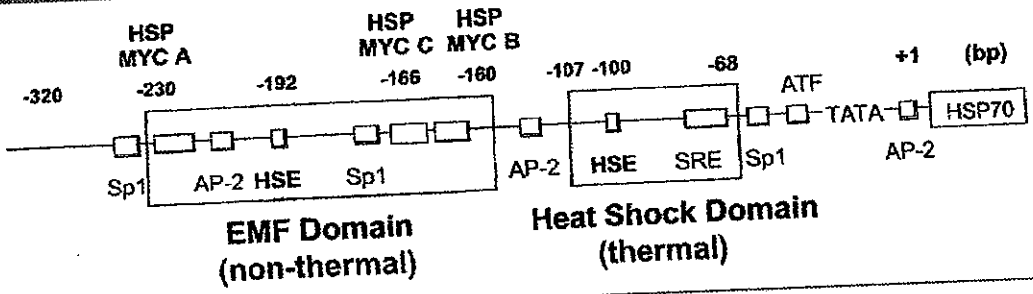

Fig. 2. A map of the EM field and thermal ('heat shock') domains on the HSP70 promoter. Binding sites within the EM field domain are indicated (HSE, AP-2, Sp1). The DNA consensus sequence that interacts with EM fields is nCTCTn and is at the three MYC binding sites (A,B,C) shown as boxes. All the locations are indicated by the numbered sequence of bases at the top of the diagram.

because of their much smaller mass, and electronic responses are assumed to be essentially complete before the heavier atomic nuclei start to react. It is, therefore, reasonable to expect EM fields to interact initially with electrons in biological systems, including DNA.

Interaction of electric and magnetic fields with electrons was indicated in studies of the Na,K-ATPase, the membrane enzyme that transports Na^+ and K^+ ions across membranes against electrochemical gradients (Blank, 2005). Low frequency electric and magnetic fields were shown to affect enzyme function differently, but both fields accelerated the reaction when the enzyme was relatively inactive. We assumed that the same force was needed at the threshold for acceleration by each field, and calculated the velocity (v) of the charge (q) that is affected in the two fields by equating the electric with the magnetic force,

$$F = qE = qvB. \quad (1)$$

It follows that $v = E/B$, the ratio of the threshold fields. The measured thresholds (Blank and Soo, 1992, 1996) were $E = 5 \times 10^{-4}$ V/m and $B = 5 \times 10^{-7}$ T (0.5 μT), giving $v = 10^3$ m/s, a speed similar to that of electrons in DNA (Wan et al., 1999).

Since electrons are affected by EM fields in the ELF range, there should be sufficient energy to stimulate electrons in DNA and other biochemical reactions. To test the effect of EM fields on reactions where we know that electrons are involved, we studied electron transfer from cytochrome c to cytochrome oxidase (Blank and Soo, 1998) and in the Belousov–Zhabotinsky (BZ) reaction, which is the oxidation of malonic acid (Blank and Soo, 2003). In all three reactions, EM fields:

- accelerate chemical reactions (including electron transfer reactions)
- compete with the intrinsic chemical forces driving the reactions, and are most effective when the intrinsic chemical forces are low.
- activate at low thresholds: Na,K-ATPase (0.2–0.3 μT), cytochrome oxidase (0.5–0.6 μT), BZ reaction (<0.5 μT); the threshold for biosynthesis is below 0.8 μT.
- show frequency optima for the two enzymes studied that are close to reaction turnover numbers (Na,K-ATPase, 60 Hz; cytochrome oxidase, 800 Hz), suggesting a tie-in with the molecular kinetics. This is not a resonance-like interaction because the optima are broad.

A study reporting no effect of EM fields on the BZ reaction (Sontag, 2006) actually strengthens the above interpretation. In that study, the EM field was not applied until the reaction was well under way for about seven minutes. In our studies, the field was applied from time zero, that is, at the mixing of the reactants. This difference is critical. We have shown that all three reactions studied respond to EM fields only when the intrinsic chemical forces are relatively weak. EM fields accelerated the Na,K-ATPase reaction only when enzyme activity was low. The same was true for cytochrome oxidase, and also can be seen from the temperature dependence of the BZ reaction. EM fields are not magic. They exert a force in competition with other forces that affect chemical kinetics, and their effect is negligible when overcome by intrinsic chemical forces. To study effects of EM fields, one must select conditions where intrinsic chemical forces are weak and the EM field is strong enough to have an effect on the kinetics.

Studies of the three biochemical reactions, show that EM fields accelerate electron transfer, and that the EM forces ($\sim 10^{-20}$ N) at the low thresholds may be strong enough to displace electrons in DNA. The force due to interaction of an electron with a magnetic field is determined by the strength of the field and the velocity of the electron. Relative change of field or motion of electron is required. The force due to an AC magnetic field acting on a 'static' electron is due to the rate of variation of the B field and is usually much smaller. The largest force on an electron results when the magnetic field is changing, as in AC, and the electron is also moving. Significant movement would be expected due to the 'flickering' of H-bonds that occurs in water (Fecko et al., 2003). This also occurs at water interfaces (McGuire and Shen, 2006), and probably in the hydration layer of DNA.

In the experiments stimulating protein synthesis, an EM force of only $\sim 10^{-20}$ N was shown to activate DNA. This force can move an isolated electron ~1 nm in 1 nsec, a distance that is greater than the length of an H-bond (~0.3 nm). The displaced charge can create conditions that lead to disaggregation by overcoming the cohesive forces, including the H-bonds, and enabling water molecules to enter any gap created by the weakened bond. In principle, this process could occur at the site where the electron has added a net negative charge, or at the site where the electron came from and left an unbalanced positive charge.

A related mechanism probably occurs in the DNA of striated muscle, where the electric fields (not EM fields) associated with action potentials stimulate the nuclei to synthesize muscle proteins in vivo (Blank, 1995). That the effect is due to the electric field stimulus is shown by the relation between the muscle proteins synthesized and the frequency of the action potentials. Under normal physiological conditions, conduction of an action potential along the muscle membrane creates an electric field estimated at ~10 V/m (Blank and

Goodman, 2004). In striated muscle, this electric field drives the currents across the nuclei adjacent to the membrane and stimulates the DNA to synthesize different muscle proteins in response to the frequency of the action potentials. The magnitude of electric field provides a large safety margin in muscle, since fields as low as 3 mV/m stimulate HL60 cells (Blank et al., 1992), and the threshold electric stimulus for the Na,K-ATPase is even lower, at ~0.5 mV/m (Blank, 2005).

Differences in the frequency response between DNA and the enzymes provide some insight into the EM field interaction mechanism. For the two enzyme reactions studied in the ELF range, the peaks of the broad frequency optima are close to reaction turnover numbers (Na,K-ATPase, 60 Hz; cytochrome oxidase, 800 Hz) and appear to be related to the molecular kinetics. That is, the applied EM fields at those particular frequencies aid the electron transfer that occurs at that frequency. Unlike the case of the enzymes, the wide range of frequencies that stimulate stress protein synthesis indicates that the characteristics of the EM signal that activate DNA are probably unrelated to an ongoing biochemical reaction.

Electrons in DNA probably interact with the H-bonded water network, where bonds are in constant motion, and they move much faster than the changing EM fields that have been studied. Electrons would be expected to move at the ~nanometer/picosecond 'flicker' rate of protons in H-bonded networks (Fecko et al., 2003), and one would expect a velocity of this magnitude. Comparing the 'flicker' rate (10^{12} Hz) to the power (60 Hz) and radio (10^{10} Hz) frequencies in DNA studies, it appears that the EM fields hardly change while an electron is in motion; they are like repeated 'DC pulses.' For this reason, all frequencies in the range where EM fields act as DC pulses affect the electrons similarly, and even the weak power frequency fields exert sufficient force to move an electron. The characteristics of the fluctuations suggest that EM field interactions extend to microwave frequencies. The picosecond 'flicker' rate (10^{12} Hz) may also represent an upper limit in the ability of EM fields to affect DNA, because there may be insufficient time to move electrons at the higher frequencies.

EM Fields Interact With Electrons in DNA

DNA is composed of two single strands in the form of a double helix or twisted ladder that has 'rungs' formed by pairs of complementary molecular bases, AT and GC. There are π-electron orbits within the base pairs that extend above and below each 'rung' of the ladder, and these overlap with their counterparts from neighboring rungs, thus creating a electron pathway through the molecule that enables charge migration/transport. Wan et al. (2000) have used a well-characterized duplex DNA consisting of a fluorescent charge donor and a charge acceptor, bridged by varying numbers of intervening base pairs. They found indications of a decrease in charge transfer rate as a function of bridge distance. Several groups have shown that DNA can transfer electrons and that electron transfer can chemically repair a thymine dimer, that is, when two adjacent thymines on the same DNA strand bond together. They have shown that cells can modulate the electrical properties of DNA using an enzyme, methyltransferase, and that electron transfer can be interrupted by inserting an insulating chemical group in the π-electron stack.

Electron migration in DNA is complicated, and the debate on the nature of the conductivity of DNA has been controversial and contentious. One model that has been used to explain charge migration is hole hopping between local amino acid sites driven by the torsional motions of the 'floppy' ribose-phosphate backbones. This model has been used to analyze experimental results for sequence-dependent long range hole transport in DNA (Ratner, 1999; Giese and Spichty, 2000; Berlin et al., 2001). Porath et al. (2000) have made direct electrical transport measurements on DNA, and have shown that DNA behaves as a linear conductor. Shao et al. (2005) have demonstrated sequence dependence on charge transport through DNA domains. DNA charge appears to be remarkably sensitive to DNA sequence and structure. The unique DNA sequence on both the HSP70 and *c-myc* promoters, an nCTCTn domain, responds to EM fields and induces upregulation of the genes. The responsiveness is dependent on the number of nCTCTn present (Lin et al., 2001). It is clear that electrons can move in DNA and that some DNA sequences are associated with the response to EM fields.

Separation of Biopolymer Chains Due to Charging

In the proposed mechanism, DNA chain separation is initiated by charging of the chain segments where electrons are displaced. The disaggregation that follows is not simply the result of electrostatic repulsion, since a large part of the energy change is associated with hydration of the newly exposed chains to the aqueous solvent. The extent of disaggregation is determined by the balance between electrostatic and hydration forces, with H-bonds between the base pairs also contributing to the bonding energy.

Biopolymer disaggregation has been studied primarily in proteins in solution, where the emphasis has been on interaction with the aqueous medium. Lauffer (1975, 1989) focused almost entirely on the hydration energy. He used the term 'entropy driven' to describe aggregation of protein subunits in aqueous media, where the large increase in entropy was due to release of many bound water molecules when subunits aggregate. The term 'entropy driven' indicates that aggregation is spontaneous (i.e., the free energy change is negative), and that it occurs with a production of heat (i.e., a positive enthalpy). The negative free energy together with a positive heat production results in a large positive entropy change.

Characterizing protein aggregation as 'entropy driven' has caused many to overlook the importance of charge. Proteins disaggregate when the pH differs from the isoelectric point and their net charge increases (e.g., Klug, 1979; Blank and Soo, 1987), while the entropy increase due to release of water molecules is the same at every pH. The effects of charge can usually be neglected at constant pH, but they must be considered when the protein ionizes due to a conformational change, as during hemoglobin oxygenation, where an analysis shows that both electrostatic (due to ionization of a histidine) and hydration energies (due to changes in the surface area in contact with water) are needed to account for the observations (Blank, 1975, 1994). The relation between molecular surface area in contact with water and the surface charge density has proven useful in understanding a number of biopolymer properties, for example, the dissociation of hemoglobin tetramers into dimers (Blank and Soo, 1987), cooperative interactions and the Hill coefficient (Blank, 1989, 1994), the high viscosities of concentrated hemoglobin solutions (Blank, 1984) and the relation between gating current and opening of voltage-gated channel proteins in excitable membranes (Blank, 1987). The idea can account for the different effects of electric and magnetic fields on the Na,K-ATPase reaction (Blank, 2005). The ability of changes in molecular charge to explain complex physiological effects, suggests that the same forces apply to DNA, and that local charging should favor local disaggregation.

Charging of DNA Segments and Chain Separation

EM fields activate DNA by affecting the competition between forces minimizing charge density and those minimizing molecular contact with water. Charge tends to increase the area occupied, since this decreases electrostatic repulsion.

Hydration energy and H-bond energy oppose an increase in area which means greater contact of DNA bases with water. The charge density on the bases is a measure of the electrostatic repulsion, and the surface area of the bases exposed to water is a measure of the hydration energy, so we can use these two measures to estimate the relative effects of electrostatic and hydration energies on the disaggregation of the two DNA chains. The displacement of an electron also affects H-bonds, but H-bond energy is small compared to hydration energies.

The DNA surface exposed to water can be estimated from model structures. The effect of charge can be estimated from measurements of hemoglobin disaggregation as a function of pH (Blank and Soo, 1987). In the hemoglobin experiments, the osmotic pressure increase enabled calculation of the surface area increase when tetramers split into dimers, while the added charge was determined by the titrated acid. It is important to note that the disaggregation equilibrium constant varied with the pH, but *the surface charge density was the same at every pH.* In hemoglobin, despite the increase in total positive or negative charge, the charge density remained at $\sim .01\ nm^{-2}$. Apparently, the surface charge density determines the balance between areas exposed to water and unexposed (aggregated) areas. One expects quantitative differences between hydration of proteins and nucleic acids, but we can assume that the energy associated with breaking of water-water bonds is the same, and that the hydration energies are probably comparable. What is different in DNA is the association with histones and other charged chemicals that would alter the equilibrium. In any case, we would expect DNA to maintain a particular charge density and start to disaggregate when the charge density exceeded that value.

The structure of DNA is quite complicated at the molecular level, but we can approximate the energetics with a simple geometric model that estimates the area exposed to solution and the surface charge density for small DNA segments before and after they disaggregate/separate. Figure 3A shows the model used to estimate surface charge density of a 4 bp DNA segment in two DNA chains, DNA I and DNA II, before disaggregation. Each segment is approximated as four cubes, and each cube of length a and facial area a^2 represents a base (B) connected to a ribose (R) phosphate (P) polymer chain that forms the backbone of DNA. The end segments are joined to other segments on the same chain, and do not contribute to the exposed area of the segment. A total of 24 faces of area a^2 are in contact with the aqueous solvent. In Figure 3B, the 4 bp segments on the two chains have disaggregated. The bases on segment II, shown as CTCT, are exposed to the aqueous solvent, as are their (hidden) conjugates GAGA on segment I. As in Figure 3A, the end segments joined to the rest of the same chain do not contribute to the exposed area of the segment, but the newly exposed bases make a total of 32 faces of area a^2 in contact with the aqueous solvent. When aggregated,

$$\text{total exposed area} = 24 \times 0.64\ nm^2 = 15.36\ nm^2. \quad (2)$$

When the 4 bp in contact split apart, they generate an additional $8 \times 0.64\ nm^2$ or $5.12\ nm^2$ for a total solvated area of $20.48\ nm^2$.

Initially, the two segments (I and II) are attached and the surface charge, Q, which can be as high as 1 per PO_4 group, is spread over $15.36\ nm^2$. If an EM field stimulus adds a single charge to the block, a charge of $Q + 1$ will now be spread over $20.48\ nm^2$. If we assume that the surface charge density has the same value as a result of a DNA split and an increase in bases exposed to solution,

$$\frac{Q}{15.36} = \frac{Q+1}{20.48}. \quad (3)$$

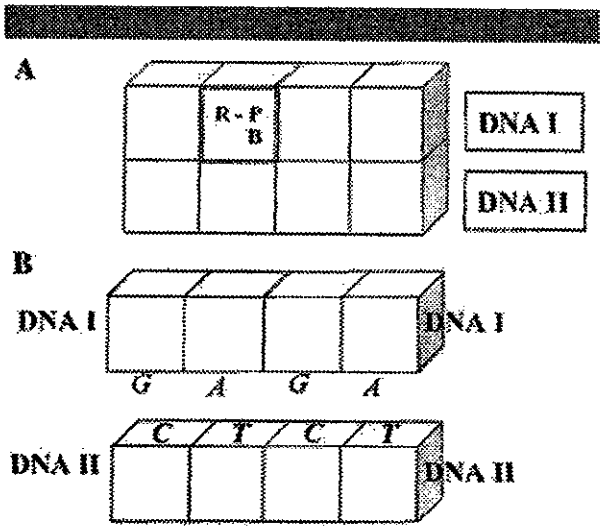

Fig. 3. A: Geometric model used to estimate surface charge density of a 4 bp DNA segment of two DNA chains, DNA I and DNA II, before separation. Each segment is approximated as four cubes, and each cube of length a and facial area a^2 represents a base (B) connected to a ribose (R) phosphate (P) polymer chain that forms the backbone of DNA. The end segments are joined to other segments, and do not contribute to the exposed area. A total of 24 faces of area a^2 are in contact with the aqueous solvent. B: When the 4 bp DNA segments on chains DNA I and DNA II separate, the bases on segment II, shown as CTCT, are exposed to the aqueous solvent, as are their (hidden) conjugates GAGA on segment I. As in subpart (A), the end segments joined to the rest of the same chain do not contribute to the exposed area, but the newly exposed bases make a total of 32 faces of area a^2 in contact with the aqueous solvent.

This leads to $Q = 3.0$, or three charges on the original 4 bp.

Repeating the calculation using different numbers of base pairs in a segment, the value $Q = 3$ appears to be a consequence of the idealized geometry we have chosen and the assumption of a constant surface charge density. The calculated values are based on approximations of molecular dimensions and neglect of interactions with histones, etc, but the value of Q is not unreasonable. Orthophosphate, an approximation for the ribose phosphate groups in DNA is about half ionized at pH 7.2. What has been demonstrated is that a polymer having the geometry of DNA can undergo aggregation-disaggregation transitions at various segment lengths with equal ease. DNA appears ready to be disaggregated and expose its code for transcription when there is a small change in the charge at a particular site. This may explain the specificity of transcription factors at particular sites and the ability of the same RNA polymerase mechanism to operate all along the chain.

Although disaggregation of DNA appears equally likely at all segment lengths, the strain of distorting the ribose-phosphate chain to pull out one base is probably too great. Also, the opening of 1 bp may not be enough to allow entry of RNA polymerase for transcription to proceed. With longer segments, there is less distortion to the DNA backbone, but more energy is needed to move the larger molecular mass after it has been hydrated. The balance between these two factors may coincide with the 4 bp unit CTCT associated with the response to EM fields. The perturbations of DNA structure due to interaction with proteins, as when bases flip out of a DNA double helix (Roberts and Cheng, 1998), can involve only a small number of base pairs.

The above mechanism offers a general rationale for disaggregation of DNA at small groups of bases, and the simple example made it appear that DNA is equally likely to disaggregate at all segment lengths and compositions. It is obvious that small differences in the actual dimensions of the individual bases and the groups that have interacted with them must affect the equilibria. The 4 bp unit CTCT associated with the response to EM fields and used in the example may be particularly effective. In addition to the low electron affinities, which enable electrons to be displaced relatively easily, the CTCT surface is 'molecularly smooth.' CTCT bases are pyrimidines and smaller than their complementary purines A and G, so a split forming CTCT and GAGA surfaces has a smaller total area than the usual mixture of pyrimidines and purines. A displaced electron at this site would have a greater effect on the charge density and create a greater driving force for separation. The smoother fit on the molecular level also leads to a lower tendency to form multiple H-bonds that increase the strength of adhesion between chains (Suehnel, 2002). Fewer multiple H-bonds would make it easier for base pairs to separate.

It is hard to make quantitative predictions, since both a 900 bp segment of the *c-myc* promoter with eight CTCT sequences, and a 70 bp region of the HSP70 promoter with only three CTCT sequences, respond to EM fields. However, in the experiments with the artificial construct, the EM field response appeared to be proportional to the number of CTCT groups present in the promoter (Lin et al., 1998). The nCTCTn sequences exist in a 3D configuration and are therefore also in contact with other DNA sequences that could be involved in the interaction. It is becoming clear from the discovery that genes could be affected by their position on the chromosomes, that overlap of genetic functional units is a widespread phenomenon. Given the fact that the DNA chain is contorted in space, and can be methylated, acetylated and phosphorylated, the positioning of the CTCT groups along the chain is probably also significant. CTCT groups separated by many base pairs may actually be quite close together in space, and some separations may allow two groups to act synergistically in helping the two chains to disaggregate.

Conclusion

Charge is a major factor controlling disaggregation of biopolymers at molecular cleavage planes. For this reason, transfer of charge in EM fields could contribute to separation of base pairs in DNA. A simple model of DNA geometry shows that an increase in local charge can cause separation of small groups of base pairs, and the low electronegativities of CTCT bases associated with the response to EM fields increase the likelihood of electron displacement. EM field initiated DNA separation can set in motion the inter-connected biochemical signaling pathways that are activated in the stress response.

Some clear implications of these ideas can be tested. The response of DNA to EM fields should vary with the charge and electron affinity of the DNA bases. Predictions about responses of DNA to charging should be testable through variations of pH by the selective binding of metal ions, histones and known transcription factors, or changes in the charge due to phosphorylation, acetylation, etc. It is also possible to test if the H-bond 'flicker' frequency in water is an upper limit for DNA response.

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EXHIBIT G

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Editorial

Starting with this issue, *Electromagnetic Biology and Medicine* institutes a new addition to the types of articles that are published. Readers will now occasionally find lengthy review articles, covering subjects in a detailed way with appropriate full referencing. This speaks to the fact that the subject matter in this research area has become so extensive as to warrant providing in-depth and historical perspectives for researchers and students.

It is particularly fitting that the first such review for this journal is by Martin Blank of Columbia University, who for nearly four decades has made many key contributions to studying how electromagnetic fields interact with biomolecules.

A. R. Liboff

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Protein and DNA Reactions Stimulated by Electromagnetic Fields

MARTIN BLANK

Department of Physiology and Cellular Biophysics, Columbia University, New York, New York, USA

The stimulation of protein and DNA by electromagnetic fields (EMF) has been problematic because the fields do not appear to have sufficient energy to directly affect such large molecules. Studies with electric and magnetic fields in the extremely low-frequency range have shown that weak fields can cause charge movement. It has also been known for some time that redistribution of charges in large molecules can trigger conformational changes that are driven by large hydration energies. This review considers examples of direct effects of electric and magnetic fields on charge transfer, and structural changes driven by such changes. Conformational changes that arise from alterations in charge distribution play a key role in membrane transport proteins, including ion channels, and probably account for DNA stimulation to initiate protein synthesis. It appears likely that weak EMF can control and amplify biological processes through their effects on charge distribution.

Keywords Electric fields; Magnetic fields; Charge transfer; Protein; Hydration energy; DNA.

The Problem

Mark Twain once defined common sense as the sense that tells you the earth is flat. For most people, that line generally evokes a guilty smile. We know the earth is not flat even as our senses deceive us into believing that it is. In the study of biological effects of electromagnetic fields (EMF), we know that we do not usually perceive effects of these fields. However, we also know that biochemical and physiological measurements show profound effects of EMF on living cells. As scientists, we try to let science guide our common sense.

To put EMF in perspective, we know that of the four fundamental physical interaction forces, EM forces are those that mainly affect living systems. One would expect that biological responses to EM forces evolved over time in optimizing the ability of cells to survive. However, it appears that biological systems are unusually sensitive to EMF in frequency ranges that are unlikely to have been experienced by

Address correspondence to Martin Blank, Department of Physiology, Columbia University, 630 West 168 Street, New York, NY 10032; E-mail: mb32@columbia.edu

living systems before the advent of modern technology. Obviously, EMF must affect the same systems and reactions as were affected by other factors that played a role in the adaptation of living systems.

One of the other factors is easy to pinpoint, an ability to influence molecular interactions with water. Water is an essential component of living systems, so much so, that the search for life beyond Earth is essentially a search for the water needed to sustain it. Water has many unusual properties, among which is an ability to interact with and dissolve ions and many biopolymers. Because water hydrates molecules and forms solutions, chemical forces play a major role in biological systems. Of course, hydration forces are ultimately electromagnetic, e.g., water dipoles interacting with ions and the charged groups on proteins, but their effects are easier to describe in chemical terms and using thermodynamic properties. Natural biopolymers such as proteins and nucleic acids in solution are hydrated, and changes in charge distribution can lead to changes in molecular conformation. Such structural changes are generally accompanied by changes in hydration and very large changes in heat and entropy.

EMF interact with molecules to cause changes in charge distribution, but when considering biological mechanisms, we must also focus on the cell as the functional unit and on the ultra-thin ($\sim$10 nm) cell membrane that surrounds the cell and controls traffic in and out of the cell. The cell is sustained by biochemical reactions, many of which involve electron transfer, while cell functions are generally carried out by membrane components and involve ions. In this review, we shall consider electron and ion transport processes in solution and across membranes. We shall also discuss the effects of EMF on two major classes of biopolymers, proteins involved in transport across membranes, and the DNA in the cell nucleus that can be stimulated to initiate protein synthesis. Charge transfer due to EMF is a likely triggering mechanism in both biopolymers. The overall effect occurs in a two-step process, in which EMF move charges within the biopolymers, and the perturbations cause the biopolymers to change their conformation to accommodate the changes in charge distribution. Many of the biological examples discussed, e.g., the multi-subunit proteins, hemoglobin and Na,K-ATPase, and the DNA that initiates stress protein synthesis, are from studies carried out in this laboratory. Recent reviews describe EMF mechanisms in Na,K-ATPase (Blank, 2005) and in DNA (Blank and Goodman, 2007).

Electron Transfer in Chemical Reactions

Electric and magnetic fields exert a force on static and moving charges, and accelerate them. The largest effects of the fields are on electrons, the unit negative charges, because of their high charge to mass ratio. At the sub-atomic level, the Born-Oppenheimer Approximation assumes that electrons respond instantaneously compared to protons and heavier atomic nuclei. Even weak EMF in the low-frequency range can affect the rates of electron transfer reactions between molecules. A 10 μT magnetic field exerts a very small force of only $\sim 10^{-20}$ newtons on a unit charge, but this force can move an isolated electron more than a bond length, $\sim$1 nm, in $\sim$1 nanosecond.

Effects on electrons in chemical reactions were detected indirectly in studies of the effects of electric and magnetic fields on the Na,K-ATPase (Blank, 2005). Each field, studied separately, accelerated the reaction when the enzyme was relatively

inactive. By assuming that the same charge was affected in the two fields, one could estimate the velocity (v) and determine the nature of the charge (q) that was critical in the action of this enzyme. If both fields exerted the same force at the threshold, we can equate the electric (E) and the magnetic (B) forces:

$$F = qE = qvB. \quad (1)$$

From this $v = E/B$, the ratio of the threshold fields, and by substituting the measured thresholds (Blank and Soo, 1992, 1996), $E = 5 \times 10^{-4}$ volts/m and $B = 5 \times 10^{-7}$ T (0.5 μT), we obtain $v = 10^3$ m/s. This very rapid velocity, similar to that of electrons in DNA (Wan et al., 1999), indicated that electrons were probably involved in the ATP splitting reaction and the ion transport mechanism of the Na,K-ATPase (Blank, 2005). An electron moving at a velocity of 10^3 m/s crosses the enzyme ($\sim 10^{-8}$ m) before the 60 Hz field has had a chance to change. This means that even though a low-frequency sine wave signal was used, the effective stimulus was actually a repeated DC pulse. This is true in all low-frequency studies that involve effects on fast moving electrons.

The magnitudes of the threshold fields that affect the Na,K-ATPase are in the very low range of mV/m electric field and μT magnetic field. The very small force of $\sim 10^{-20}$ newtons on an electron and the very small dimensions and short times, calculated above, are relevant at the molecular level for the proteins and DNA that we consider in later sections. The small magnitudes also suggest boundary conditions on the responses that can be expected from weak fields. In essence, they question the possibility of direct effects of such weak fields on much more massive ions and molecules. There just is not sufficient EMF energy to cause significant movement of ions, especially if they are hydrated. Ions are affected by the much larger DC electric fields involved in physiological membrane processes, a subject treated below.

In the search for weak fields that can cause biological effects, we realized that weak DC magnetic fields are also unlikely to affect physiological processes for the same reasons. The ability of DC magnetic fields to affect lifetimes of free radical pairs (Steiner and Ulrich, 1989) only occurs at field strengths that are several orders of magnitude higher than the AC magnetic field thresholds mentioned earlier and other studies to be discussed. This review is focused on the effects of the low levels of EMF, comparable to those in the environment, that are apt to influence biological processes, so the effects of DC magnetic fields will not be considered.

Electrons are not usually invoked in the mechanism of the Na,K-ATPase, so it was necessary to demonstrate the effects of magnetic fields on electrons in known electron transfer reactions. This was done by studying electron transfer from cytochrome C to cytochrome oxidase (Blank and Soo, 1998) and in the oxidation of malonic acid (Blank and Soo, 2003), also known as the Belousov-Zhabotinsky (BZ) reaction. In both of these reactions, as well as in the Na,K-ATPase reaction, the following was true:

- Magnetic fields accelerated the rate of the reaction at very low thresholds. The experimentally determined threshold values were Na,K-ATPase (0.2–0.3 μT), cytochrome oxidase (0.5–0.6 μT), BZ reaction (<0.5 μT).
- In all three cases, magnetic fields were most effective when the intrinsic chemical forces were low, showing that EMF competes with the intrinsic chemical forces driving the reactions. To emphasize the fact that EMF will affect a reaction only

when the intrinsic chemical forces are weak, a recent study reported no effect of magnetic fields on the BZ reaction (Sontag, 2006) under conditions where the chemical forces swamped the magnetic forces. The magnetic fields were only applied well after the reaction was under way and the chemical forces had already set the oscillatory pattern of the reaction.

It was interesting that the two enzymes studied showed frequency optima close to the reaction turnover numbers, Na,K-ATPase, 60 Hz; cytochrome oxidase, 800 Hz, suggesting that the EMF were interacting optimally when in synchrony with the molecular kinetics (Blank and Soo, 2001). As we shall see in a later section, this is not true for magnetic field interactions with DNA, which are stimulated in both the power frequency and radio frequency ranges (Blank, 2007). EMF interactions with DNA do not appear to involve electron transfer reactions with well-defined kinetics. There are no other frequency data on enzymes to add to this list; studies on the enzyme ornithine decarboxylase (Byus et al., 1987) were done at 60 Hz only. While there are very few examples from which to generalize, it is reasonable to expect frequency optima only where electron transfer reactions have well-defined kinetics.

There are additional frequency data for DNA that should be mentioned, but the experiments are quite different from the above studies and the results cannot be compared. The studies involved stimulation of DNA in striated muscle to produce specific muscle proteins by stimulating (electrical) action potentials in the attached nerves. The stimulation of DNA will be discussed in detail in a later section, but the electric fields associated with the action potentials are likely to stimulate electron movement in DNA of the muscle nuclei (Blank, 1995). The two frequencies studied in muscle, high (100 Hz) and low (10 Hz) frequency, were chosen to correspond to the frequencies of the fast muscles and slow muscles that are characterized by different contraction rates and different proteins. In the experiments, either the fast or slow muscle proteins were synthesized at the high- or low-frequency stimulation rates corresponding to the frequency of the action potentials. This clear frequency dependence on electric fields was to be expected from the muscle physiology, but it is unlikely to have come from particular electron transfer reactions as in cytochrome oxidase. It is more probable that an entire region of DNA, coding for multiple proteins, was activated simultaneously.

Many of the biochemical charge transfer reactions that occur in living cells are oxidation-reduction reactions, but by and large, they have not been the concern of biologists interested in EMF mechanisms. It is the electrochemists who study electron transfer mechanisms at electrode surfaces driven by electric fields, and who ask such questions as the number of steps in a reaction, number of electrons transferred per step, rate of each step, etc. Those concerned with biological EMF mechanisms are oriented towards cell function and focus on physical chemical processes involving membranes and ions, the topic of the following section.

Cell Membranes and Ion Transfer

The functional unit in physiological systems, the cell, is surrounded by an ultra-thin ($\sim$10 nm) cell membrane having the basic structure of a phospholipid bilayer. The bilayer serves as a matrix in which many different functional elements (e.g., enzymes, channels, transporters) are embedded in varying amounts in different tissues. In the

red cell, a relatively inactive cell, the functional elements constitute about half of the membrane (Blank et al., 1979), while in active synaptic vesicle membranes there is twice as much protein by weight as lipid. A diagram of a synaptic vesicle membrane is on the cover of the November 17, 2006 issue of *Cell*.

Cells are sustained by biochemical reactions, many of which involve electron transfer, but the charge transport processes in many cell functions (e.g., nerve, muscle conduction) primarily involve ions and the much more energetic electric fields needed to transport them. This accounts for the focus on ions and electric fields as triggers of physiological processes. The word trigger is appropriate. Electric fields transfer relatively small amounts of charge that cause changes in the membrane, which then allow the normal ion gradients to cause much larger changes in the cell. This will become clearer when we discuss the effects of electric fields on ion gradients across membranes and on ion channels in membranes.

Ion transport differs from electron transport in many ways. Ions are much more massive, have both positive and negative charges, and are stable in solution. In ion transport studies carried out in electric fields, cations and anions move in opposite directions and at different speeds because of their different sizes and degrees of hydration. These differences lead to significant *ion concentration changes* due to ion transport across ion selective membranes.

Living cells have compositions that differ markedly from the surrounding solutions, so natural membranes normally separate solutions having very different ionic compositions and concentrations. K is the main intra-cellular cation and Na is the main extra-cellular cation, so large ionic gradients exist across cell membranes (see bold faced symbols K and N in Fig. 1). Most cell membranes are cation selective, and differences in the rates of diffusion of K and Na across membranes lead to membrane potentials of about 100 mV. Ionic leaks are compensated by 'ion pumps', such as the Na,K-ATPase to be discussed in a later section, so the steady-state potentials are known as resting potentials. When nerves or muscles are activated, the changes in membrane potential are called action potentials.

Because of the differences in steady-state concentrations across the membrane, and because the permeability of the membrane to K is normally much greater than to Na, small currents due to applied electric fields can cause large changes in the ionic concentrations at the membrane surfaces. Take the examples given in Fig. 1, of an electric field across a cation selective membrane that separates a cell from its surrounding solution. Both solutions contain the cations sodium (N) and potassium (K), shown with N higher outside and K higher inside, as normally distributed in cells and with the symbols for the steady state N and K in proportion to the concentrations. (The anions are the same concentration on both sides and assumed not to cross the cation selective membrane.)

An ion current, indicated by arrows, will be carried by both ions, but in different proportions because of the steady-state ion concentrations across the membrane. In the top panel for an outward current, the major part of the current is carried by K. In the bottom panel for an inward current, the major part of the current is carried by N. The main result of a sustained DC current flow in either direction, shown in italic symbols, is a decreased cation gradient across the membrane for each cation. This means that a depolarizing current that normally stimulates a nerve and causes sodium ion flux actually decreases the concentration gradient (i.e., the chemical driving force) of the sodium ions that start the action potential. The decreased cation gradients across the membrane also decrease the membrane potential and affect

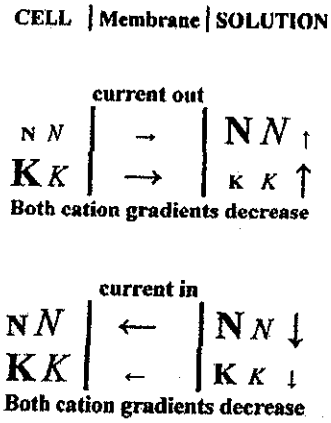

Figure 1. Changes in sodium (N) and potassium (K) concentrations at the surfaces of a cation selective membrane due to the current flow in outward and inward directions, as indicated by the arrows in the membrane. The relative sizes of the symbols N and K in the solution compartment indicate the relative concentrations in the two solutions, and the sizes of the arrows indicate the relative magnitudes of the current. The bold symbols represent the steady-state concentrations and the italic symbols show the concentrations after current flow in the two different directions. The upper diagram is for current out of the cell, when cations in the solution increase, and the lower diagram is for current into the cell, when cations in the solution decrease. Current in either direction leads to a reduction in the concentration gradients of both cations.

the distribution of charge across the membrane. As discussed in a later section, because of a direct effect on the charges as well as an indirect effect due to lowering the membrane potential, a depolarizing current opens ion channels, which are the major contributor to the increased ion fluxes. The depolarizing currents also have a direct effect through the changes in ion concentration at the membrane surfaces.

The changes in concentration at the membrane surfaces persist there, because they are dissipated slowly by diffusion into the solution. Such changes were demonstrated when the actual concentration of ions at a surface was measured by transporting surface active ions across liquid/liquid interfaces. The surface active ions carried the direct (DC) current and also indicated their presence at the interface by changes in interfacial tension (Blank and Feig, 1963). The concentration changes during current flow were significant and relatively long lived.

Intuitively, one expects that passing an alternating current (AC) through a cell might leave no net effect, because the processes during the initial half of the cycle would be canceled in the second half, when the electric field is reversed. However, it is easy to see from Fig. 1 that for cation selective cell membranes with cation gradients across them, the effects of AC on cation concentrations are additive. When considering an entire cell, the inward current directed into one side of a cell appears to be balanced by an outward current on the other side. Here again, we see from Fig. 1 that the effects on both sides of a cell are in the same direction. Cation gradients are reduced on both sides.

Because the effects on the cation concentrations are additive, even small AC electric fields lead to significant changes over time. The effects of AC currents through a simple theoretical model membrane showed that the concentrations do not increase indefinitely because of diffusion away from the surface and binding

reactions with fixed charges at the membrane surface. The effects varied with the AC frequency (Blank and Blank, 1986), depending upon the ion binding constants to fixed counter charges on the surface and ion mobilities in solution. It has been known for a long time that AC currents across nerves can reduce and block their activity. AC apparently decreases the ion gradients to the point that they can no longer drive the action potentials.

The fixed charges at a membrane surface not only can bind to the ions near the surface layer, but the change in surface charge can affect ion transport through the surface. To study the effect of the charge on a surface on the ability of ions to diffuse across the boundary, Miller and Blank (1968) used charged monolayers to show that the rate of ion transport is controlled by the charge on the surface. The effects of charged surfaces on the ability of ions to cross an interface could be explained by the expected ion concentration changes in the surface region, e.g., fixed positive charges reduce concentrations of adjacent cations and increase anion concentrations.

These studies show that ions at membrane surfaces may be important for understanding biological ion transport across membrane dimensions and in millisecond time scales. Actually, the surface concentration of ions at an axon membrane surface in the steady state is comparable to the magnitude of the ionic flux during an action potential. The number of ions stored at an axon membrane surface, having a capacitance of 10^{-6} farads/cm^2 and a resting potential of 100 mV, is about $\sim 10^{-12}$ ions/cm^2. The magnitude of the ion flows in an action potential is also about $\sim 10^{-12}$ ions/cm^2 of nerve axon membrane surface.

When discussing ion concentration changes at membrane surfaces and changes in polarization across membranes, it is important to realize that there is a major difference between the characteristic response times of chemical systems and electrical systems. In transient or non steady-state membrane processes, the two driving forces for ionic movement, the chemical potential for diffusion and the electrical potential for migration, change at very different rates. A membrane can be depolarized quite rapidly, with time constants on the order of 1–10 *micro*seconds, while chemical potentials readjust at much slower rates with time constants of about 1 *milli*second, characteristic of diffusion processes over distances on the order of cell diameters. It is therefore possible to generate unbalanced chemical gradients for short periods of time by manipulating membrane (electrical) potentials. The disparity in the response times of the two forces that drive ions across membranes can lead to unusual transient ionic fluxes.

Biological systems add an additional complication to the changes expected in physical systems, i.e., changes in ion concentration at surfaces due to depolarizing currents and due to the great disparity between the rates of change in concentration and electrical potential. In biological systems there are voltage-dependent ion channels that open when depolarized. This topic will be discussed in greater detail in a later section.

An analysis of the ion flows in excitable membranes, called the Surface Compartment Model (Blank, 1987), showed what happens when all of these factors occur in the layers of solution immediately adjacent to the membrane surfaces, specifically:

- the changes in ion concentration due to depolarizing currents (Fig. 1), ion flows under electrochemical forces (described by the same equations that apply to ions in solution), and any ion exchange between Na and K that occurred with fixed surface charges at the membrane surfaces due to changes in concentration;

- the disparity between the rates of change in ion concentration by diffusion and migration, and the much faster changes in electrical potential;
- the effects due to voltage-dependent ion channels that open by a charge transfer process shown in Fig. 3, to be discussed in detail later. The ion channels that had been incorporated into an empirical description of ion transport across membranes were complicated functions of time, while these were dependent on charge distribution.

The Surface Compartment Model was able to show that these factors could account for the unusual ionic fluxes seen in excitable membranes. It also showed how the apparent selectivity of channels could vary with different rates of opening. This description of ionic fluxes in excitable membranes offered insights into factors that contribute to the unusual fluxes and the apparent ion selectivity in channels.

It is obvious that the electrical activity that drives nerves and muscles utilize mechanisms that take advantage of ionic gradients that are normally present in living systems. These ionic gradients are built up by the action of membrane enzymes like the Na,K-ATPase and are fueled by the energy from the splitting of ATP. Consequently, it takes relatively little energy to trigger an action potential and take advantage of the energy stored in the ionic gradients across cell membranes. The ion fluxes that evoke an action potential are very weak stimuli by comparison. However, it does take energy to open the voltage gated ion channels and various transporters in the membrane. This source of energy, triggered by changes in charge, is the conformational energy stored in chemical structures. This is a probable explanation for the way ion channels are stimulated to open by depolarizing currents, and also for the way very weak EMF can stimulate responses in DNA, both of which require considerable energy.

Proteins and Hydration Energy–Hemoglobin Equilibria

The energetics of intermolecular interactions and interactions with water as a solvent determine membrane structure, as well as the changes that occur when perturbed by applied EMF. Among the early attempts to understand the energetics of chemical structures and their relation to chemical properties, Langmuir (1916) showed that the surface tension of a pure liquid could be derived from information about the interaction energy between molecules. Vaporizing a liquid breaks all bonds between molecules, while molecules at a liquid surface are not completely surrounded and miss interactions with the missing neighbors. It is the missing interactions that give rise to the surface tension. The unbalanced energy at a surface requires molecules to have extra energy to get to the surface, and that the liquid minimizes the energy and the surface area. Langmuir's success in relating surface tension to heat of vaporization indicated that nearest neighbor interactions account for most of the energy, and that the change in surface free energy (i.e., the surface tension) is a good approximation to the total free energy change.

The situation in aqueous solutions is more complex, but we have estimated the total free energy change of a molecule in solution from the changes in surface area when interacting with water. In aqueous solutions, the interactions with water are quite energetic and have a profound influence on equilibria, especially those involving proteins. Lauffer (1975, 1989) characterized the aggregation of multi-subunit

protein molecules in aqueous media using the short-hand phrase "entropy driven" to summarize the energetics of the interaction. The aggregation is spontaneous (i.e., the free energy change, ΔF, is negative) and it occurs with an evolution of heat (i.e., the enthalpy change, ΔH, is positive). The negative ΔF together with the positive ΔH means that there is a large positive entropy change. Hence, "entropy driven". The large increase in entropy is due to release of many water molecules when the hydrated proteins come into contact after releasing their bound water. The increase in ΔH is another consequence of the release of water from protein surfaces and the aggregation of the protein subunits.

This description is correct but incomplete, because aggregation is very dependent on pH while hydration is not. The pH affects molecular charge, since it is well known that proteins disaggregate as the charge increases, and they aggregate as the charge decreases. Two often quoted examples are hemoglobin (Fanelli et al., 1964) and tobacco mosaic virus protein (Klug, 1979). It is possible to extend Langmuir's approach to include an effect of charge. The aggregation of multi-subunit proteins with a decrease in molecular charge can be formulated as a simple relation between molecular charge and the area of the protein molecule in contact with aqueous solvent. The basic idea is that proteins in aqueous media minimize their surface free energy by decreasing contact with the water *and* decreasing charge. For this reason, decreases in charge drive the protein toward aggregation. However, when there is an increase in charge, the two driving forces compromise and there is an increase disaggregation. The repulsive forces between charges would increase the surface free energy, and this can only be reduced by an increase in area. Disaggregation spreads the charges and lowers the repulsion between them.

This simple model using surface free energy to account for the influence of charge on subunit assembly was shown to apply quantitatively to the protein, hemoglobin (Hb) as a function of surface charge (Blank and Soo, 1987). The actual study, the disaggregation of the Hb tetramer $(\alpha\beta)^2$ into 2 dimers $(\alpha\beta)$, where α and β are protein subunits, showed that the concentration of hemoglobin dimers increased linearly with surface charge as the pH varied from the isoelectric point. As the Hb tetramers were disaggregating, the increasing charge was being spread over an increasing protein/water interface, and the surface charge density (total charge/total molecular area) remained constant.

The same surface free energy model could also account for the unusual effects of increasing concentration of Hb on the viscosity of solutions (Blank, 1984) if one assumes that the increase in viscosity with Hb concentration is due to aggregation into larger particles. The same forces that drive the aggregation of dimers to tetramers should continue because of the same loss of area upon aggregation:

$$\alpha\beta \rightarrow (\alpha\beta)^2 \rightarrow (\alpha\beta)^3 \rightarrow (\alpha\beta)^4 \rightarrow (\alpha\beta)^5. \qquad (2)$$

At the point where the chain becomes long enough to close upon itself, there should be a steep change in the equilibrium. The closing of the chain means that an added $\alpha\beta$ has caused two links, with double the loss of interfacial area and double the free energy change. A closed chain would also account for the steep increase in viscosity, since a chain where the ends are joined is no longer as flexible and behaves more like a rigid rod.

The relation between changes in interfacial area and the free energy change enabled a semi-quantitative estimate of the energy change due to the changes in molecular shape when Hb is oxygenated. The conformational changes in Hb, documented by X-ray crystallography, enabled estimation of the interfacial area. The charge on the Hb at different pH's could be determined from titration studies, such as those in the study of disaggregation. The data enabled calculation of the acid and alkaline Bohr effects, the names given to the variation of the oxygenation equilibrium constant with pH and ionic strength (Blank, 1975).

The success of the surface free energy model in calculating the acid and alkaline Bohr effects demonstrated the predictive value of the relation between changes in surface free energy and the equilibrium constant. This idea also led to understanding the physical meaning of the empirical Hill coefficient that is widely used as a measure of cooperativity. By using the surface free energy model to estimate conformational changes (e.g., Hb), it was possible to show that the changes in free energy that affect the equilibrium constant are simply related to the Gibbs surface excess, a fundamental property in surface chemistry (Blank, 1989). According to the surface free energy model, the Hill coefficient is not empirical and is not constant. It varies with the degree of reaction, has a maximum value at the half way point, and is definitely equal to unity at both extremes. The approach to unity has been observed in the reaction between Hb and oxygen (Paul and Roughton, 1951).

The surface free energy model is a way to estimate the energy changes due to the hydration of nascent hydrophilic surfaces of biopolymers, such as proteins and nucleic acids, in terms of the surface free energies of newly formed surfaces. To make calculations, one needs estimates of surface areas and surface charge, so it has been relatively easy to apply these ideas to many properties of Hb, a well-characterized molecule. The model has also related the conformational changes of voltage-gated channel proteins (Blank, 1987, 1989) to the depolarizing currents that transfer charge across a channel, and the conformational changes of the Na,K-ATPase (Blank, 2005) and other membrane transporters to the charge movement when ATP splits. The same effects of EMF on charge movement may account for the ability of EMF to cause DNA to initiate protein synthesis (Blank and Goodman, 2007). These are examples of biological amplification that are related through the ability of small charge movements to stimulate large structural changes utilizing the energy stored in biopolymer conformation. The following three sections are devoted to Na,K-ATPase, ion channel proteins, and DNA.

Membrane Transport Proteins–Na,K-ATPase

Many of the biological transport systems embedded in membranes are multi-subunit proteins that can open to both sides of a membrane in alternate conformations. This process enables the binding of substances to one side of the protein and subsequent release to the other side after a conformation change. The opening of a transporter creates new protein water interfaces and involves changes in binding of the subunits with each other, the water and the bilayer lipids. Similar reactions occur when the protein opens on the other side. If the two open states of the protein on opposite sides of the membrane were of approximately the same energy, it would minimize the energy required for the transport. In transport, the conformation change is usually triggered by the energy released from th ATP splitting reaction.

This type of transport mechanism has been documented for many different substances. A short list of recent articles includes studies on β-galactosidase and glucose-6-phosphate (Locher et al., 2003), various drugs (Dong et al., 2005; Yin et al., 2006; Reyes and Chang, 2005), zinc (Lu and Fu, 2007), metal chelates (Pickett et al., 2007), and vitamin B12 (Hvorup et al., 2007). The mechanism is best known from its association with the Na,K-ATPase, the enzyme that "pumps" Na and K ions against their gradients across cell membranes.

The Na,K-ATPase is probably the best studied of this class of transporters, known as ABC (ATP Binding Cassette) transporters, and as such it offers insights into how ATP driven conformation changes can occur in bilayer structures. The lipid bilayer membrane is stable because of hydration forces, and the term hydrophobic interactions used to describe these forces indicates that the lipid molecules interact with each other and avoid contact with water molecules. Exposing bilayer lipid molecules to water is energetically unfavorable, so membrane transport mechanisms utilize multi-subunit proteins in the bilayer that have hydrophobic areas that can interact with lipid molecules in the bilayer and hydrophilic areas that can interact with water at the surfaces. Because of their compositions, transporters can flip their conformations from inner-face-open-to-water to outer-face-open-to-water to enable the transfer of molecules by expanding the hydrophilic areas and contracting the hydrophobic and vice versa. In the Na,K-ATPase the different conformations are determined by the binding of Na, K and ATP.

The Na,K-ATPase is composed of two polypeptide chains (α and β) that extend through the bilayer in the form of a tetramer ($\alpha_2\beta_2$). The ATPase activity resides in the α chain and is directly influenced by the ion concentrations in contact with the two sides of the enzyme (Skou, 1957; Tonomura, 1986; Lauger, 1991; Jorgensen et al., 2003). The Na,K-ATPase is activated when sodium ions bind on the inside surface and potassium ions on the outside surface. In a complete cycle, the catalytic unit splits ATP on the inside surface, and for each ATP molecule split, $3\,Na^+$ ions move from inside out and $2\,K^+$ ions from outside in.

The enzyme complex has two conformations, E_1 when Na^+ ions (and ATP) are bound on the inside, and E_2 when K^+ ions are bound on the outside. The ion binding sites are not fully accessible to ion exchange with the surrounding solutions in the two conformations (Rephaeli et al., 1986; Glynn and Karlish, 1990). Potential sensitive dyes show charge shifts at specific points in the ATP-splitting cycle (Buhler et al., 1991). A release of Na^+ ions accompanied a rapid movement of charge when binding sites open to the outer surface in the presence of Na^+ ions (Hilgemann, 1994). These data suggest that conformational changes of the Na,K-ATPase and charge shifts within the protein are involved in the mechanism. The effects of applied low frequency electric and magnetic fields on Na,K-ATPase function, presented earlier, provide additional evidence of rapid charge movement that contributes to the conformation change after the enzyme has reacted.

The key to the conformation change is the rapid shift of charge across the enzyme. Figure 2 illustrates changes in a protein channel that starts with an asymmetric charge distribution. The outside surface is normally negatively charged, and the charged groups interact with water. This expanded area of contact with water is open to the outside. A significant shift in charge causes the channel to shift from an inside facing channel to an outside facing channel. If the charges crossing the enzyme are electrons, they cross very rapidly to the opposite side of the enzyme, and the ratio of charged hydrated area and uncharged unhydrated area remains virtually unchanged. With

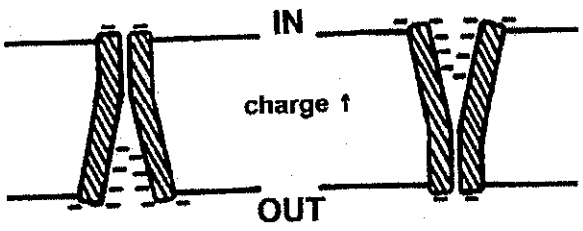

Figure 2. Changes in a protein channel that starts with an asymmetric charge distribution, and there is a large and rapid shift in charge as indicated by the arrow. The outside surface is initially negatively charged, and an expanded area of contact with water faces the outside. A large shift in charge causes the channel to change from an inside facing channel to an outside facing channel. If the charges crossing the enzyme are electrons, they cross very rapidly to the opposite side of the enzyme, and the ratio of charged hydrated area and uncharged unhydrated area remain virtually unchanged and with virtually no net change in energy.

virtually no net exothermic aggregation or endothermic disaggregation, the conformation change probably occurs with a minimum of energy change.

It is not generally accepted that ATP splitting and the accompanying ion transport involve electron transfer. However, it is quite clear from EMF measurements discussed earlier that there is a rapid flow of charge through the enzyme, resulting from the enzyme reaction. This flow of charge could trigger the sequence of conformation changes that are part of the cation transport mechanism (Blank, 2005). The effective concentrations of non-specific cation inhibitors of the Na,K-ATPase were related to the redox potentials (Britten and Blank, 1973), suggesting involvement of an electron transfer step. Many observations associate electrons with the ATPase reaction. In mitochondrial function, the ATP synthase catalyzes the same reaction and is directly coupled with electron transport. In the ATP synthase, it is possible to stop the flow of electrons in the electron transport chain with inhibitors, or to reverse the flow of electrons by changing the concentration of substrates. The electron transport chain can also be made to go in reverse when ATP is hydrolyzed and electrons are fed into the chain.

In line with the known reversibility of ATPases in mitochondria, Garrahan and Glynn (1967) were able to reverse the Na,K-ATPase reaction in red cells to generate ATP. They did this by creating a supernormal K ion gradient, thus hyperpolarizing the membrane. When the membrane potential changed from −15 mV to −85 mV, they were able to approximately double the ATP concentration from ADP and phosphate normally present. The increase in membrane potential makes the region near the catalytic portion of the Na,K-ATPase on the inner surface of the membrane more negative. The increase in H ion concentration near the enzyme would be expected to drive the reaction toward making more ATP. In any case, the experiment clearly shows the tight coupling between the ATPase reaction and ion flow across the membrane. It also shows their similar reversibility to charge flow, the Na,K-ATPase to ion flow and the mitochondrial ATP synthase to electron flow.

Charge Transfer and Ion Channel Function

In the section on ion transfer, the transient ion flows in excitable membranes were described in terms of concentration changes in the layers of solution immediately

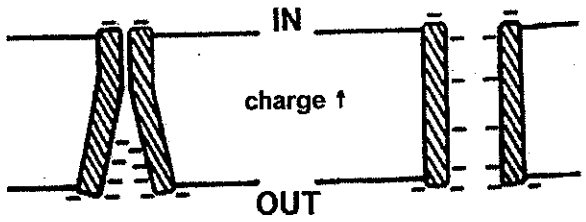

Figure 3. Changes in a protein channel that starts with an asymmetric charge distribution, and a large portion of the charge shifts rapidly, as indicated by the arrow, to spread across the length of the protein in the bilayer. If the charges crossing the enzyme are electrons, they can spread out very rapidly. The shift in charge is sufficient to open a hydrophilic channel and create a conduit for ions from inside to outside solutions. This implies that the charged parts of the protein that interact strongly with water create a continuous aqueous path. Because there is a change in the ratio of charged hydrated area and uncharged unhydrated area, this process must result in a significant change in energy. The distribution of charge depends on the membrane polarization, and if the charge movement is reversed by repolarization, the channel closes.

adjacent to the membrane surfaces. These thin regions were referred to as surface compartments and the equations describing the processes as the surface compartment model (Blank, 1987). The main processes were variations in Na and K ion concentrations due to depolarizing currents, ion exchange between ions in solution and those bound to fixed surface charges at the membrane surfaces, and the very different rates of ion concentration changes by diffusion and changes in electrical potential. Voltage-dependent ion channels that open and close depending on changes in charge distribution were included in the description, but a fuller discussion was deferred until after the section on transport mechanisms in the lipid bilayer.

The discussion of voltage dependent ion channels is easier to understand following the section on multi-subunit protein transporters that flip from inner-face-open-to-water to outer-face-open-to-water. Proteins like the Na,K-ATPase can apparently negotiate these changes with a minimum change in hydration energy by keeping the ratio of hydrated and unhydrated protein surfaces relatively constant during the charge transfer. However, this does not appear to be possible with the opening of an ion channel, where the whole length of a hydrophilic pathway through the bilayer must be open to enable the continuous flow of ions. Figure 3 shows a protein channel that starts with an asymmetric charge distribution, and where a large portion of the charge spreads across the length of the protein in the bilayer. If the charges are electrons, they can spread very rapidly. The change in the ratio of charged hydrated area and uncharged unhydrated area must result in a significant change in energy, and the energy change must be reversed when channel returns to its resting state, i.e., closes.

The surface free energy model can relate the opening of voltage gated channel proteins (Blank, 1987, 1989) to charge transfer due to the depolarizing currents, and it also provides a way to evaluate the energy changes that occur. The process shown in Fig. 3 assumes that the gating currents in excitable membranes transfer charge across the protein, and this changes the energetics of the channel protein to favor opening a channel. Since disaggregation is endothermic and aggregation exothermic, the model predicts an initial cooling as protein contacts water on channel opening, followed by heating on channel closing. The thermal changes should be quite large because of the

nature of hydration interactions and the protein surface areas involved. As described below, thermal changes occur, but not quite as predicted by Fig. 3.

Thermal measurements are generally difficult, especially when the changes are rapid and the systems small, as with nerves. It is always difficult to get an accurate measurement of temperature change when the action potentials in nerves are faster than the response time of the thermal sensors. Also, action potentials involve the opening and closing of two sets of channels at different rates. There are Na channels that enable the initial rapid depolarization, and K channels that account for the slower repolarization phase but that may open at the same time. The effect of an overlap of opening and closing on the temperature sensor further complicates the analysis. To add to the difficulties, even the easiest nerves to study contain many axons that conduct action potentials at different rates, so there is some interference because of slow and fast conducting axons. Analyzing these data is an unenviable challenge.

Despite the difficulties, thermal measurements have been made and analyzed, and there is agreement about the observations. In excitable membranes, the heat associated with excitation of nerve (Howarth et al., 1968) or electric organ (Keynes and Aubert, 1964) shows three distinct phases during an action potential. There is an initial, short-lived warming phase followed by a longer cooling phase of comparable amplitude and a still longer warming phase having the largest amplitude and most probably associated with recovery mechanisms. The net heat evolved is actually small in comparison with the initial heating and cooling, suggesting that the net heat is a measure of the dissipation due to the flow of ions down electrochemical gradients, and the chemical bond energy used to restore the ionic gradients.

It is difficult to interpret the measurements in terms of channel protein interactions, because there are multiple sources of thermal changes. These include current flow during the action potential, discharging and recharging the membrane capacitor, ion pumping during recovery, etc. The major changes of heat appear to be due to reversible processes, and the discharging and recharging of the membrane capacitor can account for about half of the reversible heat change observed. The changes in hydration energy during channel opening and closing are another source that may account for the reversible changes. It would be hard to find another source for the large negative heat, which is a major unexplained aspect of the process.

We can estimate the energy changes from channel opening and closing, assuming that the number of sodium channels per unit area of membrane is the same as in unmyelinated C fibers of rabbit vagus nerve (Howarth et al., 1968) of 110 nmol/kg wet weight. C fiber diameters range from 0.4–1.2 μm, so assuming an average diameter and a density of Na channels comparable to the squid axon (Levinson and Meves, 1975), it is possible to estimate the measured heats per gram from the estimated positive heat of 25 μcal/g and the negative heat of 22 μcal/g. If all of the ΔH were due to the reactions of the proteins in the channels, the negative heat is a better measure of the largely reversible ΔH for channel opening and closing. In that case, the reversible channel process involves a ΔH of about 6 kcal/mole of channel protein (molecular weight 270 kD), or about .02 cal/g of channel. The ΔH for the aggregation of tobacco mosaic virus protein (Klug, 1979) is about 0.7 cal/g. This implies that only about 3% of the protein surface is involved in the reactions affecting channel opening and closing. Since the discharging and recharging of the capacitor can account for about half of the reversible heat change observed, only $\sim 1\%$ of the protein surface can account for the unexplained heat.

The measured heats appear of reasonable magnitude, but the sequence is at odds with what would be expected if the simple model depicted in Fig. 3 were the only source of heat exchanged. One expects the positive ΔH for channel closing to coincide with the falling phase of the action potential, and channel opening should be associated with a negative ΔH or heat absorption. The channel is certainly more complicated than the model in Fig. 3, and so are the thermal changes. The heat evolved during the discharging of the membrane capacitor is simultaneous with the heat absorbed during channel opening. The two are also opposed during repolarization and channel closing. Furthermore, the discharging is much faster than the recharging. Undoubtedly, the thermal measurements are missing a large part of the heat exchanged, and the heat changes associated with channel opening and closing are therefore much greater than we have estimated and involve a much larger fraction of the protein surface.

In the absence of an all-inclusive and accurate analysis of all the thermal contributions to the measurements, it is nevertheless clear that an action potential is accompanied by:

- a net heat evolution as one would expect in a dissipative process;
- a reversible heat due to discharging and recharging the membrane capacitor; and
- a reversible heat of channel opening and closing due to the hydration energy associated with a small part of the protein surface.

A recent article accounts for the unexplained heat changes during an action potential by suggesting the possibility of soliton propagation in the membrane lipids as the source (Jackson, 2005; Heimburg and Jackson, 2006). The authors point out that this idea can also account for the well-known Meyer-Overton correlation between the effective concentrations of a wide range of anesthetics and their oil/water partition coefficients. The Meyer-Overton correlation is not a particularly good test, because many theories predict that correlation. In a review on anesthesia, Vandam (1966) referred to two then popular new theories of anesthesia—Pauling's clathrate formation theory and Miller's dissociation pressure of hydrates—and pointed out that any theory based on weak interactions between anesthetics and other molecules is bound to correlate with the Meyer-Overton data.

A better counter argument to the soliton proposal is probably invoking Ockham's razor rather than a detailed discussion and evaluation. Simply stated, voltage-gated ion channels are acknowledged by all to be clearly associated with the action potential, and the properties of these essential proteins may be able to account for the thermal observations without the need to turn to the properties of the matrix in which the channels are embedded. It could be that some of the optical properties ascribed to the lipids by Heimburg and Jackson are also associated with the much larger structures that appear to be parts of channels, such as the cytoplasmic components of the K channel (Long et al., 2005). Certainly, the observed changes in the thermodynamic properties are to be expected from the protein channels.

Electromagnetic Field Stimulation of DNA

One of the earliest biological effects of EMF to be described was the ability to stimulate biosynthesis (Goodman et al., 1983; Goodman and Henderson, 1988). Since those early experiments, it has been shown that EMF in both extremely low frequency

(ELF) and radio frequency (RF) ranges stimulate protein synthesis. This means that even the weak EMF in the ELF range have made DNA come apart to initiate protein synthesis. So it is no surprise that EMF can cause dose dependent, single and double strand breaks in DNA at higher field strengths and higher frequencies (Lai and Singh, 1997; REFLEX Report, 2005; Ivancsits et al., 2005; Winker et al., 2005).

The data suggest that weak EMF produce strains in DNA that can cause the chains to separate, and if the strains are large enough, cause the chains to break. Since DNA is held together by H-bonds, and since EMF are most likely to act on electrons, EMF probably act on electrons in the H-bonds to weaken the bonds. Electrons could also be affected in the H-bonds that flicker in water at a frequency $\sim 10^{15}$ Hz, and that would be expected to do so in aqueous solutions as well (Fecko et al., 2003; McGuire and Shen, 2006). This would create many transient protons and electrons in and around the DNA solution that can be accelerated by EMF.

In research focused on the stimulation of a specific stress protein, hsp70 (Goodman and Blank, 1998; Blank and Goodman, 2002, 2004), it has been possible to identify specific DNA sequences in the promoter of this protein that are needed for the EMF response (Lin et al., 1999, 2001). This was clearly demonstrated when the EMF responsive DNA sequences were transfected into the promoter of a reporter gene, and the reporter gene responded to EMF (Lin et al., 2001). The EMF responsive DNA sequences on the promoter contain sites with bases CTCT that appear to be essential. CTCT bases have low electron affinities, so electrons would be more easily displaced. Also, the CTCT are pyrimidines, and when the H-bonds split between CTCT and the GAGA (purines) bases on the complementary chain, there is a smaller smoother area that would make it easier to disaggregate.

When electrons are displaced by EMF, it can be shown that there is a favorable energy balance in the DNA disaggregation that enables the process to proceed. Strong reactions between the newly exposed DNA surfaces and water contribute to the energetics of the process. Blank and Goodman (2007) estimated the energies associated with the changes, and showed that the aggregated and disaggregated DNA structures can have equivalent energies. A simple model of disaggregation due to an increase in charge at a local site shows that an increase in area lowers the increased charge density, and that DNA cleavage would be optimal for short segments and low initial charge. The essential CTCT sites identified on the promoter may be sites of DNA cleavage or sites from which electrons have been displaced. In DNA, the initial charge can fluctuate, since electrons in DNA are not localized and are able to move as a result of the random fluctuations in H-bonded networks. This would mean that the area of DNA exposed to water molecules also fluctuates, on a slower time scale, and that some fluctuations may produce large temporary increases in local charge density. At that point, the two DNA chains would come apart to create more surface in contact with water.

The method to estimate the energy change at the DNA site associated with the response to EMF uses the same criterion as in the disaggregation of multi-subunit proteins due to charging. In proteins, where Q is the initial charge and A the area of protein exposed to water, we found that the surface charge density, Q/A, remained constant while both Q and A increased (Blank and Soo, 1987). In DNA, Q is the initial charge due to partially ionized phosphate groups and A the initial area of a DNA segment exposed to water. We assumed the surface charge density, Q/A, remained constant while both Q and A increased. This way the tendency to minimize

the surface and to spread the charge over the maximum surface (thereby minimizing the repulsion between charges) was balanced. The separation of the DNA chains enables initiation of transcription.

If ΔA is the extra area that opens up to water when 1 charge is added to a segment having an initial charge, Q, we can set the charge density before equal to the charge density after a split

$$\frac{Q}{A} = \frac{Q+1}{A+\Delta A}. \tag{3}$$

From this,

$$\Delta A = \frac{A}{Q} = \frac{1}{\text{charge density}}. \tag{4}$$

This means it is easier to open up a larger ΔA if one starts with a larger A, but not so large as to minimize the effect of adding one charge. Also, the fractional increase in open area will be greater as the charge density decreases. In any case, the opening must be large enough to allow access to the transcription enzymes. The optimal segment size may be the four base pair CTCT that was found to be associated with the EMF response.

The stimulation of DNA by magnetic fields is related to the physiological mechanism in striated muscle, where electric fields (not EM fields) associated with action potentials stimulate the DNA in muscle nuclei to synthesize muscle proteins *in vivo* (Blank, 1995). The effect is due to the electric field stimulus, since there is a clear relation between the muscle proteins synthesized and the frequency of the action potentials. Under normal physiological conditions, an action potential along a muscle membrane creates an electric field estimated at $\sim 10\,V/m$ (Blank and Goodman, 2004). In striated muscle, this electric field drives the currents across the DNA in nuclei that are normally adjacent to the membrane carrying the action potential, and the DNA is stimulated to synthesize different muscle proteins in response to the frequency of the action potentials. The magnitude of electric field provides a large safety margin in muscle, since fields as low as $3\,mV/m$ stimulate HL60 cells, and the threshold electric stimulus for the Na,K-ATPase is even lower, at $\sim 0.5\,mV/m$ (Blank and Soo, 1992).

This model based on an ability to displace charges in DNA can account for observations on activation of DNA by either electric or magnetic fields. The same effects should be stimulated by a wide range of frequencies. ELF and RF frequencies have been shown to stimulate stress protein synthesis (Blank, 2007) and because of the relation to H-bond fluctuation frequencies described earlier, there is reason to believe that frequencies up to $\sim 10^{15}\,Hz$ would be effective (Blank and Goodman, 2007).

The Proposed Mechanism in Perspective

EMF do not have sufficient energy to directly affect large protein and DNA molecules, but even weak electric and magnetic fields can cause changes in charge distribution that trigger large structural changes in proteins. Electric and magnetic fields can move both ions and electrons, but they require very different energies

because of the different masses of the charged particles. The electric fields that normally affect ions in physiological systems are orders of magnitude stronger than the magnetic fields that affect electrons. Yet, both initial reactions cause changes in charge that couple with chemical forces and provide sufficient energy to trigger physiological processes. Much of the energy in biopolymer conformations is in the form of hydration energy, and this energy can drive many of the physiological processes stimulated by EMF. The similar effects on DNA when stimulated at high or low frequencies suggests that the biological mechanisms utilize the hydration energy stored in molecular conformations, even when strong EMF forces are available.

Biological systems tend to be energy efficient even when large energy stores are available to drive these processes. The chemical changes in biopolymers triggered by charge movements frequently involve conformational changes between structures of approximately equal energy. Also, biological systems appear to use a wide range of frequencies to drive these processes. The few biochemical reactions that show a frequency dependence (Blank and Soo, 1998b) suggest synchronization of the signal with the kinetics of the reaction. On the other hand, EMF stimulation of stress protein synthesis occurs in many cells with a wide range of frequencies (Blank, 2007).

The purpose of this review has been to develop an understanding of possible biological mechanisms of EMF based on experimental results. However, it is important that the proposals should also be considered in the context of a more general discussion in the EMF literature. In the past, a frequent criticism of experimental EMF studies describing biological changes has been the absence of a mechanism to account for the effects of weak EMF. The absence of a theoretical framework was often presented as an indication that the results were not possible. Despite the clear experimental evidence of repeatable biological effects, this point of view was made to sound plausible by the relatively large energy demands of the biological phenomena ascribed to stimulation by weak EMF. The present proposal indicates a huge energy source that can account for many biological phenomena, including those stimulated by EMF.

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EXHIBIT H

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Original Contribution

Residence Near Power Lines and Mortality From Neurodegenerative Diseases: Longitudinal Study of the Swiss Population

Anke Huss, Adrian Spoerri, Matthias Egger, and Martin Röösli for the Swiss National Cohort Study

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The relation between residential magnetic field exposure from power lines and mortality from neurodegenerative conditions was analyzed among 4.7 million persons of the Swiss National Cohort (linking mortality and census data), covering the period 2000–2005. Cox proportional hazard models were used to analyze the relation of living in the proximity of 220–380 kV power lines and the risk of death from neurodegenerative diseases, with adjustment for a range of potential confounders. Overall, the adjusted hazard ratio for Alzheimer's disease in persons living within 50 m of a 220–380 kV power line was 1.24 (95% confidence interval (CI): 0.80, 1.92) compared with persons who lived at a distance of 600 m or more. There was a dose-response relation with respect to years of residence in the immediate vicinity of power lines and Alzheimer's disease: Persons living at least 5 years within 50 m had an adjusted hazard ratio of 1.51 (95% CI: 0.91, 2.51), increasing to 1.78 (95% CI: 1.07, 2.96) with at least 10 years and to 2.00 (95% CI: 1.21, 3.33) with at least 15 years. The pattern was similar for senile dementia. There was little evidence for an increased risk of amyotrophic lateral sclerosis, Parkinson's disease, or multiple sclerosis.

dementia; neurodegenerative diseases; radiation, nonionizing

Abbreviations: ALS, amyotrophic lateral sclerosis; CI, confidence interval; ELF-MF, extremely low frequency magnetic field(s); ICD-10, *International Classification of Diseases, Injuries, and Causes of Death*, Tenth Revision.

Research on the long-term effects of extremely low frequency magnetic fields (ELF-MF) has focused on cancer since Wertheimer and Leeper (1) published their results on childhood cancer and wiring configurations in 1979. In 2001, the International Agency for Research on Cancer classified exposure to residential magnetic fields above 0.4 μT as a "possible" cause of childhood leukemia (2). For noncancer endpoints, an initial report by Sobel et al. (3) on occupational ELF-MF exposure and Alzheimer's disease suggested that the risk could be substantial. Studies published subsequently have produced inconsistent results, but a recent meta-analysis (4) reported elevated risks in cohort, as well as case-control, studies. A recent review of the evidence for an association between ELF-MF and Alzheimer's disease by the World Health Organization (5) concluded that the available data were inadequate, and the topic was identified as a key research priority.

To our knowledge, no study has so far examined whether residential exposure from power lines is associated with an elevated risk of neurodegenerative diseases. Even a small association could be of high public health relevance, since a considerable number of persons are exposed to these fields. For example, 9.2% of the Swiss population live within 600 m of a 220 or 380 kV power line. We used the Swiss National Cohort, a longitudinal study of the Swiss population (6), to investigate whether living in the vicinity of power lines was associated with mortality from neurodegenerative diseases such as Alzheimer's disease, senile dementia, amyotrophic lateral sclerosis (ALS), multiple sclerosis, and Parkinson's disease.

MATERIALS AND METHODS

Study population

The present analysis was based on the 2000 national census. Mortality data were available for the period 2000–2005,

Correspondence to Dr. Anke Huss, Institute of Social and Preventive Medicine, University of Bern, Finkenhubelweg 11, CH-3012 Bern, Switzerland (e-mail: ahuss@ispm.unibe.ch).

Table 1. Number of Deaths by Cause, Recorded in Swiss Mortality Data Between December 4, 2000, and December 31, 2005

	ICD-10 Codes	Total No. of Cases[a]	No. of Included Cases[b]	% of Total Cases	Mean Age at Death (Interquartile Range), years	Female Cases, %
All causes		294,833	282,378	96	78.2 (71.6–88.5)	51
Alzheimer's disease	G30	9,758	9,228	95	85.3 (80.0–90.5)	68
Senile dementia	G30, F00, F03	29,975	28,288	94	86.9 (82.7–91.7)	68
Amyotrophic lateral sclerosis	G12.2	759	744	98	70.3 (63.5–79.0)	46
Parkinson's disease	G20–21	6,994	6,683	96	83.7 (79.6–88.8)	48
Multiple sclerosis	G35	838	773	92	67.0 (57.9–77.4)	67
Cancer of the trachea, bronchus, or lung	C33–C34	14,384	14,281	99	70.0 (62.4–78.1)	26
Cancer of the esophagus	C15	2,119	2,101	99	70.5 (61.5–79.9)	24
Alcoholic liver disease	K70	3,356	3,303	98	63.4 (55.4–71.5)	28

Abbreviation: ICD-10, *International Classification of Diseases, Injuries, and Causes of Death*, Tenth Revision.
[a] Deaths that could be linked to the census (refer to the text).
[b] Excluded were persons with unknown building coordinates or who were under 30 years of age at the start of follow-up or death.

with causes of death coded according to the *International Classification of Diseases, Injuries, and Causes of Death*, Tenth Revision (ICD-10). Enumeration in the 2000 census is nearly complete: Coverage was estimated at 98.6% (7). Deterministic and probabilistic record linkages were used to link census records to a death record or an emigration record (6). Of death records of persons older than 30 years, 95.1% could be successfully linked to a 2000 census record. At present, the database includes follow-up data until December 31, 2005.

We excluded persons aged 29 years or less at the census, as well as persons with incomplete information on building coordinates. The database contains information on age, sex, marital status, education, and occupation, as well as additional variables describing, for example, the degree of urbanization of the area or building characteristics such as the number of apartments per building. The geo-coded place of residence of the participants (i.e., Swiss-grid coordinates extracted from the Swiss building registry) is also included in the census data. In general, these coordinates pinpoint a location within a few meters of the building's midpoint. Data from the 1990 census were used to identify the place of residence at that time. The 1990 and 2000 censuses additionally include information on whether individuals had lived at the same place 5 years before the census, that is, in 1985 or in 1995. We were thus able to identify persons who had lived at their place of residence for at least 5, 10, or 15 years.

Outcomes

We considered deaths from the following neurodegenerative diseases: Alzheimer's disease, senile dementia, ALS, Parkinson's disease, and multiple sclerosis. These diseases had to be listed on the death certificate as the primary or a concomitant cause of death. The recording of neurodegenerative diseases on death certificates might be related to socioeconomic position. We therefore included outcomes that are known to be related to socioeconomic position: cancer of the trachea, bronchus, or lung; alcoholic liver disease; and all-cause mortality. The ICD-10 codes used are listed in Table 1.

Exposure

Exposure assessment was based on the distance of the place of residence to the nearest power line. We included all 220–380 kV power lines in Switzerland, over 5,100 km in total. We obtained geodata of the power lines from the Federal Inspectorate for Heavy Current Installations. Figure 1 illustrates localization of the power lines and buildings in Switzerland. We determined the shortest distance to any of the transmission lines and derived the number of persons living within the corridors around the power lines. We defined corridors of 0–<50 m, 50–<200 m, 200–<600 m, and 600 m or beyond. We determined exposure at the time of the 2000 census.

Information about the use of a building as a clinic or nursing home was available from a separate building record, which was completed by the owner of the building, and this information was then matched to the personal records of individuals. Some persons might live in a nursing home or clinic because of a neurodegenerative disease. Therefore, in order to obtain more appropriate exposure data for individuals living in such an institution in 2000, we used the exposure for the place of residence at the time of the 1990 census instead. Persons who lived in a nursing home or clinic in 1990 were excluded from the analysis.

Statistical analyses

We analyzed data using Cox proportional hazard models. We compared the risk of dying from neurodegenerative diseases across corridors and according to the duration of residence in exposed corridors (at least 5, 10, and 15 years). Person-years of observation were defined as the interval between December 4, 2000 (the date of the census), and death, emigration, or December 31, 2005.

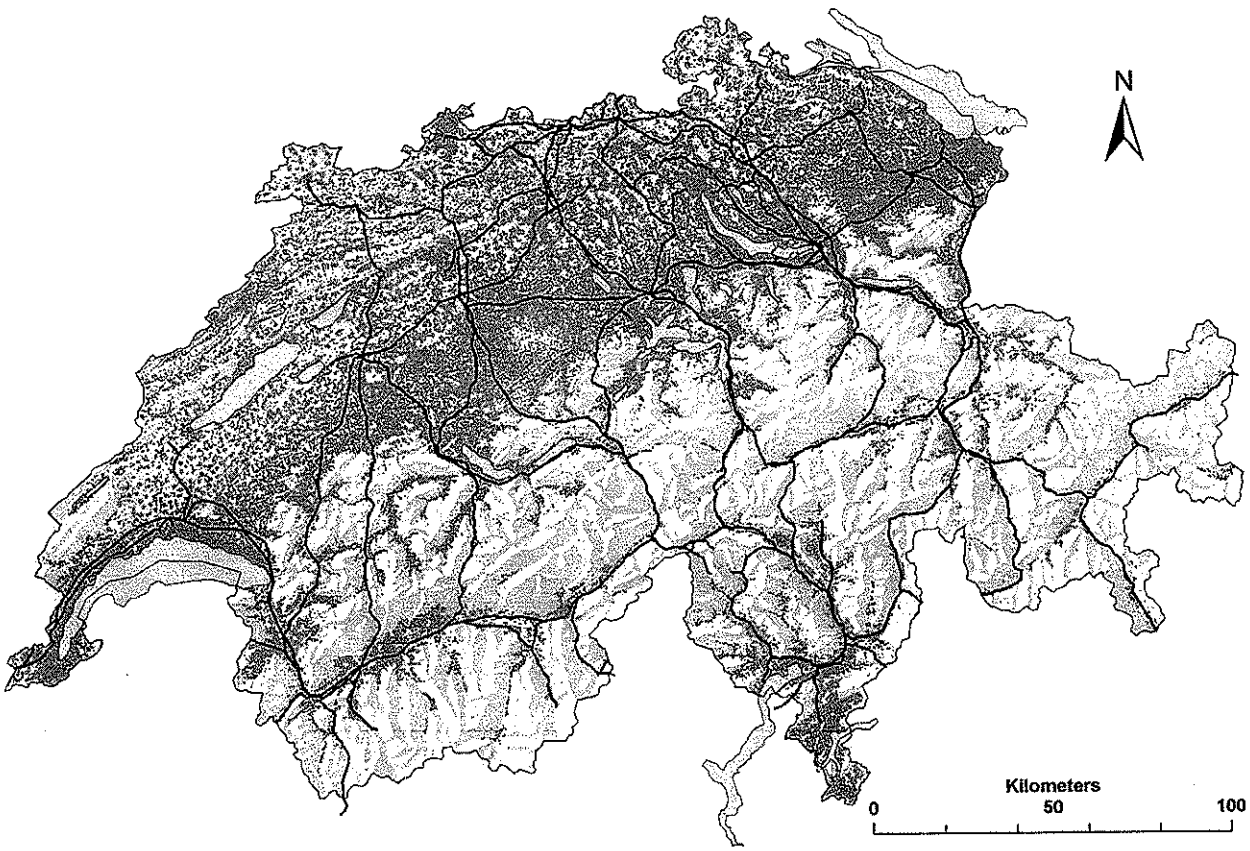

Figure 1. Power lines and buildings in Switzerland. Data sources: Federal Inspectorate for Heavy Current Installations, Fehraltdorf (power lines); Register of Buildings and Dwellings, Federal Statistical Office, Neuchâtel (building coordinates); and Federal Office of Topography swisstopo, Wabern (background map of Switzerland).

We used age as the underlying timescale in our models. All models were adjusted for sex; educational level (compulsory education, secondary level, and tertiary level); highest reported occupational attainment by code (4 levels extracted from the International Standard Classification of Occupations of 1988—1) legislators, senior officials, managers, and professionals, 2) technicians and associate professionals, clerks, service workers, and shop and market sales workers, 3) skilled agricultural and fishery workers, craft and related trades workers, plant, machine operators, and assemblers, and elementary occupations, and 4) no occupation reported); civil status (single, married, divorced, widowed); urbanization category (city, agglomeration, rural municipality); and language region (German, French, Italian). We also included the number of apartments per building into the model, a potential risk factor for magnetic field exposure due to indoor wiring (8).

Finally, because Alzheimer's disease might be associated with benzene exposure, we adjusted models for living within 50 m of a major road. We extracted proximity of the buildings to the "major road network" using data from the Swiss TeleAtlas database for this purpose. The major roads network includes motorways and motorway exits, as well as "major roads of high importance": nearly 8,700 km with 7% of the population exposed to major roads in the 50-m corridor. In sensitivity analyses, we repeated analyses for persons aged less than 85 years, by sex, and examined whether results differed between deaths where Alzheimer's disease or senile dementia had been coded as the primary or concomitant cause of death.

We tested our models successfully for the proportionality assumption using Nelson-Aalen survivor functions and statistical tests based on Schoenfeld residuals. Data were analyzed by using Stata 9 (StataCorp LP, College Station, Texas) software. Results are presented as hazard ratios with 95% confidence intervals.

The Swiss National Cohort was approved by the cantonal ethics committees of Bern and Zurich.

RESULTS

Of the 7.29 million persons recorded in the 2000 census, 2.59 million were excluded because they were under the age of 30 years at the census. Furthermore, 39,871 persons with unknown building coordinates were excluded. The cohort

Table 2. Number of Deaths, Person-Years of Follow-up, and Hazard Ratios for Alzheimer's Disease and Senile Dementia Mortality According to Distance to Power Lines, Entire Study Population and Individuals With at Least 15 Years at the Identical Place of Residence, Switzerland, 2000–2005[a]

Cause of Death	Distance to 220–380 kV Power Line, m	No. of Cases	No. of Person-Years	Crude		Adjusted	
				Hazard Ratio	95% Confidence Interval	Hazard Ratio	95% Confidence Interval
		Entire study population					
Alzheimer's disease	0–<50	20	58,423	1.18	0.76, 1.83	1.24	0.80, 1.92
	50–<200	130	363,460	1.12	0.94, 1.33	1.13	0.95, 1.34
	200–<600	572	1,688,323	0.99	0.91, 1.08	1.02	0.94, 1.11
	≥600	8,506	20,711,618	1	Referent	1	Referent
Senile dementia	0–<50	60	58,423	1.19	0.92, 1.53	1.23	0.96, 1.59
	50–<200	371	363,460	1.06	0.96, 1.18	1.08	0.97, 1.19
	200–<600	1,702	1,688,323	0.98	0.93, 1.02	0.99	0.94, 1.04
	≥600	26,155	20,711,618	1	Referent	1	Referent
	Individuals living at least 15 years at the identical place of residence						
Alzheimer's disease	0–<50	15	22,320	1.90	1.14, 3.15	2.00	1.21, 3.33
	50–<200	63	145,148	1.12	0.88, 1.44	1.15	0.89, 1.47
	200–<600	259	641,017	0.96	0.85, 1.09	1.00	0.88, 1.13
	≥600	3,861	7,698,419	1	Referent	1	Referent
Senile dementia	0–<50	33	22,320	1.40	0.99, 1.97	1.41	1.00, 1.98
	50–<200	169	145,148	1.00	0.86, 1.16	1.01	0.86, 1.17
	200–<600	819	641,017	1.00	0.93, 1.07	1.01	0.94, 1.09
	≥600	11,930	7,698,419	1	Referent	1	Referent

[a] Cox proportional hazard models were based on either 4.65 million (entire study population) or 1.75 million (individuals with at least 15 years at the identical place of residence) people, with age as the underlying timescale, crude and adjusted for sex, educational level, occupational attainment, urban-rural area, civil status, language region, number of apartments per building, and living within 50 m of a major road.

thus consisted of 4.65 million persons. During the study period, 282,378 eligible and linked deaths from all causes were recorded, including 9,228 deaths from Alzheimer's disease, 28,288 deaths from senile dementia, 773 deaths from multiple sclerosis, and 6,683 deaths from Parkinson's disease (Table 1). The total number of person-years of follow-up was 22.82 million for the whole study population and 8.51 million for persons who reported living for at least 15 years at the identical place of residence (Tables 2 and 3).

The adjusted hazard ratio of Alzheimer's disease for persons living within 50 m of a 220–380 kV power line compared with that for persons who lived at a distance of 600 m or more was 1.24 (95% confidence interval (CI): 0.80, 1.92). There was little evidence of an increased risk beyond 50 m. Analysis by exposure duration revealed a dose-response relation with respect to years of residence in the vicinity of power lines: Persons living at least 5 years within 50 m had an adjusted hazard ratio of 1.51 (95% CI: 0.91, 2.51), which increased to 1.78 (95% CI: 1.07, 2.96) for persons with at least 10 years and to 2.00 (95% CI: 1.21, 3.33) for persons with at least 15 years (Figure 2; Table 2). These adjusted hazard ratios of 2.04 (95% CI: 1.06, 3.93) and 1.96 (95% CI: 0.88, 4.38) were similar for women and men, respectively, and for persons under 85 years of age (adjusted hazard ratio = 1.94, 95% CI: 0.97, 3.89).

For senile dementia, we observed the same pattern as with Alzheimer's disease, although associations tended to be weaker. For increasing exposure time in the vicinity of power lines, the adjusted hazard ratio increased from 1.23 (95% CI: 0.96, 1.59) for any exposure duration to 1.34 (95% CI: 0.98, 1.82) for persons with at least 5 years, to 1.36 (95% CI: 0.98, 1.89) with at least 10 years, and to 1.41 (95% CI: 1.00, 1.98) with at least 15 years of residence near the power line (Table 2). For both Alzheimer's disease and senile dementia, there was little evidence for a difference in effects between deaths coded as primary and deaths coded as concomitant cause ($P_{\text{interaction}} > 0.2$).

Parkinson's disease and ALS were not associated with residence in the proximity of power lines. The adjusted hazard ratio for any duration of exposure in the 50-m corridor was 0.83 (95% CI: 0.46, 1.49) for Parkinson's disease and could not be estimated (no case occurred in the 50-m corridor) for ALS. The adjusted hazard ratio for multiple sclerosis was 1.20 (95% CI: 0.30, 4.80). Similar results were obtained when restricting analyses to persons with at least 15 years at the same place of residence (Table 3).

No increased risk in the proximity of a power line was found for all-cause mortality, cancer of the lung, bronchus, or trachea, cancer of the esophagus, or alcoholic liver disease, for any duration of residence (data not shown) or when

Table 3. Number of Deaths, Person-Years of Follow-up, and Hazard Ratios for Amyotrophic Lateral Sclerosis, Parkinson's Disease, and Multiple Sclerosis Mortality According to Distance to Power Lines, Entire Study Population and Individuals With at Least 15 Years at the Identical Place of Residence, Switzerland, 2000–2005[a]

Cause of Death	Distance to 220–380 kV Power Line, m	No. of Cases	No. of Person-Years	Crude		Adjusted	
				Hazard Ratio	95% Confidence Interval	Hazard Ratio	95% Confidence Interval
	Entire study population						
Amyotrophic lateral sclerosis	0–<50	0	58,423				
	50–<200	10	363,460	0.88	0.47, 1.64	0.85	0.46, 1.59
	200–<600	39	1,688,323	0.74	0.54, 1.02	0.72	0.52, 1.00
	≥600	695	20,711,618	1	Referent	1	Referent
Parkinson's disease	0–<50	12	58,423	0.95	0.54, 1.67	0.87	0.50, 1.56
	50–<200	99	363,460	1.15	0.94, 1.40	1.06	0.87, 1.29
	200–<600	416	1,688,323	0.98	0.90, 1.09	0.92	0.84, 1.02
	≥600	6,156	20,711,618	1	Referent	1	Referent
Multiple sclerosis	0–<50	2	58,423	1.11	0.28, 4.43	1.19	0.30, 4.79
	50–<200	16	363,460	1.38	0.84, 2.26	1.45	0.88, 2.39
	200–<600	60	1,688,323	1.12	0.86, 1.45	1.16	0.89, 1.51
	≥600	695	20,711,618	1	Referent	1	Referent
	Individuals living at least 15 years at the identical place of residence						
Amyotrophic lateral sclerosis	0–<50	0	22,320				
	50 –<200	7	145,148	1.05	0.50, 2.21	1.00	0.47, 2.11
	200–<600	29	641,017	0.97	0.66, 1.41	0.93	0.63, 1.35
	≥600	389	7,698,419	1	Referent	1	Referent
Parkinson's disease	0–<50	8	22,320	1.25	0.63, 2.51	1.15	0.57, 2.30
	50–<200	56	145,148	1.25	0.96, 1.63	1.14	0.87, 1.49
	200–<600	210	641,017	0.99	0.86, 1.14	0.93	0.81, 1.08
	≥600	3,006	7,698,419	1	Referent	1	Referent
Multiple sclerosis	0–<50	1	22,320	1.26	0.18, 8.98	1.35	0.19, 9.62
	50–<200	11	145,148	2.09	1.15, 3.82	2.19	1.19, 4.01
	200–<600	26	641,017	1.10	0.74, 1.65	1.14	0.76, 1.71
	≥600	299	7,698,419	1	Referent	1	Referent

[a] Cox proportional hazard model based on 4.65 million and 1.75 million people, with age as the underlying timescale, crude and adjusted for sex, educational level, occupational attainment, urban-rural area, civil status, language region, number of apartments per building, and living within 50 m of a major road.

restricting analyses to persons with at least 15 years at the same place of residence (Table 4).

DISCUSSION

This large study of the entire Swiss population found that persons who lived within 50 m of a 220–380 kV power line were at increased risk of death from Alzheimer's disease, compared with persons who lived farther away from power lines. The risk increased with increasing duration of residence in the 50-m corridor. Notably, the risk declined rapidly with increasing distance, with only weak evidence for an increased risk beyond 50 m. A similar pattern was observed for senile dementia. In contrast, we found no consistent association for ALS, Parkinson's disease, or multiple sclerosis. Our study thus indicates a possible association between ELF-MF exposure and risks of Alzheimer's disease and senile dementia.

Comparison with previous studies

Established risk factors for Alzheimer's disease include age and genetic factors (9). Controversy remains regarding environmental risk factors, including ELF-MF (10). The association between Alzheimer's disease and ELF-MF has generally been studied with respect to occupational exposures. Occupational exposures are typically about 0.5 μT for electricians, some machine operators, or train drivers, above 1 μT for some machine operators, and around 3 μT for electrical power installers and repairers (11). In occupational settings, increased risks of Alzheimer's disease have been reported with magnetic field exposures at levels around 0.5 μT (4). To our knowledge, an analysis of the potential

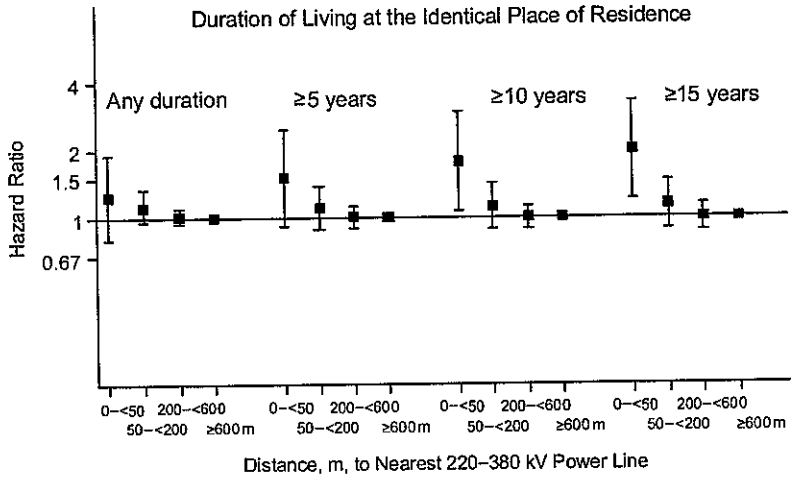

Figure 2. Mortality from Alzheimer's disease in relation to proximity to 220–380 kV power lines, Switzerland, 2000–2005. Cox proportional hazard models for persons in Switzerland who reported living at the place of residence at the time of the 2000 census or at the identical place of residence for at least 5, 10, or 15 years, using age as the underlying timescale, adjusted for sex, educational level, occupational attainment, urban-rural area, civil status, language region, number of apartments per building, and living within 50 m of a major road.

association of neurodegenerative diseases and residential exposure has not been reported in the scientific literature, even though ELF-MF exposure from power lines can be of the same magnitude as in occupational settings. In the United Kingdom, propagation of magnetic fields at levels of about 0.5 μT at a distance of 50 m to a 275 kV line was reported (12). At maximum load, these levels could, however, be considerably higher. In Switzerland, the Federal

Table 4. Number of Cases and Hazard Ratios for Comparison Outcomes of Total Mortality, Alcoholic Liver Disease, Cancer of the Esophagus, and Lung Cancer According to Persons Who Reported Living at Least 15 Years at the Identical Place of Residence, Switzerland, 2000–2005[a]

Cause of Death	Distance to 220–380 kV Power Line, m	No. of Cases	Crude		Adjusted	
			Hazard Ratio	95% Confidence Interval	Hazard Ratio	95% Confidence Interval
Total mortality	0–<50	341	1.11	1.00, 1.24	1.07	0.96, 1.19
	50–<200	2,144	1.01	0.97, 1.06	0.97	0.93, 1.01
	200–<600	10,104	1.02	1.00, 1.04	1.00	0.98, 1.02
	≥600	135,851	1	Referent	1	Referent
Alcoholic liver disease	0–<50	4	1.01	0.38, 2.70	1.11	0.41, 2.96
	50–<200	32	1.23	0.87, 1.75	1.31	0.92, 1.86
	200–<600	94	0.82	0.66, 1.01	0.87	0.70, 1.07
	≥600	1,409	1	Referent	1	Referent
Cancer of the esophagus	0–<50	1	0.37	0.05, 2.62	0.36	0.05, 2.55
	50–<200	16	0.88	0.54, 1.45	0.84	0.51, 1.38
	200–<600	77	0.94	0.75, 1.19	0.92	0.73, 1.16
	≥600	1,055	1	Referent	1	Referent
Lung cancer	0–<50	19	1.02	0.65, 1.59	1.00	0.64, 1.57
	50–<200	119	0.95	0.79, 1.14	0.94	0.78, 1.12
	200–<600	551	0.98	0.90, 1.07	0.99	0.90, 1.08
	≥600	7,248	1	Referent	1	Referent

[a] Cox proportional hazard models, using age as the underlying timescale, crude and adjusted for sex, educational level, occupational attainment, urban-rural area, civil status, language region, number of apartments per building, and living within 50 m of a major road. The study population is the same as that for Table 3.

Office for the Environment estimated that, at full load, 1 μT would not be exceeded at a distance of 60–80 m from a 380 kV line and at 40–55 m from a 220 kV line (13).

For ALS, an association between the risk of ALS and employment in electrical occupations, which is related to both magnetic field exposure and the risk of experiencing electric shock, has been reported (14). The electric shock hypothesis would be consistent with our results, as we did not observe an association with residential magnetic field exposure. In the absence of a known biologic mechanism, the World Health Organization recently concluded that the available evidence on a possible association between ELF-MF and Alzheimer's disease, as well as ALS, was inadequate (5).

Of the few studies so far that evaluated magnetic field exposure and multiple sclerosis, none reported statistically significant increased risks, which is in line with the inconsistent results observed here (15–17). Also in line with previous studies, our results for Parkinson's disease provide little evidence for an association (18).

Strengths and limitations

This study combined the mortality register data with nearly complete population data from the 2000 census, complemented with information on duration of residence from the 1990 census. With the exception of persons emigrating from Switzerland, particularly older immigrants who tend to return to their countries after retirement, mortality data are also virtually complete. Record linkage failed in some instances, but this is unlikely to be associated with residence in the vicinity of power lines. Linkage success is very high in the age group above 30 years and highest in the age group between 65 and 85 years. Because mortality from neurodegenerative diseases is negligible in younger people, we restricted our analyses to persons aged 30 years or over. In sensitivity analyses, we excluded people aged 85 years or older and obtained virtually identical results.

The development of neurodegenerative disease, as well as its recording on death certificates, may be associated with socioeconomic position. The availability of data on education and occupation and other potential confounders on an individual level is an important strength of our study. This allowed us to adjust for several indicators of socioeconomic position, but this adjustment had only very small effects on our estimates. In addition, causes of death known to be associated with socioeconomic position were included for comparison but did not show an increased risk in the 50-m corridor.

There is no registry for neurodegenerative diseases in Switzerland, and we had to rely on information given on death certificates, where neurodegenerative diseases are known to be underreported (19–21). The degree of underreporting varies by disease. Death certification of cases of ALS and multiple sclerosis has been found to be reasonably accurate (22, 23). Underreporting of Alzheimer's disease, as well as senile dementia, is more common and increases with the age of the deceased (19, 21, 24–27). Mortality rates for Alzheimer's disease have been increasing since 1995, when a specific code was introduced in the ICD-10 system, indicating that reporting of Alzheimer's disease on death certificates has become more complete in recent years. However, it is unlikely that the completeness of reporting is associated with living in the proximity of power lines.

The magnetic fields produced by power lines depend on a variety of factors, including the load characteristics, distance between conductors, and the placement of phases. Unfortunately, information on these characteristics was not available in our study. We acknowledge that the use of exposure corridors, without measurements or taking the load of the line and other factors into account, may have introduced Berkson-type error into the exposure assessment (28), and this could have reduced the power of our study. On the other hand, it is possible that our surrogate is not predictive for true exposures at all because other sources may be more important, for instance, at work or when travelling. This would imply that the observed association is due to another factor that could not be controlled for in the analysis. However, we believe that we allowed for the most important factors in the analysis, and we are not aware of other exposures that could plausibly explain the observed associations.

There is no consensus as to which exposures from overhead power lines are biologically relevant and should be measured (2). For example, ionized particles or contact currents may also be relevant (29–31). However, all of these exposures are associated with distance to a power line. We extended the corridors around power lines up to a distance of 600 m to make our results comparable with those of the study by Draper et al. (32). In contrast to their study, we found little evidence for an increased risk beyond 50 m. With respect to a potential mechanism, we can only speculate whether one of the mechanisms that have been proposed in the literature (5) might be of importance in the context of magnetic field exposure and neurodegenerative diseases. For example, induced electric fields in neural networks (electric fields induced in tissue by exposure to extremely low frequency electric and magnetic fields) have been reported to affect synaptic transmission in neural networks, as well as the radical pair mechanism (5). Increased free radical concentrations can cause oxidative damage to cellular components, which could play a role in the etiology of Alzheimer's disease.

Finally, underground cables that replace overhead power lines in some urban areas may represent an additional source of residential magnetic field exposure, but these were not considered in our study. In Switzerland, underground cables of 220–380 kV represent only around 0.8% of the grid, and we decided to omit cables from our analyses.

Public health implication

Assuming that the associations observed in this study are causal, what are the public health implications? Considering the relatively small number of cases of Alzheimer's disease and senile dementia diagnosed in the 50-m corridor (Alzheimer's disease: 20 of 9,164 (0.22%); senile dementia: 59 of 28,045 (0.21%)), it is clear that the public health impact appears limited. The true public health impact, however, is difficult to determine. Rates of Alzheimer's disease were reported to be from 2- to 8-fold higher if diagnoses were

based on clinical examination instead of death certificates (20, 24). In addition, Alzheimer's disease might go undiagnosed in another group of persons. Finally, although we found only weak evidence for an increased risk beyond 50 m, it is unlikely that there is an abrupt change in risk at 50 m. Nevertheless, our results do provide reassurance for the population living at distances of 50–600 m from a power line.

Conclusions

The results of our study support the hypothesis that magnetic field exposure plays a role in the etiology of Alzheimer's disease and senile dementia but not of ALS or other neurodegenerative diseases. Despite the large sample size covering the whole Swiss population, these findings must be interpreted with caution, because of the lack of known biologic mechanisms.

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EXHIBIT I

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ORIGINAL ARTICLE: RESEARCH

Case-only study of interactions between DNA repair genes (*hMLH1*, *APEX1*, *MGMT*, *XRCC1* and *XPD*) and low-frequency electromagnetic fields in childhood acute leukemia

YOU YANG[1], XINGMING JIN[2], CHONGHUAI YAN[1], YING TIAN[1,3], JINGYAN TANG[2], & XIAOMING SHEN[1]

[1]*Shanghai XinHua Hospital of Shanghai Jiao Tong University School of Medicine, Shanghai Institute for Pediatric Research, Shanghai Key Laboratory of Children's Environmental Health, Shanghai, China,* [2]*Shanghai Children's Medical Center of Shanghai Jiao Tong University School of Medicine, Shanghai, China, and* [3]*Department of Environmental Health, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

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Abstract

A case-only study was conducted in 123 patients with sporadic acute leukemia (AL). The locations of electric transformers and power lines were noted in each area, and their distances from the houses of the study patients were measured. The intensities of magnetic fields (B) were measured in 66 cases. Unconditional logistic regression analysis was performed adjusting for age, gender, parental education and occupation, indoor and outdoor pesticides use, presence of television sets, refrigerators and microwave ovens in children's rooms and the presence of chemical factories or telecommunication transmitters within 500 m of the houses. The results of the gene–environment analyses revealed that an interaction existed between the *XRCC1* Ex9 + 16 A allele and the presence of electric transformers and power lines within 100 m (Mean $B = 0.14$ μTeslas, μT) of the houses (interaction odds ratio, COR = 4.31, 95%CI: 1.54–12.08). The COR for the interaction of *XRCC1* Ex9 + 16A and the presence of these installations within 50 m (Mean $B = 0.18$ μT) of the houses was 4.39 (95%CI: 1.42–13.54). Our results suggest a possible association between electric transformers and power lines and the *XRCC1* Ex9 + 16A allele in patients with childhood AL.

Keywords: *Electromagnetic fields, gene–environment interactions, DNA damage, single nucleotide polymorphisms, cancer*

Introduction

Leukemias account for 25–35% of all childhood cancers [1,2]. The mechanism by which leukemia arises is likely to involve gene–environment interactions, with the environmental exposure including both endogenous and exogenous factors [3]. Exposure to residential power-frequency magnetic fields has been suspected to increase the risk of childhood leukemia, though the risk suggested by the first report [4] has not consistently been supported by the following ones [5–12]. Laboratory-based experiments and recent studies have suggested that low frequency electromagnetic fields (EMF) can influence cell proliferation and DNA damage in both normal and tumour cells through the action of free radicals [13–15]. Many of the enzymatic genes involved in DNA repair are polymorphic and can affect the capacity for DNA repair [16,17], possibly accounting for differential susceptibilities to childhood leukemia.

Genetic variation in the coding regions of the mismatch repair gene *hMLH1* (Ile-219Val) could contribute to an individual's susceptibility to leukemogenesis [18,19], though the involvement of the repair system in the pathogenesis of plasma cell dyscrasias has not yet been fully elucidated [20]. A single base A to G transition in exon 8 at position 23

Correspondence: Xiaoming Shen, Shanghai XinHua Hospital of Shanghai Jiaotong University School of Medicine, Shanghai Institute for Pediatric Research, Shanghai Key Laboratory of Children's Environmental Health, 1665 Kong Jiang Road, Shanghai 200092, China. Fax: +86 021 65791316. E-mail: xminshen@gmail.com

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upstream was identified as a polymorphism of the *hMLH1* gene, and the AA genotype may be associated with decreased activity of the encoded enzyme. Genetic variations in base excision repair genes are associated with bladder cancer risk through gene–gene and gene–environment interactions. The Asp148Glu amino acid substitution is due to a single T to G base pair change in exon 5 at position 5 downstream in the *APEX1* gene which has been well studied [21]. Silencing of the *MGMT* gene, a key gene for DNA repair, is involved in carcinogenesis. However, the effects of these polymorphisms on the levels and types of genetic damage induced by specific environmental carcinogens remains to be fully determined. Evidence suggests that the 143 Val c single nucleotide polymorphism (SNPs) (*MGMT* Ex7 + 13 G allele) may alter the functional characteristics of the *MGMT* protein, resulting in suboptimal repair of genetic damage [22]. The *XRCC1* 280His (*XRCC1* Ex9 + 16 A) alleles affect individual chromosomal aberration levels, most probably by influencing the DNA repair phenotype [17]. The Asp312Asn and Lys751Gln polymorphisms in the *XPD* gene may also alter DNA repair capacity. The hypothesis that these two *XPD* polymorphisms were associated with lung cancer risk was confirmed in a large hospital-based, case–control study among Chinese patients [23].

EMF and the aforementioned genetic polymorphisms may be associated with childhood acute leukemia (AL), in light of the fact that there is an unexplained association between exposure to the magnetic fields arising from the supply and use of electricity, and an increase in risk of childhood leukemia [24]. The present study was conducted to explore the role of interactions between EMF and DNA repair enzymes in childhood leukemia, using a case-only study in a Chinese Han population of children.

Methods

Study population and data collection

Participants in the study were all inpatients admitted to the hematological department of the Children's Medical Centre affiliated to Shanghai Jiao Tong University School of Medicine in Shanghai, China. All subjects were ethnic Han Chinese and residents of Shanghai and Zhejiang and Jiangsu province, who resided within 500 km of Shanghai. We recruited 123 patients who had been diagnosed with AL at ages < 15 between May 2006 and August 2007. Children with AL were diagnosed according to the French–American–British classification by bone marrow biopsy [25]. A combination of morphologic, immunophenotypic and cytogenetic studies is used to establish the diagnosis of acute myeloid leukemia (AML) [26,27]. After informed consent from the parents of each participant, 0.5 mL of blood was collected before chemotherapy and stored at −80°C. The study was approved by the ethics and research committee of the Shanghai Children's Medical Centre, which is affiliated with the Shanghai Jiaotong University School of Medicine.

The mothers of the patients were interviewed at the hospital by specifically trained medical doctors using a questionnaire. Visits to the present (or previous) residential areas of 66 cases were arranged, and the actual values of magnetic field intensities were measured using an EMF detector (TriField Meter, AlphaLab, USA). Questionnaires covered information about the parents' sociodemographic characteristics, the children's pre and postnatal characteristics and the familial history of cancer and autoimmune diseases. The questions related to environmental exposure covered pregnancy and the period from birth to diagnosis and detailed information including: Was there a television set/refrigerator/microwave oven in the children's rooms? Did you regularly use insecticides at home? Did you use gardening chemicals such as, fertilisers, herbicides, insecticides, fungicides, others? Were there chemical factories/telecommunication transmitters/electric transformers/power lines around your house? Electric transformers were taken to mean those typically located within a city area to serve city districts or the main power transformation stations (10, 35, 110 kV). Distances between such installations and the houses were noted. The questionnaire also addressed the parents' occupations during pregnancy and during the study subjects' childhoods.

SNP detection using the MassARRAY system

The MassARRAY technology platform (Sequenom, San Diego, California, USA) was used to detect the SNPs in *hMLH1* Ex8–23A > G (rs1799977), *APEX1* Ex5 + 5T > G (rs1130409), *MGMT*Ex7 + 13A > G (rs2308321), *XRCC1* Ex9 + 16G > A (rs25489), *XPD* Ex10–16G > A (rs1799793) and *XPD* Ex23 + 61 T > G (rs13181) [28]. The test was based on the principle of allele-specific primer extension reaction, whereas the read-out of the result was realised using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). Polymerase chain reaction (PCR) primers and extension primers were designed by MassARRAY Assay Design 2.0 software. To provide a significant improvement in overall performance, 10-mer tag (5′-ACGTTGGATG-3′) was added to the 5′ ends of each PCR primer (Table I). To start

the experiment, the polymorphic loci were amplified by PCR on a 384-well plate in a volume of 5 μL reaction mixture with 0.1 U HotStarTaq DNA polymerase (QIAGEN GmbH, Hilden, Germany). PCR thermocycles were performed as follows: a denaturation at 95°C for 15 min, 45 cycles consisting of 20 s at 95°C, 30 s at 56°C and 60 s at 72°C, followed by a final elongation step at 72°C for 3 min. The PCR products were then purified with shrimp alkaline phosphatase. In the second step, 2 μL primer extension mixture, including approximately 9 μM extension primer (Table I), 0.018 μL Thermosequenase and 0.2 μL 10× Termination Mix of ddATP, ddCTP, ddTTP and ddGTP (suitable for the each SNP) (Amersham Biosciences, Uppsala, Sweden) were added to the PCR products. The mixture was thermocycled to process the reaction protocol as follows: 94°C for 2 min; 55 cycles each of 94°C for 5 s, then 52°C for 5 s and 72°C for 5 s. After desalting, the reaction products were transferred onto a 384-well SpectroCHIP and analysed by using the MassARRAY Analyser (Sequenom).

Statistical analysis

The χ^2-goodness-of-fit test was used to test the Hardy-Weinberg equilibrium. For polymorphisms with a low variant allele frequency, the homozygote for the variant allele was combined with the heterozygote, including *hMLH1* Ex8–23A > G, *APEX1* Ex5 + 5T > G, *XRCC1* Ex9 + 16G > A and *XPD* Ex23 + 61 T > G. The low risk genotypes (in *hMLH1* genotypes, A/A allele; in *APEX1* genotypes, T/T allele; in *MGMT* genotypes, A/A allele; in *XRCC1* genotypes, the G/G allele; in *XPD* Ex23 + 61T > G genotypes, T/T allele; in *XPD* Ex10-16G > A, G/G allele) were designated as the referent category to evaluate potential effect modification. For *XRCC1*, genotypes were also combined based on a known phenotype–genotype relationship. The combination of *XRCC1* Ex9 + 16 A/G and A/A genotypes was defined as a 'poor metaboliser', whereas the G/G genotype of *XRCC1* Ex9 + 16 was termed as an 'extensive metaboliser' [16,17,29].

To estimate gene–environment interactions, a case-only design was applied. The case-only design is an efficient way of estimating gene–environment interactions, but it cannot evaluate the main effect of either [30–32]. Such interactions may be obtained in a case-only design if independence between genotypes and environment exposure is assumed. In this study, we validated this independence assumption through the analysis of a relatively large number of healthy subjects ($n = 135$, median age 5.0 years, range 0.5–15 years) randomly selected from the same population. Generally, when E is categorical or continuous, we can assess the independence assumption by fitting the following logistic regression model to the control data:

$$\text{log it } P\,(G = 1) = \eta_0 + \eta_1 E \qquad (1)$$

where a test of whether $\eta_1 = 0$ is a test of the independence assumption. We assume that the genetic mutation and environmental exposure are binary variables. G and E indicate presence of the genetic mutation or polymorphism, and environmental exposure, respectively. We perform a case-only analysis if the test is nonsignificant at the 0.05 significance level or otherwise a case–control analysis

Table I. Oligonucleotide primers for polymerase chain reaction.

Genes	Primers (5′–3′; F, forward; R, reverse; E, extension)	Fragment length (bp)
hMLH1 Ex8-23A > G	F: ACGTTGGATGCTGATGTTAGGACACTACCC R: ACGTTGGATGTCGACATACCGACTAACAGC E: GACTAACAGCATTTCCAAAGA	102
APEX1 Ex5 + 5T > G	F: ACGTTGGATGCACCTCTTGATTGCTTTCCC R: ACGTTGGATGACAATCACCCGGCCTTCCTG E: CCTCCTGATCATGCTCCTC	120
MGMT Ex7 + 13A > G	F: ACGTTGGATGCTGCTGCAGACCACTCTGT R: ACGTTGGATGAGATCCCTGACTGACAGTGG E: ACCTGTCTTCCAGGTCCCCATCCTC	100
XRCC1 Ex9 + 16G > A	F: ACGTTGGATGTTTGCCTGTCACTGCCCCCT R: ACGTTGGATGTTTGTCTTCTCCAGTGCCAG E: TGTCGTCTCCAGTGCCAGCTCCAACTC	105
XPD Ex10-16G > A	F: ACGTTGGATGACGGACGCCCACCTGGCCAA R: ACGTTGGATGAGGCGGGAAAGGGACTGGG E: CTGCGCACCCTGCAGCACTTCGT	112
XPD Ex23 + 61 T > G	F: ACGTTGGATGAGCAGCTAGAATCAGAGGAG R: ACGTTGGATGCACCAGGAACCGTTTATGGC E: GCAATCTGCTCTATCCTCT	113

is used. When the log-odds ratio (η_1) assessing independence is zero, the case-only design is unbiased and very efficient. In our study, the odds ratio assessing independence between electric transformers and power lines and *XRCC1* was closer to 1 (estimate 1.37, $P=0.656$).

Case-only odds ratios for the relevant interactions and 95% confidence intervals (95%CI) were estimated using unconditional logistic regression. Possible confounding variables included for age, gender, parental education and occupation, indoor and outdoor pesticides use, television sets, refrigerators and microwave ovens in children's rooms and chemical factories or telecommunication transmitters within 500 m of the houses. Participants with missing values for any of the variables in the regression model were omitted from the analysis. The Mann–Whitney test was used for comparison between magnetic field intensities at residences for 66 cases in terms of distances from residences to the electric transformers.

All *P*-values are two-sided, and all analyses were carried out using SPSS software packages (version 11, SPSS, USA).

Results

Characteristics of children with AL

The characteristics of children with AL are shown in Table II. Of the participants, 66.67% were boys and 33.33% were girls; 56.10% of the cases were 2–7 years old, with the median age 5.0 years (range 0.5–14 years). Ninety-nine of the patients had acute lymphoid leukemia (ALL), and 24 had AML. All those exposed to electric transformers were also exposed to the power lines (Table III) and two cases exposed only to power lines are not included in the table. Mean peak values for magnetic field intensities were 0.13 μT ($n=19$), 0.14 μT ($n=13$) and 0.18 μT ($n=9$) for houses within 500, 100 and 50 m of electric transformers and power lines, respectively. There were significant changes in magnetic field intensity with different distances (500 m, 100 m, 50 m) between the houses and the electric transformers or power lines in 66 cases (Table IV).

Table II. Characteristics of study patients with childhood AL.

Variables	Cases no. (%)
Sex	
Boys	82 (66.67)
Girls	41 (33.33)
Age (years)	
<2	14 (11.38)
2–7	69 (56.10)
8–14	40 (32.52)
Maternal education	
≤High school	70 (56.91)
>High school	53 (43.09)
Paternal education	
≤High school	78 (63.41)
>High school	45 (36.59)
Socioprofessional categories*	
Professional, technical workers, administrators and managers	23 (18.70)
Clerical, sales and services workers	55 (44.72)
Factory and agricultural workers	45 (36.58)
Television set in children's rooms	
No	27 (21.95)
Yes	96 (78.05)
Refrigerator in children's rooms	
No	120 (97.56)
Yes	3 (2.44)
Microwave oven in children's rooms	
No	122 (99.19)
Yes	1 (0.81)
Home insecticide use	
Never	61 (49.59)
Ever	62 (50.41)
Garden pesticide use	
Never	120 (97.56)
Ever	3 (2.44)

*Socioprofessional categories: best occupational activity of child's mother or father.

Table III. Distances between installations and the houses.

Installations around the houses	Cases no. (%)		
Electric transformers and power lines	Within 500 m	Within 100 m	Within 50 m
No	84 (68.29)	92 (74.80)	103 (83.74)
Yes	39 (31.71)	31 (25.20)	20 (16.26)
Telecom transmitter	Within 500 m	Within 100 m	Within 50 m
No	103 (83.74)	116 (94.31)	121 (98.37)
Yes	20 (16.26)	7 (5.69)	2 (1.63)
Chemical factory	Within 500 m	Within 100 m	Within 50 m
No	109 (88.62)	114 (92.68)	119 (96.75)
Yes	14 (11.38)	9 (7.32)	4 (3.25)

Table IV. Peak values of magnetic field intensity with different distances from the houses to electric transformers and power lines for 66 cases, median (range) μT.

Cut-offs of the distances	Houses within distance ≤cut-offs	Houses within distance > cut-offs	*P*-value*
500 m	0.02 (0.02–0.8)	0.02 (0.01–0.2)	0.005
100 m	0.02 (0.02–0.8)	0.02 (0.01–0.3)	0.006
50 m	0.02 (0.02–0.8)	0.02 (0.01–0.3)	0.014

*Comparisons of magnetic field intensity for houses within distance ≤cut-offs and houses within distance > cut-offs from electric transformers and power lines; by Mann–Whitney test.

The effect of gene–environment interactions on childhood acute lymphoma

COR for gene–environment interactions between DNA repair enzyme genotypes and electric transformers and power lines is shown in Table V. Case-only analyses revealed that an interaction existed between electric transformers and power lines situated within 100 m of the houses and the presence of the *XRCC1* Ex9 + 16A allele in childhood AL (COR = 4.31, 95%CI: 1.54–12.08), while the COR for the interaction between *XRCC1* Ex9 + 16A and their occurrence within 50 m of the houses was increased further (COR = 4.39, 95%CI: 1.42–13.54). No significant interactions between the proximity of the electric transformers and power lines and other genotypes were observed. No significant interactions were observed between genotypes and the presence of television sets, refrigerators or microwave ovens in children's rooms, pesticides use or the presence of chemical factories or telecommunication transmitter within 500 m of the houses.

Discussion

Epidemiological studies have suggested that gene–environment interactions were associated with cancer susceptibility [33–35]. Therefore, investigation of the role of gene–environment interactions involving low penetrance genes may help to elucidate the cause of common sporadic cancers. In the present study, we examined gene–environment interactions for six polymorphisms of DNA repair genes and generators of EMF using a case-only design.

Table V. Gene–environment interactions for combination of *XRCC1* Ex9 + 16G > A and electric transformers and power lines in childhood AL.

Electric transformers and power lines	*XRCC1* Ex9 + 16G > A		OR (95%CI)*
	G/G	A/G+A/A	
Within 500 m from houses			
No	66	16	1.00 (Ref)
Yes	25	16	2.37 (0.94–5.97)
Within 100 m from houses			
No	75	17	1.00 (Ref)
Yes	16	15	4.31 (1.54–12.08)#
Within 50 m from houses			
No	82	21	1.00 (Ref)
Yes	9	11	4.39 (1.42–13.54)#

*Unconditional logistic regression adjusted for age, gender, parental education and occupation, indoor and outdoor pesticides use, television set, refrigerator and microwave oven in children's room, the chemical factory, telecom transmitter around the houses in 500 meters.
#$P < 0.01$.

Our gene and environment combined analyses suggest that a significant interaction, which could potentially increase the risk of childhood AL, exists between the *XRCC1* Ex9 + 16A and the proximity of electric transformers and power lines. No previous reports have described an association between this gene–environment interaction and childhood leukemia risk to date. However, a gene–environment interaction in childhood AL has been reported between the *CYP1A1**2A variant allele, *GSTM1* deletion and maternal smoking during pregnancy, *NQO1* polymorphism and coffee drinking [36], DNA repair genes and X-rays [37] and *CYP1A1*m1 and *CYP1A1*m2 mutations and insecticides [38]. Previous studies have also suggested an association between leukemogenesis in children and DNA repair, and thus pointing to an the effect of environmental exposure [18,39]. Moreover, it was indicated that modification of genetic susceptibility in vulnerable subjects may be involved in the effect of extremely low frequency magnetic fields [40]. Although studies have been carried out on various biological aspects of childhood AL, very little has been done with regard to the role of genetic polymorphisms in the DNA repair gene *XRCC1*, and other members of the various DNA repair pathways, in susceptibility to the disease. It was reported that individuals with the XRCC1 399Gln allele were associated with increased risk for this disease [41–43]. Risk of ALL among children with XRCC1 codon 194 variant genotypes were disputable [42,43]. Molecular epidemiologic research provides compelling evidence that environmental factors are major contributors to human carcinogenesis and that the risk of developing cancer is strongly influenced by genetically determined differences [44,45]. Our results indicated that carriers of *XRCC1* Ex9 + 16A allele were at increased risk from electric transformers and power lines. No study has been published regarding the combination of *XRCC1* and electric transformers and power lines in association with childhood leukemia risk. It has been reported that EMF contributes to DNA damage through the action of free radical species [13–15]. Functional studies using lymphocytes suggested that the 280His polymorphism reduced genomic stability [16,17]. DNA damage and chromosome breaks which are inadequately repaired, ultimately lead to the initiation and progression of disease [3]. Carriers of the *XRCC1* Ex9 + 16A allele exposed to electric transformers may experience a similar process. The risk of leukemia was further increased with shorter distance between the houses and the electric

transformers and power lines. However, our current knowledge of the role of these enzymes in the DNA repair process is based primarily on *in vitro* models, which might not reflect the complex biological situation, and the biological interpretation remains to be understood. Larger studies are needed to explore the interactions between the susceptibility genes and electric transformers, and to identify the underlying mechanisms of childhood leukemia.

One of the limitations of the case-only design is that, in order to identify gene–environment interactions, independence between genotypes and environmental exposure must be assumed. However, relying on tests of independence in the controls is not effective because of their generally low power to detect meaningful departures from independence [46]. Another limitation of the current study was the potential for biased recall by patients' parents. Though the mothers of the cases were interviewed in hospital, face-to-face, by specifically trained medical doctors using a questionnaire, we did not visit the residential areas of all cases and actual values for magnetic field intensities were measured only for those cases visited, because of limited time and loss of contact in some cases. The possibility of some recall bias in our findings cannot, therefore, be ruled out.

Although our studies were limited by EMF exposure information, the small number of exposed subjects and an independence assumption, it is still noteworthy that children with the *XRCC1* Ex9 + 16A polymorphism could be particularly sensitive to the carcinogenic effects of EMF. Future research should include improved exposure assessments, evaluation of risk in relation to age at exposure and an investigation of genetic-environmental interactions using a case–control design. There is the potential to prevent some childhood leukemias by reducing or eliminating EMF exposure for children with *XRCC1* Ex9 + 16A.

In summary, our results demonstrated a possible association between electric transformers and power lines and the XRCC1 Ex9 + 16A allele in patients with childhood AL. Residence near electric transformers and power lines, at distances $\leq$100 m, and magnetic fields >0.14 μT, may be considered a risk factor for the development of AL in children with *XRCC1* Ex9 + 16A genotypes. These preliminary exploratory results need to be confirmed by further, larger studies.

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