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BioInitiative Report:
A Rationale for a Biologically-based Public Exposure Standard for Electromagnetic Fields (ELF and RF)

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PREFACE

The Organizing Committee thanks the participants of the BioIniative Working Group for their integrity and intellectual courage in dealing with this controversial and important topic; and for devoting the time and energy to produce their chapters. The information and conclusions in each chapter are the responsibilities of the authors of that chapter.

The Group has produced what the authors hope will be a benchmark for good science and public health policy planning. It documents bioeffects, adverse health effects and public health conclusions about impacts of non-ionizing radiation (electromagnetic fields including extremely-low frequency ELF-EMF and radiofrequency/microwave or RF-EMF fields).

Societal decisions about this body of science have global implications. Good public health policy depends on acting soon enough, but not without cause, and with enough information to guide intelligent actions. To a great degree, it is the definition of the standard of evidence used to judge the scientific reports that shapes this debate. Disagreement about when the evidence is sufficient to take action has more to do with the outcome of various reviews and standard-setting proceedings than any other single factor. Whatever "standard of

evidence" is selected to assess the strength of the science will deeply influence the outcome of decisions on public policy.

We are at a critical juncture in this world-wide debate. The answers lie not only in the various branches of science; but necessarily depend on the involvement of public health and policy professionals, the regulatory, legal and environmental protection sectors, and the public sector.

This has been a long-term collaboration of international scientists employing a multi-disciplinary approach to problem assessment and solving. Our work has necessarily relied on tools and approaches across the physical, biological and engineering sciences; and those of the environmental scientist and public health professional. Only when taken together can we see the whole and begin to take steps that can prevent possible harm and protect future generations.

Signed: _David Carpenter_   Signed: _Cindy Sage_

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BioInitiative: A Rationale for a Biologically-based Exposure Standard for Electromagnetic Radiation

SECTION 1

SUMMARY FOR THE PUBLIC

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Table of Contents

I. SUMMARY FOR THE PUBLIC

A. Introduction

You cannot see it, taste it or smell it, but it is one of the most pervasive environmental exposures in industrialized countries today. Electromagnetic radiation (EMR) or electromagnetic fields (EMFs) are the terms that broadly describe exposures created by the vast array of wired and wireless technologies that have altered the landscape of our lives in countless beneficial ways. However, these technologies were designed to maximize energy efficiency and convenience; not with biological effects on people in mind. Based on new studies, there is growing evidence among scientists and the public about possible health risks associated with these technologies.

Human beings are bioelectrical systems. Our hearts and brains are regulated by internal bioelectrical signals. Environmental exposures to artificial EMFs can interact with fundamental biological processes in the human body. In some cases, this can cause discomfort and disease. Since World War II, the background level of EMF from electrical sources has risen exponentially, most recently by the soaring popularity of wireless technologies such as cell phones (two billion and counting in 2006), cordless phones, WI-FI and WI-MAX networks. Several decades of international scientific research confirm that EMFs are biologically active in animals and in humans, which could have major public health consequences.

In today's world, everyone is exposed to two types of EMFs: (1) extremely low frequency electromagnetic fields (ELF) from electrical and electronic appliances and power lines and (2) radiofrequency radiation (RF) from wireless devices such as cell phones and cordless phones, cellular antennas and towers, and broadcast transmission towers. In this report we will use the term EMFs when referring to all electromagnetic fields in general; and the terms ELF and RF when referring to the specific type of exposure. They are both types of non-ionizing radiation, which means that they do not have sufficient energy to break off electrons from their orbits around atoms and ionize (charge) the atoms, as do x-rays, CT scans, and other forms of ionizing radiation.   A glossary and definitions are provided in Section 18 to assist you. Some handy definitions you will probably need when reading about ELF and RF in this summary section (the language for measuring it) are shown with the references for this section.

B. Purpose of the Report

This report has been written by 14 (fourteen) scientists, public health and public policy experts to document the scientific evidence on electromagnetic fields. Another dozen outside reviewers have looked at and refined the Report.

The purpose of this report is to assess scientific evidence on health impacts from electromagnetic radiation below current public exposure limits and evaluate what changes in these limits are warranted now to reduce possible public health risks in the future.

Not everything is known yet about this subject; but what is clear is that the existing public safety standards limiting these radiation levels in nearly every country of the world look to be thousands of times too lenient. Changes are needed.

New approaches are needed to educate decision-makers and the public about sources of exposure and to find alternatives that do not pose the same level of possible health risks, while there is still time to make changes.

A working group composed of scientists, researchers and public health policy professionals (The BioInitiative Working Group) has joined together to document the information that must be considered in the international debate about the adequacy (or inadequacy) of existing public exposure standards.

This Report is the product of an international research and public policy initiative to give an overview of what is known of biological effects that occur at low-intensity EMFs exposures (for both radiofrequency radiation RF and power-frequency ELF, and various forms of combined exposures that are now known to be bioactive). The Report examines the research and current standards and finds that these standards are far from adequate to protect public health.

Recognizing that other bodies in the United States, United Kingdom, Australia, many European Union and eastern European countries as well as the World Health Organization are actively debating this topic, the BioInitiative Working Group has conducted a independent science and public health policy review process. The report presents solid science on this issue, and makes recommendations to decision-makers and the public. Conclusions of the individual authors, and overall conclusions are given in Table 2-1 (BioInitiative Overall Summary Chart).

Eleven (11) chapters that document key scientific studies and reviews identifying low-intensity effects of electromagnetic fields have been written by members of the BioInitiative Working Group. Section 16 and 17 have been prepared by public health and policy experts. These sectoins discusses the standard of evidence which should be applied in public health planning, how the scientific information should be evaluated in the context of prudent public health policy, and identifies the basis for taking precautionary and preventative actions that are proportionate to the knowledge at hand. They also evaluate the evidence for ELF that leads to a recommendation for new public safety limits (not precautionary or preventative actions, as need is demonstrated).

Other scientific review bodies and agencies have reached different conclusions than we have by adopting standards of evidence so unreasonably high as to exclude any conclusions likely to lead to new public safety limits. Some groups are actually recommending a relaxation of the existing

(and inadequate) standards.   Why is this happening? One reason is that exposure limits for ELF and RF are developed by bodies of scientists and engineers that belong to professional societies who have traditionally developed recommendations; and then government agencies have adopted those recommendations. The standard-setting processes have little, if any, input from other stakeholders outside professional engineering and closely-related commercial interests. Often, the industry view of allowable risk and proof of harm is most influential, rather than what public health experts would determine is acceptable.

Main Reasons for Disagreement among Experts

1) Scientists and public health policy experts use very different definitions of the standard of evidence used to judge the science, so they come to different conclusions about what to do. Scientists do have a role, but it is not exclusive and other opinions matter.
2) We are all talking about essentially the same scientific studies, but use a different way of measuring when "enough is enough" or "proof exists".
3) Some experts keep saying that all studies have to be consistent (turn out the same way every time) before they are comfortable saying an effect exists.
4) Some experts think that it is enough to look only at short-term, acute effects.
5) Other experts say that it is imperative we have studies over longer time (showing the effects of chronic exposures) since that is what kind of world we live in.
6) Some experts say that everyone, including the very young, the elderly, pregnant women, and people with illnesses have to be considered – others say only the average person (or in the case of RF, a six-foot tall man) matter.
7) There is no unexposed population, making it harder to see increased risk of diseases.
8) The lack of consensus about a single biological mechanism of action.
9) The strength of human epidemiological studies reporting risks from ELF and RF exposures, but animal studies don't show a strong toxic effect.
10) Vested interests have a substantial influence on the health debate.

Public Policy Decisions
Safety limits for public exposure to EMFs need to be developed on the basis of interaction among not only scientists, but also public health experts, public policy makers and the general public.

"In principle, the assessment of the evidence should combine with judgment based on other societal values, for example, costs and benefits, acceptability of risks, cultural preferences, etc. and result in sound and effective decision-making. Decisions on these matters are eventually taken as a function of the views, values and interests of the stakeholders participating in the process, whose opinions are then weighed depending on several factors. Scientific evidence perhaps carries, or should carry, relatively heavy weight, but grants no exclusive status; decisions will be evidence-based but will also be based on other factors." (1)

The clear consensus of the BioInitiative Working Group members is that the existing public safety limits are inadequate for both ELF and RF.

> **These proposals reflect the evidence that a positive assertion of safety with respect to chronic exposure to low-intensity levels of ELF and RF cannot be made.   As with many other standards for environmental exposures, these proposed limits may not be totally protective, but more stringent standards are not realistic at the present time.   Even a small increased risk for cancer and neurodegenerative diseases translates into an enormous public health consequence. Regulatory action for ELF and preventative actions for RF are warranted at this time to reduce exposures and inform the public of the potential for increased risk; at what levels of chronic exposure these risks may be present; and what measures may be taken to reduce risks.**

C. Problems with Existing Public Health Standards (Safety Limits)

Today's public exposure limits for telecommunications are based on the presumption that heating of tissue (for RF) or induced electric currents in the body (for ELF) are the only concerns when living organisms are exposed to RF. These exposures can create tissue heating that is well known to be harmful in even very short-term doses. As such, thermal limits do serve a purpose. For example, for people whose occupations require them to work around radar facilities or RF heat-sealers, or for people who install and service wireless antenna tower, thermally-based limits are necessary to prevent damage from heating (or, in the case of power-frequency ELF from induced current flow in tissues). In the past, scientists and engineers developed exposure standards for electromagnetic radiation based what we now believe are faulty assumptions that the right way to measure how much non-ionizing energy humans can tolerate (how much exposure) without harm is to measure only the heating of tissue (RF) or induced currents in the body (ELF).

In the last few decades, it has been established beyond any reasonable doubt that bioeffects and some adverse health effects occur at far lower levels of RF and ELF exposure where no heating (or induced currents) occurs at all; some effects are shown to occur at several hundred thousand times below the existing public safety limits where heating is an impossibility.

> **It appears it is the INFORMATION conveyed by electromagnetic radiation (rather than heat) that causes biological changes - some of these biological changes may lead to loss of wellbeing, disease and even death.**

Effects occur at non-thermal or low-intensity exposure levels thousands of times below the levels that federal agencies say should keep the public safe. For many new devices operating with wireless technologies, the devices are exempt from any regulatory standards. The existing standards have been proven to be inadequate to control against harm from low-intensity, chronic exposures, based on any reasonable, independent assessment of the scientific literature. It means that an entirely new basis (a biological basis) for new exposure standards is needed. New standards need to take into account what we have learned about the effects of ELF and RF (all non-ionizing electromagnetic radiation and to design new limits based on biologically-demonstrated effects that are important to proper biological function in living organisms.   It is vital to do so because the explosion of new sources has created unprecedented levels of artificial

electromagnetic fields that now cover all but remote areas of the habitable space on earth. Mid-course corrections are needed in the way we accept, test and deploy new technologies that expose us to ELF and RF in order to avert public health problems of a global nature.

Recent opinions by experts have documented deficiencies in current exposure standards. There is widespread discussion that thermal limits are outdated, and that biologically-based exposure standards are needed. Section 4 describes concerns expressed by WHO, 2007 in its ELF Health Criteria Monograph; the SCENIHR Report, 2006 prepared for the European Commission; the UK SAGE Report, 2007; the Health Protection Agency, United Kingdom in 2005; the NATO Advanced Research Workshop in 2005; the US Radiofrequency Interagency Working Group in 1999; the US Food and Drug Administration in 2000 and 2007; the World Health Organization in 2002; the International Agency for Cancer Research (IARC, 2001), the United Kingdom Parliament Independent Expert Group Report on Mobile Phones – Stewart Report, 2000) and others.

A pioneer researcher, the late Dr. Ross Adey, in his last publication in Bioelectromagnetic Medicine (P. Roche and M. Markov, eds. 2004) concluded:

> "*There are major unanswered questions about possible health risks that may arise from exposures to various man-made electromagnetic fields where these human exposures are intermittent, recurrent, and may extend over a significant portion of the lifetime of the individual.*"

> "*Epidemiological studies have evaluated ELF and radiofrequency fields as possible risk factors for human health, with historical evidence relating rising risks of such factors as progressive rural electrification, and more recently, to methods of electrical power distribution and utilization in commercial buildings. Appropriate models describing these bioeffects are based in nonequilibrium thermodynamics, with nonlinear electrodynamics as an integral feature. Heating models, based in equilibrium thermodynamics, fail to explain an impressive new frontier of much greater significance. ..... Though incompletely understood, tissue free radical interactions with magnetic fields may extend to zero field levels.*" (2)

There may be no lower limit at which exposures do not affect us. Until we know if there is a lower limit below which bioeffects and adverse health impacts do not occur, it is unwise from a public health perspective to continue "business-as-usual" deploying new technologies that increase ELF and RF exposures, particularly involuntary exposures.

II. SUMMARY OF THE SCIENCE

A. Evidence for Cancer

1. Childhood Leukemia

The evidence that power lines and other sources of ELF are consistently associated with higher rates of childhood leukemia has resulted in the International Agency for Cancer Research (an arm of the World Health Organization) to classify ELF as a Possible Human Carcinogen (in the Group 2B carcinogen list). Leukemia is the most common type of cancer in children.

There is little doubt that exposure to ELF causes childhood leukemia.

The exposure levels for increased risk are quite low – just above background or ambient levels and much lower than current exposure limits. The existing ICNIRP limit is 1000 mG (904 mG in the US) for ELF. Increased risk for childhood leukemia starts at levels almost one thousand times below the safety standard. Leukemia risks for young boys are reported in one study to double at only 1.4 mG and above (7) Most other studies combine older children with younger children (0 to 16 years) so that risk levels do not reach statistical significance until exposure levels reach 2 mG or 3 mG.   Although some reviews have combined studies of childhood leukemia in ways that indicate the risk level starts at 4 mG and above; this does not reflect many of the studies reporting elevated risks at the lower exposure levels of 2 mG and 3 mG.

2. Other Childhood Cancers

Other childhood cancers have been studied, including brain tumors, but not enough work has been done to know if there are risks, how high these risks might be or what exposure levels might be associated with increased risks. The lack of certainty about other childhood cancers should not be taken to signal the "all clear"; rather it is a lack of study.

The World Health Organization ELF Health Criteria Monograph No 322 (2007) says that other childhood cancers "cannot be ruled out". (8)

There is some evidence that other childhood cancers may be related to ELF

exposure but not enough studies have been done.

Several recent studies provide even stronger evidence that ELF is a risk factor for childhood leukemia and cancers later in life. In the first study (9), children who were recovering in high-

ELF environments had poorer survival rates (a 450% increased risk of dying if the ELF fields were 3 mG and above). In the second study, children who were recovering in 2 mG and above ELF environments were 300% more likely to die than children exposed to 1 mG and below. In this second study, children recovering in ELF environments between 1 and 2 mG also had poorer survival rates, where the increased risk of dying was 280%. (10) These two studies give powerful new information that ELF exposures in children can be harmful at levels above even 1 mG. The third study looked what risks for cancer a child would have later in life, if that child was raised in a home within 300 meters of a high-voltage electric power line. (11) For children who were raised for their first five years of life within 300 meters, they have a life-time risk that is 500% higher for developing some kinds of cancers.

> **Children who have leukemia and are in recovery have poorer survival rates if their ELF exposure at home (or where they are recovering) is between 1mG and 2 mG in one study; over 3 mG in another study.**

Given the extensive study of childhood leukemia risks associated with ELF, and the relatively consistent findings that exposures in the 2 mG to 4 mG range are associated with increased risk to children, a 1 mG limit for habitable space is recommended for new construction. While it is difficult and expensive to retrofit existing habitable space to a 1 mG level, and is also recommended as a desirable target for existing residences and places where children and pregnant women may spend prolonged periods of time.

> **New ELF public exposure limits are warranted at this time, given the existing scientific evidence and need for public health policy intervention and prevention.**

3. Brain Tumors and Acoustic Neuromas

Radiofrequency radiation from cell phone and cordless phone exposure has been linked in more than one dozen studies to increased risk for brain tumors and/or acoustic neuromas (a tumor in the brain on a nerve related to our hearing).

> **People who have used a <u>cell phone</u> for ten years or more have higher rates of malignant brain tumor and acoustic neuromas. It is worse if the cell phone has been used primarily on one side of the head.**

For brain tumors, people who have used a cell phone for 10 years or longer have a 20% increase in risk (when the cell phone is used on both sides of the head). For people who have used a cell phone for 10 years or longer predominantly on one side of the head, there is a 200% increased

risk of a brain tumor. This information relies on the combined results of many brain tumor/cell phone studies taken together (a meta-analysis of studies).

> **People who have used a <u>cordless phone</u> for ten years or more have higher rates of malignant brain tumor and acoustic neuromas.   It is worse if the cordless phone has been used primarily on one side of the head.**

The risk of brain tumor (high-grade malignant glioma) from cordless phone use is 220% higher (both sides of the head). The risk from use of a cordless phone is 470% higher when used mostly on only one side of the head.

For acoustic neuromas, there is a 30% increased risk with cell phone use at ten years and longer; and a 240% increased risk of acoustic neuroma when the cell phone is used mainly on one side of the head. These risks are based on the combined results of several studies (a meta-analysis of studies).

For use of cordless phones, the increased risk of acoustic neuroma is three-fold higher (310%) when the phone is mainly used on one side of the head.

> **The current standard for exposure to the emissions of <u>cell phones and cordless phones</u> is not safe considering studies reporting long-term brain tumor and acoustic neuroma risks.**

Other indications that radiofrequency radiation can cause brain tumors comes from exposures to low-level RF <u>other than</u> from cell phone or cordless phone use. Studies of people who are exposed in their work (occupational exposure) show higher brain tumor rates as well.   Kheifets (1995) reported a 10% to 20% increased risk of brain cancer for those employed in electrical occupations. This meta-analysis surveyed 29 published studies of brain cancer in relation to occupational EMFs exposure or work in electrical occupations. (6). The evidence for a link between other sources of RF exposure like working at a job with EMFs exposure is consistent with a moderately elevated risk of developing brain tumors.

4. *Other Adult Cancers*

There are multiple studies that show statistically significant relationships between occupational exposure and leukemia in adults (see Chapter 11), in spite of major limitations in the exposure assessment. A very recent study by Lowenthal et al. (2007) investigated leukemia in adults in relation to residence near to high-voltage power lines. While they found elevated risk in all adults living near to the high voltage power lines, they found an OR of 3.23 (95% CI = 1.26-8.29) for individuals who spent the first 15 years of life within 300 m of the power line. This study provides support for two important conclusions: adult leukemia is also associated with EMF exposure, and exposure during childhood increases risk of adult disease.

A significant excess risk for adult brain tumors in electrical workers and those adults with occupational EMF exposure was reported in a meta-analysis (review of many individual studies) by Kheifets et al., (1995). This is about the same size risk for lung cancer and secondhand smoke (US DHHS, 2006). A total of 29 studies with populations from 12 countries were included in this meta-analysis. The relative risk was reported as 1.16 (CI = 1.08 – 1.24) or a 16% increased risk for all brain tumors. For gliomas, the risk estimate was reported to be 1.39 (1.07 – 1.82) or a 39% increased risk for those in electrical occupations. A second meta-analysis published by Kheifets et al., ((2001) added results of 9 new studies published after 1995. It reported a new pooled estimate (OR = 1.16, 1.08 – 1.01) that showed little change in the risk estimate overall from 1995.

The evidence for a relationship between exposure and breast cancer is relatively strong in men (Erren, 2001), and some (by no means all) studies show female breast cancer also to be elevated with increased exposure (see Chapter 12). Brain tumors and acoustic neuromas are more common in exposed persons (see Chapter 10). There is less published evidence on other cancers, but Charles et al. (2003) report that workers in the highest 10% category for EMF exposure were twice as likely to die of prostate cancer as those exposed at lower levels (OR 2.02, 95% CI = 1.34-3.04). Villeneuve et al. (2000) report statistically significant elevations of non-Hodgkin's lymphoma in electric utility workers in relation to EMF exposure, while Tynes et al. (2003) report elevated rates of malignant melanoma in persons living near to high voltage power lines. While these observations need replication, they suggest a relationship between exposure and cancer in adults beyond leukemia.

In total the scientific evidence for adult disease associated with EMF exposure is sufficiently strong for adult cancers that preventive steps are appropriate, even if not all reports have shown exactly the same positive relationship. This is especially true since many factors reduce our ability to see disease patterns that might be related to EMF exposure: there is no unexposed population for comparison, for example, and other difficulties in exposure assessment, The evidence for a relationship between EMF exposure and adult cancers and neurodegenerative diseases is sufficiently strong at present to merit preventive actions to reduce EMF exposure.

5. Breast Cancer

There is rather strong evidence from multiple areas of scientific investigation that ELF is related to breast cancer. Over the last two decades there have been numerous epidemiological studies (studies of human illness) on breast cancer in both men and women, although this relationship remains controversial among scientists. Many of these studies report that ELF exposures are related to increased risk of breast cancer (not all studies report such effects, but then, we do not expect 100% or even 50% consistency in results in science, and do not require it to take reasonable preventative action).

> **The evidence from studies on women in the workplace rather strongly suggests that ELF is a risk factor for breast cancer for women with long-term exposures of 10 mG and higher.**

Breast cancer studies of people who work in relatively high ELF exposures (10 mG and above) show higher rates of this disease. Most studies of workers who are exposed to ELF have defined high exposure levels to be somewhere between 2 mG and 10 mG; however this kind of mixing of

relatively low to relatively high ELF exposure just acts to dilute out real risk levels. Many of the occupational studies group exposures so that the highest group is exposed to 4 mG and above. What this means is that a) few people are exposed to much higher levels and b) illness patterns show up at relatively low ELF levels of 4 mG and above. This is another way of demonstrating that existing ELF limits that are set at 933-1000 mG are irrelevant to the exposure levels reporting increased risks.

Laboratory studies that examine human breast cancer cells have shown that ELF exposure between 6 mG and 12 mG can interfere with protective effects of melatonin that fights the growth of these breast cancer cells. For a decade, there has been evidence that human breast cancer cells grow faster if exposed to ELF at low environmental levels. This is thought to be because ELF exposure can reduce melatonin levels in the body. The presence of melatonin in breast cancer cell cultures is known to reduce the growth of cancer cells. The absence of melatonin (because of ELF exposure or other reasons) is known to result in more cancer cell growth.

Laboratory studies of animals that have breast cancer tumors have been shown to have more tumors and larger tumors when exposed to ELF and a chemical tumor promoter at the same time. These studies taken together indicate that ELF is a likely risk factor for breast cancer, and that ELF levels of importance are no higher than many people are exposed to at home and at work. A reasonable suspicion of risk exists and is sufficient evidence on which to recommend new ELF limits; and to warrant preventative action.

> **Given the very high lifetime risks for developing breast cancer, and the critical importance of prevention; ELF exposures should be reduced for all people who are in high ELF environments for prolonged periods of time.**

Reducing ELF exposure is particularly important for people who have breast cancer. The recovery environment should have low ELF levels given the evidence for poorer survival rates for childhood leukemia patients in ELF fields over 2 mG or 3 mG. Preventative action for those who may be at higher risk for breast cancer is also warranted (particularly for those taking tamoxifen as a way to reduce the risk of getting breast cancer, since in addition to reducing the effectiveness of melatonin, ELF exposure may also reduce the effectiveness of tamoxifen at these same low exposure levels). There is no excuse for ignoring the substantial body of evidence we already have that supports an association between breast cancer and ELF exposure; waiting for conclusive evidence is untenable given the enormous costs and societal and personal burdens caused by this disease.

> **Studies of human breast cancer cells and some animal studies show that ELF is likely to be a risk factor for breast cancer. There is supporting evidence for a link between breast cancer and exposure to ELF that comes from cell and animal studies, as well as studies of human breast cancers.**

These are just some of the cancer issues to discuss. It may be reasonable now to make the assumption that all cancers, and other disease endpoints might be related to, or worsened by exposures to EMFs (both ELF and RF).

If one or more cancers are related, why would not all cancer risks be at issue? It can no longer be said that the current state of knowledge rules out or precludes risks to human health. The enormous societal costs and impacts on human suffering by not dealing proactively with this issue require substantive public health policy actions; and actions of governmental agencies charged with the protection of public health to act on the basis of the evidence at hand.

B. Changes in the Nervous System and Brain Function

Exposure to electromagnetic fields has been studies in connection with Alzheimer's disease, motor neuron disease and Parkinson's disease. (4) These diseases all involve the death of specific neurons and may be classified as neurodegenerative diseases. There is evidence that high levels of amyloid beta are a risk factor for Alzheimer's disease, and exposure to ELF can increase this substance in the brain. There is considerable evidence that melatonin can protect the brain against damage leading to Alzheimer's disease, and also strong evidence that exposure to ELF can reduce melatonin levels. Thus it is hypothesized that one of the body's main protections against developing Alzheimer's disease (melatonin) is less available to the body when people are exposed to ELF. Prolonged exposure to ELF fields could alter calcium (Ca2+) levels in neurons and induce oxidative stress (4).   It is also possible that prolonged exposure to ELF fields may stimulate neurons (particularly large motor neurons) into synchronous firing, leading to damage by the buildup of toxins.

Evidence for a relationship between exposure and the neurodegenerative diseases, Alzheimer's and amyotrophic lateral sclerosis (ALS), is strong and relatively consistent (see Chapter 12). While not every publication shows a statistically significant relationship between exposure and disease, ORs of 2.3 (95% CI = 1.0-5.1 in Qio et al., 2004), of 2.3 (95% CI = 1.6-3.3 in Feychting et al., 2003) and of 4.0 (95% CI = 1.4-11.7 in Hakansson et al., 2003) for Alzheimer's Disease, and of 3.1 (95% CI = 1.0-9.8 in Savitz et al., 1998) and 2.2 (95% CI = 1.0-4.7 in Hakansson et al., 2003) for ALS cannot be simply ignored.

> **Alzheimer's disease is a disease of the nervous system. There is strong evidence that long-term exposure to ELF is a risk factor for Alzheimer's disease.**

Concern has also been raised that humans with epileptic disorders could be more susceptible to RF exposure. Low-level RF exposure may be a stressor based on similarities of neurological effects to other known stressors; low-level RF activates both endogenous opioids and other substances in the brain that function in a similar manner to psychoactive drug actions. Such effects in laboratory animals mimic the effects of drugs on the part of the brain that is involved in addiction.

Laboratory studies show that the nervous system of both humans and animals is sensitive to ELF and RF. Measurable changes in brain function and behavior occur at levels associated with new technologies including cell phone use. Exposing humans to cell phone radiation can change

brainwave activity at levels as low as 0.1 watt per kilogram SAR (W/Kg)*** in comparison to the US allowable level of 1.6 W/Kg and the International Commission for Non-ionizing Radiation Protection (ICNIRP) allowable level of 2.0 W/Kg. It can affect memory and learning. It can affect normal brainwave activity. ELF and RF exposures at low levels are able to change behavior in animals.

> **There is little doubt that electromagnetic fields emitted by cell phones and cell phone use affect electrical activity of the brain.**

Effects on brain function seem to depend in some cases on the mental load of the subject during exposure (the brain is less able to do two jobs well simultaneously when the same part of the brain is involved in both tasks). Some studies show that cell phone exposure speeds up the brain's activity level; but also that the efficiency and judgment of the brain are diminished at the same time. One study reported that teenage drivers had slowed responses when driving and exposed to cell phone radiation, comparable to response times of elderly people. Faster thinking does not necessarily mean better quality thinking.

> **Changes in the way in which the brain and nervous system react depend very much on the specific exposures. Most studies only look at short-term effects, so the long-term consequences of exposures are not known.**

Factors that determine effects can depend on head shape and size, the location, size and shape of internal brain structures, thinness of the head and face, hydration of tissues, thickness of various tissues, dialectric constant of the tissues and so on. Age of the individual and state of health also appear to be important variables. Exposure conditions also greatly influence the outcome of studies, and can have opposite results depending on the conditions of exposure including frequency, waveform, orientation of exposure, duration of exposure, number of exposures, any pulse modulation of the signal, and when effects are measured (some responses to RF are delayed). There is large variability in the results of ELF and RF testing, which would be expected based on the large variability of factors that can influence test results. However, it is clearly demonstrated that under some conditions of exposure, the brain and nervous system functions of humans are altered. The consequence of long-term or prolonged exposures have not been thoroughly studied in either adults or in children.

> **The consequence of prolonged exposures to children, whose nervous systems continue to develop until late adolescence, is unknown at this time. This could have serious implications to adult health and functioning in society if years of exposure of the young to both ELF and RF result in diminished capacity for thinking, judgment, memory, learning, and control over behavior.**

People who are chronically exposed to low-level wireless antenna emissions report symptoms such as problems in sleeping (insomnia), fatigue, headache, dizziness, grogginess, lack of concentration, memory problems, ringing in the ears (tinnitus), problems with balance and orientation, and difficulty in multi-tasking. In children, exposures to cell phone radiation have resulted in changes in brain oscillatory activity during some memory tasks. Although scientific studies as yet have not been able to confirm a cause-and-effect relationship; these complaints are widespread and the cause of significant public concern in some countries where wireless technologies are fairly mature and widely distributed (Sweden, Denmark, France, Germany, Italy, Switzerland, Austria, Greece, Israel).   For example, the roll-out of the new 3rd Generation wireless phones (and related community-wide antenna RF emissions in the Netherlands) caused almost immediate public complaints of illness.(5)

Conflicting results from those few studies that have been conducted may be based on the difficulty in providing non-exposed environments for testing to compare to environments that are intentionally exposed. People traveling to laboratories for testing are pre-exposed to a multitude of RF and ELF exposures, so they may already be symptomatic prior to actual testing. Also complicating this is good evidence that RF exposures testing behavioral changes show delayed results; effects are observed after termination of RF exposure. This suggests a persistent change in the nervous system that may be evident only after time has passed, so is not observed during a short testing period.

> **The effects of long-term exposure to wireless technologies including emissions from cell phones and other personal devices, and from whole-body exposure to RF transmissions from cell towers and antennas is simply not known yet with certainty. However, the body of evidence at hand suggests that bioeffects and health impacts can and do occur at exquisitely low exposure levels: levels that can be thousands of times below public safety limits.**

The evidence reasonably points to the potential for serious public health consequences (and economic costs), which will be of global concern with the widespread public use of, and exposure to such emissions. Even a small increase in disease incidence or functional loss of cognition related to new wireless exposures would have a large public health, societal and economic consequences. Epidemiological studies can report harm to health only after decades of exposure, and where large effects can be seen across "average" populations; so these early warnings of possible harm should be taken seriously now by decision-makers.

C. Effects on Genes (DNA)

Cancer risk is related to DNA damage, which alters the genetic blueprint for growth and development.   If DNA is damaged (the genes are damaged) there is a risk that these damaged cells will not die. Instead they will continue to reproduce themselves with damaged DNA, and this is one necessary pre-condition for cancer. Reduced DNA repair may also be an important part of this story. When the rate of damage to DNA exceeds the rate at which DNA can be repaired, there is the possibility of retaining mutations and initiating cancer. Studies on how ELF and RF may affect genes and DNA is important, because of the possible link to cancer.

Even ten years ago, most people believed that very weak ELF and RF fields could not possibly have any effect at all on DNA and how cells work (or are damaged and cannot do their work properly). The argument was that these weak fields are do not possess enough energy (are not physically strong enough) to cause damage. However, there are multiple ways we already know about where energy is not the key factor in causing damage. For example, exposure to toxic chemicals can cause damage. Changing the balance of delicate biological processes, including hormone balances in the body, can damage or destroy cells, and cause illness. In fact, many chronic diseases are directly related to this kind of damage that does not require any heating at all. Interference with cell communication (how cells interact) may either cause cancer directly or promote existing cancers to grow faster.

Using modern gene-testing techniques will probably give very useful information in the future about how EMFs targets and affects molecules in the body. At the gene level, there is some evidence now that EMFs (both ELF and RF) can cause changes in how DNA works. Laboratory studies have been conducted to see whether (and how) weak EMFs fields can affect how genes and proteins function. Such changes have been seen in some, but not all studies.

Small changes in protein or gene expression might be able to alter cell physiology, and might be able to cause later effects on health and well-being. The study of genes, proteins and EMFs is still in its infancy, however, by having some confirmation at the gene level and protein level that weak EMFs exposures do register changes may be an important step in establishing what risks to health can occur.

What is remarkable about studies on DNA, genes and proteins and EMFs is that there should be no effect at all if it were true that EMFs is too weak to cause damage. Scientists who believe that the energy of EMFs is insignificant and unlikely to cause harm have a hard time explaining these changes, so are inclined to just ignore them. The trouble with this view is that the effects are occurring. Not being able to explain these effects is not a good reason to consider them imaginary or unimportant.

The European research program (REFLEX) documented many changes in normal biological functioning in tests on DNA (3). The significance of these results is that such effects are directly related to the question of whether human health risks might occur, when these changes in genes and DNA happen. This large research effort produced information on EMFs effects from more than a dozen different researchers. Some of the key findings included:

> *"Gene mutations, cell proliferation and apoptosis are caused by or result in altered gene and protein expression profiles. The convergence of these events is required for the development of all chronic diseases."* (3)

> *"Genotoxic effects and a modified expression of numerous genes and proteins after EMF exposure could be demonstrated with great certainty."* (3)

> *"RF-EMF produced genotoxic effects in fibroblasts, HL-60 cells, granulosa cells of rats and neural progenitor cells derived from mouse embryonic stem cells."* (Participants 2, 3 and 4). (3)

> *"Cells responded to RF exposure between SAR levels of 0.3 and 2 W/Kg with a significant increase in single- and double-strand DNA breaks and in micronuclei frequency."* (Participants 2, 3 and 4). (3)

"In HL-60 cells an increase in intracellular generation of free radicals accompanying RF-EMF exposure could clearly be demonstrated." (Participant 2). (3)

"The induced DNA damage was not based on thermal effects and arouses consideration about the environmental safety limits for ELF-EMF exposure." (3)

"The effects were clearly more pronounced in cells from older donors, which could point to an age-related decrease of DNA repair efficiency of ELF-EMF induced DNA strand breaks." (3)

Both ELF and RF exposures can be considered genotoxic (will damage DNA) under certain conditions of exposure, including exposure levels that are lower than existing safety limits.

D. Effects on Stress Proteins (Heat Shock Proteins)

In nearly every living organism, there is a special protection launched by cells when they are under attack from environmental toxins or adverse environmental conditions. This is called a stress response, and what are produced are stress proteins (also known as heat shock proteins). Plants, animals and bacteria all produce stress proteins to survive environmental stressors like high temperatures, lack of oxygen, heavy metal poisoning, and oxidative stress (a cause of premature aging).   We can now add ELF and RF exposures to this list of environmental stressors that cause a physiological stress response.

Very low-level ELF and RF exposures can cause cells to produce stress proteins, meaning that the cell recognizes ELF and RF exposures as harmful. This is another important way in which scientists have documented that ELF and RF exposures can be harmful, and it happens at levels far below the existing public safety standards.

An additional concern is that if the stress goes on too long, the protective effect is diminished. There is a reduced response if the stress goes on too long, and the protective effect is reduced. This means the cell is less protected against damage, and it is why prolonged or chronic exposures may be quite harmful, even at very low intensities.

The biochemical pathway that is activated is the same for ELF and for RF exposures, and it is non-thermal (does not require heating or induced electrical currents, and thus the safety standards based on protection from heating are irrelevant and not protective).   ELF exposure levels of only 5 to 10 mG have been shown to activate the stress response genes (Table 2, Section 6). The specific absorption rate or SAR is not the appropriate measure of biological threshold or dose, and should not be used as the basis for a safety standard, since SAR only regulates against thermal damage.

17

E. Effects on the Immune System

The immune system is another defense we have against invading organisms (viruses, bacteria, and other foreign molecules). It protects us against illness, infectious diseases, and tumor cells. There are many different kinds of immune cells; each type of cell has a particular purpose, and is launched to defend the body against different kinds of exposures that the body determines might be harmful.

> **There is substantial evidence that ELF and RF can cause inflammatory reactions, allergy reactions and change normal immune function at levels allowed by current public safety standards.**

The body's immune defense system senses danger from ELF and RF exposures, and targets an immune defense against these fields, much like the body's reaction in producing stress proteins. These are additional indicators that very low intensity ELF and RF exposures are a) recognized by cells and b) can cause reactions as if the exposure is harmful. Chronic exposure to factors that increase allergic and inflammatory responses on a continuing basis are likely to be harmful to health. Chronic inflammatory responses can lead to cellular, tissue and organ damage over time. Many chronic diseases are thought to be related to chronic problems with immune system function.

The release of inflammatory substances, such as histamine, are well-known to cause skin reactions, swelling, allergic hypersensitivity and other conditions that are normally associated with some kind of defense mechanism. The human immune system is part of a general defense barrier that protects against harmful exposures from the surrounding environment.   When the immune system is aggravated by some kind of attack, there are many kinds of immune cells that can respond. Anything that triggers an immune response should be carefully evaluated, since chronic stimulation of the immune system may over time impair the system's ability to respond in the normal fashion.

Measurable physiological changes (mast cell increases in the skin, for example that are markers of allergic response and inflammatory cell response) are triggered by ELF and RF at very low intensities. Mast cells, when activated by ELF or RF, will break (degranulate) and release irritating chemicals that cause the symptoms of allergic skin reactions.

There is very clear evidence that exposures to ELF and RF at levels associated with cell phone use, computers, video display terminals, televisions, and other sources can cause these skin reactions. Changes in skin sensitivity have been measured by skin biopsy, and the findings are remarkable. Some of these reactions happen at levels equivalent to those of wireless technologies in daily life. Mast cells are also found in the brain and heart, perhaps targets of immune response by cells responding to ELF and RF exposures, and this might account for some of the other symptoms commonly reported (headache, sensitivity to light, heart arrythmias and other cardiac symptoms). Chronic provocation by exposure to ELF and RF can lead to immune dysfunction, chronic allergic responses, inflammatory diseases and ill health if they occur on a continuing basis over time.

These clinical findings may account for reports of persons with electrical hypersensitivity, which is a condition where there is intolerance for any level of exposure to ELF and/or RF. Although there is not yet a substantial scientific assessment (under controlled conditions, if that is even possible); anecdotal reports from many countries show that estimates range from 3% to perhaps 5% of populations, and it is a growing problem. Electrical hypersensitivity, like multiple chemical sensitivity, can be disabling and require the affected person to make drastic changes in work and living circumstances, and suffer large economic losses and loss of personal freedom. In Sweden, electrohypersensitivity (EHS) is officially recognized as fully functional impairment (i.e., it is not regarded as a disease – see Section 6, Appendix A).

F.   Plausible Biological Mechanisms

Plausible biological mechanisms are already identified that can reasonably account for most biological effects reported for exposure to RF and ELF at low-intensity levels (oxidative stress and DNA damage from free radicals leading to genotoxicity; molecular mechanisms at very low energies are plausible links to disease, e.g., effect on electron transfer rates linked to oxidative damage, DNA activation linked to abnormal biosynthesis and mutation).   It is also important to remember that traditional public health and epidemiological determinations do not require a proven mechanism before inferring a causal link between EMFs exposure and disease (12). Many times, proof of mechanism is not known before wise public health responses are implemented.

"Obviously, melatonin's ability to protect DNA from oxidative damage has implications for many types of cancer, including leukemia, considering that DNA damage due to free radicals is believed to be the initial oncostatic event in a majority of human cancers [Cerutti et al., 1994]. In addition to cancer, free radical damage to the central nervous system is a significant component of a variety of neurodegenerative diseases of the aged including Alzheimer's disease and Parkinsonism. In experimental animal models of both of these conditions, melatonin has proven highly effective in forestalling their onset, and reducing their severity [Reiter et al., 2001]."   (13)

> **Oxidative stress through the action of free radical damage to DNA is a plausible biological mechanism for cancer and diseases that involve damage from ELF to the central nervous system.**

G.   Another Way of Looking at EMFs: Therapeutic Uses

Many people are surprised to learn that certain kinds of EMFs treatments actually can heal. These are medical treatments that use EMFs in specific ways to help in healing bone fractures, to heal wounds to the skin and underlying tissues, to reduce pain and swelling, and for other post-surgical needs. Some forms of EMFs exposure are used to treat depression.

EMFs have been shown to be effective in treating conditions of disease at energy levels far below current public exposure standards. This leads to the obvious question. How can scientists dispute

the harmful effects of EMF exposures while at the same time using forms of EMF treatment that are proven to heal the body?

> **Medical conditions are successfully treated using EMFs at levels below current public safety standards, proving another way that the body recognizes and responds to low-intensity EMF signals. Otherwise, these medical treatments could not work. The FDA has approved EMFs medical treatment devices, so is clearly aware of this paradox.**

Random exposures to EMFs, as opposed to EMFs exposures done with clinical oversight, could lead to harm just like the unsupervised use of pharmaceutical drugs. This evidence forms a strong warning that indiscriminate EMF exposure is probably a bad idea.

> **No one would recommend that drugs used in medical treatments and prevention of disease be randomly given to the public, especially to children. Yet, random and involuntary exposures to EMFs occur all the time in daily life.**

The consequence of multiple sources of EMFs exposures in daily life, with no regard to cumulative exposures or to potentially harmful combinations of EMFs exposures means several things. First, it makes it very difficult to do clinical studies because it is almost impossible to find anyone who is not already exposed. Second, people with and without diseases have multiple and overlapping exposures – this will vary from person to person.

Just as ionizing radiation can be used to effectively diagnose disease and treat cancer, it is also a cause of cancer under different exposure conditions. Since EMFs are both a cause of disease, and also used for treatment of disease, it is vitally important that public exposure standards reflect our current understanding of the biological potency of EMF exposures, and develop both new public safety limits and measures to prevent future exposures.

III.   EMF EXPOSURE AND PRUDENT PUBLIC HEALTH PLANNING

> • **The scientific evidence is sufficient to warrant regulatory action for ELF; and it is substantial enough to warrant preventative actions for RF.**
>
> • **The standard of evidence for judging the emerging scientific evidence necessary to take action should be proportionate to the impacts on health and well-being**
>
> • **The exposures are widespread.**
>
> • **Widely accepted standards for judging the science are used in this assessment.**

Public exposure to electromagnetic radiation (power-line frequencies, radiofrequency and microwave) is growing exponentially worldwide. There is a rapid increase in electrification in developing countries, even in rural areas. Most members of society now have and use cordless phones, cellular phones, and pagers. In addition, most populations are also exposed to antennas in communities designed to transmit wireless RF signals. Some developing countries have even given up running land lines because of expense and the easy access to cell phones. Long-term and cumulative exposure to such massively increased RF has no precedent in human history. Furthermore, the most pronounced change is for children, who now routinely spend hours each day on the cell phone. Everyone is exposed to a greater or lesser extent. No one can avoid exposure, since even if they live on a mountain-top without electricity there will likely be exposure to communication-frequency RF exposure. Vulnerable populations (pregnant women, very young children, elderly persons, the poor) are exposed to the same degree as the general population. Therefore it is imperative to consider ways in which to evaluate risk and reduce exposure. Good public health policy requires preventative action proportionate to the potential risk of harm and the public health consequence of taking no action.

IV. RECOMMENDED ACTIONS

A. Defining new exposure standards for ELF

This chapter concludes that new ELF limits are warranted based on a public health analysis of the overall existing scientific evidence. The public health view is that new ELF limits are needed now. They should reflect environmental levels of ELF that have been demonstrated to increase

risk for childhood leukemia, and possibly other cancers and neurological diseases. ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor. It is no longer acceptable to build new power lines and electrical facilities that place people in ELF environments that have been determined to be risky. These levels are in the 2 to 4 milligauss* (mG) range, not in the 10s of mG or 100s of mG. The existing ICNIRP limit is 1000 mG (904 mG in the US) for ELF is outdated and based on faulty assumptions.   These limits are can no longer be said to be protective of public health and they should be replaced. A safety buffer or safety factor should also be applied to a new, biologically-based ELF limit, and the conventional approach is to add a safety factor lower than the risk level.

While new ELF limits are being developed and implemented, a reasonable approach would be a 1 mG planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG limit for all other new construction. It is also recommended for that a 1 mG limit be established for existing habitable space for children and/or women who are pregnant (because of the possible link between childhood leukemia and *in utero* exposure to ELF). This recommendation is based on the assumption that a higher burden of protection is required for children who cannot protect themselves, and who are at risk for childhood leukemia at rates that are traditionally high enough to trigger regulatory action. This situation in particular warrants extending the 1 mG limit to existing occupied space. "Establish" in this case probably means formal public advisories from relevant health agencies. While it is not realistic to reconstruct all existing electrical distribution systems, in the short term; steps to reduce exposure from these existing systems need to be initiated, especially in places where children spend time, and should be encouraged. These limits should reflect the exposures that are commonly associated with increased risk of child hood leukemia (in the 2 to 5 mG range for all children, and over 1.4 mG for children age 6 and younger). Nearly all of the occupational studies for adult cancers and neurological diseases report their highest exposure category is 4 mG and above, so that new ELF limits should target the exposure ranges of interest, and not necessarily higher ranges.

Avoiding chronic ELF exposure in schools, homes and the workplace above levels associated with increased risk of disease will also avoid most of the possible bioactive parameters of ELF discussed in the relevant literature.

B. Defining preventative actions for reduction in RF exposures

Given the scientific evidence at hand (Chapter 17), the rapid deployment of new wireless technologies that chronically expose people to pulsed RF at levels reported to cause bioeffects, which in turn, could reasonably be presumed to lead to serious health impacts, is of public health concern.   Section 17 summarizes evidence that has resulted in a public health recommendation that preventative action is warranted to reduce or minimize RF exposures to the public. There is suggestive to strongly suggestive evidence that RF exposures may cause changes in cell membrane function, cell communication, cell metabolism, activation of proto-oncogenes and can trigger the production of stress proteins at exposure levels below current regulatory limits. Resulting effects can include DNA breaks and chromosome aberrations, cell death including death of brain neurons, increased free radical production, activation of the endogenous opioid system, cell stress and premature aging, changes in brain function including memory loss, retarded learning, slower motor function and other performance impairment in children, headaches and fatigue, sleep disorders, neurodegenerative conditions, reduction in melatonin secretion and cancers (Chapters 5, 6, 7, 8, 9, 10, and 12).

As early as 2000, some experts in bioelectromagnetics promoted a 0.1 µW/cm2 limit (which is 0.614 Volts per meter) for ambient outdoor exposure to pulsed RF, so generally in cities, the public would have adequate protection against involuntary exposure to pulsed radiofrequency (e.g., from cell towers, and other wireless technologies). The Salzburg Resolution of 2000 set a target of 0.1 µW/cm2 (or 0.614 V/m) for public exposure to pulsed radiofrequency. Since then, there are many credible anecdotal reports of unwellness and illness in the vicinity of wireless transmitters (wireless voice and data communication antennas) at lower levels. Effects include sleep disruption, impairment of memory and concentration, fatigue, headache, skin disorders, visual symptoms (floaters), nausea, loss of appetite, tinnitus, and cardiac problems (racing heartbeat), There are some credible articles from researchers reporting that cell tower -level RF exposures (estimated to be between 0.01 and 0.5 µW/cm2) produce ill-effects in populations living up to several hundred meters from wireless antenna sites.

This information now argues for thresholds or guidelines that are substantially below current FCC and ICNIPR standards for whole body exposure. Uncertainty about how low such standards might have to go to be prudent from a public health standpoint should not prevent reasonable

efforts to respond to the information at hand.   No lower limit for bioeffects and adverse health effects from RF has been established, so the possible health risks of wireless WLAN and WI-FI systems, for example, will require further research and no assertion of safety at any level of wireless exposure (chronic exposure) can be made at this time. The lower limit for reported human health effects has dropped 100-fold below the safety standard (for mobile phones and PDAs); 1000- to 10,000-fold for other wireless (cell towers at distance; WI-FI and WLAN devices). The entire basis for safety standards is called into question, and it is not unreasonable to question the safety of RF at any level.

A cautionary target level for pulsed RF exposures for ambient wireless that could be applied to RF sources from cell tower antennas, WI-FI, WI-MAX and other similar sources is proposed. The recommended cautionary target level is 0.1 microwatts per centimeter squared (μW/cm2)** (or 0.614 Volts per meter or V/m)** for pulsed RF where these exposures affect the general public; this advisory is proportionate to the evidence and in accord with prudent public health policy. A precautionary limit of 0.1 μW/cm2 should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers and PDAs and other sources of radiofrequency radiation. An outdoor precautionary limit of 0.1 μW/cm2 would mean an even lower exposure level inside buildings, perhaps as low as 0.01 μW/cm2. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however, for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of WI-FI technologies, we also recommend that wired alternatives to WI-FI be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts.   This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future.

Broadcast facilities that chronically expose nearby residents to elevated RF levels from AM, FM and television antenna transmission are also of public health concern given the potential for very high RF exposures near these facilities (antenna farms).   RF levels can be in the 10s to several 100's of μW/cm2 in residential areas within half a mile of some broadcast sites (for example,

Lookout Mountain, Colorado and Awbrey Butte, Bend, Oregon). Such facilities that are located in, or expose residential populations and schools to elevated levels of RF will very likely need to be re-evaluated for safety.

For emissions from wireless devices (cell phones, personal digital assistant or PDA devices, etc) there is enough evidence for increased risk of brain tumors and acoustic neuromas now to warrant intervention with respect to their use. Redesign of cell phones and PDAs could prevent direct head and eye exposure, for example, by designing new units so that they work only with a wired headset or on speakerphone mode.

These effects can reasonably be presumed to result in adverse health effects and disease with chronic and uncontrolled exposures, and children may be particularly vulnerable. The young are also largely unable to remove themselves from such environments. Second-hand radiation, like second-hand smoke is an issue of public health concern based on the evidence at hand.

V.   CONCLUSIONS

• We cannot afford 'business as usual" any longer.   It is time that planning for new power lines and for new homes, schools and other habitable spaces around them is done with routine provision for low-ELF environments . The business-as-usual deployment of new wireless technologies is likely to be risky and harder to change if society does not make some educated decisions about limits soon. Research must continue to define what levels of RF related to new wireless technologies are acceptable; but more research should not prevent or delay substantive changes today that might save money, lives and societal disruption tomorrow.

 • New regulatory limits for ELF are warranted. ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor. It is no longer acceptable to build new power lines and electrical facilities that place people in ELF environments that have been determined to be risky (at levels generally at 2 mG and above).

• While new ELF limits are being developed and implemented, a reasonable approach would be a 1 mG planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG limit for all other new construction, It is also recommended for that a 1 mG limit be established for existing habitable space for children and/or women who are pregnant . This recommendation is based on the assumption that a higher burden of protection is required for children who cannot protect themselves, and who are at risk for childhood leukemia at rates that are traditionally high enough to trigger regulatory action. This situation in particular warrants extending the 1 mG limit to existing occupied space. "Establish" in this case probably means formal public advisories from relevant health agencies.

• While it is not realistic to reconstruct all existing electrical distributions systems, in the short term; steps to reduce exposure from these existing systems need to be initiated, especially in places where children spend time, and should be encouraged.

• A precautionary limit of 0.1 (μW/cm2 (which is also 0.614 Volts per meter) should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers and PDAs and other sources of radiofrequency radiation. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however, for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of WI-FI technologies, we also recommend that wired alternatives to WI-FI be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts.   This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future.

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Some Quick Definitions for Units of Measurement of ELF and RF

*Milligauss (mG)

A milligauss is a measure of ELF intensity and is abbreviated mG. This is used to describe electromagnetic fields from appliances, power lines, interior electrical wiring.

**Microwatts per centimeter squared (µW/cm2)

Radiofrequency radiation in terms of power density is measured in microwatts per centimeter squared and abbreviated (µW/cm2). It is used when talking about emissions from wireless facilities, and when describing ambient RF in the environment. The amount of allowable RF near a cell tower is 1000 µW/cm2 for some cell phone frequencies, for example.

***Specific Absorption Rate (SAR is measured in watts per kilogram or W/Kg)

SAR stands for specific absorption rate. It is a calculation of how much RF energy is absorbed into the body, for example when a cell phone or cordless phone is pressed to the head. SAR is expressed in watts per kilogram of tissue (W/Kg). The amount of allowable energy into 1 gram of brain tissue from a cell phone is 1.6 W/Kg in the US. For whole body exposure, the exposure is 0.8 W/Kg averaged over 30 minutes for the general public. International standards in most countries are similar, but not exactly the same.

Table 1-1 BioInitiative Report Overall Conclusions

OVERALL SUMMARY OF CONCLUSIONS

• The existing ICNIRP and FCC limits for public and occupational exposure to ELF and RF are insufficiently protective of public health.

• Biologically-based public and occupational exposure standards for extra-low frequency and radiofrequency radiation are recommended to address bioeffects and potential adverse health effects of chronic exposure to ELF and RF. These effects are now widely reported to occur at exposure levels significantly below most current national and international limits.

• A biologically-based exposure limit is one that is protective against ELF and RF intensity and modulation factors which, with chronic exposure, can reasonably be presumed to result in significant impacts to health and well-being.

• Research is needed (but should not delay) regulatory action for ELF and <u>substantive</u> *preventative action* for RF proportionate to potential health and wellbeing risks from chronic exposure.

• A biologically-based exposure limit should reflect current scientific knowledge of bioeffects and health effects, and impose new limits based on preventative action as defined by the Precautionary Principle (EEA, 2001).

• Biologically-based exposure standards shall be protective against exposures levels of ELF and RF that affect or change normal biological functioning of organisms (humans). They shall not be based solely on energy absorption or thermal levels of energy input, or resulting tissue heating. They shall be protective against chronic exposure responses.

• The existing standards are based on thermal (heating) limits, and do not address non-thermal (or low-intensity) exposures which are widely reported to cause bioeffects, some likely leading to adverse health effects with chronic exposure.

• Biological effects may include both potential adverse health effects and loss of homeostasis and well-being.

• Biologically-based exposure standards are needed to prevent disruption of normal body processes. Effects are reported for DNS damage (genotoxicity that is directly linked to integrity of the human genome), cellular communication, cellular metabolism and repair, cancer surveillance within the body; and for protection against cancer and neurological diseases. Also reported are neurological effects including impairment of sleep and sleep architecture, cognitive function and memory; depression; cardiac effects; pathological leakage of the blood-brain barrier; and impairment of normal immune function, fertility and reproduction.

• Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different effects. In addition, in order to understand the biological consequences of EMF exposure, one must know whether the effect is cumulative, whether compensatory responses result, and when homeostasis will break down.

• Plausible biological mechanisms that can account for genotoxicity (DNA damage) are already well known (oxidative damage via free-radical actions) although it should also be said that there is not yet proof. *However, proof of mechanism is not required to set prudent public health policy, nor is it mandatory to set new guidelines or limits if adverse health effects occur at lower-than-existing IEEE and ICNIRP standards.*

Table 1-1 BioInitiative Report Overall Conclusions

OVERALL SUMMARY OF CONCLUSIONS (continued

• The SCENIHR report (2007) states that "for breast cancer and cardiovascular disease, recent research has indicated that an association with EMF is unlikely." The WHO ELF Health Criteria Monograph (2007) states "The evidence does not support an association between ELF exposure and cardiovascular disease" and "(T)he evidence for breast cancer was also considered to be effectively negative, while for other diseases it was judged to be inadequate." Neither conclusion is supported by any finding by IARC that would classify EMF as Class 4 (Not A Carcinogen), so it is premature for either group to dismiss the evidence for EMF as a potential risk factor for either breast cancer or for cardiovascular disease.

• The standard for taking action should be precautionary; action should not be deferred while waiting for final proof or causal evidence to be established that EMF is harmful to health and well-being.

• There is great public concern over increasing levels of involuntary exposure to radiofrequency and ELF-modulated radiofrequency exposures from new wireless technologies; there is widespread public resistance to radiofrequency and extra-low frequency radiation exposures which are allowable under current, thermally-based exposure standards.

• There is inadequate warning and notice to the public about possible risks from wireless technologies in the marketplace, which is resulting in adoption and use of technologies that may have adverse health consequences which are still unknown to the public. There is no "informed consent".

• No positive assertion of safety can be made by governments that continue to support and enforce exposure limits for RF and ELF based on ICNIRP or IEEE criteria (or the equivalent). Governments that are considering proposals to relax existing RF and ELF standards should reject these proposals given the weight of scientific evidence that is available; and the clear disconnect between existing public safety limits and their responsibility to provide safe and healthful living environments for all segments of affected populations.

Section 5   Genotoxicity Based on Proteomics

• EMF exposure can change gene and/or protein expression in certain types of cells, even at intensities lower than ICNIRP recommended values.

• The biological consequences of most of the changed genes/proteins are still unclear, and need to be further explored.

• The EMF research community should pay equal attention to the negative reports as to the positive ones. Not only the positive findings need to be replicated, all the negative ones are also needed to be validated.

• The IEEE and WHO data bases do not include the majority of ELF studies (only 6 of 14 in the WHO; 0 of 16 in IEEE); they do include the majority of the RF studies (14 of 16).

Table 1-1 BioInitiative Report Overall Conclusions

Section 6   Genotoxicity (DNA Damage from RF and ELF)

• Toxicity to the genome can lead to a change in cellular functions, cancer, and cell death. One can conclude that under certain conditions of exposure RF is genotoxic. Data available are mainly applicable only to cell phone radiation exposure. One study reports that RF at levels equivalent to the vicinity of base stations and RF- transmission towers is genotoxic and could cause DNA damage (Phillips et al., 1998).

• RF may be considered genotoxic (cause DNA damage). Of 28 total studies on radiofrequency radiation (RF) and DNA damage, 14 studies reported effects (50%) and 14 reported no significant effect (50%). Of 29 total studies on radiofrequency radiation and micronucleation, 16 studies reported effects (55%) and 13 reported no significant effect (45%). Of 21 total studies on chromosome and genome damage from radiofrequency radiation, 13 studies (62%) reported effects and 8 studies (38%) reported no significant effects.

• During cell phone use, a relatively constant mass of tissue in the brain is exposed to radiation at relatively high intensity (peak SAR of 4 - 8 W/kg). Several studies have reported DNA damage at lower than 4 W/kg.

• Since critical genetic mutations in one single cell are sufficient to lead to cancer and there are millions of cells in a gram of tissue, *it is **inconceivable*** that the base of the IEEE SAR standard was changed from averaged over 1 gram of tissue to 10 grams.

• Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different consequences. In order to understand the biological consequence of exposure, one must understand whether the effect is cumulative, whether compensatory responses result and when homeostasis will break down.   The choice of cell type or organism studied can also influence the outcome.

• Extremely-low frequency (ELF) has also been shown to be genotoxic and cause DNA damage. Of 41 relevant studies of genotoxicity and ELF exposure, 27 studies (66%) report DNA damage and 14 studies (44%) report no significant effect.

Table 1-1 BioInitiative Report Overall Conclusions

Section 7: Stress Response

• Scientific research on stress proteins has shown that the public is not being protected from potential damage that can be caused by exposure to EMF, both power frequency (ELF) and radio frequency (RF).

• Cells react to an EMF as potentially harmful by producing stress proteins (heat shock proteins or hsp).

• Direct interaction of ELF and RF with DNA has been documented and both activate the synthesis of stress proteins.

• The biochemical pathway that is activated is the same pathway in both ELF and RF and it is non-thermal.

• Many biological systems are affected by EMFs (meaning both ELF and RF trigger stress proteins).

• Many frequencies are active. Field strength and exposure duration thresholds are very low.

• Molecular mechanisms at very low energies are plausible links to disease (e.g., effect on electron transfer rates linked to oxidative damage, DNA activation linked to abnormal biosynthesis and mutation).   Cells react to an EMF as potentially harmful.

• Many lines of research now point to changes in DNA electron transfer as a plausible mechanism of action as a result of non-thermal ELF and RF.

• The same biological reaction (production of stress proteins) to an EMF can be activated in more than one division of the EM spectrum.

• Direct interaction of ELF and RF with DNA has been documented and both activate the synthesis of stress proteins.

• Thresholds triggering stress on biological systems occur at environment levels on the order of 0.5 to 1.0 μT for ELF.

• DNA damage (e.g., strand breaks), a cause of cancer, occurs at levels of ELF and RF that are below the safety limits. Also, there is no protection against cumulative effects stimulated by different parts of the EM spectrum.

• The scientific basis for EMF safety limits is flawed when the same biological mechanisms are activated in ELF and RF ranges at vastly different levels of the Specific Absorption Rate (SAR). Activation of DNA to synthesize stress proteins (the stress response) is stimulated in the ELF at a non-thermal SAR level that is over a billion times lower than the same process activated by RF at the thermal level.

• There is a need for a biological standard to replace the thermal standard and to also protect against cumulative effects across the EM spectrum.

• Based on studies of stress proteins, the specific absorption rate (SAR) is not the appropriate measure of biological threshold or dose, and should not be used as a basis for a safety standard since it regulates against thermal effects only.

Table 1-1 BioInitiative Report Overall Conclusions

Section 8   Effects on Immune Function

• Both human and animal studies report large immunological changes with exposure to environmental levels of electromagnetic fields (EMFs). Some of these exposure levels are equivalent to those of e.g. wireless technologies in daily life.

• Measurable physiological changes (mast cells increases, for example) that are bedrock indicators of allergic response and inflammatory conditions are stimulated by EMF exposures.

• Chronic exposure to such factors that increase allergic and inflammatory responses on a continuing basis may be harmful to health.

• It is possible that chronic provocation by exposure to EMF can lead to immune dysfunction, chronic allergic responses, inflammatory responses and ill health if they occur on a continuing basis over time. This is an important area for future research.

 • Specific findings from studies on exposures to various types of modern equipment and/or EMFs report over-reaction of the immune system; morphological alterations of immune cells; profound increases in mast cells in the upper skin layers, increased degranulation of mast cells and larger size of mast cells in electrohypersensitive individuals; presence of biological markers for inflammation that are sensitive to EMF exposure at non-thermal levels; changes in lymphocyte viability; decreased count of NK cells; decreased count of T lymphocytes; negative effects on pregnancy (uteroplacental circulatory disturbances and placental dysfunction with possible risks to pregnancy); suppressed or impaired immune function; and inflammatory responses which can ultimately result in cellular, tissue and organ damage.

• Electrical hypersensitivity is reported by individuals in the United States, Sweden, Switzerland, Germany. Denmark and many other countries of the world. Estimates range from 3% to perhaps 10% of populations, and appears to be a growing condition of ill-health leading to lost work and productivity.

• The WHO and IEEE literature surveys do not include all of the relevant papers cited here, leading to the conclusion that evidence has been ignored in the current WHO ELF Health Criteria Monograph; and the proposed new IEEE C95.1 RF public exposure limits (April 2006).

• The current international public safety limits for EMFs do not appear to be sufficiently protective of public health at all, based on the studies of immune function. New, biologically-based public standards are warranted that take into account low-intensity effects on immune function and health that are reported in the scientific literature.

Table 1-1   BioInitiative Report Overall Conclusions

Section 9   Neurology and Behavioral Effects

• Effects on neurophysiological and cognitive functions are quite well established.

• Studies on EEG and brain evoked-potentials in humans exposed to cellular phone radiation predominantly showed positive effects (i.e., positive means the exposure has the ability to change brainwave activity even at exposure levels where no effect would be expected, based on traditional understanding and safety limits).

• There is little doubt that electromagnetic fields emitted by cell phones and cell phone use affect electrical activity in the brain.

• The behavioral consequences of these neuroelectrophysiological changes are not always predictable and research on electrophysiology also indicates that effects are dependent on the mental load of the subjects during exposure, e.g., on the complexity of the task that a subject is carrying out.

 • Most of the studies carried out so far are short-term exposure experiments, whereas cell phone use causes long-term repeated exposure of the brain.

• In most of the behavioral experiments, effects were observed after the termination of RF exposure. In some experiments, tests were made days after exposure. This suggests a persistent change in the nervous system after exposure to RF.

• In many instances, neurological and behavioral effects were observed at a SAR less than 4 W/kg. This directly contradicts the basic assumption of the IEEE guideline criterion.

• Caution should be taken in concluding that a neurological effect resulted solely from the action of RF on the central nervous system because it is well known that the functions of the central nervous system can be affected by activity in the peripheral nervous system.

Table 1-1 BioInitiative Report Overall Conclusions

Section 10   Brain Tumors and Acoustic Neuromas

• Studies on brain tumors and use of mobile phones for $\geq$ 10 years gave a consistent pattern of an increased risk for acoustic neuroma and glioma.

• Cell phone use > 10 years give a consistent pattern of an increased risk for acoustic neuroma and glioma, most pronounced for high-grade glioma. The risk is highest for ipsilateral exposure.

Section 10   Brain Tumors and RF - Epidemiology

• Only a few studies of long-term exposure to low levels of RF fields and brain tumors exist, all of which have methodological shortcomings including lack of quantitative exposure assessment. Given the crude exposure categories and the likelihood of a bias towards the null hypothesis of no association, *the body of evidence is consistent with a moderately elevated risk.*

• Occupational studies indicate that long-term exposure at workplaces may be associated with an elevated brain tumor risk.

• Although the population attributable risk is low (likely below 4%), still more than 1,000 cases per year in the US can be attributed to RF exposure at workplaces alone. Due to the lack of conclusive studies of environmental RF exposure and brain tumors the potential of these exposures to increase the risk cannot be estimated.

• Overall, the evidence suggests that long-term exposure to levels generally below current guideline levels still carry the risk of increasing the incidence of brain tumors.

• Epidemiological studies as reviewed in the IEEE C95.1 revision (2006) are deficient to the extent that the entire analysis is professionally unsupportable. IEEEs dismissal of epidemiological studies that link RF exposure to cancer endpoints should be disregarded, as well as any IEEE conclusions drawn from this flawed analysis of epidemiological studies.

Table 1-1   BioInitiative Report Overall Conclusions

Brain Tumors and Acoustic Neuromas

Additional Data from Section 10

• Mobile phone use increases the risk of acoustic neuroma for persons using a mobile phone 10 years or longer by 30% (when used on both sides of head) to 240% (habitually used on one side of head).   This information relies on a meta-analysis of several major studies. For acoustic neuroma studies by Lönn et al., (2004), Christensen et al., (2004) Schoemaker et al., (2005) and Hardell et al., (2006a) all giving results for at least 10 years latency period or more. Overall OR = 1.3, 95 % CI = 0.6-2.8 was obtained increasing to OR = 2.4, 95 % CI = 1.1-5.3 for ipsilateral mobile phone use (Lönn et al., 2004, Schoemaker et al., 2005, Hardell et al., 2006).

• There is observational support for the association between acoustic neuroma and the use of mobile phones since some studies report that the tumor is often located in an anatomical area with high exposure during calls with cellular or cordless phones (Hardell et al., 2003).

• Mobile phone use increases the risk of brain tumors (glioma) for persons using a mobile phone 10 years or longer by 20% (when used on both sides of head) to 200% (habitually used on one side of head). This information relies on a meta-analysis of several major studies. For glioma OR = 1.2, [95 % CI = 0.8-1.9] was calculated (Lönn et al., 2005, Christensen et al., 2005, Hepworth et al., 2006, Schüz et al., 2006, Hardell et al., 2006b, Lahkola et al., 2007). Ipsilateral use yielded OR = 2.0, [95 % CI = 1.2-3.4](Lönn et al., 2005, Hepworth et al., 2006, Hardell et al., 2006b, Lahkola et al., 2007).

• Cordless phone use is also associated with an increased risk for acoustic neuromas and brain tumors (both low-and high-grade gliomas (Hardell et al., 2006 a,b).

• The increased risk of acoustic neuroma from use of a cordless phone for ten years or more was reported to be 310% higher risk (when the cordless phone habitually used on the same-side of the head) in Hardell et al., 2006a.

• The increased risk of high-grade glioma from use of a cordless phone for ten years or more was reported to be 220% higher risk (when cordless used on both sides of head) to 470% higher risk (when cordless used habitually on same side of head) in Hardell et al., 2006b.

• The increased risk of low-grade glioma from use of a cordless phone for ten years or more was reported to be 60% higher risk (when cordless used on both sides of head) to 320% higher risk (when cordless used habitually on same side of head) in Hardell et al., 2006b.

• The current standard for exposure to microwaves during mobile phone use and for cordless phone use is not safe considering studies reporting long-term brain tumor risk.

Table 1-1 BioInitiative Report Overall Conclusions

Section 11   Leukemia

- The balance of evidence suggests that childhood leukemia is associated with exposure to power frequency EMFs either during early life or pregnancy.

- Considering only average ELF (MF flux densities) the population attributable risk is low to moderate. However there is a possibility that other exposure metrics are much more strongly related to childhood leukemia and may account for a substantial proportion of cases. The population attributable fraction ranges between 1-4% (Kheifets et al., 2007); 2-4% (Greenland & Kheifets 2006); and 3.3% (Greenland, 2001) assuming only exposures above 3 to 4 mG (0.3 – 0.4 μT) are relevant. However, if it is not average ELF (average MF flux density) that is the metric causally related to childhood leukemia the attributable fraction can be much higher. Up to 80% of childhood leukemia may be caused by exposure to ELF.

- Other childhood cancers except leukemia have not been studied in sufficient detail to allow conclusions about the existence and magnitude of the risk.

- IEEE guideline levels are designed to protect from short-term immediate effects, long-term effects, such as cancer are evoked by levels several orders of magnitudes below current guideline levels.

- Measures should be implemented to guarantee that exposure due to transmission and distribution lines is below an average of about 1 mG (0.1 μT) and precautionary measures are warranted that can reduce all aspects of exposure.

Table 1-1 BioInitiative Report Overall Conclusions

Section 12 Melatonin, Alzheimers Disease and Breast Cancer

• There is strong epidemiologic evidence that long-term exposure to ELF magnetic field (MF) is a risk factor for Alzheimers disease.

• There is now evidence that 1) high levels of peripheral amyloid beta are a risk factor for AD and 2) medium to high MF exposure can increase peripheral amyloid beta. High brain levels of amyloid beta are also a risk factor for AD and medium to high MF exposure to brain cells likely also increases these cells' production of amyloid beta.

• There is considerable *in vitro* and animal evidence that melatonin protects against Alzheimer's disease. Therefore it is certainly possible that low levels of melatonin production are associated with an increase in the risk of AD.

• There are insufficient studies to formulate an opinion as to whether radiofrequency MF exposure is a risk factor for AD.

• Some studies on EMF show reduced melatonin levels, There is sufficient evidence from *in vitro* and animal studies, from human biomarker studies, from occupational and light-at-night studies, and a single longitudinal study with appropriate collection of urine samples to conclude that high MF exposure may be a risk factor for breast cancer.

• There is rather strong evidence from case-control studies that longterm, high occupational exposure ($\geq$ 10 mG or 1.0 μT)) to ELF magnetic fields is a risk factor for breast cancer.

• Seamstresses are, in fact, one of the most highly MF exposed occupations, with exposure levels generally above 10 mG (1.0 μT) over a significant proportion of the workday. They have also been consistently found to be at higher risk of Alzheimer's disease and (female) breast cancer. This occupation deserves attention in future studies.

• There are no studies of RF magnetic fields on breast cancer that do not exclude ELF magnetic field, so that predictions of RF magnetic field alone on breast cancer cannot be assessed at this time.

Table 1-1 BioInitiative Report Overall Conclusions

Section 13   Melatonin – Cell and Animal Studies

• An association between power-frequency electromagnetic fields (ELF) and breast cancer is strongly supported in the scientific literature by a constellation of relevant scientific papers providing mutually-reinforcing evidence from cell and animal studies.

• ELF at environmental levels negatively affects the oncostatic effects of both melatonin and tamoxifen on human breast cancer cells at common environmental levels of ELF exposure at 6 to 12 mG (0.6 to 1.2 μT).   Epidemiological studies over the last two decades have reported increased risk of male and female breast cancer with exposures to residential and occupational levels of ELF. Animal studies have reported increased mammary tumor size and incidence in association with ELF exposure.

• ELF limits for public exposure should be revised to reflect increased risk of breast cancer at environmental levels possibly as low as 2 mG or 3 mG (o.2 to 0.3 μT); certainly as low as 4 mG (0.4 μT).

Section 14   Effects of Modulation of Signal

• There is substantial scientific evidence that some modulated fields (pulsed or repeated signals) are bioactive, which increases the likelihood that they could have health impacts with chronic exposure even at very low exposure levels.

• Modulation signals may interfere with normal, non-linear biological processes.

• Modulation is a fundamental factor that should be taken into account in new public safety standards; at present it is not even a contributing factor.

• To properly evaluate the biological and health impacts of exposure to modulated RF (carrier waves), it is also essential to study the impact of the modulating signal (lower frequency fields or ELF-modulated RF).

.• Current standards have ignored modulation as a factor in human health impacts, and thus are inadequate in the protection of the public in terms of chronic exposure to some forms of ELF-modulated RF signals.

• The current IEEE and ICNIRP standards are not sufficiently protective of public health with respect to chronic exposure to modulated fields (particularly new technologies that are pulse-modulated and heavily used in cellular telephony).

Table 1-1 BioInitiative Report Overall Conclusions

Section 14 **Effects of Modulation of Signal** (continued)

• The collective papers on modulation appear to be omitted from consideration in the recent WHO and IEEE science reviews. This body of research has been ignored by current standard setting bodies that rely only on traditional energy-based (thermal) concepts.

• More research is needed to determine which modulation factors, and combinations are bioactive and deleterious at low intensities, and are likely to result in disease-related processes and/or health risks; however this should not delay preventative actions supporting public health and wellness.

• If signals need to be modulated in the development of new wireless technologies, for example, it makes sense to use what existing scientific information is available to avoid the most obviously deleterious exposure parameters and select others that may be less likely to interfere with normal biological processes in life.

• The current membership on Risk Assessment committees needs to be made more inclusive, by adding scientists experienced with the research reporting non-thermal biological effects.

• The current practice of segregating scientific investigations (and resulting public health limits) by artificial divisions of frequency needs to be changed because this approach dramatically dilutes the impact of the basic science results and eliminates consideration of modulation signals, thereby reducing and distorting the weight of evidence in any evaluation process.

Section 15 **Therapeutic Uses of EMF at Low-Intensity Levels**

• EMFs are both a cause of disease, and also used for treatment of disease (at levels far below existing public exposure standards).

• Electromagnetic fields are widely used in therapeutic medical applications.

• Proof of effectiveness has been demonstrated in numerous clinical applications of low-intensity ELF and RF.

• EMFs have been shown to be effective in treating conditions of disease at energy levels far below current public exposure standards.

• Indiscriminate EMF exposure is ill advised at even at common environmental levels.

• Multiple sources of EMF exposure in daily life, and cumulative exposures to potentially harmful combinations of EMF are ignored – we don't even study it properly yet.

Table 1-1 BioInitiative Report Overall Conclusions

Section 16   The Precautionary Principle

• The Precautionary Principle has been developed to help justify public policy action on the protection of health where there are plausible, serious and irreversible hazards from current and future exposures and where there are many uncertainties and much scientific ignorance. EMF is characterized by such circumstances.

• The lessons from the histories of most well known hazards show that precautionary- based yet proportionate measures taken in response to robust early warnings can avoid the kinds of costs incurred by asbestos, smoking, PCBs ,X rays etc. Such lessons are relevant to the EMF issue.

• Policymakers need to be aware of the systematic biases within the environmental health science against finding a true hazard, in order to not compromise scientific integrity. However, this bias can lead to the health of people or environments being compromised.

• The Precautionary Principle introduces the use of different levels of proof (or strengths of evidence) to justify actions to reduce exposure, where the level of proof chosen depends upon the nature and distribution of the costs of being wrong in acting, or not acting; the benefits of the agent or substance in question; the availability of alternatives, etc. Waiting for high levels of scientific proof of causality, or for knowledge about mechanisms of action, can be very expensive in terms of compensation, health care, job losses, reductions in public trust of scientists etc.

• The level of proof chosen to justify action does not determine any particular policy measure, or type of action. This is dependent on factors such as the costs of different measures, equity, the origins of the risk, ie voluntary or imposed, etc.

• There is a need to involve stakeholders in helping to frame problems for risk assessments and to choose appropriate levels of proof and types of actions to reduce exposure.

Table 1-1 BioInitiative Report Overall Conclusions

Section 17:   Key Scientific Evidence and Public Health Policy Recommendations

• We cannot afford 'business as usual" any longer.   It is time that planning for new power lines and for new homes, schools and other habitable spaces around them is done with provision for low-ELF environments.   The business-as-usual deployment of new wireless technologies is likely to be risky and harder to change if society does not make some educated decisions about limits soon. Research must continue to define what levels of RF related to new wireless technologies are acceptable; but more research should not prevent or delay substantive changes today that might save money, lives and societal disruption tomorrow.

 • New regulatory limits for ELF are warranted. ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor. It is no longer acceptable to build new power lines and electrical facilities that place people in ELF environments that have been determined to be risky (at levels generally at 2 mG (0.2 μT) and above).

 • While new ELF limits are being developed and implemented, a reasonable approach would be a 1 mG (0.1 μT) planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG (0.2 μT) limit for all other new construction,   It is also recommended for that a 1 mG (0,1 μT) limit be established for existing habitable space for children and/or women who are pregnant . This recommendation is based on the assumption that a higher burden of protection is required for children who cannot protect themselves, and who are at risk for childhood leukemia at rates that are traditionally high enough to trigger regulatory action. This situation in particular warrants extending the 1 mG (0.1 μT) limit to existing occupied space. "Establish" in this case probably means formal public advisories from relevant health agencies.

• While it is not realistic to reconstruct all existing electrical distributions systems, in the short term; steps to reduce exposure from these existing systems need to be initiated, especially in places where children spend time, and should be encouraged.

• A precautionary limit of 0.1 μW/cm2 (which is also 0.614 Volts per meter) should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers and PDAs and other sources of radiofrequency radiation. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however, for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of WI-FI technologies, we also recommend that wired alternatives to WI-FI be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts. This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future.

Table 1-1 BioInitiative Report Overall Conclusions

Section 17:   Key Scientific Evidence and Public Health Policy Recommendations (continued)

• New public safety limits should be developed and implemented for ELF (50 Hz and 60 Hz electrical power frequencies). ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor.

• Guidance should be provided to electric utilities on the need to reduce ELF exposures in siting and construction of new power lines and substations. Mitigation of existing sources of ELF over 1 mG (0.1 μT) should be encouraged, particularly where children and women who are pregnant, or who may be come pregnant spend significant portions of their time.

• Requests for measurement and monitoring of ELF and RF should be provided by utilities (for power line and household ELF) and by employers (for workplace ELF and RF) ,and those who request information should receive full results of such surveys on request.

• International health organizations and agencies should issue public health advisories for those exposed to levels of ELF and RF implicated with increased risks from cancer/neurodegenerative diseases and memory/learning/immune/stress responses. These advisories should address both residential and occupational exposures.

• Reliable, unbiased information should be developed and distributed through a clearinghouse that is available to the public. Scientific, public health and policy option information should be provided for independent review at an affordable cost to the public. Research articles and prudent avoidance strategies should be made available in many languages.

• Cell phones and other wireless devices should be redesigned to operate only on speaker-phone mode or text message mode.

• Restrictions should be placed on the sale and advertising of cell phones and other wireless devices to children age 0 to 18 years.

• All countries should continue to provide wired phone service; and should be strongly discouraged from phasing it out; including pay telephones in public places.

• Manufacturers of devices that operate with wireless features should be required to carry SAR level information and warning labels on the outside packaging (not hidden inside). Wireless devices that create elevated RF levels for the user should be required to warn the user of possible adverse effects on memory and learning, cognitive function, sleep disruption and insomnia, mood disorders, balance, headache, fatigue, ringing in the ears (tinnitus), immune function, and other adverse symptoms of use.

• Warning labels on cell phones and PDAs (personal digital assistant devices) and other wireless devices are needed to alert users to excessively high ELF emissions from the switching battery pack, and require labels to list mitigation measures to reduce exposure (do not wear on or near body in "ON-Receive" position; use only with earpiece or on speaker mode, etc).

• Disclosure should be provided to the public on the location and operating characteristics of all wireless antenna sites in a fashion easily accessible to the public so informed choices can be made about where to live, shop, work and go to school. Such information should mandatorily include cumulative RF/MW exposures based on calculations from FCC OET Bulletin 65 (or equivalent) at ground level and second story level in increments of 50 feet outward from the facility to a power density of 0.1 μW/cm2 or 0.614 V/m. Signage for the public should be a mandatory condition of approval for all sites, and should be kept current.   Public agencies that approve and monitor wireless sites should require the applicant to identify locations of wireless facilities.

Table 1-1 BioInitiative Report Overall Conclusions

Section 17:   Key Scientific Evidence and Public Health Policy Recommendations (continued)

• Mobile phone - free and WI-FI-free public areas should be established in areas where the public congregates and can have a reasonable expectation of safety; including airports, public shopping, hospitals, libraries, medical clinics, convalescent homes and assisted living facilities, theatres, restaurants, parks, etc.

• Health agencies and school districts should strongly discourage or prohibit cell towers on or near (within 1000' of) school properties, should delay any new WLAN installations in school classrooms, pre-schools and day-care facilities; and should either remove or disable existing wireless facilities, or be required to offer classrooms with no RF exposure to those families who choose not to have their children involuntarily exposed.

SECTION 2:   **STATEMENT OF THE PROBLEM**

Background and Objectives

This Report is the product of an international research and public policy initiative to document what is known of biological effects that occur at low-intensity EMF exposures (for both radiofrequency radiation RF and power-frequency ELF, and various forms of combined exposures that are now known to be bioactive). The Report has been written to document the reasons why current public exposure standards for non-ionizing electromagnetic radiation are no longer good enough to protect public health.

A working group composed of scientists, researchers and public health policy professionals (The BioInitiative Working Group) has joined together to document the information that must be considered in the international debate about the adequacy (or inadequacy) of existing public exposure standards.

Recognizing that other bodies in the United States, United Kingdom, Australia, many European Union and eastern European countries as well as the World Health Organization are actively debating this topic, the BioInitiative Working Group has conducted a independent science and public health policy review process.

Objectives

1) To establish a working group

2) To evaluate literature reviews for IEEE (2006) and WHO (2007) initiatives on standards that have resulted in (or continue to recommend) no change in thermally-based public exposure limits.

3) To identify systematic screening-out techniques that consequently under-report, omit or overlook results of scientific studies reporting low-intensity bioeffects and/or potential health effects.

4) To document key scientific studies and reviews that identify low-intensity effects for which any new human exposure standards should provide safety limits.

5) To document key "chains of evidence" that must be taken into account in new human exposure standards (melatonin and free-radical production effects on DNA damage and/or repair; stress protein induction at low-intensity levels; etc.)

6) To write a rationale for a biologically-based human exposure standard,

7) To identify "next steps" in advancing biologically-based exposure standards that are protective of public health; that are derived in traditional public health approaches.

Eleven (11) chapters documenting key scientific studies and reviews that identify low-intensity effects of electromagnetic fields have been produced by the members of the BioInitiative Working Group; four additional chapters are provided that discuss public health considerations, how the scientific information should be evaluated in the context of prudent public health policy, and discussing the basis for taking precautionary and preventative actions that are proportionate to the knowledge at hand. Other scientific review bodies and agencies have reached different conclusions by adopting standards of evidence so unreasonably high as to exclude any finding of scientific concern, and thus justify retaining outdated thermal standards. The clear consensus of the BioInitiative Working Group members is that the existing public safety limits are inadequate. New approaches to development of public safety standards are needed based on biologically-based effects, rather than based solely on RF heating (or induced currents in the case of ELF). The Report concludes with recommended actions that are proportionate to the evidence and in accord with prudent public health policy.

The Report also presents information about what level of scientific evidence is sufficient to make changes now. It addresses the questions:

- What is "proof"? Do we need proof before we take any action? Is an unreasonably high and overly-restrictive definition of "proof" what is keeping some governments from facing the evidence that the need for new public exposure limits is demonstrated?
- What is sufficient evidence? How much evidence is needed? Do we have it yet?
- Do scientists and public health experts differ on when action is warranted? If so, how?
- What is the prudent course of action when the consequence of doing nothing is likely to have serious global consequences on public health, confidence in governments and social/economic resources?
- What are the costs of guessing wrong and under-reacting? Or, of over-reacting?
- Whose opinions should count in the process of deciding about health risks and harm?
- Is the global, governmental process addressing these questions transparent and responsive to public concerns? Or, is it a cosmetic process giving the illusion of transparency and democratic participation? Are some countries ostracized for views

and actions that are more protective of public health? How can we equitably decide on the appropriate level of public protection within each country, when it is obvious that some countries would be best off spending their time and money on basic medical needs and infrastructure improvements to save lives, when others need to look at prevailing disease endpoints relevant to their populations, and wish to act accordingly?

- How has the effort for global harmonization of ELF and RF exposure standards thwarted the efforts of individual countries to read, reason and choose?
- How much control have special interests exerted over harmonization goals and safety standards? How much over scientific funding, research design, dissemination of research results and media control? Are the interests of the public being conserved?
- What actions are proportionate to the knowledge we now have? What is preventative action and how does it differ from precautionary action?

It describes what the existing exposure standards are, and how some international governmental bodies are standing by the old exposure standards despite evidence that change is needed.

A good way to compare what kind of actions should be taken now is to look at what has been done with other environmental toxicants. It is well-established that public health decision-makers should act before it is too late to prevent damage that can reasonably be expected now; especially where the harm may be serious and widespread. Some actions that can prevent future harm are identified. The basis for taking action now rather than later is explained. This report can serve as a basis for arguing the scientific and public health policy reasons that changes are needed. It documents information for decision-makers and the public who want to understand what is already known biological effects occuring at low-intensity exposures; and why it is reasonable to expect our governmental agencies to develop new, biologically-based exposure standards that protect the public.

Problems with Existing Public Health Standards (Safety Limits)

Today's public exposure limits are based on the presumption that heating is the only concern when living organisms are exposed to RF and ELF. These exposures can create tissue heating that is well known to be harmful in even very short-term doses. As such, thermal limits do serve a purpose. For example, for people whose occupations require them to work around electrical power lines or heat-sealers, or for people who install and service wireless antenna towers; thermally-based limits are necessary to prevent damage from heating (or, in the case of ELF -

from induced currents in tissues). In the past, scientists and engineers developed exposure standards for electromagnetic radiation based what we now believe are faulty assumptions that the right way to measure how much non-ionizing energy humans can tolerate (how much exposure) without harm is to measure only the heating of tissue (for – induced currents in the body). In the last few decades, it has been established beyond any reasonable doubt that bioeffects and some adverse health effects occur at far lower levels of RF and exposure where no heating occurs at all; some effects are shown to occur at several hundred thousand times below the existing public safety limits where heating is an impossibility. Effects occur at non-thermal or low-intensity exposure levels far below the levels that federal agencies say should keep the public safe. For many new devices operating with wireless technologies, the devices are exempt from any regulatory standards. The existing standards have been proven to be inadequate to control against harm from low-intensity, chronic exposures, based on any reasonable, independent assessment of the scientific literature. It means that an entirely new basis (a biological basis) for new exposure standards is needed. New standards need to take into account what we have learned about the effects of non-ionizing electromagnetic fields and to design new limits based on biologically-demonstrated effects that are important to proper biological function in living organisms.   It is vital to do so because the explosion of new sources has created unprecedented levels of artificial electromagnetic fields that now cover all but remote areas of the habitable space on earth. Mid-course corrections are needed in the way we accept, test and deploy new technologies that expose us to ELF and RF in order to avert public health problems of a global nature.

At least three decades of scientific study and observation of effects on humans and animals shows that non-thermal exposure levels can result in biologically-relevant effects. There should be no effects occurring at all. Yet, clearly they do occur. This means the standards for protecting public health are based on the wrong premise - that only what heats tissue can result in harm. It does appear that it is the INFORMATION conveyed by electromagnetic radiation, rather than the heat, which causes biological changes, some of which may lead to unwellness, illness and even death, According to Adey (2004):

> *"There are major unanswered questions about possible health risks that may arise from human exposures to various man-made electromagnetic fields where these exposures are intermittent, recurrent, and may extend over a significant portion of the lifetime of an*

individual. Current equilibrium thermodynamic models fail to explain an impressive spectrum of observed bioeffects at non-thermal exposure levels."

Recent opinions by experts have documented deficiencies in current exposure standards. There is widespread discussion that thermal limits are outdated, and that biologically-based exposure standards are needed. Section 4 describes concerns expressed by WHO, 2007 in its Health Criteria Monograph; the SCENIHR Report, 2006 prepared for the European Commission; the UK SAGE Report, 2007; the Health Protection Agency, United Kingdom in 2005; the NATO Advanced Research Workshop in 2005; the US Radiofrequency Interagency Working Group in 1999; the US Food and Drug Administration in 2000 and 2007; the World Health Organization in 2002; the World Health Organization International Agency for Cancer Research (IARC, 2001), the United Kingdom Parliament Independent Expert Group Report (Stewart Report, 2000) and others.

A pioneer researcher, the late Dr. Ross Adey, in his last publication in Bioelectromagnetic Medicine (P. Roche and M. Markov, eds. 2004) concluded:

> "*There are major unanswered questions about possible health risks that may arise from exposures to various man-made electromagnetic fields where these human exposures are intermittent, recurrent, and may extend over a significant portion of the lifetime of the individual.*"[1]

> "*Epidemiological studies have evaluated and radiofrequency fields as possible risk factors for human health, with historical evidence relating rising risks of such factors as progressive rural electrification, and more recently, to methods of electrical power distribution and utilization in commercial buildings. Appropriate models describing these bioeffects are based in nonequilibrium thermodynamics, with nonlinear electrodynamics as an integral feature. Heating models, based in equilibrium thermodynamics, fail to explain an impressive new frontier of much greater significance. ….. Though incompletely understood, tissue free radical interactions with magnetic fields may extend to zero field levels. (Adey, 2004)*

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SECTION 3:   **THE EXISTING PUBLIC EXPOSURE STANDARDS**

The US Federal Communications Commission (FCC) Exposure Standard Recommendations

In the United States, the Federal Communications Commission (FCC) enforces limits for both occupational exposures (in the workplace) and public exposures. The exposure limits are variable according to the frequency (in megahertz) and the duration of exposure time (6 minutes for occupational and 30 minutes for public exposures). Table 3.1 show exposure limits for occupational and uncontrolled public access to radiofrequency radiation such as is emitted from AM, FM, television and wireless sources through the air. As an example, 583 microwatts/cm2 (μW/cm2) is the public limit for the 875 MHz cell phone wireless frequency and 1000 μW/cm2 is the limit for PCS frequencies in the 1800 – 1950 MHz range averaged over 30 minutes. The limits in Table 3.1 would pertain to exposures in the vicinity of transmitting antennas (not devices like cell phones, for which exposure limits are shown in Table 3.2).

The FCC is required by the National Environmental Policy Act of 1969 to evaluate the effect of emissions from FCC-regulated transmitters on the quality of the human environment. At the present time there is no federally-mandated radio frequency (RF) exposure standard. However, several non-government organizations, such as the American National Standards Institute (ANSI), the Institute of Electrical and Electronics Engineers, Inc. (IEEE), and the National Council on Radiation Protection and Measurements (NCRP) have issued recommendations for human exposure to RF electromagnetic fields. The FCC has endorsed these recommendations, and enforces compliance.   http://www.fcc.gov/oet/rfsafety/

Table 3.1 FCC LIMITS FOR MAXIMUM PERMISSIBLE EXPOSURE (MPE)

(A) Limits for Occupational/Controlled Exposure

Frequency Range (MHz)	Electric Field Strength (E) (V/m)	Magnetic Field Strength (H) (A/m)	Power Density (S) (mW/cm2)	Averaging Time $[E]^2$ $[H]^2$ or S (minutes)
0.3-3.0	614	1.63	(100)*	6
3.0-30	1842/f	4.89/f	(900/f2)*	6
30-300	61.4	0.163	1.0	6
300-1500			f/300	6
1500-100,000			5	6

_____

(B) FCC Limits for General Population/Uncontrolled Exposure

Frequency Range (MHz)	Electric Field Strength (E) (V/m)	Magnetic Field Strength (H) (A/m)	Power Density (S) (mW/cm2)	Averaging Time $[E]^2$ $[H]^2$ or S (minutes)
0.3-3.0	614	1.63	(100)*	30
3.0-30	824/f	2.19/f	(180/f2)*	30
30-300	27.5	0.073	0.2	30
300-1500	--	--	f/1500	30
1500-100,000	--	--	1.0	30

_____

f = frequency in MHz   *Plane-wave equivalent power density

NOTE 1: ***Occupational/controlled*** limits apply in situations in which persons are exposed as a consequence of their employment provided those persons are fully aware of the potential for exposure and can exercise control over their exposure. Limits for occupational/controlled exposure also apply in situations when an individual is transient through a location where occupational/controlled limits apply provided he or she is made aware of the potential for exposure.

NOTE 2: ***General population/uncontrolled*** exposures apply in situations in which the general public may be exposed, or in which persons that are exposed as a consequence of their employment may not be fully aware of the potential for exposure or can not exercise control over their exposure.

Source: OET, 1997.

FCC Guidelines for Cell and PCS Phones (and other radiofrequency emitting

devices)

Cell phones and portable transmitting devices that operate in the Cellular Radiotelephone Service, the Personal Communications Services (PCS), the Satellite Communications Services, the Maritime Services (ship earth stations only) and the Specialized Mobile Radio (SMR) Service are subject to routine environmental (not health) evaluation for RF exposure prior to equipment authorization or use by the FCC. Section 2.1093 of the FCC's Rules (47 CFR §2.1093) that apply to "portable" devices. For purposes of these requirements a portable device is defined as a transmitting device designed to be used so that the radiating structure(s) of the device is/are within 20 centimeters of the body of the user (OET, 1997).

Cell phones and some other wireless communication devices are regulated by the FCC according to their emissions, which depend on the amount of power absorbed into the body. The metric for measurement is specific absorption rate (SAR) and is expressed in watts per kilogram of tissue. The limit for absorption of radiofrequency radiation is limited to 1.6 W/kG within 1 gram of human tissue. This limit has been recommended for change (relaxation) by the IEEE in April of 2006. If adopted by the FCC, this amount of heat or 1.6 W/Kg would be measured over 10 times as much tissue (10 grams) so that far higher heating is possible from these devices over small amounts of tissue (would be far less strict that the current limit, if adopted). More cell phone and related PDA devices would then comply be able with the looser standard, and the public could potentially receive much higher radiofrequency radiation exposures, and it would be in compliance (legal).

"The SAR criteria to be used are specified below and apply for portable devices transmitting in the frequency range from 100 kHz to 6 GHz. The limits used for evaluation are based generally on criteria published by the Institute of Electrical and Electronics Engineers, Inc., (IEEE) for localized specific absorption rate ("SAR") in Section 4.2 of "IEEE Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields, 3 kHz to 300 GHz," ANSI/IEEE C95.1-1992.

These criteria for SAR evaluation are similar to those recommended by the National Council on Radiation Protection and Measurements (NCRP) in "Biological Effects and Exposure Criteria for Radiofrequency Electromagnetic Fields," NCRP Report No. 86, Section 17.4.5. Copyright NCRP, 1986, Bethesda, Maryland 20814."

 (1) FCC Limits for Occupational/Controlled exposure: 0.4 W/kg as averaged over the whole-body and spatial peak SAR not exceeding 8 W/kg as averaged over any 1 gram of tissue (defined as a tissue volume in the shape of a cube). Exceptions are the hands, wrists, feet and ankles where the spatial peak SAR shall not exceed 20 W/kg, as averaged over any 10 grams of tissue (defined as a tissue volume in the shape of a cube). Occupational/Controlled limits apply when persons are exposed as a consequence of their employment provided these persons are fully aware of and exercise control over their

exposure. Awareness of exposure can be accomplished by use of warning labels or by specific training or education through appropriate means, such as an RF safety program in a work environment (OET, 1997).

(2) FCC Limits for General Population/Uncontrolled exposure: 0.08 W/kg as averaged over the whole-body and spatial peak SAR not exceeding 1.6 W/kg as averaged over any 1 gram of tissue (defined as a tissue volume in the shape of a cube). Exceptions are the hands, wrists, feet and ankles where the spatial peak SAR shall not exceed 4 W/kg, as averaged over any 10 grams of tissue (defined as a tissue volume in the shape of a cube). General Population/Uncontrolled limits apply when the general public may be exposed, or when persons that are exposed as a consequence of their employment may not be fully aware of the potential for exposure or do not exercise control over their exposure. Warning labels placed on consumer devices such as cellular telephones will not be sufficient reason to allow these devices to be evaluated subject to limits for occupational/controlled exposure (OET, 1997).

In the United States, two professional societies - the Institute of Electrical and Electronics Engineers, Inc. (IEEE) and the National Council for Radiation Protection and Measurements (NCRP) develop recommendations for safety standards. . The IEEE charter calls itself the world's leading professional association for the advancement of technology, as well as the instigator of public safety standards. The IEEE recommendations have historically been endorsed by the American National Standards Institute (ANSI) and finally considered by the FCC for implementation. The US Federal Communications Commission (FCC) may then take the recommendations and adopt them as mandatory exposure limits. Several standard-setting processes have occurred like this in the last few decades.

The most recent IEEE recommendations for 3 kHz to 300 GHz were developed in 2006 (IEEE, 2006). Rather than lower the existing limits for radiofrequency and microwave radiation exposure, they greatly increase the exposure limits. This is perplexing since it ignores or discounts a large body of scientific evidence clearly documenting biologically-relevant changes at levels LOWER (much lower) than the existing standards.

ICNIRP Guidelines (International Radiofrequency Guidelines)

In April 1998, the International Commission on Non-Ionizing Radiation Protection (ICNIRP) published guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields in the frequency range up to 300 GHz.. These guidelines replaced previous advice issued in 1988 and 1990. The main objective of the ICNIRP Guidelines is to establish guidelines for limiting EMF exposure that will provide protection against known adverse health effects (ICNIRP, 1998). An adverse health effect is defined by ICNIRP as one which causes detectable impairment of the health of the exposed individual or of his or her offspring; a biological effect, on the other hand, may or may not result in an adverse health effect.

The guidelines presented in Table 3.2 apply to occupational and public exposure.

Table 3.2   ICNIRP Basic restrictions for time varying electric and magnetic fields for frequencies up to 10 GHz.

Exposure characteristics	Frequency range	Current density for head and trunk (mA m^{-2})(rms)	Whole-body average SAR (W kg^{-1})	Localized SAR (head and trunk) (W kg^{-1})	Localized SAR (limbs) (W kg^{-1})
Occupational exposure	up to 1 Hz	40	—	—	—
	1–4 Hz	40/f	—	—	—
	4 Hz–1 kHz	10	—	—	—
	1–100 kHz	f/100	—	—	—
	100 kHz–10 MHz	f/100	0.4	10	20
	10 MHz–10 GHz		0.4	10	20
General public exposure	up to 1 Hz	8	—	—	—
	1–4 Hz	8/f	—	—	—
	4 Hz–1 kHz	2	—	—	—
	1–100 kHz	f/500	—	—	—
	100 kHz–10 MHz	f/500	0.08	2	4
	10 MHz–10 GHz		0.08	2	4

Notes:

1. f is the frequency in hertz.
2. Because of electrical inhomogeneity of the body, current densities should be averaged over a cross-section of 1 cm^2 perpendicular to the current direction.
3. For frequencies up to 100 kHz, peak current density values can be obtained by multiplying the rms value by $\sqrt{2}$ (~1.414). For pulses of duration t_p the equivalent frequency to apply in the basic restrictions should be calculated as $f = 1/(2t_p)$. For frequencies up to 100 kHz and for pulsed magnetic fields, the maximum current density associated with the pulses can be calculated from the rise/fall times and the maximum rate of change of magnetic flux density. The induced current density can then be compared with the appropriate basic restriction.
4. All SAR values are to be averaged over any 6-minute period.
5. Localized SAR averaging mass is any 10 g of contiguous tissue; the maximum SAR so obtained should be the value used for the estimation of exposure.
6. For pulses of duration t_p the equivalent frequency to apply in the basic restrictions should be calculated as $f = 1/(2t_p)$. Additionally, for pulsed exposures, in the frequency range 0.3 to 10 GHz and for localized exposure of the head, in order to limit or avoid auditory effects caused by thermoelastic expansion, an additional basic restriction is recommended. This is that the SA should not exceed 10 mJ kg^{-1} for workers and 2 mJ kg^{-1} for the general public averaged over 10 g tissue.

In the frequency range from a few Hz to 1 kHz, for levels of induced current density above 100 mA m^{-2}, the thresholds for acute changes in central nervous system excitability and other acute effects such as reversal of the visually evoked potential are exceeded. In view of the safety considerations above, it was decided that, for frequencies in the range 4 Hz to 1 kHz, occupational exposure should be limited to fields that induce current densities less than 10 mA m^{-2}, i.e., to use a safety factor of 10. For the general public an additional factor of 5 is applied, giving a basic exposure restriction of 2 mA m^{-2}. Below 4 Hz and above 1 kHz, the basic restriction on induced current density increases progressively.
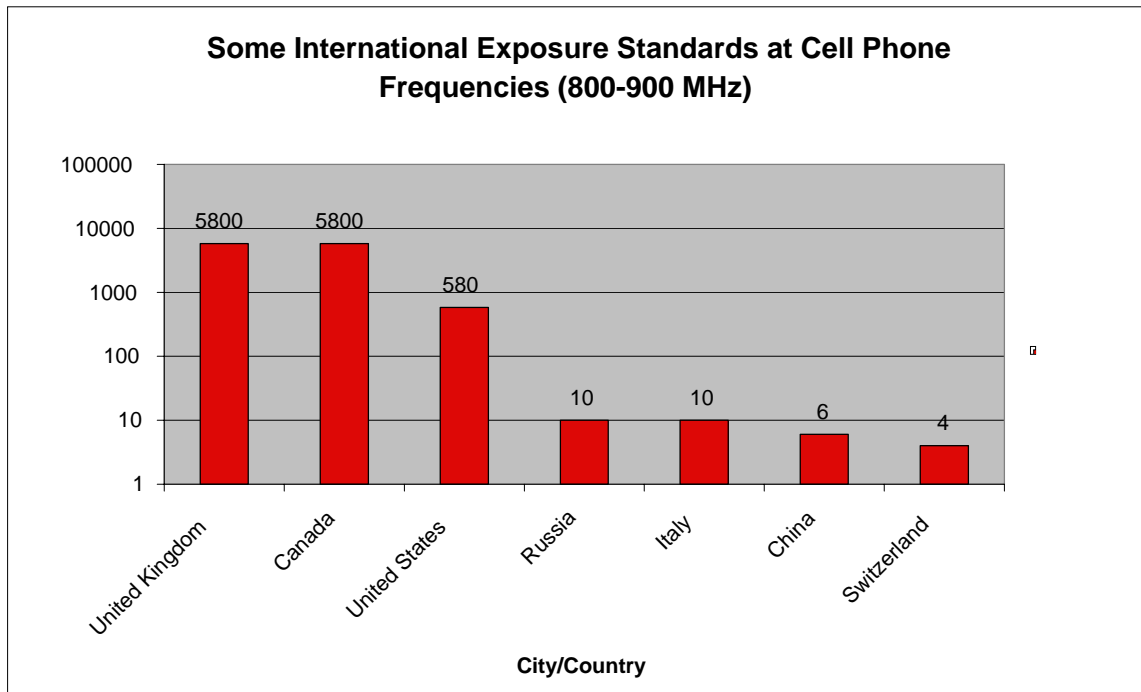
ICNRP maintains that guidelines for limiting exposure have been developed following a thorough review of all published scientific literature (ICNIRP, 1998).

"The criteria applied in the course of the review were designed to evaluate the credibility of the various reported findings (Repacholi and Stolwijk 1991; Repacholi and Cardis 1997); only established effects were used as the basis for the proposed exposure restrictions. Induction of cancer from long-term EMF exposure was not considered to be established, and so these guidelines are based on short-term, immediate health effects such as stimulation of peripheral nerves and muscles, shocks and burns caused by touching conducting objects, and elevated tissue temperatures resulting from absorption of energy during exposure to EMF. In the case of potential long-term effects of exposure, such as an increased risk of cancer, ICNIRP concluded that available data are insufficient to provide a basis for setting exposure restrictions, although epidemiological research has provided suggestive, but unconvincing, evidence of an association between possible carcinogenic effects and exposure at levels of 50/60 Hz magnetic flux densities substantially lower than those recommended in these guidelines. In-vitro effects of short-term exposure to ELF or ELF amplitude-modulated EMF are summarized. Transient cellular and tissue responses to EMF exposure have been observed, but with no clear exposure–response relationship. These studies are of limited value in the assessment of health effects because many of the responses have not been demonstrated in vivo. Thus, in-vitro studies alone were not deemed to provide data that could serve as a primary basis for assessing possible health effects of EMF. " (ICNIRP, 1998)   http://www.icnirp.de

Guidelines and Limits (Other Countries)

On the other hand, some countries in the world have established new, low-intensity based exposure standards that respond to studies reporting effects that do not rely on heating. Consequently, new exposure guidelines are hundreds or thousands of times lower than those of IEEE and ICNIRP. Table 3.3 shows some of the countries that have lowered their limits, for example, in the cell phone frequency range of 800 MHz to 900 MHz. The levels range from 10 microwatts per centimeter squared in Italy and Russia to 4.2 microwatts per centimeter squared in Switzerland.   In comparison, the United States and Canada limit such exposures to only 580 microwatts per centimeter squared (at 870 MHz) and then averaged over a time period (meaning that higher exposures are allowed for shorter times, but over a 30 minute period, the average must be 580 microwatts per centimeter squared or less at this frequency). The United Kingdom allows one hundred times this level, or 5800 microwatts per centimeter squared.   Higher frequencies have higher safety limits, so that at 1000 MHz, for example, the limit is 1000 microwatts per centimeter squared (in the United States). Each individual frequency in the radiofrequency radiation range needs to be calculated. These are presented as reference points only. Emerging scientific evidence has encouraged some countries to respond by adopting planning targets, or interim action levels that are responsive to low-intensity or non-thermal radiofrequency radiation bioeffects and health impacts.

Table 3.3 Some International Exposure Standards at Cell Phone Frequencies

Some International Exposure Standards at Cell Phone Frequencies (800-900 MHz)

City/Country	Value
United Kingdom	5800
Canada	5800
United States	580
Russia	10
Italy	10
China	6
Switzerland	4

Professional bodies from technical societies like IEEE and ICNIRP continue to support "thermal-only" guidelines routinely defend doing so a) by omitting or ignoring study results reporting bioeffects and adverse impacts to health and wellbeing from a very large body of peer-reviewed, published science because it is not yet "proof" according to their definitions; b) by defining the proof of "adverse effects" at an impossibly high a bar (scientific proof or causal evidence) so as to freeze action; c) by requiring a conclusive demonstration of both "adverse effect" and risk before admitting low-intensity effects should be taken into account; e) by ignoring low-intensity studies that report bioeffects and health impacts due to modulation; f) by conducting scientific reviews with panels heavily burdened with industry experts and under-represented by public health experts and independent scientists with relevant low-intensity research experience; g) by limiting public participation in standard-setting deliberations; and other techniques that maintain the status quo.

Much of the criticism of the existing standard-setting bodies comes because their contributions are perceived as industry-friendly (more aligned with technology investment and dissemination of new technologies) rather than public health oriented. The view of the Chair of the latest IEEE standard-setting ICES Eleanor Adair is made clear by Osepchuk and Petersen (2003) who write in the abstract of their paper "*her goal and the goal of ICES is to establish rational standards that will make future beneficial applications of RF energy credible to humanity.*" Authors Osepchuk and Petersen note that "*(I)t is important that safety standards be rational and avoid excessive safety margins.*" The authors specifically dismiss the body of evidence for low-intensity effects with "*(A)lthough the literature reporting "athermal" bioeffects of exposure to*

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microwave/RF energy (other than electrostimulation) is included in the review process, it has been found to be inconsistent and not useful for purposes of standard-setting."

This report addresses the substantial body of evidence reporting low-intensity effects from electromagnetic fields (both power-frequency fields in the ELF range, and radiofrequency/microwave fields at exposure levels that do not involve any heating. It also addresses the inconsistency in the literature quoted as the basis for retaining thermal-only exposure standards (see particularly the Genotoxics Section 6 where half of more of the published papers report negative effects and half positive effects).

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OET 1997. Office of Engineering Technology, Federal Communications Commission Bulletin 65 97-01, August 1997. **http://www.fcc.gov/oet/rfsafety**

Osepchuk JM Petersen RC. 2003. Historical Review of RF Exposure Standards and the International Committee on Electromagnetic Safety (ICES). Bioelectromagnetics Supplement 6:S7-16. Osepchuk is a former employee of Raytheon. Petersen is a former employee of Bell Labs and Lucent Technologies. Both are independent industry consultants in their retirement.

SECTION 4: EVIDENCE FOR INADEQUACY OF THE STANDARDS

Evidence for judging the adequacy (or inadequacy) of the existing ICNIRP and IEEE C95.1 radiofrequency radiation standards can be taken from many relevant sources. The ICNIRP standards are similar to the IEEE (except for the new C95.1 -2006) revisions by IEEE SC-4), and these discussions can be used to evaluate both sets of public exposure standards for adequacy (or inadequacy).

An important screen for assessment of how review bodies conduct their science reviews and resulting conclusions on the adequacy of ELF and RF exposure limits depends on embedded assumptions. The singularly most important embedded assumption is whether these bodies assume from the beginning that only conclusive scientific evidence (proof) will be sufficient to warrant change; or whether actions should be taken on the basis of a growing body of evidence which provides early but consequential warning of (but not yet proof) of possible risks.

As a result of current international research and scientific discussion on whether the prevailing RF and ELF standards are adequate for protection of public health, there are many recent developments to provide valuable background on the uncertainty about whether current standards adequately protect the public.

World Health Organization Draft Framework for Electromagnetic Fields

The International EMF Project was established by WHO in 1996. Its mission was to *"pool resources and knowledge concerning the effects of exposure to EMF and make a concerted effort to identify gaps in knowledge, recommend focused research programmes that allow better health risk assessments to be made, conduct updated critical reviews of the scientific literature, and work towards an international consensus and solutions on the health concerns."* (WHO September 1996 Press Release - Welcome to the International EMF Project)

The stated role of the WHO Precautionary Framework on EMF Health Risk Research (Radiation and Environment Health) has termed its objectives as follows;

- to anticipate and respond to possible threats before introduction of an agent or technology
- to address public concerns that an uncertain health risk is minimized after introduction of an agent
- to develop and select options proportional to the degree of scientific certainty, the severity of harm, the size and nature of the affected population and the cost.

The role of WHO is advisory only to the countries of Europe but it is an important function and can significantly affect decision-making on public health issues. It provides analysis and recommendations on various topics of health and environment, for consideration by member countries of the EU. Given the EU Article 174 policy requires a precautionary approach to judging health and environmental risks, and given that the

charter of WHO is to serve the needs of the EU, one would think it essential that the WHO EMF Program health criteria results should be guided by and tailored to compliance with Article 174.   This needs to occur in the assessment of the scientific literature (e.g., not requiring studies to provide scientific proof or causal scientific evidence but paying attention to and acting on the evidence, and the trend of the evidence at hand) and in its environmental health criteria recommendations. If the WHO EMF Program instead chooses to use the definitions of adverse impact and risk based on reacting to nothing short of conclusive scientific evidence, it fails to comply with the over-arching EU principle of health.

The World Health Organization has issued a draft framework to address the adequacy of scientific information, and accepted definitions of bioeffect, adverse health effect and hazard (WHO EMF Program Framework for Developing EMF Standards, Draft, October 2003). These definitions are not subject to the whim of organizations preparing public exposure standard recommendations. The WHO definition states that:

"(A)nnoyance or discomforts caused by EMF exposure may not be pathological per se, but, if substantiated, can affect the physical and mental well-being of a person and the resultant effect may be considered as an adverse health effect. A health effect is thus defined as a biological effect that is detrimental to health or well-being. According to the WHO Constitution, health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity."   **www.who.int/peh-emf**

The European Union Treaties Article 174

The EU policy (Article 174-2) requires that the precautionary principle be the basis for environmental protection for the public, and that protecting public health and taking preventative action before certainty of harm is proven is the foundation of the Precautionary Principle.   It is directly counter to the principles used by ICNIRP and IEEE in developing their recommendations for exposure standards. Both bodies require proof of adverse effect and risk before amending the exposure standards; this Treaty requires action to protect the public when a reasonable suspicion of risk exists (precautionary action).

Article 174 (2) [ex Article 130r]

1. Community policy on the environment shall contribute to pursuit of the following objectives:
—preserving, protecting and improving the quality of the environment;
—protecting human health;
—prudent and rational utilisation of natural resources;
—promoting measures at international level to deal with regional or worldwide environmental problems.

2. Community policy on the environment shall aim at a high level of protection taking into account the diversity of situations in the various regions of the Community. It shall

be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should as a priority be rectified at source and that the polluter should pay. In this context, harmonization measures answering environmental protection requirements shall include, where appropriate, as a safeguard clause allowing Member States to take provisional measures, for non-economic environmental reasons, subject to a Community inspection procedure.

3. In preparing its policy on the environment, the Community shall take account of:

—available scientific and technical data;
—environmental conditions in the various regions of the Community;
—the potential benefits and costs of action or lack of action;
—the economic and social development of the Community as a whole and the balanced development of its regions.

http://www.law.harvard.edu/library/services/research/guides/international/eu/eu_legal_research_treaties.php

WHO ELF Environmental Health Criteria Monograph, June 2007

In 2007. the WHO EMF Program released its ELF Health Criteria Monograph and held a workshop in Geneva, Switzerland June 20-21st.

ELF Health Criteria Monograph

12.6 Conclusions

Acute biological effects have been established for exposure to ELF electric and magnetic fields in the frequency range up to 100 kHz that may have adverse consequences on health. Therefore, exposure limits are needed. International guidelines exist that have addressed this issue. Compliance with these guidelines provides adequate protection.

Consistent epidemiological evidence suggests that chronic low-intensity ELF magnetic field exposure is associated with an increased risk of childhood leukaemia. **However, the evidence for a causal relationship is limited, therefore exposure limits based upon epidemiological evidence are not recommended, but some precautionary measures are warranted.** (emphasis added).

The Monograph finds no reason to change the designation of EMF as a 2B (Possible) Human Carcinogen as defined by the International Agency for Cancer Research (IARC). In finding that ELF-EMF is classifiable as a possible carcinogen, it is inconsistent to conclude that no change in the exposure limits is warranted. If the Monograph confirms, as other review bodies have, that childhood leukemia occurs at least as low as the 3 mG to 4 mG exposure range, then ICNIRP limits of 1000 mG for 50 Hz and 60 Hz ELF exposures are clearly too high and pose a risk to the health of children.

The WHO Fact Sheet summarizes some of the Monograph findings but adds further recommendations.

"Potential long-term effects*"*

Much of the scientific research examining long-term risks from ELF magnetic field exposure has focused on childhood leukaemia. In 2002, IARC published a monograph classifying ELF magnetic fields as "possibly carcinogenic to humans. This classification was based on pooled analyses of epidemiological studies demonstrating a consistent pattern of a two-fold increase in childhood leukaemia associated with average exposure to residential power-frequency magnetic field above 0.3 to 0.4 µT. **The Task Group concluded that additional studies since then do not alter the status of this classification."** *(emphasis added)*

"International exposure guidelines*"*

"Health effects related to short-term, high-level exposure have been established and form the basis of two international exposure limit guidelines (ICNIRP, 1998; IEEE, 2002). At present, these bodies consider the scientific evidence related to possible health effects from long-term, low-level exposure to ELF fields insufficient to justify lowering these quantitative exposure limits."

"Regarding long-term effects, given the weakness of the evidence for a link between exposure to ELF magnetic fields and childhood leukaemia, the benefits of exposure reduction on health are unclear. In view of this situation, the following recommendations are given:

*   1) Government and industry should monitor science and promote research programmes to further reduce the uncertainty of the scientific evidence on the health effects of ELF field exposure. Through the ELF risk assessment process, gaps in knowledge have been identified and these form the basis of a new research agenda.*

*   2) Member States are encouraged to establish effective and open communication programmes with all stakeholders to enable informed decision-making. These may include improving coordination and consultation among industry, local government, and citizens in the planning process for ELF EMF-emitting facilities.*

*   3) When constructing new facilities and designing new equipment, including appliances, low-cost ways of reducing exposures may be explored. Appropriate exposure reduction measures will vary from one country to another. However, policies based on the adoption of arbitrary low exposure limits are not warranted."*

The last bullet in the WHO ELF Fact Sheet does not come from the Monograph, nor is it consistent with conclusions of the Monograph. The Monograph does call for prudent avoidance measures, one of which could reasonably be to establish numeric planning targets or interim limits for new and upgraded transmission lines and appliances used by children, for example. Countries should not be dissuaded by WHO staff, who unlike the authors of the Monograph, go too far in defining appropriate boundaries for countries that may wish to implement prudent avoidance in ways that best suit their population needs, expectations and resources.   www.who.int/peh-emf/project/en

4

World Health Organization Report on Children's Health and Environment

Environmental Issue Report Number 29 from the World Health Organization (2002) cautions about the effects of radiofrequency radiation on children's health. As part of a publication on "Children's Health and Environment: A Review of Evidence" the World Health Organization (WHO) wrote:

> *"The possible adverse health effects in children associated with radiofrequency fields have not been fully investigated."*
>
> *"Because there are suggestions that RF exposure may be more hazardous for the fetus and child due to their greater susceptibility, prudent avoidance is one approach to keeping children's exposure as low as possible."*
>
> *"Further research is needed to clarify the potential risks of ELF-EMF and radiofrequency fields for children's health."*

International Agency for Research on Cancer (IARC)

A 2001 report by the WHO International Agency for Research on Cancer (IARC) concluded that ELF-EMF power frequency fields are a Category 2B (Possible) Human Carcinogen. These are power-frequency electromagnetic fields (50-Hz and 60-Hz electric power frequency fields).

The World Health Organization (WHO) is conducting the International Electromagnetic Fields (EMF) Project to assess health and environmental effects of exposure to static and time varying electric and magnetic fields in the frequency range of 1 – 300 gigahertz (GHz). Project goals include the development of international guidelines on exposure limits. This work will address radio and television broadcast towers, wireless communications transmission and telecommunications facilities, and associated devices such as mobile phones, medical and industrial equipment, and radars. It is a multi-year program that began in 1996 and will end in 2005.   www.who.int/peh-emf

SCENIHR Opinion (European Commission Study of EMF and Human Health)

An independent Scientific Committee on newly emerging risks commissioned by the European Union released an update of its 2001 opinion on electromagnetic fields and human health in 2007. "The Committed addressed questions related to potential risks associated with interaction of risk factors, synergistic effects, cumulative effects, anti-microbial resistance, new technologies such as nanotechnologies, medical devices, tissue engineeringm blood products, fertility reduction, cancer of endocrine organs, physical hazards such as noise and electromagnetic fields and methodologies for assessing new risks." SCENIHR, 2007

SCENIHR Conclusions on Extremely low frequency fields (ELF fields)

The previous conclusion that ELF magnetic fields are possibly carcinogenic, chiefly based on childhood leukaemia results, is still valid. There is no generally accepted mechanism to explain how ELF magnetic field exposure may cause leukaemia.

For breast cancer and cardiovascular disease, recent research has indicated that an association is unlikely. For neurodegenerative diseases and brain tumours, the link to ELF fields remains uncertain. A relation between ELF fields and symptoms (sometimes referred to as electromagnetic hypersensitivity) has not been demonstrated.

SCENIHR Conclusions on Radiofrequency Radiation fields (RF fields)

Since the adoption of the 2001 opinion, extensive research has been conducted regarding possible health effects of exposure to low intensity RF fields. This research has investigated a variety of possible effects and has included epidemiologic, in vivo, and in vitro research. The overall epidemiologic evidence suggests that mobile phone use of less than 10 years does not pose any increased risk of brain tumour or acoustic neuroma. For longer use, data are sparse, since only some recent studies have reasonably large numbers of long-term users. Any conclusion therefore is uncertain and tentative. From the available data, however, it does appear that there is no increased risk for brain tumours in long-term users, with the exception of acoustic neuroma for which there is limited evidence of a weak association. Results of the so-called Interphone study will provide more insight, but it cannot be ruled out that some questions will remain open.

SCENIHR Conclusions on Sensitivity of Children

Concerns about the potential vulnerability of children to RF fields have been raised because of the potentially greater susceptibility of their developing nervous system; in addition, their brain tissue is more conductive than that of adults since it has a higher water content and ion concentration, RF penetration is greater relative to head size, and they have a greater absorption of RF energy in the tissues of the head at mobile telephone frequencies. Finally, they will have a longer lifetime exposure.

Few relevant epidemiological or laboratory studies have addressed the possible effects of RF field exposure on children. Owing to widespread use of mobile phones among children and adolescents and relatively high exposures to the brain, investigation of the potential effect of RF fields in the development of childhood brain tumour is warranted. The characteristics of mobile phone use among children, their potential biological vulnerability and longer lifetime exposure make extrapolation from adult studies problematic.

There is an ongoing debate on possible differences in RF absorption between children and adults during mobile phone usage, e.g. due to differences in anatomy (Wiart et al. 2005, Christ and Kuster, 2005). Several scientific questions like possible differences of the dielectric tissue parameters remain open. The anatomical development of the nervous system is finished around 2 years of age, when children do not yet use mobile phones although baby phones have recently been introduced. Functional development, however, continues up to adult age and could be disturbed by RF fields.

Health Protection Agency (Formerly the NRPB - United Kingdom)

The National Radiation Protection Board or NRPB (2004) concluded, based on a review of the scientific evidence, that the most coherent and plausible basis from which guidance could be developed on exposures to ELF concerned weak electric field interactions in the brain and CNS (NRPB, 2004). A cautious approach was used to indicate thresholds for possible adverse health effects.

> *"Health Effects - It was concluded from the review of scientific evidence (NRPB, 2004b) that the most coherent and plausible basis from which guidance could be developed on exposures to ELF EMFs concerned weak electric field interactions in the brain and CNS (NRPB, 2004). A cautious approach was used to indicate thresholds for possible adverse health effects."*

> *"The brain and nervous system operate using highly complex patterns of \electrical signals. Therefore, the basic restrictions are designed to limit the electric fields and current densities in these tissues so as to not adversely affect their normal functioning. The adverse effects that might occur cannot easily be characterized according to presenting signs or symptoms of disease or injury. They represent potential changes to mental processes such as attention and memory, as well as to regulatory functions with in the body. Thus, the basic <u>restrictions should not be regarded as precisely determined values below which no adverse health effects can occur</u> and above which clearly discernible effects will happen. The do, however, indicate an increasing likelihood of effects occurring as exposure increases above the basic restriction values."*

> *"From the results of the epidemiological investigations, there remain concerns about a possible increased risk of child leukaemia associated with exposure to magnetic fields above about 0.4 uT (4 mG). In this regard, it is important to consider the possible need for further precautionary measures."*

This recent statement by the UK Health Protection Agency clearly indicates that the current guidelines may not be protective of public health. Yet, the reference levels used in the United Kingdom remain at 5000 mG for 50 Hz power frequency fields for occupational exposure and 1000 mG for public exposure.

US Government Radiofrequency Interagency Working Group Guidelines Statement

The United States Radiofrequency Interagency Working Group (RFIAWG) cited concerns about current federal standards for public exposure to radiofrequency radiation in 1999 (Lotz, 1999 for the Radiofrequency Interagency Working Group)

> "*Studies continue to be published describing biological responses to nonthermal ELF-modulated RF radiation exposures that are not produced by CW (unmodulated) radiation. These studies have resulted in concern that 'exposure guidelines based on thermal effects, and using information and concepts (time-averaged dosimetry, uncertainty factors) that mask any differences between intensity-modulated RF radiation exposure and CW exposure, do not directly address public exposures, and therefore may not adequately protect the public.*"

The United States government Federal Radiofrequency Interagency Working Group has reviewed the existing ANSI/IEEE RF thermal-based exposure standard upon which the FCC limit is based. This Working Group was made up of representatives from the US government's National Institute for Occupational Safety and Health (NIOSH), the Federal Communications Commission (FCC), Occupational Health and Safety Administration (OSHA), the Environmental Protection Agency (US EPA), the National Telecommunication and Information Administration, and the US Food and Drug Administration (FDA).

On June 17, 1999, the RFIAWG issued a Guidelines Statement that concluded the present RF standard "may not adequately protect the public". The RFIAWG identified fourteen (14) issues that they believe are needed in the planned revisions of ANSI/IEEE RF exposure guidelines including "to provide a strong and credible rationale to support RF exposure guidelines". In particular, the RFIAWG criticized the existing standards as not taking into account chronic, as opposed to acute exposures, modulated or pulsed radiation (digital or pulsed RF is proposed at this site), time-averaged measurements that may erase the unique characteristics of an intensity-modulated RF radiation that may be responsible for reported biologic effects, and stated the need for a comprehensive review of long-term, low-level exposure studies, neurological-behavioral effects and micronucleus assay studies (showing genetic damage from low-level RF).

The existing federal standards may not be protective of public health in critical areas. The areas of improvement where changes are needed include: a) selection of an adverse effect level for chronic exposures not based on tissue heating and considering modulation effects; b) recognition of different safety criteria for acute and chronic exposures at non-thermal or low-intensity levels; c) recognition of deficiencies in using time-averaged measurements of RF that does not differentiate between intensity-modulated RF and continuous wave (CW) exposure, and *therefore may not adequately protect the public.*

As of 2007, requests to the RFIAWG on whether these issues have been satisfactorily resolved in the new 2006 IEEE recommendations for RF public safety limits have gone unanswered (BioInitiative Working Group, 2007).

United Kingdom - Parliament Independent Expert Group Report (Stewart Report)

The Parliament of the United Kingdom commissioned a scientific study group to evaluate the evidence for RF health and public safety concerns. In May of 2000, the United Kingdom Independent Expert Group on Mobile Phones issued a report underscoring concern that standards are not protective of public health related to both mobile phone use and exposure to wireless communication antennas.

Conclusions and recommendations from the Stewart Report (for Sir William Stewart) indicated that the Group has some reservation about continued wireless technology expansion without more consideration of planning, zoning and potential public health concerns. Further, the Report acknowledges significant public concern over community siting of mobile phone and other communication antennas in residential areas and near schools and hospitals.

> *"Children may be more vulnerable because of their developing nervous system, the greater absorption of energy in the tissue of the head and a longer lifetime of exposure."*
>
> *"The siting of base stations in residential areas can cause considerable concern and distress. These include schools, residential areas and hospitals."*
>
> *" There may be indirect health risks from living near base stations with a need for mobile phone operators to consult the public when installing base stations."*
>
> *"Monitoring should be expecially strict near schools, and that emissions of greatest intensity should not fall within school grounds."*
>
> *"The report recommends "a register of occupationally exposed workers be established and that cancer risks and mortality should be examined to determine whether there are any harmful effects."*   *(*IEGMP, 2000)

Food and Drug Administration (US FDA)

The Food and Drug Administration announced on March 28, 2007 it is contracting with the National Academy of Science to conduct a symposium and issue a report on additional research needs related to possible health effects associated with exposure to radio frequency energy similar to those emitted by wireless communication devices.   The National Academy of Sciences will organize an open meeting of national and international experts to discuss the research conducted to date, knowledge gaps, and additional research needed to fill those gaps. The workshop will consider the scientific literature and ongoing research from an international perspective in order to avoid duplication, and in recognition of the international nature of the scientific community and of the wireless industry.

Funding for the project will come from a Cooperative Research and Development Agreement (CRADA) between the Food and Drug Administration's Center for Devices

and Radiological Health and the Cellular Telecommunications and Internet Association
(CTIA).   http://www.fda.gov/cellphones/index.html

National Institutes for Health - National Toxicology Program

The National Toxicology Program (NTP) is a part of the National Institute for
Environmental Health Sciences, National Institutes for Health.   Public and agency
comment has been solicited on whether to add radiofrequency radiation to its list of
substances to be tested by NTP as carcinogens. In February 2000 the FDA made a
recommendation to the NPT urging that RF be tested for carcinogenicity
(www.fda.gov.us). The recommendation is based in part on written testimony stating:

> " *Animal experiments are crucial because meaningful data will not be available from
> epidemiological studies for many years due to the long latency period between exposure
> to a carcinogen and the diagnosis of a tumor.*
>
> *"There is currently insufficient scientific basis for concluding either that wireless
> communication technologies are safe or that they pose a risk to millions of users."*
>
> *"FCC radiofrequency radiation guidelines are based on protection from acute injury
> from thermal effects of RF exposure* and may not be protective *against any non-thermal
> effects of chronic exposures."*

In March of 2003, the National Toxicology Program issued a Fact Sheet regarding its
toxicology and carcinogenicity testing of radiofrequency/microwave radiation. These
studies will evaluate radiofrequency radiation in the cellular frequencies.

> "*The existing exposure guidelines are based on protection from acute injury from thermal
> effects of RF exposure. Current data are insufficient to draw definitive conclusions
> concerning the adequacy of these guidelines to be protective against any non-thermal
> effects of chronic exposures. "*

US Food and Drug Administration

In February of 2000, Russell D. Owen, Chief of the Radiation Biology Branch of the
Center for Devices and Radiological Health, US Food and Drug Administration (FDA)
commented that there is:

> *"currently insufficient scientific basis for concluding whether wireless
> communication technologies pose any health risk."*
>
> *"Little is known about the possible health effects of repeated or long-term
> exposures to low level RF of the sort emitted by such devices."*

> *"Some animal studies suggest the possibility for such low-level exposures to increase the risk of cancer…"*

Dr. Owen's comments are directed to users of cell phones, but the same questions are pertinent for long-term RF exposure to radiofrequency radiation for the larger broadcast transmissions of television, radio and wireless communications (Epidemiology Vol. 1, No. 2 March 2000 Commentary). The Food and Drug Administration signed an agreement (CRADA agreement) to provide funding for immediate research into RF health effects, to be funded by the Cellular Telephone Industry of America. The FDA no longer assures the safety of users. No completion date has been set.

National Academy of Sciences - National Research Council

An Assessment of Non-Lethal Weapons Science and Technology by the Naval Studies Board, Division of Engineering and Physical Sciences (National Academies Press (2002) has produced a report that confirms the existence of non-thermal bioeffects from information transmitted by radiofrequency radiation at low intensities that cannot act by tissue heating (prepublication copy, page 2-13).

In this report, the section on Directed-Energy Non-Lethal Weapons it states that:

> *"The first radiofrequency non-lethal weapons, VMADS, is based on a biophysical susceptibility known empirically for decades. More in-depth health effects studies were launched only after the decision was made to develop that capability as a weapon. The heating action of RF signals is well understood and can be the basis for several additional directed-energy weapons. <u>Leap-ahead non-lethal weapons technologies will probably be based on more subtle human/RF interactions in which the signal information within the RF exposure causes an effect other than simply heating: for example, stun, seizure, startle and decreased spontaneous activity.</u> Recent developments in the technology are leading to ultrawideband, very high peak power and ultrashort signal capabilities, suggesting the the phase space to be explored for subtle, uyet potentially effective non-thermal biophysical susceptibilities is vast. Advances will require a dedicated effort to identify useful susceptibilities."*
>
> Page 2-13 of the prepublication report (emphasis added)

This admission by the Naval Studies Board confirms several critical issues with respect to non-thermal or low-intensity RF exposures. First, it confirms the existence of bioeffects from non-thermal exposure levels of RF. Second, it identifies that some of these non-thermal effects can be weaponized with bioeffects that are incontrovertibly adverse to health (stun, seizure, startle, decreased spontaneous activity). Third, it confirms that there has been knowledge for decades about the susceptibility of human beings to non-thermal levels of RF exposure. Fourth, it provides confirmation of the concept that radiofrequency interacts with humans based on the RF information content (signal information) rather than heating, so it can occur at subtle energy levels, not at high levels associated with tissue heating. Finally, the report indicates that a dedicated

scientific research effort is needed to really understand and refine non-thermal RF as a weapon, but it is promising enough for continued federal funding.

The IEEE (United States)

IEEE ICES SCC-28 SC-4 Subcommittee (Radiofrequency/Microwave Radiation)
Members of the ICES SCC-28 SC-4 committee presented their views and justifications in a Supplement to the Bioelectromagnetics Journal (2003). It offers a window into the thinking that continues to support thermal-only risks, and on which the current United States IEEE recommendations have been made. The United States Federal Communications Commission (FCC) has historically based its federally-mandated public and occupational exposure standards on the recommendations of the IEEE.

Radiofrequency/Microwave Radiation
IEEE's original biological benchmark for setting human exposure standards (on which most contemporary human standards are based) is disruption of food-motivated learned behavior in subject animals. For RF, it was based on short, high intensity RF exposures that were sufficient to result in changes in animal behavior.

"The biological endpoint on which most contemporary standards are based is disruption of food-motivated learned behavior in subject animals. The threshold SAR for behavioral disruption has been found to reliably occur between 3 and 9 W/kg across a number of animal species and frequencies; a whole-body average SAR of 4 W/kg is considered the threshold below which adverse effects would not be expected. To ensure a margin of safety, the threshold SAR is reduced by a safety factor of 10 and 50 to yield basic restrictions of 0.4 W/kg and 0.08 W/kg for exposures in controlled (occupational) and uncontrolled (public) environments, respectively." (Osepchuk and Petersen, 2003).

The development of public exposure standards for RF is thus based on acute, but not chronic exposures, fails to take into account intermittent exposures, fails to consider special impacts of pulsed RF and ELF-modulated RF, and fails to take into account bioeffects from long-term, low-intensity exposures that may lead to adverse health impacts over time.

BEMS Supplement 6 (Journal of the Bioelectromagnetics Society)

BEMS Supplement 6 was prepared in support of the IEEE SC-4 committee RF recommendations. In explaining and defending revised recommendations on RF limits contained within C.95.1, some key members took out space in Bioelectromagnetics (the Journal of the Bioelectromagnetic Society) to present papers ostensibly justifying a relaxation of the existing IEEE RF standards, rather than making the standards more conservative to reflect the emerging scientific evidence for both bioeffects and adverse health impacts.

Several clues are contained in the BEMS Supplement 6 to understand how the SC-4 IEEE C.95 revision working group and the ICES could arrive at a decision to not to recommend tighter limits on RF exposure. Not one but two definitions of "adverse effect" are

12

described, one by Osepchuk/Petersen (2003) and another by the working group itself (D'Andrea et al, 2003). Both set a very high bar for demonstration of proof, and both are ignored in the final recommendations by the SC-4 Subcommittee.

Second, many of the findings presented in the papers by individual authors in the BEMS Supplement 6 do report that RF exposures are linked to bioeffects and to adverse effects; but these findings are evidently ignored or dismissed by the SC-4 Subcommittee, ICES and by the eventual adoption of these recommendations by the full IEEE membership (in 2006). Even with a very high bar of evidence set by the SC-4 Subcommittee (and two somewhat conflicting definitions of adverse effect against which all scientific papers were reviewed and analyzed); there is clear sign that the "deal was done' regardless of even some of the key Subcommittee member findings reporting such effects at exposure levels below the existing limits.* sidebar

The SC-4 Subcommittee has developed a new and highly limited definition on RF effects, adverse effects and hazards that is counter to the WHO Constitution Principle on Health. The definition as presented by D'Andrea et al (2003, page S138) is based on the SC-4 IEEE C.95 revision working group definition of adverse effect:

"An adverse effect is a biological effect characterized by a harmful change in health. For example, such changes can include organic disease, impaired mental function, behavioral disfunction, reduced longevity, and defective or deficient reproduction. Adverse effects do not include: biological effects without detrimental health effect, changes in subjective feelings of well-being that are a result of anxiety about RF effects or impacts of RF infrastructure that are not related to RF emissions, or indirect effects caused by electromagnetic interference with electronic devices. An adverse effects exposure level is the condition or set of conditions under which an electric, magnetic or electromagnetic field has an adverse effect."

Further, the working group extended its definition to include that of Michaelson and Lin (1987) which states:

"If an effect is of such an intense nature that it compromises the individual's ability to function properly or overcomes the recovery capability of the individual, then the 'effect' may be considered a hazard. In any discussion of the potential for 'biological effects' from exposure to electromagnetic energies we must first determine whether any 'effect' can be shown; and then determine whether such an observed 'effect' is hazardous."

The definition of adverse effect according to Osepchuk and Petersen (2003) reported in the same BEMS Supplement 6 is:

"An adverse biological response is considered any biochemical change, functional impairment, or pathological lesion that could impair performance and reduce the ability of an organism to respond to additional challenge. Adverse biological responses should be distinguished from biological responses in general, which could be adaptive or compensatory, harmful, or beneficial. "

In contrast, the World Health Organization draft framework has accepted definitions of bioeffect, adverse health effect and hazard (WHO EMF Program Framework for Developing EMF Standards, Draft, October 2003). These definitions are not subject to the whim of organizations preparing public exposure standard recommendations. The WHO definition states that:

"(A)nnoyance or discomforts caused by EMF exposure may not be pathological per se, but, if substantiated, can affect the physical and mental well-being of a person and the resultant effect may be considered as an adverse health effect. A health effect is thus defined as a biological effect that is detrimental to health or well-being. According to the WHO Constitution, health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity."

The SC-4 definitions require proof that RF has caused organic disease or other cited effects that qualify. The burden of proof is ultimately shifted to the public, that bears the burden of unacknowledged health effects and diseases, where the only remedy is proof of illness over a large population of affected individuals, over a significant amount of time, and finally, delays until revisions of the standards can be implemented. The results of studies and reviews in the BEMS Supplement 6 already acknowledge the existence of bioeffects and adverse effects that occur at non-thermal exposure levels (below current FCC and ICNIRP standards that are supposedly protective of public health. However, they go on to ignore their own findings, and posit in advance that adverse effects seen today will, even with chronic exposure, not conclusively reveal disease or dysfunction tomorrow at exposure levels below the existing standards.

Sidebar: Quotes from BEMS Supplement 6

a) Studies and reviews where bioeffects likely to lead to adverse health effects with chronic exposure are reported;
b) adverse effects which are already documented;
c) studies where non-thermal RF effects are reported and unexplained;
d) effects are occurring below current exposure limits, and
e) conclusions by authors they cannot draw conclusions about hazards to human health

These quotes appear in articles presented by the IEEE SC-4 Subcommittee in BEMS Supplement 6. Despite these acknowledged gaps in information, lack of consistency in studies, abundant conflicting evidence documenting low level RF effects that can resulting serious adverse health impacts (DNA damage, cognitive impairment, neurological deficits, cancer, etc), and other clear instances of denial of ability to predict human health outcomes, the IEEE SC-4 Subcommittee has proposed recommendations to relax the existing limits.

D'Andrea et al., 2003a (Behavioral and Cognitive Effects of Microwave Exposure S39-S62)

"Reports of change of cognitive function (memory and learning) in humans and laboratory animals are in the scientific literature. Mostly, these are thermally mediated effects, but other low level effects are not so easily explained by thermal mechanisms." S39 Abstract
Elwood in Epidemiological Studies of Radiofrequency Exposures and Human Cancer (S63-S73)

"Studies are unable to confidently exclude any possibility of increased risk of cancer." S63 Abstract.
"Further research to clarify the situation is justified. Priorities include further studies of leukemia in both adults and children, and of cranial tumors in relationship to mobile phone use." S63 Abstract
"Although the epidemiological evidence in total suggests no increased risk of cancer, the results cannot be unequivocally interpreted in terms of cause and effect." S63 Abstract

D'Andrea et al., 2003b (Microwave Effects on the Nervous System S107-S147

"Low-level exposures that report alterations of the (blood-brain barrier) BBB remain controversial." S10 Abstract

"Research with isolated brain tissue has provided new results that do not seem to rely on thermal mechanisms." S107 Abstract

"Studies of individuals who are reported to be sensitive to electric and magnetic fields are discussed." S107 Abstract

"In this review of the literature, it is difficult to draw any conclusions concerning hazards to human health." S107 Abstract

"At lower levels of exposure biological effects may still occur but thermal mechanisms are not ruled out." S107 Abstract

"Based on a review of the literature presented here, it is difficult to draw conclusions concerning hazards to human health." " At lower levels of exposure, biological effects may still occur but thermal mechanisms are not ruled out." " There are too few studies to draw conclusions about the health effects of the low level findings" (on morphological effects of RF on animals).

"Other studies report low level effects where thermal mechanisms cannot explain the results." (effects of MW on neurochemistry).

"Additional work is needed to further evaluate the effects of RF exposure on working memory and cognition." (S138-S139)

Conclusions:
"Some reports of biological effects that cannot be explained by thermal mechanisms are in the scientific literature. These will require much more research to fully understand the mechanisms involved. Regardless of the mechanism, reports of effects that are at or below current recommended safety guidelines deserve rapid evaluation." (S140)

Proceedings of the NATO Advanced Research Workshop – Mechanisms of the Biological Effect on Extra High Power Pulses (EHPP) and UNESCO/WHO/IUPAB Seminar "Molecular and Cellular Mechanisms of Biological Effects of EMF" held March 2005, Yerevan, Armenia.

The proceedings conclude that "*the authors agreed with one main conclusion from these meeting(s): that in the future worldwide harmonization of standards have to be based on biological responses, rather than computed values*". The authors included 47 scientists, engineers, physicians and policy makers from 21 countries from Europe, North and South America, and Asia.

> *"The ICNIRP Guidelines for radiofrequency electromagnetic exposure are based only on thermal effects, and completely neglects the possibility of non-thermal effect."*

> *"The guidelines of the International Commission on Non-Ionizing Radiation Protection (ICNIRP) specify the quantative characteristics of EMF used to specify the basic restrictions are current density, specific absorption rate (SAR) and power density, i.e., the energetic characteristics of EMF. However, experimental data on energy-dependency of biological effects by EMF have shown that the SAR approach, very often, neither adequately describes or explains the real value of EMF-induced biological effects on cells and organisms, for at least two reasons: a) the non-linear character of EMF-induced bioeffects due to the existence of amplitude, frequency and 'exposure time-windows' and b) EMF-induced bioeffects significantly depend on physical and chemical composition of the surrounding medium."* (Preface pages XI – XIII).

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SECTION 5

EVIDENCE FOR EFFECTS ON GENE AND PROTEIN

EXPRESSION

(Transcriptomic and Proteomic Research)

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Prepared for the BioInitiative Working Group

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TABLE OF CONTENTS

I. INTRODUCTION

Daily exposure to electromagnetic fields (EMF), including extremely low frequency magnetic fields (ELF MF) and radiofrequency (RF) EMF, in the environment has raised public concerns about whether they have harmful consequences on human health. Several epidemiological studies suggest that exposure to EMF might associate with an elevated risk of cancer and other diseases in humans (reviewed in [Feychting et al., 2005]). To explain and/or support epidemiological observations, many laboratory studies have been conducted, but the results were controversial and no clear conclusion could be drawn to assess EMF health risk.

It is reasoned that one of the priorities in EMF research is to elucidate the biological effects of EMF exposure and the underlining mechanisms of action. Gene and protein are key players in organisms, and it has been assumed that any biological impact of EMF must be mediated by alterations in gene and protein expression [Phillips et al., 1992; Wei et al., 1990]. For example, heat shock protein, c-myc, and c-jun have been identified as EMF responsive genes and/or proteins in certain biological systems. In order to reveal the global effects of EMF on gene and protein expression, transcriptomics and proteomics, as high-throughput screening techniques (HTSTs), were eventually employed in EMF research with an intention to screen potential EMF-responsive genes and/or proteins without any bias. In 2005, WHO organized a Workshop on Application of Proteomics and Transcriptomics in EMF Research in Helsinki, Finland to discuss the related problems and solutions in this field [Leszczynski 2006; Leszczynski and Meltz 2006]. Later the journal Proteomics published a special issue devoted to the application of proteomics and transcriptomics to EMF research. This review aims to summarize the current research progress and discuss the applicability of HTSTs in the field.

II.   ELF MF

II A.   TRANSCRIPTOMICS

Binninger and Ungvichian firstly measured purified mRNA levels of total RNA from MF- and sham-exposed yeast cells and reported that the levels of a significant proportion of mRNAs were altered in response to continuous exposure to 20   T 60 Hz MF over a period of approximately 15 cell generations (24 h) [Binninger and Ungvichian 1997]. Unfortunately, no reproducible genes (polypetides) were identified in this study although the authors consistently found different proportions of transcripts whose abundances were altered in all four replication experiments.

Wu *et al*. have applied differential display reverse transcriptase–polymerase chain reaction (DD-RT-PCR) and Northern blotting to screen MF-responsive gene in Daudi cells. The cells were exposed to 0.8 mT of 50 Hz MF for 24 h. The authors screened out two candidate genes in Daudi cells and one was identified as a MF-responsive gene *ceramide glucosyltransferase*. They further found time-dependent changes in the transcription of *ceramide glucosyltransferase* induced by 0.8 mT MF [Wu et al., 2000]. With the help of DD-RT-PCR, Olivares-Banuelos *et al* reported that exposure to 0.7 mT 60 Hz MF for 7 days，4 h a day (2 h in the morning and 2 h in the afternoon), changed the global transcription profile of chromaffin cells. Eight RT-PCR products which correspond to six genes were identified, including *phosphoglucomutase*-1, *neurofibromatosis-2 interacting protein*, *microtubule associated protein*-2, *thiamine pyrophosphokinase*, and two hypothetical proteins (RNOR02022103 and ROR01044577). In addition, the authors found that presumed regulatory regions of these genes contained CTCT-clusters [Olivares-Banuelos et al., 2004], which has been identified as an electromagnetic field-responsive DNA element regulating gene expression [Goodman and Blank 2002].

Balcer-Kubiczek *et al*. have applied the two-gel cDNA library screening method (BIGEL) to screen MF-responsive genes, in which the gel arrays contained a total of

960 cDNAs selected at random from the cDNA library. The HL 60 cells were exposed to 2 mT of 60 Hz square wave MF for 24 h. Four candidate genes were shown responsive to the MF exposure, but could not be confirmed by following Northern analysis. Furthermore, the authors found that these four candidates and another four selected genes (*MYC*, *HSP70*, *RAN* and *SOD1*) did not react to either square wave or sine wave 60 Hz MF at 2 mT for 24 h [Balcer-Kubiczek et al., 2000]. However, the cellular responses to square wave and sine wave 60 Hz MF might be different. In order to systematically evaluate the effect of 60 Hz MF on gene expression in HL 60 cells, it is necessary for the authors to screen 60 Hz sine wave MF responsive candidate genes in HL 60 cells with BIGEL method as well, and then, perform validation with Northern blotting for these candidates.

Using cDNA arrays containing 588 cancer-related genes, Loberg *et al*. analyzed gene expression in normal (HME) and transformed (HBL-100) human mammary epithelial cells and human promyelocytic leukemia (HL60) cells after exposure to 60 Hz MF at intensity of 0.01 or 1.0 mT for 24 h. The authors reported that several genes were identified in MF-exposed cells whose expressions were increased by at least two folds or decreased by 50% or more, but no gene was found to be differentially expressed in each of three independent exposures for any cell type, and no relationship between exposure intensity and differential gene expression was found [Loberg et al., 2000].

In order to obtain a more global evaluation, genome-wide microarray screening methods were applied to identify genes responding to ELF MF in certain types of cells. By application of cDNA microarray, Nakasono *et al*. have investigated the effect of 50 Hz MF below 300 mT on gene expression in yeast. The authors reported that several genes were found differentially expressed in yeast cells with medium to low confidence level (CL) after exposure to 10, 150 and 300 mT for 24 h. Among these genes, seven showed a dose-response relationship in the normalized ratio data and three genes showed a reproducible change for all three intensities. They also proposed that these genes should be re-examined by methods with greater sensitivity or by quantitative methods, such as real-time PCR. On the other hand, no high-confidence expression

changes were observed for genes that are involved in heat-shock response, DNA repair, respiration, protein synthesis, or cell cycle. Thus, they concluded that 50 Hz MF up to 300 mT did not appear to affect gene expression linked to either defined cell processes stated above or unknown cell responses in investigated model eukaryotic cells [Nakasono et al., 2003]. Unfortunately, only single experiment for array analysis was performed in this study.

Recently, a similar study was conducted by Luceri *et al*. to investigate the global gene response to 50 Hz MF in human lymphocytes and yeast cells. These two types of cells were exposed to MF at intensity of 100 T, 10 T and 1 T for 18 h. As a result, in lymphocytes, one gene was found down-regulated at 100 T, one down-regulated gene and two up-regulated genes were screened out at 10 T, and no gene was detected changed at 1 T. As to the yeast cells, the results showed 2, 15 and 2 genes as differentially expressed (mainly down-regulated) after exposure to 100, 10 and 1 T, respectively, in which SPS100 gene was consistently up-regulated after exposure to 50 Hz MF at all three intensities. But no genes were found differentially expressed when the authors analyzed the data by other statistical methods. Thus, the authors concluded that 50 Hz MF did not affect gene expression in these two types of cells and the variations of a few genes mentioned above could be due to experimental noise [Luceri et al., 2005]. However, it is necessary to examine the candidates, especially the SPS100 gene, to validate whether they were real "un-responsive" genes.

In Henderson's report, human umbilical vein endothelial cells (HUVEC) were exposed to various patterns and intensities of 50 Hz MF, including continuous exposure at a two intensities (10 and 700 T), intermittent exposure (60 min on/ 30 min off) at a single intensity (700 T), and continuous exposure to a variable-intensity fields (10-30 T). The transcriptional response of the cells was investigated using oligonucleotide microarrays containing up to 30, 000 unique features. Although different genes were identified where their expressions appeared to be affected by exposure to MF in individual experiments, none of these genes were regulated in the same manner in

subsequent repetition experiments [Henderson et al., 2006].

Antonini *et al* reported that intermittent exposure (5 min on/5 min off) to 50 Hz MF at flux densities of 2 mT for 16 h could change gene expression in human neuroblastoma cell line SH-SY5Y by application of whole-genome Human Unigene RZPD-2 cDNA array which contains about 75, 000 cDNA clones. Several genes were found down- or up-regulated at least five-fold after ELF MF exposure and the authors concluded that SH-SY5Y cells were sensitive to ELF MF [Antonini et al., 2006]. However, no reports indicated that these differentially expressed genes were confirmed by other methods.

Lupke *et al* investigated the effect of ELF MF on gene expression profiling in human umbilical cord blood-derived monocytes using the same Unigene RZPD-2. The results indicated that 0.1 mT 50 Hz MF exposure for 45 minutes altered the expressions of 986 genes involved in metabolism, cellular physiological processes, signal transduction, and immune response, among them, five genes were significantly regulated. Furthermore, the authors analyzed several genes by real-time RT-PCR and one ELF MF candidate responsive gene IL15RA was confirmed. However, this study only did single array analysis for pooling sample from 78 donors and two independent real-time RT-PCR analyses for samples from 5 and 6 different donors. The authors did not report the examinations of other candidates with real-time RT-PCR analysis [Lupke et al., 2006].

II B.   PROTEOMICS

Nakasono *et al*. has investigated the effects of protein expression in model system such as *Escherichia coli* and *Saccharomyces cerevisiae* using two dimensional gels electrophoresis (2-DE) method. When the bacterial cells were exposed to each MF at 5-100 Hz under aerobic conditions (6.5 h) or at 50 Hz under anaerobic conditions (16 h) at the maximum intensity (7.8 to 14 mT), no reproducible changes were observed in the 2D gels. However, the stress-sensitive proteins did respond to most stress factors, including temperature change, chemical compounds, heavy metals, and nutrients. The authors concluded that the high-intensity ELF MF (14 mT at power frequency) did not act as a general stress factor [Nakasono and Saiki 2000]. When using *Saccharomyces cerevisiae* as a model system, Nakasono *et al*. reported that no reproducible changes in the 2D gels were observed in yeast cells after exposure to 50 Hz MF at the intensity up to 300 mT for 24 h [Nakasono et al., 2003]. In this study, only three sets of gels from three independent experiments were analyzed.

Li *et al*. have performed a proteomics approach to investigate the changes of protein expression profile induced by ELF MF in human breast cancer cell line MCF-7. With help of 2-DE and data analysis on nine gels for each group, 44 differentially expressed protein spots were screened in MCF-7 cells after exposure to 0.4 mT 50 Hz MF for 24 h. Three proteins were identified by LC-IT Tandem MS as RNA binding protein regulatory subunit, proteasome subunit beta type 7 precursor, and translationally controlled tumor protein, respectively [Li et al 2005]. Further investigations, such as Western blotting, are required to confirm these ELF responsive candidate proteins.

Using 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) technology and MS in a blind study, Sinclair *et al* have investigated the effects of ELF MF on the proteomes of wild type *Schizosaccharomyces pombe* and a Sty1p deletion mutant which displays increased sensitivity to a variety of cellular stresses. The yeast cells were exposed to 50 Hz EMF at field strength of 1 mT for 60 min. While this study

identified a number of protein isoforms that displayed significant differential expressions across experimental conditions, there was no correlation between their patterns of expression and the ELF MF exposure regimen. The authors concluded that there were no significant effects of ELF MF on the yeast proteome at the sensitivity afforded by 2D-DIGE. They hypothesized that the proteins identified in the experiments must be sensitive to subtle changes in culture and/or handling conditions. Based on their experience, they suggested to the community that the interpretation of proteomic data in a biological context should be treated with caution [Sinclair et al., 2006].

II C.   SUMMARY

Generally, recent studies on global gene and protein expression responding to ELF MF have been conducted in different biological systems by applications of HTSTs. Only a few studies reported to identify ELF MF responsive genes successfully. For example Wu *et al*. identified *ceramide glucosyltransferase* as a MF-responsive gene in Daudi cells [Wu et al., 2000] and Olivares-Banuelos *et al.* identified six ELF MF genes in chromaffin cells [Olivares-Banuelos et al., 2004] with the help of DD-RT-PCR and Northern blotting analysis; by combining cDNA array analysis with real-time RT-PCR confimation, Lupke *et al*. identified IL15RA as ELF MF responsive genes in human monocytes [Lupke et al., 2006]. Although many transcriptome and proteome analysis showed that ELF MF exposure could change gene and/or protein expression in certain cell types [Antonini et al., 2006; Binninger and Ungvichian 1997; Li et al., 2005], there are lack of confirmation to determine if they are real ELF MF responsive genes or proteins. Therefore, it is a priority to conduct confirmation experiments to demonstrate the author's findings.

As to those negative reports, few or no genes and proteins were found significantly changed according to their statistical analysis and screening standards. But these few genes and proteins were neither reproducible [Henderson et al., 2006; Nakasono et al.,

2003; Sinclair et al., 2006]nor confirmed by other methods [Balcer-Kubiczek et al., 2000], and the changes were not related to ELF MF exposure [Loberg et al., 2000; Luceri et al., 2005; Nakasono et al., 2003]. Therefore, these studies are also needed to be replicated or verified.

III. RF EMF

III A . TRANSCRIPTOMICS

In an initial study utilizing membrane-based cDNA microarray, Harvey and French studied the effects of 864.3 MHz (CW) on HMC-1 human monocytes. The exposure was carefully controlled and averaged at an SAR of 7 W/kg, almost double the exposure level of established adverse effects. Three 20 min exposures were performed at 4-h intervals daily for 7 days. cDNA microarray analyses revealed consistent alterations in steady-state mRNA levels of 3 of the 558 genes represented on the membranes including one proto-oncogene *c-kit* (increased), one apoptosis-associated gene *DAD-1* (decreased) and one potential tumor suppressor gene *NDPK* (decreased) [Harvey and French 1999]. However, there were considerable variabilities between the two experiments reported and the fold change of each differentially expressed gene was small (< 1.5 folds). Meanwhile, the authors did not use other methods to confirm the results.

Pacini *et al*. investigated the effect of gene expression in human skin fibroblasts by using cDNA arrays including 82 genes, and reported that exposure to GSM 902.4 MHz RF EMF at an average SAR of 0.6 W/kg for 1 h increased the expression of 14 genes which function in mitogenic signal transduction, cell growth and apoptosis controlling. The authors further demonstrated a significant increase in DNA synthesis and intracellular mitogenic second messenger formation which were matched the high expression of MAP kinase family genes [Pacini et al., 2002]. The authors suggested that the RF EMF exposure has significant biological effects on human skin fibroblasts.

However, only one experiment was performed in array analysis and no more experiment was made by the authors to confirm the array analysis result.

With help of cDNA microarray, Leszczynski *et al*. reported that exposure to GSM 900 MHz RF EMF at an average SAR of 2.4 W/kg for 1 h changed expression of 3600 genes, including down-regulated genes involved in forming the Fas/TNFa apoptotic pathway in human endothelial cell line EA.hy926 [Leszczynski et al., 2004]. The authors performed three separate experiments in array analysis, but no confirmation experiments were conducted to validate the array analysis result. Recently, Leszczynski group compared the global gene response of two human endothelial cells, EA.hy926 and its variant EA.hy926v1 to RF EMF and reported that the same genes were differently affected by the exposure to GSM 900 MHz RF EMF at an average SAR of 2.8 W/kg for 1 h in each of the cell lines [Nylund and Leszczynski 2006]. Similarly, no reports indicated that the differentially expressed genes in this study were confirmed by other methods.

Lee *et al*. used the serial analysis of gene expression (SAGE) method to measure the RF EMF effect on genome scale gene expression in HL 60 cells. The cells were exposed to 2.45 GHz RF EMF at an average SAR of 10 W/kg for 2 h and 6 h. The authors observed that 221 genes and 759 genes altered their expression after 2 h exposure and 6 h exposure respectively. Functional classification of the affected genes revealed that apoptosis-related genes were among the up-regulated ones and the cell cycle genes among the down-regulated ones, but no significant increase in the expression of heat shock genes were found [Lee et al., 2005]. However, the SAGE experiment was repeated only once and only one control with 2 h sham exposure was used. No confirmation experiment was reported to validate these differentially expressed genes.

Huang *et al*. investigated the effect of 1763 MHz RF EMF on gene expression in Jurkat cells by Applied Biosystems 1700 full genome expression microarray. The authors

found that 68 genes were differentially expressed in the cells after exposure to RF EMF at SAR of 10 W/kg for 1 h and harvested immediately or after 5 h [Huang et al., 2006]. The authors repeated sets of experiment five times to collect biological triplicates in every sample but the differentially expressed genes were not confirmed by other methods.

Whitehead *et al.* have performed *in vitro* experiments with C3H 10T(1/2) mouse cells to determine whether Frequency Division Multiple Access (FDMA) or Code Division Multiple Access (CDMA) modulated RF radiations can induce changes in gene expression using the Affymetrix U74Av2 GeneChip. The GenesChip data showed the number of probe sets with an expression change greater than 1.3-fold was less than or equal to the expected number of false positives in C3H 10T(1/2) mouse cells after 835.62 MHz FDMA or 847.74 MHz CDMA modulated RF EMF exposure at SAR of 5 W/kg for 24 h. The authors concluded that the 24 h exposures to FDMA or CDMA RF radiation at 5 W/kg had no statistically significant effect on gene expression [Whitehead et al., 2006a; Whitehead et al., 2006b]. However, the authors did not demonstrate that these differentially expressed genes were real "false positive" with other methods.

In Gurisik's report, human neuroblastoma cells (SK-N-SH) were exposed to GSM 900 MHz RF signal at SAR of 0.2 W/kg for 2 h and recovered without field for 2 h post-exposure. Gene expression were examined by Affymetrix Human Focus Gene Arrays including 8400 genes and followed by real-time RT-PCR of the genes of interest. Only six genes were found to be slightly down-regulated in response to RF exposure comparing with mock-exposed cells. Furthermore, these genes can not be confirmed by real-time RT-PCR analysis. Thus, the authors concluded that the RF EMF exposure applied in this study could not change gene expression in SK-N-SH cells [Gurisik et al., 2006]. However, the array analysis experiment was repeated only once and only one array for exposure or sham exposure group.

Qutob *et al* have assessed the ability of exposure to a 1.9 GHz pulse-modulated RF field

to affect global gene expression in U87MG glioblastoma cells by application of Agilent Human 1A (v1) oligonucleotide 22K microarray slides. The U87MG cells were exposed to 1.9 GHz pulse-modulated (50 Hz, 1/3 duty cycle) RF field at an average SAR of 0.1, 1.0 and 10.0 W/kg for 4 hours, and incubated for an additional 6 hours. The authors found no evidence that exposure to RF fields under different exposure conditions can affect gene expression in cultured U87MG cells. In this paper, the authors performed five experiments, each containing a single replicate and some of genes were confirmed as real "un-effected genes" [Qutob et al., 2006].

Zeng *et al*. have investigated gene expression profile in MCF-7 after exposing to GSM 1800 MHz RF EMF using Affymetrix Genechip U133A. The result showed that no gene with 100% consistency change were found in MCF-7 cells after intermittent exposure (5 min on/ 10 min off) to RF EMF at an average SAR of 2.0 W/kg for 24 h while five genes with 100% consistency change were found in MCF-7 at same exposure conditions but at SAR of 3.5 W/kg. However, these five differentially transcribed genes could not be further confirmed by real-time RT-PCR assay. Thus, this study did not provide evidence that RF EMF exposure can produce distinct effects on gene expression in the MCF-7 cells [Zeng et al., 2006].

Remondini *et al*. have investigated the effect of RF EMF on gene expression profile in six different cell lines or primary cells, and found various types of cell reacted differently in RF EMF exposure). RF EMF exposure changed gene expression in 900 MHz-exposed EA.hy926 endothelial cells (22 up-regulations, ten down-regulations), 900 MHz-exposed U937 lymphoblastoma cells (32 up-regulations, two down-regulations), and 1800 MHz-exposed HL-60 leukemia cells (11 up-regulations, one down-regulation) while NB69 neuroblastoma cells, T-lymphocytes, and CHME5 microglial cells did not show significant changes in gene expression. The authors concluded that there were alterations in gene expression in some human cells types exposed to RF-EMF but these chenges depended on the type of cells and RF-EMF signal [Remondini et al., 2006]. However, these RF responsive candidate genes in

different types of cells were not confirmed yet.

Very recently, Zhao *et al*. have investigated the effects of RF EMF on gene expression of *in vitro* cultured rat neuron with Affymetrix Rat Neurobiology U34 array. Among 1200 candidate genes, 24 up-regulted genes and 10 down-regulated genes were identified after 24-h intermittent exposure (5 min on/ 10 min off) at an average SAR of 2.0 W/kg, which are associated with multiple cellular functions. The changes of most of genes were successfully validated by real-time RT-PCR, including genes involved in cytoskeleton, signal transduction pathway, metabolism [Zhao et al., 2007].

Belyaev et al. analyzed gene expression profile in RF exposed animals. Rats were exposed or sham exposed to GSM 915 MHz at whole body average SAR of 0.4 mW/g for 2 h and total RNA was extracted from cerebellum. Gene expression profiles were obtained by Affymetrix U34 GeneChips representing 8800 rat genes and analyzed with the Affymetrix Microarray Suite (MAS) 5.0 software. The results showed that 11 genes were up-regulated in a range of 1.34-2.74 folds and one gene was down-regulated 0.48-fold. The induced genes encode proteins with diverse functions including neurotransmitter regulation, blood-brain barrier (BBB), and melatonin production [Belyaev et al., 2006]. In this study, triplicate arrays were applied for three exposed samples or three sham exposed samples. But the differentially expressed genes were not confirmed by other methods.

III B . PROTEOMICS

Leszczynski *et al*. have provided perhaps some of the most relevant *in vitro* data by studying the effects of GSM 900 MHz RF EMF exposure [Leszczynski et al., 2002; Nylund and Leszczynski 2004; Nylund and Leszczynski 2006]. Firstly, the EA.hy926 cells were exposed to RF EMF at SAR of 2.0 W/kg over a one-hour period and the data indicated the RF exposure changed protein expression at a proteome scale, and up-regulated the level of HSP 27 protein and induced its hyper-phosphorylation. The

activation of p38 mitogen activated kinase (MAPK) was partially responsible for the phosphorylation of the HSP. They confirmed HSP27 protein expression, phosphorylation and cellular distribution by independent protein analytical techniques including western blotting and indirect immunofluorescence [Leszczynski et al., 2002]. Secondly, the group screened 38 proteins with statistically significantly altered expression in the same cell line after GSM 900 MHz exposure at SAR of 2.4 W/kg for 1 h. An isoform of vimentin was confirmed as a responsive protein by Western blotting and indirect immunofluorescence. The authors concluded that the cytoskeleton might be one of the mobile phone radiation-responding cytoplasmic structures [Nylund and Leszczynski 2004]. Furthermore, they compared *in vitro* response to GSM 900 MHz RF EMF in EA.hy926 with its variant EA.hy926v1 by examination of protein expression using 2-DE. The results showed protein expression profiles were altered in both examined cell lines after RF EMF exposure. However, the affected proteins were differently in each of the cell lines, 38 and 45 differentially expressed proteins were found in EA.hy926 and EA.hy926v1 respectively. Several differentially expressed proteins in EA.hy926 cells were confirmed by other methods, but no differentially expressed protein in EA.hy926v1 cells was confirmed. Base on the transcriptome and proteome analysis data, the authors concluded that the response might be genome- and proteome-dependent [Nylund and Leszczynski 2006]. One thing should be mentioned that all the 2-DE analyses in Leszczynski group reports were replicated ten times.

Zeng *et al*. systematically explored the effects of 1800 MHz RF EMF on protein expression in MCF-7 cells by 2-DE, and revealed that a few but different proteins were differentially expressed under continuous or intermittent RF EMF exposure at SAR of 3.5 W/kg for 24 h or less, implying that the observed effects might have occurred by chance. By combination with the transcriptomics analysis data, this study did not provide convincing evidence that RF EMF exposure could produce distinct effects on gene and protein expression in the MCF-7 cells. The authors supposed that the MCF-7 cells may be less sensitive to RF EMF exposure [Zeng et al., 2006]. However, in this study, only triplicate gels were performed in each exposure condition experiment.

III C . SUMMARY

The effects of RF EMF on global gene and protein expression have been investigated in different biological systems, and most of studies were focused on the mobile phone utilization frequency (800-2000 MHz) at relative low exposure density (average SAR near 2.0 W/kg). Some studies reported negative results of RF EMF exposure on gene expression. For example, Whitehead *et al*. did not find differentially expressed genes in RF exposed C3H 10T(1/2) mouse cells [Whitehead et al., 2006a; Whitehead et al., 2006b]. Remondini *et al*. reported that NB69 cells, T lymphocytes, and CHME5 cells did not show significant changes in gene expression after RF EMF exposure [Remondini et al., 2006]. In Gurisik *et al.* [Gurisik et al., 2006]and Zeng *et al.* [Zeng et al., 2006]study, although they screened out several RF EMF-responsive candidate genes, they could not confirm these genes by real-time RT-PCR method.

Meanwhile, several groups claimed that RF EMF exposure can change gene and protein expression profile in certain types of cells and identified certain EMF responsive genes and proteins. Only one report found RF EMF exposure changed gene expression profile in neurons and most of changed genes were confirmed by real-time RT-PCR [Zhao et al 2007]. As to proteome analysis, only two groups have analyzed protein expression by proteomic approaches, including 2-DE and Mass Spectrum. Zeng *et al*. systematically explored the effects of 1800 MHz RF EMF on protein expression in MCF-7 cells by 2-DE, and revealed that a few but different proteins were differentially expressed under different exposure conditions, implying that the observed effects might have occurred by chance [Zeng et al., 2006]. However, in this study, only triplicate gels were performed in each exposure condition experiment. In contrast, Leszczynski group identified two RF EMF responsive proteins in EA.hy926 cells, i.e. HSP27 [Leszczynski et al., 2002] and vimentin [Leszczynski et al., 2004] with help of 2-DE and MS analysis. This group further confirmed the expression and cellular distribution of HSP27 and vimentin in RF exposed EA.hey926 cells by other methods including Western blotting and indirect immunofluorescence staining. Furthermore, they reported the changes of these RF EMF molecular targets had

down-stream impact on cell physiology [Leszczynski et al., 2002; Leszczynski et al., 2004].

Generally, it seems that the response of a cell to RF EMF exposure depends on exposure condition, cell type, and/or the cell's genome- and proteome [[Remondini et al., 2006; Nylund and Leszczynski 2006].

IV.   Overall Conclusion

Based on current available literature, it is justified to conclude that EMF exposure can change gene and/or protein expression in certain types of cells, even at intensities lower than ICNIRP recommended values. However, the biological consequences of most of the changed genes/proteins are still unclear, and need to be further explored. Thus, it is not the time point yet to assess the health impact of EMF based on the gene and protein expression data.   The IEEE and WHO data bases do not include the majority of ELF studies; they do include the majority of the RF studies.

Currently, controversial data exist in the literature. The EMF research community should pay equal attention to the negative reports as to the positive ones. Not only the positive findings need to be replicated, all the negative ones are also needed to be validated.

It is noteworthy that low intensity EMF is a weak physical stimulus for a cell or organism, and high throughput screening techniques (HTSTs) would sacrifice its sensitivity to ensure its high throughput. It has been recognized there is methodological defects while analyzing weak effect with HTSTs, such as reproducibility and variability. Thus, more experimental replications are needed to reduce the ratio of noise over signal. Meanwhile, confirmation study must be included to assure the validity of the data.

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SECTION 6

EVIDENCE FOR GENOTOXIC EFFECTS
(RFR AND ELF Genotoxicity)

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TABLE OF CONTENTS

I. Introduction

Toxicity to the genome can lead to a change in cellular functions, cancer, and cell death. A large number of studies have been carried out to investigate the effects of electromagnetic field (EMF) exposure on DNA and chromosomal structures. The single-cell gel electrophoresis (comet assay) has been widely used to determine DNA damages: single and double strand breaks and cross-links. Studies have also been carried out to investigate chromosomal conformation and micronucleus formation in cells after exposure to EMF.

II. Radiofrequency radiation (RFR) and DNA damage (*28 total studies – 14 reported effects (50%) and 14 reported no significant effect (50%)*)

II A. DNA studies that reported effects*:*

The following is a summary of the research data reported in the literature.

Aitken et al. [2005] exposed mice to 900-MHz RFR at a specific absorption rate (SAR) of 0.09 W/kg for 7 days at 12 h per day. DNA damage in caudal epididymal spermatozoa was assessed by quantitative PCR (QPCR) as well as alkaline and pulsed-field gel electrophoresis postexposure. Gel electrophoresis revealed no significant change in single- or double-DNA strand breakage in spermatozoa. However, QPCR revealed statistically significant damage to both the mitochondrial genome ($p < 0.05$) and the nuclear β-globin locus ($p < 0.01$).

Diem et al [2005] exposed human fibroblasts and rat granulosa cells to mobile phone signal (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; during 4, 16 and 24 h; intermittent 5 min on/10min off or continuous). RFR exposure induced DNA single- and double-strand breaks as measured by the comet assay. Effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect in the than continuous exposure.

Gandhi and Anita [2005] reported increases in DNA strand breaks and micronucleation in lymphocytes obtained from cell phone users.

Garaj-Vrhovac et al [1990] reported changes in DNA synthesis and structure in Chinese hamster cells after various durations of exposure to 7.7 GHz field at 30 mW/cm^2.

Lai and Singh [1995; 1996; 1997a; 2005] and Lai et al. [1997] reported increases in single and double strand DNA breaks in brain cells of rats exposed for 2 hrs to 2450-MHz field at 0.6-1.2 W/kg.

Lixia et al. [2006] reported an increase in DNA damage in human lens epithelial cells at 0 and 30 min after 2 hrs of exposure to 1.8 GHz field at 3 W/kg.

Markova et al. [2005] reported that GSM signals affected chromatin conformation and gama-H2AX foci that colocalized in distinct foci with DNA double strand breaks in human lymphocytes.

Narasimhan and Huh [1991] reported changes in lambdaphage DNA suggesting single strand breaks and strand separation.

Nikolova et al. [2005] reported a low and transient increase in DNA double strand break in mouse embryonic stem cells after acute exposure to 1.7- GHz field.

Paulraj and Behari [2006] reported an increased in single strand breaks in brain cells of rats after 35 days of exposure to 2.45 and 16.5 GHz fields at 1 and 2.01 W/kg.

Phillips et al. [1998] found increase and decrease in DNA strand breaks in cells exposure to various forms of cell phone radiation.

Sun et al. [2006] reported an increase in DNA single strand breaks in human lens epithelial cells after 2 hrs of exposure to 1.8 GHz field at 3 and 4 W/kg. The DNA damages caused by 4 W/kg field were irreversible.

Zhang et al. [2002] reported that 2450-MHz field at 5 mW/cm^2 did not induce DNA and chromosome damage in human blood cells after 2 hrs of exposure, but could increase DNA damage effect induced by mitomycin-C.

Zhang et al. [2006] reported that 1800-MHz field at 3.0 W/kg induced DNA damage in Chinese hamster lung cells after 24 hrs of exposure.

II B. DNA studies that reported no significant effect:

Chang et al. [2005] using the Ames assay found no significant change in mutation frequency in bacteria exposed for 48 hrs at 4W/kg to an 835-MHz CDMA signal.

Hook et al. [2004] showed that 24-hr exposure of Molt-4 cells to CDMA, FDMA, iDEN or TDMA modulated RF radiation did not significantly alter the level of DNA damage.

Lagroye et al. [2004a] reported no significant change in DNA strand breaks in brain cells of rats exposed for 2 hrs to 2450-MHz field at 1.2 W/kg.

Lagroye et al. [2004b] found no significant increases in DNA-DNA and DNA-protein cross-link in C3H10T(1/2) cells after a 2-hr exposure to CW 2450 MHz field at 1.9 W/kg.

Li et al. [2001] reported no significant change in DNA strand breaks in murine C3H10T(1/2) fibroblasts after 2 hrs of exposure to 847.74 and 835.02 MHz fields at 3-5 W/kg.

Maes et al. [1993, 1996, 1997, 2000, 2001, 2006] published a series of papers on in vitro genotoxic effects of radiofrequency radiation and interaction with chemicals. Their mostly found no significant effect.

Malyapa et al. [1997a,b, 1998] reported no significant change in DNA strand-breaks in cells exposed to 2450-Hz and various forms of cell phone radiation. Both in vitro and in vivo experiments were carried out.

McNamee et al. [2002a,b, 2003] found no significant increase in DNA breaks and micronucleus formation in human leukocytes exposed for 2 hrs to 1.9 GHz field at SAR up to 10 W/kg.

Sakuma et al. [2006] exposed human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs to mobile communication radiation for 2 and 24 hrs. No significant change in DNA strand breaks were observed up to 800 mW/kg.

Stronati et al. [2006] showed that 24 hrs of exposure to 935-MHz GSM basic signal at 1 or 2 W/Kg did not cause DNA strand breaks in human blood cells.

Tice et al. [2002] measured DNA single strand breaks in human leukocytes using the comet assay after exposure to various forms of cell phone signals. Cells were exposed at 37±1°C, for 3 or 24 h at average specific absorption rates (SARs) of 1.0-10.0 W/kg. Exposure for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes.

Vershaeve et al. [2006] long-term exposure (2 hrs/day, 5 days/week for 2 years) of rats to 900 MHz GSM signal at 0.3 and 0.9 W/kg did not significantly affect levels of DNA strand breaks in cells.

Vijayalaximi et al [2000] reported no significant increase in single strand breaks in human lymphocytes after 2 hrs of exposure to 2450-MHz field at 2 W/kg.

Zeni et al. [2005] reported that a 2-hr exposure to 900-MHz GSM signal at 0.3 and 1 W/kg did not significantly affect levels of DNA strand breaks in human leukocytes.

III. Micronucleus studies (29 Total studies: 16 reported effects (55%) and 13 reported no significant effect (45%))

III A.   Micronucleus studies that reported effects:

Balode [1996] obtained blood samples from female Latvian Brown cows from a farm close to and in front of the Skrunda Radar and from cows in a control area. Micronuclei in peripheral erythrocytes were significantly higher in the exposed cows.

Busljeta et al. [2004] exposed male rats to 2.45 GHz RFR fields for 2 hours daily, 7 days a week, at 5-10 mW/cm^2 for up to 30 days. Erythrocyte count, haemoglobin and haematocrit were increased in peripheral blood on irradiation days 8 and 15. Anuclear cells and erythropoietic precursor cells were significantly decreased in the bone marrow on day 15, but micronucleated cells were increased.

D'Ambrosio et al. [2002] exposed human peripheral blood to 1.748 GHz continuous wave (CW) or phase-modulated wave (GMSK) for 15 min at a maximum specific absorption rate of ∾5 W/kg. No changes were found in cell proliferation kinetics after exposure to either CW or GMSK fields. Micronucleus frequency result was not affected by CW exposure but a statistically significant increase in micronucleus was found following GMSK exposure.

Ferreira et al. [2006] found that rat offspring exposed to radiation from a cellular phone during their embryogenesis showed a significant increase in micronucleus frequency.

Fucic et al. [1992] reported increase in frequencies of micronuclei in the lymphocytes of humans exposed to microwaves.

Gandhi and Singh [2005] analyzed short term peripheral lymphocyte cultures for chromosomal aberrations and the buccal mucosal cells for micronuclei. They reported an increase in the number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes.

Garaj-Vrhovac et al [1992] exposed human whole-blood samples to continuous-wave 7.7 GHz radiation at power density of 0.5, 10 and 30 mW/cm^2 for 10, 30 and 60 min. In all experimental conditions, the frequencies of all types of chromosomal aberrations (dicentric and ring chromosomes) and micronucleus were significantly higher than in the control samples.

Garaj-Vrhovac et al. [1999] investigated peripheral blood lymphocytes of 12 subjects occupationally exposed to microwave radiation. Results showed an increase in frequency of micronuclei as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls.

Haider et al. [1994] exposed plant cuttings bearing young flower buds for 30 h on both sides of a slewable curtain antenna (300/500 kW, 40-170 V/m) and 15 m (90 V/m) and 30 m (70 V/m) distant from a vertical cage antenna (100 kW) as well as at the neighbors living near the broadcasting station (200 m, 1-3 V/m). Laboratory controls were maintained for comparison. Higher micronucleus frequencies than in laboratory controls were found for all exposure sites in the immediate vicinity of the antennae,

Tice et al. [2002] measured micronucleus frequency in human leukocytes using the comet assay after exposure to various forms of cell phone signals. Cells were exposed at 37±1°C, for 3 or 24 h at average specific absorption rates (SARs) of 1.0-10.0 W/kg. Exposure for 3 h did not induce a significant increase in micronucleated lymphocytes. However, exposure to each of the signals for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase in the frequency of micronucleated lymphocytes. The magnitude of the response (approximately four fold) was independent of the technology, the presence or absence of voice modulation, and the frequency.

Trosic et al. [2001] investigated the effect of a 2450-MHz microwave irradiation on alveolar macrophage kinetics and formation of multinucleated giant cells after whole body irradiation of rats at 5-15 mW/cm^2. A group of experimental animals was divided in four subgroups that received 2, 8, 13 and 22 irradiation treatments of two hours each. The animals were killed on experimental days 1, 8, 16, and 30. Multinucleated cells were significantly increased in treated animals. The increase in number of nuclei per cell was time- and dose-dependent. Macrophages with two nucleoli were more common in animals treated twice or eight times. Polynucleation was frequently observed after 13 or 22 treatments.

Trosic et al. [2002] exposed adult male Wistar for 2 h a day, 7 days a week for up to 30 days to continuous 2450-MHz microwaves at a power density of 5-10mW/cm^2. Frequency of micronuclei in polychromatic erythrocytes showed a significant increase in the exposed animals after 2, 8 and 15 days of exposure compared to sham-exposed control.

Trosic et al. [2004] investigated micronucleus frequency in bone marrow red cells of rats exposed to a 2450-MHz continuous–wave microwaves for 2 h daily, 7 days a week, at a power density of 5-10 mW/cm^2 (whole body SAR 1.25 +/- 0.36 (SE) W/kg). The frequency of micronucleated polychromatic erythrocytes was significantly increased on experimental day 15.

Trosic et al. [2006] exposed rats 2 h/day, 7 days/week to 2450-MHz microwaves at a whole-body SAR of 1.25 +/- 0.36W/kg. Control animals were included in the study. Bone marrow micronucleus frequency was increased on experimental day 15, and

polychromatic erythrocytes micronucleus frequency in the peripheral blood was increased on day 8.

Zotti-Martelli et al. [2000] exposed human peripheral blood lymphocytes in G(0) phase to electromagnetic fields at different frequencies (2.45 and 7.7 GHz) and power densities (10, 20 and 30 mW/cm^2) for 15, 30 or 60min. The results showed for both radiation frequencies an induction of micronuclei as compared to control cultures at a power density of 30mW/cm^2 and after an exposure of 30 and 60 min.

Zotti-Martelli et al. [2005] exposed whole blood samples from nine different healthy donors for 60, 120 and 180 min to continuous-wave 1800-MHz microwaves at power densities of 5, 10 and 20 mW/cm^2. A statistically significant increase of micronucleus in lymphocytes was observed dependent on exposure time and power density. A considerable decrease in spontaneous and induced MN frequencies was measured in a second experiment.

III B. Micronucleus studies that reported no significant effects:

Bisht et al. [2002] exposed C3H 10T½ cells to 847.74 MHz CDMA (3.2 or 4.8 W/kg) or 835.62 MHz FDMA (3.2 or 5.1 W/kg) RFR for 3, 8, 16 or 24 h. No exposure condition was found to result in a significant increase relative to sham-exposed cells either in the percentage of binucleated cells with micronuclei or in the number of micronuclei per 100 binucleated cells.

Juutilainen et al. [2007] found no significant change in micronucleus frequency in erythrocytes of mice after long-term exposure to various mobile phone frequencies.

Koyama et al. [2004] exposed Chinese hamster ovary (CHO)-K1 cells to 2450-MHz microwaves for 2 h at average specific absorption rates (SARs) of 5, 10, 20, 50, 100, and 200 W/kg. Micronucleus frequency in cells exposed at SARs of 100 and 200 W/kg were significantly higher when compared with sham-exposed controls. They speculated that the effect observed was a thermal effect.

Port et al. [2003] reported that exposure of HL-60 cells to EMFs 25 times higher than the ICNIRP reference levels for occupational exposure did not induce any significant changes in apoptosis, micronucleation, abnormal morphologies and gene expression.

Scarfi et al [2006] exposed human peripheral blood lymphocytes to 900 MHz GSM signal at specific absorption rates of 0, 1, 5 and 10 W/kg peak values. No significant change in micronucleus frequency was observed.

Vijayalaximi et al. [1997a] exposed human blood to continuous-wave 2450- MHz microwaves, either continuously for a period of 90 min or intermittently for a total exposure period of 90 min (30 min on and 30 min off, repeated three times). The mean power density at the position of the cells was 5.0 mW/cm^2 and mean specific absorption rate was 12.46 W/kg. There were no significant differences between RFR-exposed and sham-exposed lymphocytes with respect to; (a) mitotic indices; (b) incidence of cells showing chromosome damage; (c) exchange aberrations; (d) acentric fragments; (e) binucleate lymphocytes, and (f) micronuclei.

Vijayalaximi et al. [1997b] exposed C3H/HeJ mice for 20 h/day, 7 days/week, over 18 months to continuous-wave 2450 MHz microwaves at a whole-body average specific absorption rate of 1.0 W/kg. At the end of the 18 months, peripheral blood and bone marrow smears were examined for the extent of genotoxicity as indicated by the

presence of micronuclei in polychromatic erythrocytes. The results indicate that the incidence of micronuclei/1,000 polychromatic erythrocytes was not significantly different between groups exposed to RF radiation and sham-exposed groups.

Vijayalaximi et al. [1999] exposed CF-1 male mice to ultra-wideband electromagnetic radiation (UWBR) for 15 min at an estimated whole-body average specific absorption rate of 37 mW/kg. Peripheral blood and bone marrow smears were examined to determine the extent of genotoxicity, as assessed by the presence of micronuclei (MN) in polychromatic erythrocytes (PCE). There was no evidence for excess genotoxicity in peripheral blood or bone marrow cells of mice exposed to UWBR.

Vijayalaximi et al. [2001a] reported that there was no evidence for the induction of micronuclei in peripheral blood and bone marrow cells of rats exposed for 24h to 2450-MHz continuous-wave microwaves at a whole body average SAR of 12 W/kg.

Vijayalaximi et al. [2001b] reported that there is no evidence for the induction of chromosomal aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 835.62 MHz RF radiation at SARs of 4.4 or 5.0 W/kg.

Vijayalaximi et al. [2001c] reported no evidence for induction of chromosome aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 847.74 MHz RF radiation (CDMA) at SARs of 4.9 or 5.5 W/kg.

Vijayalaximi et al. [2003] exposed timed-pregnant Fischer 344 rats (from nineteenth day of gestation) and their nursing offspring (until weaning) to a far-field 1.6 GHz Iridium wireless communication signal for 2 h/day, 7 days/week at power density of 0.43 mW/cm^2 and whole-body average specific absorption rate of 0.036 to 0.077 W/kg (0.10 to 0.22 W/kg in the brain). This was followed by chronic, head-only exposures of male and female offspring to a near-field 1.6 GHz signal for 2 h/day, 5 days/week, over 2 years. Near-field exposures were conducted at an SAR of 0.16 or 1.6 W/kg in the brain. At the end of 2 years, all rats were necropsied. Bone marrow smears were examined for the extent of genotoxicity, assessed from the presence of micronuclei in polychromatic erythrocytes. There was no evidence for excess genotoxicity in rats that were chronically exposed to 1.6 GHz microwaves compared to sham-exposed and cage controls.

Zeni et al. [2003] investigated the induction of micronucleus in human peripheral blood lymphocytes after exposure to electromagnetic fields at various duration of exposure, specific absorption rate (SAR), and signal [continuous-wave (CW) or GSM (Global System of Mobile Communication)-modulated signal]. No statistically significant difference was detected in any case.

IV. Chromosome and genome effects (21 studies total: 13 reported effects (62%) and 8 reported no significant effect (38%))

IV A. Chromosome and genome studies that reported effects:

Belyaev et al. [I992] studied the effect of low intensity microwaves on the conformational state of the genome of X-irradiated E. coli cells by the method of viscosity anomalous time dependencies. A power density of 1 microW/cm^2 is sufficient to suppress radiation-induced repair of the genome conformational state.

Belyaev et al. [1996] studied the effect of millimeter waves on the genome conformational state of E. coli AB1157 by the method of anomalous viscosity time dependencies in the frequency range of 51.64-51.85 GHz. Results indicate an electron-conformational interactions.

Belyaev et al. [2005] investigated response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to microwaves from GSM mobile phone (915 MHz, specific absorption rate 37 mW/kg), and power frequency magnetic field (50 Hz, 15 microT peak value). Changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected.

Belyaev et al. [2006] investigated whether exposure of rat brain to microwaves of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression at a specific absorption rate (SAR) of 0.4 mW/g for 2 h. Data showed that GSM MWs at 915 MHz did not induce DNA double stranded breaks detectable by pulsed-field gel electrophoresis or changes in chromatin conformation, but affected expression of genes in rat brain cells.

Gadhia et al. [2003] reported a significant increase in dicentric chromosomes in blood cells among mobile users who were smoker–alcoholic as compared to nonsmoker–nonalcoholic; the same held true for controls of both types.

Garaj-Vrhovac et al. [1990] exposed V79 Chinese hamster cells to continuous-wave 7.7 GHz RFR at power density of 30 mW/cm^2 for 15, 30, and 60 min. Results suggest that the radiation causes changes in the synthesis as well as in the structure of DNA molecules.

Garaj-Vrhovac et al. [1991] exposed V79 Chinese hamster fibroblast cells to continuous wave 7.7 GHz radiation at power density of 0.5 mW/cm^2 for 15, 30 and 60 min. There was a significantly higher frequency of specific chromosome aberrations such as dicentric and ring chromosomes in irradiated cells.

Mashevich et al. [2003] found that human peripheral blood lymphocytes exposed to continuous 830-MHz electromagnetic fields (1.6-8.8 W/kg for 72 hr) showed a SAR-dependent chromosome aneuploidy, a major "somatic mutation" leading to genomic instability and thereby to cancer. The aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in the SAR dependent genetic toxicity. The effects were non-thermal.

Ono et al. (2004) exposed pregnant mice intermittently at a whole-body averaged specific absorption rate of 0.71 W/kg (10 seconds on, 50 seconds off which is 4.3 W/kg during the 10 seconds exposure) for 16 hours a day, from the embryonic age of 0 to 15 days. At 10 weeks of age, mutation frequencies at the lacZ gene in spleen, liver, brain, and testis were examined. Quality of mutation assessed by sequencing the nucleotides of mutant DNAs revealed no appreciable difference between exposed and non-exposed samples.

Sarimov et al. [2004] reported that exposure to microwaves of 895-915 MHz at 5.4 mW/kg resulted in statistically significant changes in condensation of chromatin in

human lymphocytes. Effects are similar to stress response, differ at various frequencies, and vary among donors.

Sarkar et al. [1994] exposed mice to 2450-MHz microwaves at a power density of 1 mW/cm^2 for 2 h/day over a period of 120, 150 and 200 days. Rearrangement of DNA segments were observed in testis and brain of exposed animals.

Semin et al. [1995] exposed DNA samples at 18°C at 10 different microwave frequencies (4- to 8 GHz, 25 ms pulses, 0.4 to 0.7 mW/cm^2 peak power, 1- to 6-Hz repetition rate, no heating). Irradiation at 3 or 4 Hz and 0.6 mW/cm^2 peak power clearly increased the accumulated damage to the DNA secondary structure (P< .00001). However, changing the pulse repetition rate to 1, 5, 6 Hz, as well as changing the peak power to 0.4 or 0.7 mW/cm^2 did not induce significant effect. Thus, the effect occurred only within narrow 'windows' of the peak intensities and modulation frequencies.

Sykes et al. [2001] exposed mice daily for 30 min to plane-wave fields of 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms for 1, 5 or 25 days. Three days after the last exposure, spleen sections were screened for DNA inversion events. There was no significant difference between the control and treated groups in the 1- and 5-day exposure groups, but there was a significant reduction in inversions below the spontaneous frequency in the 25-day exposure group. This observation suggests that exposure to RF radiation can lead to a perturbation in recombination frequency which may have implications for recombination repair of DNA.

IV. B. Chromosome and genome studies that reported no significant effects:

Antonopoulos et al. [1997] found no significant change in cell cycle progression and the frequencies of sister-chromatid exchanges in human lymphocytes exposed to electromagnetic fields of 380, 900 and 1800 MHz.

Ciaravino et al. [1991] reported that RFR did not affect changes in cell progression caused by adriamycin, and the RFR did not change the number of sister chromatid exchanges that were induced by the adriamycin.

Garson et al. [1991] analyzed lymphocytes from Telecom Australia radio-linemen who had all worked with RFR in the range 400 kHz-20 GHz with exposures at or below the Australian occupational limits. There was no significant increase in chromosomal damage in circulating lymphocytes.

Gos et al. [2000] exposed actively growing and resting cells of the yeast Saccharomyces cerevisiae to 900-MHz Global System for Mobile Communication (GSM) pulsed modulation format signals at specific absorption rates (SAR) of 0.13 and 1.3 W/kg. They reported no significant effect of the fields on forward mutation rates on the frequency of petite formation, on rates of intrachromosomal deletion formation, or on rates of intragenic recombination in the absence or presence of the genotoxic agent methyl methansulfonate.

Kerbacher et al (1990) reported that exposure to pulsed 2450-MHz microwaves for 2 h at an SAR of 33.8 W/kg did not significantly cause chromosome aberrations in CHO cells. The radiation also did not interact with Mitomycin C and Adriamycin.

Komatsubara et al. [2005] reported that exposure to 2.45-GHz microwaves for 2 h with up to 100 W/kg SAR CW and an average 100 W/kg PW (a maximum SAR of 900 W/kg) did not induce chromosomal aberrations in mouse m5S cells.

Meltz et al. [1990] reported no significant mutagenic effect of exposure to 2.45-GHz RFR (40 W/kg) alone and interaction with proflavin, a DNA-intercalating drug, in L5178Y mouse leukemic cells.

Roti-Roti et al. [2001] reported no significant effect of exposure to radiofrequency radiation in the cellular phone communication range (835.62 MHz frequency division multiple access, FDMA; 847.74 MHz code division multiple access, CDMA) on neoplastic transformation frequency using the in vitro C3H 10T(1/2) cell transformation assay system.

Takahashi et al. [2002] exposed mice to 1.5 GHz EMF in the head region at 2.0, 0.67, and 0 W/kg specific absorption rate for 90 min/day, 5 days/week, for 4 weeks. No mutagenic effect in mouse brain cells was detected.

V. Conclusions

From this literature survey, since only 50% of the studies reported effects, it is apparent that there is no consistent pattern that radiofrequency radiation exposure could induce genetic damages/changes in cells and organisms. However, one can conclude that under certain conditions of exposure, radiofrequency radiation is genotoxic. Data available are mainly applicable only to cell phone radiation exposure. Other than the study by Phillips et al [1998], there is no indication that RFR at levels that one can experience in the vicinity of base stations and RF-transmission towers could cause DNA damage.

During cell phone use, a relatively constant mass of tissue in the brain is exposed to the radiation at relatively high intensity (peak SAR of 4 - 8 W/kg). Several studies reported DNA damage at lower than 4 W/kg. This questions the wisdom of the IEEE Committee in using 4 W/kg as the threshold of effect for exposure-standard setting. Furthermore, since critical genetic mutations in one single cell are sufficient to lead to cancer and there are millions of cells in a gram of tissue, it is inconceivable that the base of SAR standard was changed from averaged over 1 gm of tissue to 10 gm. (The limit of localized tissue exposure has been changed from 1.6 W/kg averaged over 1 gm of tissue to 2 W/kg over 10 gm of tissue. Since distribution of radiofrequency energy is non-homogenous inside tissue, this change allows a higher peak level of exposure.) What actually needed is a better refinement of SAR calculation to identify 'peak values' of SAR inside the brain,

Aside from influences that are not directly related to experimentation [Huss et al., 2007], many factors could influence the outcome of an experiment in bioelectromagnetics research.

Any effect of EMF has to depend on the energy absorbed by a biological entity and on how the energy is delivered in space and time. Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different effects. In addition, in order to understand the biological consequence of EMF exposure, one must know whether the effect is cumulative, whether compensatory responses result, and when homeostasis will break down. The contributions of these physical factors are discussed in a talk presented in

Vienna, Austria in 1998. The paper is posted in many websites (e.g., http://www.wave-guide.org/library/lai.html).

Thus, differences in outcomes of the research on genotoxic effects of RFR could be explained by the many different exposure conditions used in the studies. An example is the study of Phillips et al. [1998] showing that different cell phone signals could cause different effects on DNA (i.e., an increase in strand breaks with exposure to one type of signal and a decrease with another). This is further complicated by the fact that some of the studies listed above used very poor exposure procedures with very limited documentation of exposure parameters, e.g., using a cell phone to expose cells and even animals. Data from these experiments are questionable.

Another source of influence on an experimental outcome is the cell or organism studied. Many different biological systems were used in the genotoxicity studies. Different cell types [Hoyto et al., 2007] and organisms [Anderson et al., 2000; DiCarlo and Litovitz, 1999] may respond differently to EMF.

A few words have to be said on the 'comet assay', since it was used in most of the EMF studies to determine DNA damage. Different versions of the assay have been developed. These versions have different detection sensitivities and can be used to measure different aspects of DNA strand breaks. A comparison of data from experiments using different versions of the assay may be misleading. Another concern is that most of the 'comet assay' studies were carried out by experimenters who had no prior experience on the assay. My experience with the 'comet assay' is that it is a very sensitive assay and requires great care in performing. Thus, different detection sensitivities could result from different experimenters, even following the same procedures. One way to solve this experimental variation problem is for each researcher or laboratory to report their sensitivity of the 'comet assay', e.g., threshold of detecting strand breaks in human lymphocytes exposed to x-rays. This information is generally not available from the EMF-genotoxicity studies. However, in one incidence, an incredibly high sensitivity was even reported [Malyapa et al., 1998], suggesting the inexperience of the researchers on the assay.

A drawback in the interpretation and understanding of experimental data from bioelectromagnetic research is that there is no general acceptable mechanism on how EMF affects biological systems. The mechanism by which RFR causes genetic effect is unknown. Since the energy level is not sufficient to cause direct breakage of chemical bonds within molecules, the effects are probably indirect and secondary to other induced-chemical changes in the cell.

One possibility is via free radical formation inside cells. Free radicals kill cells by damaging macromolecules, such as DNA, protein and membrane. Several reports have indicated that electromagnetic fields (EMF) enhance free radical activity in cells [e.g., Lai and Singh, 1997a, b; 2004; Oral et al., 2006; Simko, 2007], particularly via the Fenton reaction [Lai and Singh, 2004]. The Fenton reaction is a catalytic process of iron

to convert hydrogen peroxides, a product of oxidative respiration in the mitochondria, into hydroxyl free radical, which is a very potent and toxic free radical.

EMF

iron

H_2O_2

$OH^{\bullet}$

mitochondria

Cellular damage
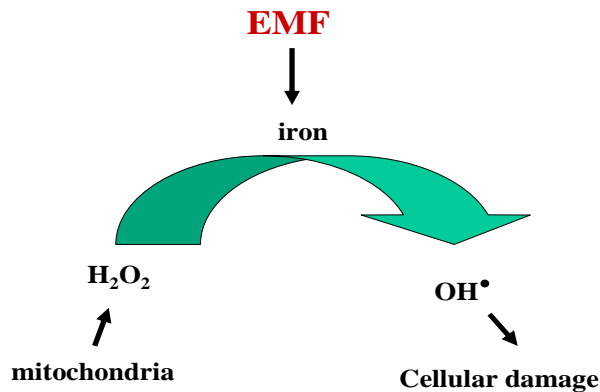
THE FENTON REACTION

What is interesting that extremely-low frequency EMF has also been shown to cause DNA damage (see the list of papers on ELF EMF and DNA at the end of this chapter). Free radicals have also been implicated in this effect of ELF EMF. This further supports the view that EMF affects DNA via an indirect secondary process, since the energy content of ELF EMF is much lower than that of RFR.

Effects via the Fenton reaction predict how a cell would respond to EMF:

1. Cells that are metabolic active would be more susceptible to the effect because more hydrogen peroxide is generated by the mitochondria to fuel the reaction.

2. Cells that have high level of intracellular free iron would be more vulnerable. Cancer cells and cells undergoing abnormal proliferation have high concentration of free iron because they uptake more iron and have less efficient iron storage regulation. Thus, these cells could be selectively damaged by EMF, and EMF could potentially be used for the treatment of cancer and hyperplasia diseases. The effect could be further enhanced if one could shift anaerobic glycolysis of cancer cells to oxidative glycolysis. There is quite a large database of information on the effects of EMF (mostly in the ELF range) on cancer cells and tumors. The data tend to indicate that EMF could retard tumor growth and kill cancer cells.

3. Since the brain is exposed to rather high levels of EMF during cell phone use, the consequences of EMF-induced genetic damage in brain cells are of particular importance. Brain cells have high level of iron. Special molecular pumps are present on nerve cell nucleus membrane to pump iron into the nucleus. Iron atoms have been found to intercalate within DNA molecules. In addition, nerve cells have a low capability for DNA repair and DNA breaks could accumulate. Another concern is the presence of superparamagnetic iron-particles (magnetites) in body tissues,

particularly in the brain. These particles could enhance free radical activity in cells and cellular-damaging effects of EMF. These factors make nerve cells more vulnerable to EMF. Thus, the effect of EMF on DNA could conceivably be more significant on nerve cells than on other cell types of the body. Since nerve cells do not divide and are not likely to become cancerous, more likely consequences of DNA damage in nerve cells are changes in functions and cell death, which could either lead to or accelerate the development of neurodegenerative diseases. Double strand breaks, if not properly repaired, are known to lead to cell death. Cumulative DNA damage in nerve cells of the brain has been associated with neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases. However, another type of brain cells, the glial cells, can become cancerous, resulting from DNA damage. The question is whether the damaged cells would develop into tumors before they are killed by EMF due to over accumulation of genetic damages. The outcome depends on the interplay of these different physical and biological factors: an increase, decrease, or no significant change in cancer risk could result.

4.   On the other hand, cells with high antioxidant potentials would be less susceptible to EMF. These include the amount of antioxidants and anti-oxidative enzymes in the cells. Furthermore, the effect of free radicals could depend on the nutritional status of an individual, e.g., availability of dietary antioxidants, consumption of alcohol, and amount of food consumption. Various life conditions, such as psychological stress and strenuous physical exercise, have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can also speculate that some individuals may be more susceptible to the effects of EMF exposure.

More research has to be carried out to prove the involvement of the free radicals in the biological effects of EMF. However, the Fenton reaction obviously can only explain some the genetic effects observed. For example, RF- and ELF EMF-induced DNA damages have been reported in normal lymphocytes, which contain a very low concentration of intracellular free iron.

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APPENDIX 6-A
Abstracts on Effects of Extremely Low Frequency (ELF) EMF on DNA

27 (E)- effect reported; 14 (NE)- no significant effect reported

Ahuja YR, **Vijayashree B**, **Saran R**, **Jayashri EL**, **Manoranjani JK**, **Bhargava SC**. **In vitro effects of low-level, low-frequency electromagnetic fields on DNA damage in human leucocytes by comet assay.** Indian J Biochem Biophys. 36(5):318-322, 1999. (E)

The sources for the effects of electromagnetic fields (EMFs) have been traced to time-varying as well as steady electric and magnetic fields, both at low and high to ultra high frequencies. Of these, the effects of low-frequency (50/60 HZ) magnetic fields, directly related to time-varying currents, are of particular interest as exposure to some fields may be commonly experienced. In the present study, investigations have been carried out at low-level (mT) and low-frequency (50 Hz) electromagnetic fields in healthy human volunteers. Their peripheral blood samples were exposed to 5 doses of electromagnetic fields (2,3,5,7 and 10mT at 50 Hz) and analysed by comet assay. The results were compared to those obtained from unexposed samples from the same subjects. 50 cells per treatment per individual were scored for comet-tail length which is an estimate of DNA damage. Data from observations among males were pooled for each flux density for analysis. At each flux density, with one exception, there was a significant increase in the DNA damage from the control value. When compared with a similar study on females carried out by us earlier, the DNA damage level was significantly higher in the females as compared to the males for each flux density.

Cantoni O, **Sestili P**, **Fiorani M**, **Dacha M**. **Effect of 50 Hz sinusoidal electric and/or magnetic fields on the rate of repair of DNA single strand breaks in cultured mammalian cells exposed to three different carcinogens: methylmethane sulphonate, chromate and 254 nm U.V. radiation.** Biochem Mol Biol Int. 38(3):527-533, 1996. (NE)

Treatment of cultured mammalian cells with three different carcinogens, namely methylmethane sulphonate (MMS), chromate and 254 U.V. radiation, produces DNA single strand breaks (SSB) in cultured mammalian cells. The rate of removal of these lesions is not affected by exposure to 50 Hz electric (0.2 - 20 kV/m), magnetic (0.0002-0.2 mT), or combined electric and magnetic fields. These results indicate that, under the experimental conditions utilized in this study, 50 Hz electric, magnetic and electromagnetic fields (over a wide range of intensities) do not affect the machinery involved in the repair of DNA SSBs generated by different carcinogens in three different cultured mammalian cell lines, making it unlikely that field exposure enhances the ability of these carcinogens to induce transformation via inhibition of DNA repair.

Chahal R, **Craig DQ**, **Pinney RJ**. **Investigation of potential genotoxic effects of low frequency electromagnetic fields on Escherichia coli.** J Pharm Pharmacol. 45(1):30-33, 1993. (NE)

Exposure of growing cells of Escherichia coli strain AB1157 to a frequency of 1 Hz with field strengths of 1 or 3 kV m-1 did not affect spontaneous or ultraviolet light (UV)-induced mutation frequencies to rifampicin resistance. Neither did growth in the presence of charge alter the sensitivities of strains AB1157, TK702 umuC or TK501 umuC uvrB to UV. Similarly, although the resistance of strains TK702 umuC and TK501 umuC uvrB to UV was increased by the presence of plasmid pKM101, which carries DNA repair genes, pregrowth of plasmid-containing strains in electric fields did not increase UV resistance. Finally, growth in a low frequency field in the presence of sub-inhibitory concentrations of mitomycin C did not affect mitomycin C-induced mutation frequencies. It is concluded that low frequency electromagnetic fields do not increase spontaneous mutation, induce DNA repair or increase the mutagenic effects of UV or mitomycin C.

Chow K, **Tung WL** **Magnetic field exposure enhances DNA repair through the induction of DnaK/J synthesis.** FEBS Lett. 478(1-2):133-136, 2000. (E)

In contrast to the common impression that exposure to a magnetic field of low frequency causes mutations to organisms, we have demonstrated that a magnetic field can actually enhance the efficiency of DNA repair. Using Escherichia coli strain XL-1 Blue as the host and plasmid pUC8 that had been mutagenized by hydroxylamine as the vector for assessment, we found that bacterial transformants that had been exposed to a magnetic field of 50 Hz gave lower percentages of white colonies as compared to transformants that had not been exposed to the magnetic field. This result was indicative that the efficiency of DNA repair had been improved. The improvement was found to be mediated by the induced overproduction of heat shock proteins DnaK/J (Hsp70/40).

Delimaris J, **Tsilimigaki S**, **Messini-Nicolaki N**, **Ziros E**, **Piperakis SM** Effects of pulsed electric fields on DNA of human lymphocytes. Cell Biol Toxicol. 22(6):409-415, 2006. (E)

The effects of pulsed electric fields of low frequency (50 Hz) on DNA of human lymphocytes were investigated. The influence of additional external factors, such as hydrogen peroxide (H_2O_2) and gamma-irradiation, as well as the repair efficiency in these lymphocytes, was also evaluated. The comet assay, a very sensitive and rapid method for detecting DNA damage at the single cells level was the method used. A significant amount of damage was observed after exposure to the electric fields, compared to the controls. After 2 h incubation at 37 degrees C, a proportion of damage was repaired. H_2O_2 and gamma-irradiation increased the damage to lymphocytes exposed to pulsed electric fields according to the dose used, while the amount of the repair was proportional to the damage.

Fairbairn DW, **O'Neill KL** **The effect of electromagnetic field exposure on the formation of DNA single strand breaks in human cells.** Cell Mol Biol (Noisy-le-grand). 40(4):561-567, 1994. (NE)

Electromagnetic fields (EMF) have been reported to be associated with human cancers in a number of epidemiological studies. Agents that are associated with cancer affect DNA in an adverse manner. This is a report of a DNA damage study in human cells exposed to EMFs. Single strand breaks in DNA are proposed to be necessary events in both mutagenesis and carcinogenesis. The single cell gel assay is a sensitive and accurate technique that was used in this study for single strand break detection. The EMF exposure system used here appeared to have no direct effect on DNA damage induction in a series of experiments. Moreover, EMF did not have a significant effect in potentiating DNA damage in cells treated with oxidative stresses.

Fiorani M, **Cantoni O**, **Sestili P**, **Conti R**, **Nicolini P**, **Vetrano F**, **Dacha M**. **Electric and/or magnetic field effects on DNA structure and function in cultured human cells.** Mutat Res. 282(1):25-29, 1992. (NE)

Exposure of cultured K562 cells to 50 Hz electric (0.2-20 kV/m), magnetic (0.002-2 G), or combined electric and magnetic fields for up to 24 h did not result in the production of detectable DNA lesions, as assayed by the filter elution technique. The rate of cell growth was also unaffected as well as the intracellular ATP and NAD+ levels. These results indicate that, under the experimental conditions utilized in this study, 50 Hz electric, magnetic and electromagnetic fields are not geno- and cyto-toxic in cultured mammalian cells.

Frazier ME, **Reese JA**, **Morris JE**, **Jostes RF**, **Miller DL** **Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis of DNA repair of induced, single-strand breaks.** Bioelectromagnetics. 11(3):229-234, 1990. (NE)

DNA damage was induced in isolated human peripheral lymphocytes by exposure at 5 Gy to 60Co radiation. Cells were permitted to repair the DNA damage while exposed to 60-Hz fields or while sham-exposed. Exposed cells were subjected to magnetic (B) or electric (E) fields, alone or in combination, throughout their allotted repair time. Repair was stopped at specific times, and the cells were immediately lysed and then analyzed for the presence of DNA single-strand breaks (SSB) by the alkaline-elution technique. Fifty to 75 percent of the induced SSB were repaired 20 min after exposure, and most of the remaining damage was repaired after 180 min. Cells were exposed to a 60-Hz ac B field of 1 mT; an E field of 1 or 20 V/m; or combined E and B fields of 0.2 V/m and 0.05 mT, 6 V/m and 0.6 mT, or 20 V/m and 1 mT. None of the exposures was observed to affect significantly the repair of DNA SSB.

Hong R, **Zhang Y**, **Liu Y**, **Weng EQ**. [Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 23(6):414-417, 2005. (E)

[Article in Chinese]

OBJECTIVE: To study the effects of 50 Hz electromagnetic fields (EMFs) on DNA of testicular cells and sperm chromatin structure in mice. METHODS: Mice were exposed to 50 Hz, 0.2 mT or 6.4 mT electromagnetic fields for 4 weeks. DNA strand breakage in testicular cells was detected by single-cell gel electrophoresis assay. Sperm chromatin structure was analyzed by sperm chromatin structure assay with flow cytometry. RESULTS: After 50 Hz, 0.2 mT or 6.4 mT EMFs exposure, the percentage of cells with DNA migration in total testicular cells increased from the control level of 25.64% to 37.83% and 39.38% respectively. The relative length of comet tail and the percentage of DNA in comet tail respectively increased from the control levels of 13.06% +/- 12.38% and 1.52% +/- 3.25% to 17.86% +/- 14.60% and 2.32% +/- 4.26% after 0.2 mT exposure and to 17.88% +/- 13.71% and 2.35% +/- 3.87% after 6.4 mT exposure (P < 0.05). Exposure to EMFs had not induced significant changes in S.D.alphaT and XalphaT, but COMPalphaT (cells outside the main population of alpha t), the percentage of sperms with abnormal chromatin structure, increased in the two exposed groups. CONCLUSION: 50 Hz EMFs may have the potential to induce DNA strand breakage in testicular cells and sperm chromatin condensation in mice.

Ivancsits S, **Pilger A**, **Diem E**, **Jahn O**, **Rudiger HW**.**Cell type-specific genotoxic effects of intermittent extremely low-frequency electromagnetic fields.** Mutat Res. 583(2):184-188, 2005. (E)

The issue of adverse health effects of extremely low-frequency electromagnetic fields (ELF-EMFs) is highly controversial. Contradictory results regarding the genotoxic potential of ELF-EMF have been reported in the literature. To test whether this controversy might reflect differences between the cellular targets examined we exposed cultured cells derived from different tissues to an intermittent ELF-EMF (50 Hz sinusoidal, 1 mT) for 1-24h. The alkaline and neutral comet assays were used to assess ELF-EMF-induced DNA strand breaks. We could identify three responder (human fibroblasts, human melanocytes, rat granulosa cells) and three non-responder cell types (human lymphocytes, human monocytes, human skeletal muscle cells), which points to the significance of the cell system used when investigating genotoxic effects of ELF-EMF.

Ivancsits S, **Diem E**, **Jahn O**, **Rudiger HW**. **Age-related effects on induction of DNA strand breaks by intermittent exposure to electromagnetic fields.** Mech Ageing Dev. 124(7):847-850, 2003. (E)

Several studies indicating a decline of DNA repair efficiency with age raise the question, if senescence per se leads to a higher susceptibility to DNA damage upon environmental exposures. Cultured fibroblasts of six healthy donors of different age exposed to intermittent ELF-EMF (50 Hz sinus, 1 mT) for 1-24 h exhibited different basal DNA strand break levels correlating with age. The cells revealed a maximum response at 15-19 h of exposure. This response was clearly more pronounced in cells from older donors, which could point to an age-related decrease of DNA repair efficiency of ELF-EMF induced DNA strand breaks.

Ivancsits S, **Diem E**, **Pilger A**, **Rudiger HW**, **Jahn O**. **Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts.** Mutat Res. 519(1-2):1-13, 2002. (E)

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

Jajte J, **Zmyslony M**, **Palus J**, **Dziubaltowska E**, **Rajkowska E**. **Protective effect of**

melatonin against in vitro iron ions and 7 mT 50 Hz magnetic field-induced DNA damage in rat lymphocytes. Mutat Res. 483(1-2):57-64, 2001. (E)

We have previously shown that simultaneous exposure of rat lymphocytes to iron ions and 50Hz magnetic field (MF) caused an increase in the number of cells with DNA strand breaks. Although the mechanism of MF-induced DNA damage is not known, we suppose that it involves free radicals. In the present study, to confirm our hypothesis, we have examined the effect of melatonin, an established free radicals scavenger, on DNA damage in rat peripheral blood lymphocytes exposed in vitro to iron ions and 50Hz MF. The alkaline comet assay was chosen for the assessment of DNA damage. During pre-incubation, part of the cell samples were supplemented with melatonin (0.5 or 1.0mM). The experiments were performed on the cell samples incubated for 3h in Helmholtz coils at 7mT 50Hz MF. During MF exposure, some samples were treated with ferrous chloride ($FeCl_2$, 10microg/ml), while the rest served as controls. A significant increase in the number of cells with DNA damage was found only after simultaneous exposure of lymphocytes to $FeCl_2$ and 7mT 50Hz MF, compared to the control samples or those incubated with $FeCl_2$ alone. However, when the cells were treated with melatonin and then exposed to iron ions and 50Hz MF, the number of damaged cells was significantly reduced, and the effect depended on the concentration of melatonin. The reduction reached about 50% at 0.5mM and about 100% at 1.0mM. Our results indicate that melatonin provides protection against DNA damage in rat lymphocytes exposed in vitro to iron ions and 50Hz MF (7mT). Therefore, it can be suggested that free radicals may be involved in 50Hz magnetic field and iron ions-induced DNA damage in rat blood lymphocytes. The future experimental studies, in vitro and in vivo, should provide an answer to the question concerning the role of melatonin in the free radical processes in the power frequency magnetic field.

Kindzelskii AL, **Petty HR**. **Extremely low frequency pulsed DC electric fields promote neutrophil extension, metabolic resonance and DNA damage when phase-matched with metabolic oscillators.** Biochim Biophys Acta. 1495(1):90-111, 2000. (E)

Application of extremely low frequency pulsed DC electric fields that are frequency- and phase-matched with endogenous metabolic oscillations leads to greatly exaggerated neutrophil extension and metabolic resonance wherein oscillatory NAD(P)H amplitudes are increased. In the presence of a resonant field, migrating cell length grows from 10 to approximately 40 microm, as does the overall length of microfilament assemblies. In contrast, cells stop locomotion and become spherical when exposed to phase-mismatched fields. Although cellular effects were not found to be dependent on electrode type and buffer, they were sensitive to temporal constraints (phase and pulse length) and cell surface charge. We suggest an electromechanical coupling hypothesis wherein applied electric fields and cytoskeletal polymerization forces act together to overcome the surface/cortical tension of neutrophils, thus promoting net cytoskeletal assembly and heightened metabolic amplitudes. Metabolic resonance enhances reactive oxygen metabolic production by neutrophils. Furthermore, cellular DNA damage was observed after prolonged metabolic resonance using both single cell gel electrophoresis ('comet' assay) and 3'-OH DNA labeling using terminal deoxynucleotidyl transferase. These

results provide insights into transmembrane signal processing and cell interactions with weak electric fields.

Lai H, **Singh NP**. **Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells.** Bioelectromagnetics. 18(2):156-165, 1997. (E)

Acute (2 h) exposure of rats to a 60 Hz magnetic field (flux densities 0.1, 0.25, and 0.5 mT) caused a dose-dependent increase in DNA strand breaks in brain cells of the animals (assayed by a microgel electrophoresis method at 4 h postexposure). An increase in single-strand DNA breaks was observed after exposure to magnetic fields of 0.1, 0.25, and 0.5 mT, whereas an increase in double-strand DNA breaks was observed at 0.25 and 0.5 mT. Because DNA strand breaks may affect cellular functions, lead to carcinogenesis and cell death, and be related to onset of neurodegenerative diseases, our data may have important implications for the possible health effects of exposure to 60 Hz magnetic fields.

Lai H, **Singh NP**. **Magnetic-field-induced DNA strand breaks in brain cells of the rat.** Environ Health Perspect. 112(6):687-694, 2004. (E)

In previous research, we found that rats acutely (2 hr) exposed to a 60-Hz sinusoidal magnetic field at intensities of 0.1-0.5 millitesla (mT) showed increases in DNA single- and double-strand breaks in their brain cells. Further research showed that these effects could be blocked by pretreating the rats with the free radical scavengers melatonin and N-tert-butyl-alpha-phenylnitrone, suggesting the involvement of free radicals. In the present study, effects of magnetic field exposure on brain cell DNA in the rat were further investigated. Exposure to a 60-Hz magnetic field at 0.01 mT for 24 hr caused a significant increase in DNA single- and double-strand breaks. Prolonging the exposure to 48 hr caused a larger increase. This indicates that the effect is cumulative. In addition, treatment with Trolox (a vitamin E analog) or 7-nitroindazole (a nitric oxide synthase inhibitor) blocked magnetic-field-induced DNA strand breaks. These data further support a role of free radicals on the effects of magnetic fields. Treatment with the iron chelator deferiprone also blocked the effects of magnetic fields on brain cell DNA, suggesting the involvement of iron. Acute magnetic field exposure increased apoptosis and necrosis of brain cells in the rat. We hypothesize that exposure to a 60-Hz magnetic field initiates an iron-mediated process (e.g., the Fenton reaction) that increases free radical formation in brain cells, leading to DNA strand breaks and cell death. This hypothesis could have an important implication for the possible health effects associated with exposure to extremely low-frequency magnetic fields in the public and occupational environments.

Lai H, **Singh NP**. **Melatonin and N-tert-butyl-alpha-phenylnitrone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells.** J Pineal Res. 22(3):152-162, 1997. (E)

In previous research, we have found an increase in DNA single- and double-strand breaks in brain cells of rats after acute exposure (two hours) to a sinusoidal 60-Hz magnetic field. The present experiment was carried out to investigate whether treatment with melatonin and the spin-trap compound N-tert-butyl-alpha-phenylnitrone (PBN) could

block the effect of magnetic fields on brain cell DNA. Rats were injected with melatonin (1 mg/kg, sc) or PBN (100 mg/kg, ip) immediately before and after two hours of exposure to a 60-Hz magnetic field at an intensity of 0.5 mT. We found that both drug treatments blocked the magnetic field-induced DNA single- and double-strand breaks in brain cells, as assayed by a microgel electrophoresis method. Since melatonin and PBN are efficient free radical scavengers, these data suggest that free radicals may play a role in magnetic field-induced DNA damage.

Li SH, **Chow KC**. **Magnetic field exposure induces DNA degradation.** Biochem Biophys Res Commun. 280(5):1385-1388, 2001. (E)

In our earlier experiments, we discovered that magnetic field exposure could bring both stabilizing and destabilizing effects to the DNA of Escherichia coli, depending on our parameters of assessment, and both of these effects were associated with the induced synthesis of the heat shock proteins Hsp70/Hsp40 (DnaK/DnaJ). These contradicting results prompted us to explore in this study the effect of magnetic field exposure on the DNA stability in vivo when the heat shock response of the cell was suppressed. By using plasmid pUC18 in E. coli as the indicator, we found that without the protection of the heat shock response, magnetic field exposure indeed induced DNA degradation and this deleterious effect could be diminished by the presence of an antioxidant, Trolox C. In our in vitro test, we also showed that the magnetic field could potentiate the activity of oxidant radicals.

Lopucki M, **Schmerold I**, **Dadak A**, **Wiktor H**, **Niedermuller H**, **Kankofer M**. **Low dose magnetic fields do not cause oxidative DNA damage in human placental cotyledons in vitro.** Virchows Arch. 446(6):634-639, 2005. (NE)

The biological impact of low dose magnetic fields generated by electric appliances present in the human environment is still uncertain. In this study, human placentas served as a model tissue for the evaluation of the potential effect of oscillating low intensity magnetic fields on the concentration of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in cellular DNA. Cotyledons were dissected from placentas obtained immediately after physiological labours and exposed to magnetic fields (groups MF A, 2 mT, 50 Hz and MF B, 5 mT, 50 Hz) or sham exposed (group C) during an in vitro perfusion of 3 h. Cellular DNA was isolated, hydrolyzed and analyzed by HPLC. Native nucleosides were monitored at 254 nm and 8-OH-dG by electrochemical detection. Results were expressed as mumol 8-OH-dG/mol deoxyguanosine (dG). The concentrations of 8-OH-dG in group C, MF A and MF B were 28.45+/-15.27 micromol/mol dG, 62.80+/-31.91 mumol/mol dG, and 27.49+/-14.23 micromol/mol dG, respectively, demonstrating no significant difference between the groups. The results suggest that placental tissues possess a capacity to protect DNA against oxidative alterations by magnetic field of intensities previously shown to produce radical mediated DNA damage in rat brain cells in vivo and imbalances in electrolyte release of cotyledons under in vitro conditions.

Lourencini da Silva R, **Albano F**, **Lopes dos Santos LR**, **Tavares AD Jr**, **Felzenszwalb I**. **The effect of electromagnetic field exposure on the formation of DNA lesions.** Redox Rep. 5(5):299-301, 2000. (E)

In an attempt to determine whether electromagnetic field (EMF) exposure might lead to DNA damage, we exposed SnCl2-treated pBR322 plasmids to EMF and analysed the resulting conformational changes using agarose gel electrophoresis. An EMF-dependent potentiation of DNA scission (i.e. the appearance of relaxed plasmids) was observed. In confirmation of this, plasmids pre-exposed to EMF also were less capable of transforming Escherichia coli. The results indicate that EMF, in the presence of a transition metal, is capable of causing DNA damage. These observations support the idea that EMF, probably through secondary generation of reactive oxygen species, can be clastogenic and provide a possible explanation for the observed correlation between EMF exposure and the frequency of certain types of cancers in humans.

Luceri C, **De Filippo C**, **Giovannelli L**, **Blangiardo M**, **Cavalieri D**, **Aglietti F**, **Pampaloni M**, **Andreuccetti D**, **Pieri L**, **Bambi F**, **Biggeri A**, **Dolara P**. Extremely low-frequency electromagnetic fields do not affect DNA damage and gene expression profiles of yeast and human lymphocytes. Radiat Res. 164(3):277-285, 2005. (NE)

We studied the effects of extremely low-frequency (50 Hz) electromagnetic fields (EMFs) on peripheral human blood lymphocytes and DBY747 Saccharomyces cerevisiae. Graded exposure to 50 Hz magnetic flux density was obtained with a Helmholtz coil system set at 1, 10 or 100 microT for 18 h. The effects of EMFs on DNA damage were studied with the single-cell gel electrophoresis assay (comet assay) in lymphocytes. Gene expression profiles of EMF-exposed human and yeast cells were evaluated with DNA microarrays containing 13,971 and 6,212 oligonucleotides, respectively. After exposure to the EMF, we did not observe an increase in the amount of strand breaks or oxidated DNA bases relative to controls or a variation in gene expression profiles. The results suggest that extremely low-frequency EMFs do not induce DNA damage or affect gene expression in these two different eukaryotic cell systems.

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

Several recent studies have reported that whole-body exposure of rodents to power frequency magnetic fields (MFs) can result in DNA single- and double-strand breaks in the brains of these animals. The current study was undertaken to investigate whether an acute 2h exposure of a 1 mT, 60 Hz MF could elicit DNA damage, and subsequently apoptosis, in the brains of immature (10-day-old) mice. DNA damage was quantitated at 0, 2, 4, and 24h after exposure using the alkaline comet assay. Apoptosis was quantitated in the external granule cell layer (EGCL) of the immature mouse cerebellum at 0 and 24h after exposure to MF by the TdT-mediated dUTP nick-end labeling (TUNEL) assay. Four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. While increased DNA damage was detected by tail ratio at

2h after MF exposure, no supporting evidence of increased DNA damage was detected by the other parameters. In addition, no similar differences were observed using these parameters at any of the other post-exposure times. No increase in apoptosis was observed in the EGCL of MF-exposed mice, when compared to sham mice. Taken together, these results do not support the hypothesis that acute MF exposure causes DNA damage in the cerebellums of immature mice.

McNamee JP, **Bellier PV**, **Chauhan V**, **Gajda GB**, **Lemay E**, **Thansandote A**. Evaluating DNA damage in rodent brain after acute 60 Hz magnetic-field exposure. Radiat Res. 164(6):791-797, 2005. (NE)

In recent years, numerous studies have reported a weak association between 60 Hz magnetic-field exposure and the incidence of certain cancers. To date, no mechanism to explain these findings has been identified. The objective of the current study was to investigate whether acute magnetic-field exposure could elicit DNA damage within brain cells from both whole brain and cerebellar homogenates from adult rats, adult mice and immature mice. Rodents were exposed to a 60 Hz magnetic field (0, 0.1, 1 or 2 mT) for 2 h. Then, at 0, 2 and 4 h after exposure, animals were killed humanely, their brains were rapidly removed and homogenized, and cells were cast into agarose gels for processing by the alkaline comet assay. Four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. For each species, a significant increase in DNA damage was detected by each of the four parameters in the positive control (2 Gy X rays) relative to the concurrent nonirradiated negative and sham controls. However, none of the four parameters detected a significant increase in DNA damage in brain cell homogenates from any magnetic-field exposure (0- 2 mT) at any time after exposure. The dose-response and time-course data from the multiple animal groups tested in this study provide no evidence of magnetic-field-induced DNA damage.

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

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

Radiat. Biol., 71: 75-79, 1997). From these results, it appears that exposure to the high density ELFMF at more than 50 mT may potentiate X-ray-induced DNA strand breaks.

Moretti M, **Villarini M**, **Simonucci S**, **Fatigoni C**, **Scassellati-Sforzolini G**, **Monarca S**, **Pasquini R**, **Angelucci M**, **Strappini M Effects of co-exposure to extremely low frequency (ELF) magnetic fields and benzene or benzene metabolites determined in vitro by the alkaline comet assay.** Toxicol Lett. 157(2):119-128, 2005. (E)

In the present study, we investigated in vitro the possible genotoxic and/or co-genotoxic activity of 50 Hz (power frequency) magnetic fields (MF) by using the alkaline single-cell microgel-electrophoresis (comet) assay. Sets of experiments were performed to evaluate the possible interaction between 50 Hz MF and the known leukemogen benzene. Three benzene hydroxylated metabolites were also evaluated: 1,2-benzenediol (1,2-BD, catechol), 1,4-benzenediol (1,4-BD, hydroquinone), and 1,2,4-benzenetriol (1,2,4-BT). MF (1 mT) were generated by a system consisting of a pair of parallel coils in a Helmholtz configuration. To evaluate the genotoxic potential of 50 Hz MF, Jurkat cell cultures were exposed to 1 mT MF or sham-exposed for 1h. To evaluate the co-genotoxic activity of MF, the xenobiotics (benzene, catechol, hydroquinone, and 1,2,4-benzenetriol) were added to Jurkat cells subcultures at the beginning of the exposure time. In cell cultures co-exposed to 1 mT (50 Hz) MF, benzene and catechol did not show any genotoxic activity. However, co-exposure of cell cultures to 1 mT MF and hydroquinone led to the appearance of a clear genotoxic effect. Moreover, co-exposure of cell cultures to 1 mT MF and 1,2,4-benzenetriol led to a marked increase in the genotoxicity of the ultimate metabolite of benzene. The possibility that 50 Hz (power frequency) MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

Nikolova T, **Czyz J**, **Rolletschek A**, **Blyszczuk P**, **Fuchs J**, **Jovtchev G**, **Schuderer J**, **Kuster N**, **Wobus AM**. **Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells.** ASEB J. 19(12):1686-1688, 2005. (E)

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure

for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

Reese JA, **Jostes RF**, **Frazier ME**. **Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis for DNA single-strand breaks.** Bioelectromagnetics. 9(3):237-247, 1998. (NE)

Chinese hamster ovary (CHO) cells were exposed for 1 h to 60-Hz magnetic fields (0.1 or 2 mT), electric fields (1 or 38 V/m), or to combined magnetic and electric fields (2 mT and 38 V/m, respectively). Following exposure, the cells were lysed, and the DNA was analyzed for the presence of single-strand breaks (SSB), using the alkaline elution technique. No significant differences in numbers of DNA SSB were detected between exposed and sham-exposed cells. A positive control exposed to X-irradiation sustained SSB with a dose-related frequency. Cells exposed to nitrogen mustard (a known cross-linking agent) and X-irradiation demonstrated that the assay could detect cross-linked DNA under our conditions of electric and magnetic field exposures.

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

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

administered concurrently. More research is necessary, however, before data from this in vitro study can be applied to in vivo systems.

Scarfi MR, **Sannino A**, **Perrotta A**, **Sarti M**, **Mesirca P**, **Bersani F**. **Evaluation of genotoxic effects in human fibroblasts after intermittent exposure to 50 Hz electromagnetic fields: a confirmatory study.** Radiat Res. 164(3):270-276, 2005. (NE)

The aim of this investigation was to confirm the main results reported in recent studies on the induction of genotoxic effects in human fibroblasts exposed to 50 Hz intermittent (5 min field on/10 min field off) sinusoidal electromagnetic fields. For this purpose, the induction of DNA single-strand breaks was evaluated by applying the alkaline single-cell gel electrophoresis (SCGE)/comet assay. To extend the study and validate the results, in the same experimental conditions, the potential genotoxicity was also tested by exposing the cells to a 50 Hz powerline signal (50 Hz frequency plus its harmonics). The cytokinesis-block micronucleus assay was applied after 24 h intermittent exposure to both sinusoidal and powerline signals to obtain information on cell cycle kinetics. The experiments were carried out on human diploid fibroblasts (ES-1). For each experimental run, exposed and sham-exposed samples were set up; positive controls were also provided by treating cells with hydrogen peroxide or mitomycin C for the comet or micronucleus assay, respectively. No statistically significant difference was detected in exposed compared to sham-exposed samples in any of the experimental conditions tested (P > 0.05). In contrast, the positive controls showed a statistically significant increase in DNA damage in all cases, as expected. Accordingly, our findings do not confirm the results reported previously for either comet induction or an increase in micronucleus frequency.

Schmitz C, **Keller E**, **Freuding T**, **Silny J**, **Korr H**. **50-Hz magnetic field exposure influences DNA repair and mitochondrial DNA synthesis of distinct cell types in brain and kidney of adult mice.** Acta Neuropathol (Berl). 107(3):257-264, 2004. (E)

Despite several recent investigations, the impact of whole-body magnetic field exposure on cell-type-specific alterations due to DNA damage and DNA repair remains unclear. In this pilot study adult mice were exposed to 50-Hz magnetic field (mean value 1.5 mT) for 8 weeks or left unexposed. Five minutes after ending exposure, the mice received [(3)H]thymidine and were killed 2 h later. Autoradiographs were prepared from paraffin sections of brains and kidneys for measuring unscheduled DNA synthesis and mitochondrial DNA synthesis, or in situ nick translation with DNA polymerase-I and [(3)H]dTTP. A significant (P<0.05) increase in both unscheduled DNA synthesis and in situ nick translation was only found for epithelial cells of the choroid plexus. Thus, these two independent methods indicate that nuclear DNA damage is produced by long-lasting and strong magnetic field exposure. The fact that only plexus epithelial cells were affected might point to possible effects of magnetic fields on iron transport across the blood-cerebrospinal fluid barrier, but the mechanisms are currently not understood. Mitochondrial DNA synthesis was exclusively increased in renal epithelial cells of distal convoluted tubules and collecting ducts, i.e., cells with a very high content of mitochondria, possibly indicating increased metabolic activity of these cells.

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

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

Stronati L, **Testa A**, **Villani P**, **Marino C**, **Lovisolo GA**, **Conti D**, **Russo F**, **Fresegna AM**, **Cordelli E** **Absence of genotoxicity in human blood cells exposed to 50 Hz magnetic fields as assessed by comet assay, chromosome aberration, micronucleus, and sister chromatid exchange analyses.** Bioelectromagnetics. 25(1):41-48, 2004. (NE)

In the past, epidemiological studies indicated a possible correlation between the exposure to ELF fields and cancer. Public concern over possible hazards associated with exposure to extremely low frequency magnetic fields (ELFMFs) stimulated an increased scientific research effort. More recent research and laboratory studies, however, have not been able to definitively confirm the correlation suggested by epidemiological studies. The aim of this study was to evaluate the effects of 50 Hz magnetic fields in human blood cells exposed in vitro, using several methodological approaches for the detection of genotoxicity. Whole blood samples obtained from five donors were exposed for 2 h to 50 Hz, 1 mT uniform magnetic field generated by a Helmholtz coil system. Comet assay, sister chromatid exchanges (SCE), chromosome aberrations (CA), and micronucleus (MN) tests were used to assess DNA damage, one hallmark of malignant cell transformation. The effects of a combined exposure with X-rays were also evaluated. Results obtained do not show any significant difference between ELFMFs exposed and unexposed samples. Moreover, no synergistic effect with ionizing radiation has been observed. A slight but significant decrease of cell proliferation was evident in ELFMFs treated samples and samples subjected to the combined exposure.

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

DNA migration, using single cell gel electrophoresis (comet assay), was studied on brain

cells of CBA mice exposed continuously to 50 Hz, 0.5 mT magnetic fields (MF) for 2 hrs, 5 days or 14 days. No differences were observed in the groups MF-exposed for 2 hrs and 5 days compared with controls. However, in the group exposed to MF for 14 days, a significantly extended cell DNA migration was observed ($0.02 < p < 0.05$). These changes together with results from previous studies indicate that magnetic fields may have genotoxic effects in brain cells.

Testa A, **Cordelli E**, **Stronati L**, **Marino C**, **Lovisolo GA**, **Fresegna AM**, **Conti D**, **Villani P**. **Evaluation of genotoxic effect of low level 50 Hz magnetic fields on human blood cells using different cytogenetic assays.** Bioelectromagnetics. 25(8):613-619, 2004. (NE)

The question whether extremely low frequency magnetic fields (ELFMFs) may contribute to mutagenesis or carcinogenesis is of current interest. In order to evaluate the possible genotoxic effects of ELFMFs, human blood cells from four donors were exposed in vitro for 48 h to 50 Hz, 1 mT uniform magnetic field generated by a Helmholtz coil system. Comet assay (SCGE), sister chromatid exchanges (SCE), chromosome aberrations (CAs), and micronucleus (MN) test were used to assess the DNA damage. ELF pretreated cells were also irradiated with 1 Gy of X-ray to investigate the possible combined effect of ELFMFs and ionizing radiation. Furthermore, nuclear division index (NDI) and proliferation index (PRI) were evaluated. Results do not evidence any DNA damage induced by ELFMF exposure or any effect on cell proliferation. Data obtained from the combined exposure to ELFMFs and ionizing radiation do not suggest any synergistic or antagonistic effect.

Villarini M, **Moretti M**, **Scassellati-Sforzolini G**, **Boccioli B**, **Pasquini R**. Effects of co-exposure to extremely low frequency (50 Hz) magnetic fields and xenobiotics determined in vitro by the alkaline comet assay. Sci Total Environ. 361(1-3):208-219, 2006. (E)

In the present study, we used human peripheral blood leukocytes from 4 different donors, to investigate in vitro the possible genotoxic and/or co-genotoxic activity of extremely low frequency magnetic fields (ELF-MF) at 3 mT intensity. Two model mutagens were used to study the possible interaction between ELF-MF and xenobiotics: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 4-nitroquinoline N-oxide (4NQO). Primary DNA damage was evaluated by the alkaline single-cell microgel-electrophoresis ("comet") assay. Control cells (leukocytes not exposed to ELF-MF, nor treated with genotoxins) from the different blood donors showed a comparable level of basal DNA damage, whereas the contribution of individual susceptibility toward ELF-MF and the tested genotoxic compounds led to differences in the extent of DNA damage observed following exposure to the genotoxins, both in the presence and in the absence of an applied ELF-MF. A 3 mT ELF-MF alone was unable to cause direct primary DNA damage. In leukocytes exposed to ELF-MF and genotoxins, the extent of MNNG-induced DNA damage increased with exposure duration compared to sham-exposed cells. The opposite was observed in cells treated with 4NQO. In this case the extent of 4NQO-induced DNA damage was somewhat reduced in leukocytes exposed to ELF-MF compared to sham-exposed cells. Moreover, in cells exposed to ELF-MF an increased

concentration of GSH was always observed, compared to sham-exposed cells. Since following GSH conjugation the genotoxic pattern of MNNG and 4NQO is quite different, an influence of ELF-MF on the activity of the enzyme involved in the synthesis of GSH leading to different activation/deactivation of the model mutagens used was hypothesized to explain the different trends observed in MNNG and 4NQO genotoxic activity in the presence of an applied ELF-MF. The possibility that ELF-MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

Williams PA, **Ingebretsen RJ**, **Dawson RJ**. 14.6 mT ELF magnetic field exposure yields no DNA breaks in model system Salmonella, but provides evidence of heat stress protection. Bioelectromagnetics. 27(6):445-450, 2006. (NE)

In this study, we demonstrate that common extremely low frequency magnetic field (MF) exposure does not cause DNA breaks in this Salmonella test system. The data does, however, provide evidence that MF exposure induces protection from heat stress. Bacterial cultures were exposed to MF (14.6 mT 60 Hz field, cycled 5 min on, 10 min off for 4 h) and a temperature-matched control. Double- and single-stranded DNA breaks were assayed using a recombination event counter. After MF or control exposure they were grown on indicator plates from which recombination events can be quantified and the frequency of DNA strand breaks deduced. The effect of MF was also monitored using a recombination-deficient mutant (recA). The results showed no significant increase in recombination events and strand breaks due to MF. Evidence of heat stress protection was determined using a cell viability assay that compared the survival rates of MF exposed and control cells after the administration of a 10 min 53 degrees C heat stress. The control cells exhibited nine times more cell mortality than the MF exposed cells. This Salmonella system provides many mutants and genetic tools for further investigation of this phenomenon.

Winker R, **Ivancsits S**, **Pilger A**, **Adlkofer F**, **Rudiger HW**. **Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields.** Mutat Res. 585(1-2):43-49, 2005. (E)

Environmental exposure to extremely low-frequency electromagnetic fields (ELF-EMFs) has been implicated in the development of cancer in humans. An important basis for assessing a potential cancer risk due to ELF-EMF exposure is knowledge of biological effects on human cells at the chromosomal level. Therefore, we investigated in the present study the effect of intermittent ELF electromagnetic fields (50 Hz, sinusoidal, 5'field-on/10'field-off, 2-24 h, 1 mT) on the induction of micronuclei (MN) and chromosomal aberrations in cultured human fibroblasts. ELF-EMF radiation resulted in a time-dependent increase of micronuclei, which became significant after 10 h of intermittent exposure at a flux density of 1 mT. After approximately 15 h a constant level of micronuclei of about three times the basal level was reached. In addition, chromosomal aberrations were increased up to 10-fold above basal levels. Our data strongly indicate a clastogenic potential of intermittent low-frequency electromagnetic fields, which may lead to considerable chromosomal damage in dividing cells.

Wolf FI, **Torsello A**, **Tedesco B**, **Fasanella S**, **Boninsegna A**, **D'Ascenzo M**, **Grassi C**, **Azzena GB**, **Cittadini A**. **50-Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: possible involvement of a redox mechanism.** Biochim Biophys Acta. 1743(1-2):120-129, 2005. (E)

HL-60 leukemia cells, Rat-1 fibroblasts and WI-38 diploid fibroblasts were exposed for 24-72 h to 0.5-1.0-mT 50-Hz extremely low frequency electromagnetic field (ELF-EMF). This treatment induced a dose-dependent increase in the proliferation rate of all cell types, namely about 30% increase of cell proliferation after 72-h exposure to 1.0 mT. This was accompanied by increased percentage of cells in the S-phase after 12- and 48-h exposure. The ability of ELF-EMF to induce DNA damage was also investigated by measuring DNA strand breaks. A dose-dependent increase in DNA damage was observed in all cell lines, with two peaks occurring at 24 and 72 h. A similar pattern of DNA damage was observed by measuring formation of 8-OHdG adducts. The effects of ELF-EMF on cell proliferation and DNA damage were prevented by pretreatment of cells with an antioxidant like alpha-tocopherol, suggesting that redox reactions were involved. Accordingly, Rat-1 fibroblasts that had been exposed to ELF-EMF for 3 or 24 h exhibited a significant increase in dichlorofluorescein-detectable reactive oxygen species, which was blunted by alpha-tocopherol pretreatment. Cells exposed to ELF-EMF and examined as early as 6 h after treatment initiation also exhibited modifications of NF kappa B-related proteins (p65-p50 and I kappa B alpha), which were suggestive of increased formation of p65-p50 or p65-p65 active forms, a process usually attributed to redox reactions. These results suggest that ELF-EMF influence proliferation and DNA damage in both normal and tumor cells through the action of free radical species. This information may be of value for appraising the pathophysiologic consequences of an exposure to ELF-EMF.

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

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

Yokus B, **Cakir DU**, **Akdag MZ**, **Sert C**, **Mete N**. **Oxidative DNA damage in rats exposed to extremely low frequency electro magnetic fields.** Free Radic Res. 39(3):317-323, 2005. (E)

Extremely low frequency (ELF) electromagnetic field (EMF) is thought to prolong the life of free radicals and can act as a promoter or co-promoter of cancer. 8-hydroxy-2'-deoxyguanosine (8OHdG) is one of the predominant forms of radical-induced lesions to DNA and is a potential tool to asses the cancer risk. We examined the effects of extremely low frequency electro magnetic field (ELF-EMF) (50 Hz, 0.97 mT) on 8OHdG levels in DNA and thiobarbituric acid reactive substances (TBARS) in plasma. To examine the possible time-dependent changes resulting from magnetic field, 8OHdG and TBARS were quantitated at 50 and 100 days. Our results showed that the exposure to ELF-EMF induced oxidative DNA damage and lipid peroxidation (LPO). The 8OHdG levels of exposed group (4.39+/-0.88 and 5.29+/-1.16 8OHdG/dG.10(5), respectively) were significantly higher than sham group at 50 and 100 days (3.02+/-0.63 and 3.46+/-0.38 8OHdG/dG.10(5)) ($p<0.001$, $p<0.001$). The higher TBARS levels were also detected in the exposure group both on 50 and 100 days ($p<0.001$, $p<0.001$). In addition, the extent of DNA damage and LPO would depend on the exposure time ($p<0.05$ and $p<0.05$). Our data may have important implications for the long-term exposure to ELF-EMF which may cause oxidative DNA damage.

Zmyslony M, **Palus J**, **Jajte J**, **Dziubaltowska E**, **Rajkowska E**. **DNA damage in rat lymphocytes treated in vitro with iron cations and exposed to 7 mT magnetic fields (static or 50 Hz).** Mutat Res. 453(1):89-96, 2000. (E)

The present study was undertaken to verify a hypothesis that exposure of the cells to static or 50 Hz magnetic fields (MF) and simultaneous treatment with a known oxidant, ferrous chloride, may affect the oxidative deterioration of DNA molecules.The comet assay was chosen for the assessment of DNA damage. The experiments were performed on isolated rat lymphocytes incubated for 3h in Helmholtz coils at 7 mT static or 50 Hz MF. During MF exposure, part of the cell samples were incubated with 0.01 microM H_2O_2 and another one with 10 microg/ml FeCl(2,) the rest serving as controls.Lymphocyte exposure to MF at 7 mT did not increase the number of cells with DNA damage in the comet assay. Incubation of lymphocytes with 10 microg/ml FeCl(2) did not produce a detectable damage of DNA either. However, when the FeCl(2)-incubated lymphocytes were simultaneously exposed to 7 mT MF, the number of damaged cells was significantly increased and reached about 20% for static MF and 15% for power frequency MF. In the control samples about 97% of the cells did not have any DNA damage.It is not possible at present to offer a reasonable explanation for the findings of this investigation - the high increase in the number of lymphocytes showing symptoms of DNA damage in the comet assay, following simultaneous exposure to the combination of two non-cytotoxic factors -10 microg/ml FeCl(2) and 7 mT MF. In view of the obtained results we can only hypothesise that under the influence of simultaneous exposure to FeCl(2) and static or 50 Hz MF, the number of reactive oxygen species generated by iron cations may increase substantially. Further studies will be necessary to confirm this hypothesis and define the biological significance of the observed effect.

Zmyslony M, **Palus J**, **Dziubaltowska E**, **Politanski P**, **Mamrot P**, **Rajkowska E**, **Kamedula M**. **Effects of in vitro exposure to power frequency magnetic fields on UV-induced DNA damage of rat lymphocytes.** Bioelectromagnetics. 25(7):560-562, 2004. (E)

The mechanisms of biological effects of 50/60 Hz (power frequency) magnetic fields (MF) are still poorly understood. There are a number of studies indicating that MF affect biochemical processes in which free radicals are involved, such as the biological objects' response to ultraviolet radiation (UVA). Therefore, the present study was aimed to assess the effect of 50 Hz MFs on the oxidative deterioration of DNA in rat lymphocytes irradiated in vitro by UVA. UVA radiation (150 J/m2) was applied for 5 min for all groups and 50 Hz MF (40 microT rms) exposure was applied for some of the groups for 5 or 60 min. The level of DNA damage was assessed using the alkaline comet assay, the fluorescence microscope, and image analysis. It has been found that the 1 h exposure to MF caused an evident increase in all parameters consistent with damaged DNA. This suggest that MF affects the radical pairs generated during the oxidative or enzymatic processes of DNA repair.

SECTION 7

Evidence for Stress Response
(Stress Proteins)

**Health Risk of Electromagnetic Fields:
Research on the Stress Response**

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A Scientific Perspective on Health Risk of Electromagnetic Fields: Research on the Stress Response

Table of Contents

I.   Abstract

The stress response is a protective cellular mechanism that is characterized by stress protein synthesis. The stress response, by its very nature, shows that *cells react to EMFs as potentially harmful*. The stress response is an important protective mechanism that enables cells from animals, plants and bacteria to survive environmental stressors with the aid of heat shock proteins (hsp). It is stimulated by both non-thermal power (ELF), and non-thermal radiofrequency (RF) as well as thermal radio (RF) frequency EMFs, so the greatly differing energies are not critical in activating the DNA to synthesize proteins. Direct interaction of both ELF and RF EMFs with DNA is likely, since specific DNA sequences are sensitive to EMFs and retain their sensitivity when transferred to artificial molecular constructs. Basic science research is essential for determining the biological parameters needed to assess health risks of electromagnetic fields (EMFs) and the molecular mechanisms that explain them. However, the adversarial nature of the debate about risk has clouded the evaluation of the science. To clarify the results of research on EMF stimulation of the stress response, it is necessary to consider the scientific context as well as the research. There is ample evidence that ELF and RF fields activate DNA in cells and cause damage at exposure levels that are considered 'safe' (i.e., below current exposure limits that are based on tissue heating as measured in Specific Absorption Rate or SAR). Because non-thermal EMFs are biologically active and potentially harmful, new safety standards must be developed to protect against possible damage at non-thermal levels, and the standards must be defined in terms of a non-thermal biological dose. Fewer than one quarter of the relevant references listed in Table 1 appear in the IEEE list leading to the newly revised IEEE C95.1 recommendations (April, 2006).

II.   Stress Proteins - Conclusions (Heat Shock Proteins)

Conclusion: Scientific research has shown that the public is not being protected from potential damage that can be caused by exposure to EMF, both power frequency (ELF) and radio frequency (RF).

Conclusion: DNA damage (e.g., strand breaks), a cause of cancer, occurs at levels

of ELF and RF that are below the safety limits. Also, there is no protection against cumulative effects stimulated by different parts of the EM spectrum.

<u>Conclusion:</u> The scientific basis for EMF safety limits is flawed when the same biological mechanisms are activated in ELF and RF ranges at vastly different levels of the Specific Absorption Rate (SAR). Activation of DNA to synthesize stress proteins (the stress response), is stimulated in the ELF at a non-thermal SAR level that is over a billion times lower than the same process activated in the RF at the thermal level.

<u>Conclusion:</u> There is a need for a biological standard to replace the thermal standard and to also protect against cumulative effects across the EM spectrum.

III.   ELF and RF activation of the stress response

Much detailed information about the stress response will be presented in the following sections and in the tables, but the most important finding to keep in mind is that *both ELF and RF fields activate the synthesis of stress proteins*. All cells do not respond to EMF, but activation of the same cellular mechanism by both thermal and non-thermal stimuli in a variety of cells shows that both ELF and RF are biologically active and that a biological 'dose' of EMF cannot be described in terms of SAR (Blank and Goodman, 2004a). SAR is irrelevant for non-thermal ELF responses, where energy thresholds are many orders of magnitude lower than in RF. A new definition of EMF dose is necessary for describing a safety limit, and SAR must be replaced by a measure of exposure that can be defined in biological terms.

The stress response, by its very nature, shows that *cells react to EMFs as potentially harmful*. The stress response is an important protective mechanism that enables cells from animals, plants and bacteria to survive environmental stressors, such as sharp increases in temperature (originally called 'heat shock'), hypoxia, and dissolved toxic heavy metals like Cd^{+2} and oxidative species that can damage proteins and DNA ('oxidative stress'). The stress response is evolutionarily conserved in essentially all eukaryotic and prokaryotic organisms, but not all stressors are effective in all cells, and different stress proteins are activated under different conditions. Stress proteins are a family of about 20 different proteins, ranging in size from a few kilodaltons to over 100kD. The 27kD and 70kD protein families are the most common and most frequently studied.

Kültz (2005) has called the stress response a '... defense reaction of cells to damage that environmental forces inflict on macromolecules.', based on evidence from gene analysis showing that the stress response is a reaction to molecular damage. The genes activated as a group along with stress genes, which Kültz calls the 'universally conserved proteome', are those associated with sensing and repairing damage to DNA and proteins.

Stress proteins help damaged proteins refold to regain their conformations, and also act as "chaperones" for transporting cellular proteins to their destinations in cells. The molecular damage stimulated by non-thermal ELF fields occurs in the absence of an increase in temperature. ELF energy thresholds are estimated to be about 10^{-12} W/kg, over a billion times lower than the thermal stimuli that cause damage in the RF range (Blank and Goodman, 2004a).

The classic stress response to a sharp increase in temperature (i.e., 'heat shock') is associated with a biochemical pathway where transcription factors known as heat shock factors, HSFs, translocate from the cytoplasm to the nucleus, trimerize and bind to DNA at the heat shock elements (HSEs) in the promoters of the genes. The promoter is the DNA segment where protein synthesis is initiated and it is not part of the coding region. The HSEs contain specific nucleotide sequences, nGAAn, that are the consensus sequences for thermal stimuli. The binding of HSFs to HSEs, etc is similar for heat shock in plant, animal and bacterial cells. ELF range EMFs have been shown to follow the same sequence of events in inducing stress response proteins in human cells, including breast (MCF7, HTB124), leukemia (HL60), epithelial cells, as well as E. coli and yeast cells.

Studies done with chick embryos and cells from *Drosophila* and *Sciara* salivary gland chromosomes have produced graphic evidence of the effects of EMF. In *Drosophila* and *Sciara* salivary gland chromosomes, EMF causes the formation of 'puff's, enlarged regions along the chromosome, at loci associated with activation of heat shock genes. This is followed by elevated concentrations of transcripts at the sites and eventually stress protein synthesis (Goodman and Blank, 1998). The changes in chromosome morphology are characteristic of the stress response to both EMF and elevated temperature. Chick embryos develop hearts that stop beating when the oxygen concentration is lowered, but that can be protected and kept beating if stress proteins have been induced by ELF fields (DiCarlo et al, 1998) and in the RF range (Shallom et al, 2002).

The cellular response pathways to EMF have been characterized in the ELF range (Goodman and Blank, 2002), and have been found to share some of the characteristics of heat shock stress, such as the movement of heat shock factor monomers from the cytoplasm to the nucleus. The biochemical mechanism that is activated, the MAPK signaling pathway, differs from the thermal pathway (Goodman and Blank, 2002), but is the same as the non-thermal pathway in the RF range (Leszczynski et al, 2002).

The HSP70 gene is activated within minutes in cells exposed to ELF fields (Lin et al, 1997), and is accompanied by the binding of HSFs to the specific nucleotide sites in the promoter of the gene. However, different segments of the DNA promoter function as HSEs. Research in the ELF range has shown that the promoter of the major stress protein, hsp70, has two domains that respond to two different physical stimuli, EMF and an increase in temperature (Lin et al, 1999). The stimulus-specific domains have different DNA sequences that cannot be interchanged. The ***DNA consensus sequences that respond to EMF are nCTCTn*** (Lin et al, 1997; 1999). These differ from the nGAAn consensus sequences for thermal stimuli. The existence of two different consensus sequences that respond to EMF and temperature increase, respectively, are molecular

evidence of different pathways that respond to non-thermal and thermal stimuli.

In another series of experiments, a DNA sequence from the promoter of an EMF sensitive gene was included in a construct containing a reporter gene, either chloramphenicol amino transferase (CAT) or luciferase. In each case, the construct proved to be EMF sensitive and reacted when an ELF field was applied (Lin et al, 2001). The ability to transfer EMF sensitive DNA sequences that subsequently respond to an EMF is further evidence linking the cellular response to a DNA structure.

In heat shock, the stress response is activated when extracellular signals affect receptors in the plasma membrane. This probably does not happen with an EMF, which can easily penetrate throughout the cell and whose actions are therefore not limited to the membrane. One can transfer the EMF response by transferring the DNA consensus sequences (Lin et al, 2001), so it is likely that the activation mechanism involves direct EMF interaction with the DNA consensus sequences. The cell based signal transduction pathways of the heat shock response are involved in regulation of the EMF stimulated process, probably through the feedback control mechanisms that respond to the stress proteins synthesized or the mRNA concentrations that code for them (Lin et al, 1998).

Repeated induction of the stress response in a cell has been shown to induce cytoprotection, a reduced response associated with restimulation (Blank and Goodman, 1998). This is analogous to thermotolerance, the reduced response to an increase in temperature after an initial heat shock response. Experiments with developing chick embryos show similar habituation to repeated stimulation in the ELF range (DiCarlo et al, 2002). There are different effects of continuous and intermittent EMF exposures that show feedback control features in the EMF stimulated stress response (Lin et al, 1997). This autoregulatory reaction is an indication that the thermotolerance mechanism is inherent in the response to a single stimulus as well.

It has now been shown in many laboratories that RF also stimulates the cellular stress response and cells start to synthesize stress proteins in many different kinds of cells (e.g., Kwee et al, 2001; Shallom et al, 2002; Leszczynski et al, 2002; Weisbrot et al, 2004). Cotgreave (2005) included many cells that did not synthesize stress proteins in response to RF stimulation in his summary of data. The listings in Table 1 contain additional positive and negative results. It is quite clear that certain cell lines do not respond to EMF by synthesizing stress proteins. The reasons are not known, but the changes in cells in tissue culture and in cancer cells may render some of them unable to respond to EMF. In addition to mutations in cell lines, pre-exposure to ambient ELF and RF fields in the laboratory can also affect an ability to respond. What we can say in summary at this stage is that:

> • the stress response has been demonstrated in many cells and linked to changes in the DNA and chromosomes.

• there are similarities in stress protein synthesis stimulated in the non-thermal ELF and thermal RF frequency ranges.

• the biochemical mechanism that is activated is the same non-thermal pathway in both ELF and RF, and is not associated with the thermal response.

IV.   DNA activation mechanisms: EMFs and electrons

We think of DNA as a very stable polymer that stores and transmits genetic information from generation to generation. However, DNA must also come apart relatively easily to enable the continuous protein synthesis that is needed to sustain living cells. Usually, this process is started when specialized proteins called transcription factors bind to DNA. However, both ELF and RF fields also stimulate DNA to start protein synthesis. EMF stimulation of stress protein synthesis indicates activation of DNA, even by relatively weak non-thermal ELF. This raises the possibility that EMF can cause other changes in DNA that interfere with the copying and repair processes in DNA, and that can lead to mutations and cancer.

Protein synthesis starts when the two chains of DNA come apart to make an mRNA copy of the amino acid code for a particular protein. This occurs at the specific DNA segment where the transcription factor binds, and in forming a bond changes the electron distribution. Since recent research has shown electron conduction in DNA (Wan et al, 1999; 2000; Ratner, 1999; Porath et al, 2000; Giese and Spichty, 2000), it is possible that EMF affects electron distribution and movement in DNA, and helps it to come apart to initiate protein synthesis, not unlike the action of a transcription factor. Charge transport through DNA depends on the DNA sequence (Shao et al, 2005), and there are reasons to believe that EMFs would cause the DNA to come apart at the EMF consensus sequence, nCTCTn (Blank and Goodman, 2002).

The ability of relatively small perturbations to stimulate DNA to initiate biosynthesis is consistent with larger perturbations that lead to DNA strand breaks. Several experimental studies have reported both single and double strand breaks in DNA and other chromosome damage after exposure to ELF fields (Lai and Singh, 1997a; Ivancsits et al, 2005, Diem et al, 2005; Winker et al, 2005). Ivancsits et al (2005) found DNA damage in fibroblasts, melanocytes and rat granulosa cells, but not in lymphocytes, monocytes and skeletal muscle cells. Single and double strand breaks and other DNA damage after exposure to RF fields have also been reported (Phillips et al, 1998; Sarimov et al, 2004; Lai and Singh, 2005).

The Ivancsits, Diem and Winker studies cited above are part of the REFLEX Project, a collaboration of twelve laboratories in seven countries of the European Union (REFLEX, 2004). The group found that both ELF and RF exposures, below the current safety limits, modified the expression of many genes and proteins. They also reported DNA damage (e.g., strand breaks, micronuclei, chromosomal damage) due to ELF fields at exposures

of 35µT. Similar genotoxic effects were produced in fibroblasts, granulosa cells and HL60 cells by RF fields at SARs between 0.3 and 2W/kg. The expression and phosphorylation of the stress protein hsp27 was one of the many proteins affected.

The REFLEX Project Report (2004) is available on the internet and well worth consulting as a source of much information about the effects on cells *in vitro* due to the ELF and RF exposures we encounter in our environment. The Report has an introduction by Ross Adey, one of the last things he wrote, telling us about the importance of establishing "...essential exposure metrics ... based on mechanisms of field interactions in tissues". One needs a biological metric in order to characterize EMF exposure.

The possibility that EMFs could cause greater damage to DNA in the RF range and at longer exposures was demonstrated by Phillips et al (1998) who reported more DNA breaks when cells were exposed at higher SARs. They suggested that the rate at which DNA damage can be repaired (or eliminated by apoptosis) is limited, and when the rate of damage at the higher SARs exceeds the repair rate, there is the possibility of retaining mutations and initiating carcinogenesis. Chow and Tung (2000) reported that exposure to a 50Hz magnetic field enhances DNA repair through the induction of DnaK/J synthesis. The eternal struggle in cells and organisms between the forces tending to break things down (catabolism) and those tending to build up and repair (anabolism) probably accounts for much of the variability one finds in experiments with cells as well as with people.

The changes in DNA initiated by ELF fields cannot be explained by thermal effects. Electric and magnetic fields interact with charges and magnetic dipoles, and fundamental mechanisms must ultimately be based on these interactions. From the data in Table 2, it is clear that relatively little energy is needed for effects on electron transfer (Blank and Goodman, 2002; 2004b; Blank, 2005). The low energies needed to perturb DNA in the ELF range suggest that the mechanism involves electrons, e.g., probably in the H-bonds that hold the two chains of DNA together. Electrons have very high charge to mass ratio and are most likely to be affected even by weak electric and magnetic fields.

There are many indications that electrons are involved in EMF reactions with DNA. In experiments that stimulate the stress response, the estimated force of $\sim 10^{-21}$ newtons that activates DNA can move a free electron about the length of a H-bond ($\sim$.3nm) in 1ns. The calculated electron velocity is comparable to electron velocities measured in DNA (Wan et al, 1999; 2000), and is also expected if electrons move at the ~nanometer/picosecond flickering rate of protons in H-bonded networks (Fecko et al, 2003) that would be present at normally hydrated DNA sites. Electrons can tunnel nanometer distances in proteins (Gray and Winkler, 2003), and experiments have shown comparable electron movement in DNA (Wan et al, 1999; 2000). Electrons might be expected to move more readily from the CTCT bases in the consensus sequence, because of their low electron affinities. Finally, ELF fields have been shown to accelerate electron transfer in oxidation-reduction reactions (Blank and Soo, 1998; 2003).

The fact that the same non-thermal mechanism is activated in ELF and RF ranges

emphasizes that it is not the total energy associated with the EMF that is critical, but rather the regular oscillations of the stimulating force. As already mentioned earlier, the energy associated with each wave (i.e., energy/cycle) is more or less independent of the frequency. If the same energy is needed to reach threshold in both ELF and RF, the many repetitions at the higher frequency cause the non-thermal threshold to be reached in a shorter time and the total energy absorbed over time to increase with frequency. Even in the ELF range, where SAR levels are very low, the stress response is activated by short exposures to fields of less than 1μT, while single and double strand breaks in DNA have been reported at longer exposures to higher field strengths ~0.1mT (Lai and Singh, 2005). The two mechanisms appear to be related in that breaks in DNA appear to result from free radical mechanisms that also involve electron transfer reactions (Lai and Singh, 1997b).

The reaction of EMFs with DNA differs from those listed in Table 2 in that they appear to occur with equal ease at the widely differing frequencies in ELF and RF ranges. The frequency dependence of a reaction provides information about how time constants of charge transfer processes are affected by fields, and the frequency responses of the few EMF sensitive biological systems that have been studied suggest that fields are most effective at frequencies that are close to the natural rhythms of the processes affected (Blank and Soo, 2001a; Blank and Goodman, 2004b; Blank, 2005). Frequency optima for the enzymes, Na,K-ATPase and cytochrome oxidase, differ by an order of magnitude with maximums at about 60Hz and 800Hz, respectively (Blank and Soo, 2001a), in both cases close to the observed frequency maximum of the enzyme reaction. The rate constant of the BZ reaction is about 250Hz, the frequency of the rate limiting step in a multi-step process with at least 10 sub-reactions (Blank and Soo, 2003).

The electrons in DNA that are affected by EMFs are probably not engaged in electron transfer reactions. They respond to frequencies that range from ELF to RF and are more likely to be tied to the wide frequency range of fluctuations than to the frequency of a particular reaction. The displacement of electrons in DNA would charge small groups of base pairs and lead to disaggregation forces overcoming H-bonds, separating the two chains and enabling transcription. Studies have shown that biopolymers can be made to disaggregate when the molecular charge is increased (Blank, 1994; Blank and Soo, 1987). This explanation would also apply to the effect of applied electric fields that also activate DNA. Electric fields exert a force on electrons, and have been shown to stimulate protein synthesis in HL60 cells (Blank et al, 1992), E coli (Laubitz et al, 2006) and muscle *in vivo* (Blank, 1995). The genes for the hsp70 stress protein are more likely to be activated since they have been shown to be 'bookmarked' on the DNA chain, that is, more exposed to externally applied forces (Xing et al, 2005).

The outline of a plausible mechanism to account for EMF activation of DNA through interaction with electrons has relied on evidence from many lines of research. This mechanism may or may not hold up under further testing, but the experimental facts it is based on have been verified. It has been clearly demonstrated that exposure of cells to non-thermal power and thermal radio frequency EMFs, at levels deemed to be safe for human exposure, activate DNA production of stress proteins and could increase the

number of DNA breaks. There is ample experimental evidence to support the possibility of DNA damage at non-thermal levels of exposure, and the need for greater protection.

V.   The critical role of scientific research

The connection between the results of scientific research and assessing EMF risk does not appear to be working well. We all agree that EMFs are unsafe at the level where they cause electrocution, and that we must protect against that possibility. We also agree that if other risks are associated with EMFs, we must identify them and determine the exposure levels at which they occur. This task requires that we define a biological dose of EMF, and that we obtain information about cellular mechanisms activated at different doses. As we have seen, the currently accepted measure of EMF dose, the specific absorption rate (SAR), is definitely not a measure of the effective biological dose when stress protein synthesis can be stimulated by SAR levels that differ by many orders of magnitude in the ELF and RF ranges (Blank and Goodman, 2004a). Yet, there is strong opposition to accepting the consequences of these experimental facts.

Regarding EMF mechanisms, we still have much to learn, but we know that the energy and field strength thresholds of many biological reactions are very low (Table 2). These findings indicate that safe exposure levels for the public should be substantially lowered, if only as a precautionary measure. Even when stated in vague terms, so as to require little more than lip service, a precautionary policy has not yet been recommended by the WHO. Thus, the two main problems of research on EMF risk, defining a biological dose and the desired level of exposure protection, remain to be solved.

Scientific research can contribute to defining a biological dose, but the desired level of exposure protection is a more complicated issue. Guidance for EMF policy on exposure protection has come primarily from epidemiology studies of health risks associated with power lines in the case of ELF, and cell phones in the case of RF. Basic research studies do not provide insight into the effects of long term exposures that are so important in determining risk, and they appear to have been used almost entirely to probe biochemical mechanisms that might underlie health risks identified in epidemiology studies. However, the research does overcome a basic weakness of epidemiology studies, an inability to determine a causal relation and to rule out effects of possible confounders. Epidemiology studies can correlate EMF exposure and health effects in human populations, and show quantitative dose-response relations, but it is only when coupled with basic research on molecular mechanisms that one can test and establish the scientific plausibility of effects of exposure. This scientific capability has become more important with recent advances in research on DNA, where mutations associated with initiation and promotion of cancer can be identified. EMF laboratory research has also played an indirect role in the practical aspects of risk by showing that:

- many biological systems are affected by EMFs,
- EMFs compete with intrinsic forces in a system, so effects can be variable,

- many frequencies are active,
- field strength and exposure duration thresholds are very low,
- molecular mechanisms at very low energies are plausible links to disease (e.g., effect on electron transfer rates linked to oxidative damage, DNA activation linked to abnormal biosynthesis and mutation).

Research on the stress response, a protective mechanism that involves activation of DNA and protein synthesis, was not included in previous scientific reviews prior to evaluating safety standards, and thus provides additional insights into EMF interactions (Blank and Goodman, 2004a). Activation of this protective mechanism by non-thermal as well as thermal EMF frequencies has demonstrated:

- the reality and importance of non-thermal effects of EMFs,

- that cells react to an EMF as potentially harmful,

- the same biological reaction to an EMF can be activated in more than one division of the EM spectrum,

- direct interaction of ELF and RF with DNA has been documented and both activate the synthesis of stress proteins,

- the biochemical pathway that is activated is the same pathway in both ELF and RF and it is non-thermal,

- thresholds triggering stress on biological systems occur at environment levels on the order of 0.5 to 1.0 µT for ELF,

- many lines of research now point to changes in DNA electron transfer as a plausible mechanism of action as a result of non-thermal ELF and RF.

Given these findings, the *specific absorption rate (SAR) is not the appropriate measure of biological threshold or dose*, and should not be used as a basis for a safety standard since it regulates against thermal effects only.

Cellular processes are unusually sensitive to non-thermal ELF frequency fields. The thresholds for a number of biological systems are shown in Table 2, and many are in the range of 0.5 to 1.0 µT, not very much higher than the usual environmental backgrounds of ~0.1µT. The low biological thresholds in the non-thermal ELF range undermine claims that an EMF must increase the temperature in order to cause changes in cells. They also show that many biochemical reactions can be affected by relatively low field strengths, similar to those in the environment. -Non-thermal ELF fields can also cause DNA damage, and therefore add to health and safety concerns.

In addition to very low thresholds, exposure durations do not have to be very long to be effective. Litovitz et al (1991, 1993), working with the enzyme ornithine decarboxylase,

have shown a full response to an EMF when cells were exposed for only 10sec. This occurred with ELF sine waves or ELF modulated 915MHz sine waves. The exposure had to be continuous, since gaps in the sine wave resulted in a reduced response. Interference with the sine wave in the form of superimposed ELF noise also reduced the response (Mullins et al, 1998). The interfering effect of noise has been shown in the RF range by Lai and Singh (2005), who reported that noise interferes with the ability of an RF signal to cause breaks in DNA strands. The decreased effect when noise is added to a signal is yet another indication that EMF energy is not the critical factor in causing a response.

The finding that the stress response threshold can be stimulated in both ELF and RF frequency ranges appears to suggest that the threshold is independent of EMF energy. Energy increases with the frequency, so compared to an ELF energy of ~1a.u. (arbitrary unit of energy), the energy at RF is ~10^{11}a.u. Actually, it is the energy/cycle that is independent of frequency. A typical ELF cycle at 10^2Hz lasts 10^{-2}sec and a typical RF cycle at 10^{11}Hz lasts 10^{-11}sec. Because the energy is spread over a different number of cycles each second in the two ranges, the same value of ~10^{-2} a.u./cycle applies to both ELF and RF ranges.

An early review of the stress response in the ELF range (Goodman and Blank, 1998) summarized basic findings, and a more recent review by Cotgreave (2005) has provided much additional information, primarily on the RF range. Table 1 summarizes both ELF and RF studies (mainly frequencies 50Hz, 60Hz, 900MHz, 1.8GHz) relevant to stimulation of DNA and stress protein synthesis in many different cells. The list is not exhaustive, but the citations represent the different frequencies and biological systems, as well as the diversity of results in the literature. As already noted by Cotgreave (2005), the stress response does not occur in reaction to EMFs in all cells. A paper by Jin et al (2000), to be discussed later, shows that even the same cell line can give opposite results in the same laboratory. The stress response is an important topic in its own right, but its importance for EMF research is that it offers insights into EMF interaction mechanisms in the stimulation of DNA. On the practical level, the stress response has shown the need to replace the SAR standard to take into account non-thermal biological effects.

Differences in experimental results shown in Table 1 are not uncommon when studying phenomena that are not as yet well understood, and this frequently gives rise to controversy. In EMF research, however, other factors have contributed to a controversial scientific atmosphere. The following sections on the scientific context, as well as a critique of the review by Cotgreave, will show how discussion of the stress response and the absence of discussion on related topics have compromised the evaluation of the science. The discussion of stress response stimulation in ELF and RF ranges together with ideas on DNA mechanisms, has important implications regarding EMF risk and safety.

VI.   The troubling context of today's science

The need to include basic research findings in assessment of health risks is clear, but it is

equally important to make sure that these findings are properly evaluated. No less an authority on science than Donald Kennedy (2006), the current Editor of *Science*, wrote "...how competitive the scientific enterprise has become, and the consequential incentive to push (or shred) the ethical envelope". He was referring primarily to the controversial religious/ political atmosphere over such issues as evolution, stem cell research, etc, but he could just as easily have included economic factors. In the following quote, editors of the *Journal of the American Medical Association* (JAMA 284:2203-2208, 2000) pointed out distortions in the proof of effectiveness of drugs in studies supported by the drug industry:

> "*There is a growing body of literature showing that faculty who have industry ties are more likely to report results that are favorable to a corporate sponsor, are more likely to conduct research that is of lower quality, and are less likely to disseminate their results to the scientific community*".

Even *The Wall Street Journal* (Jan 9, 2007), which generally presents favorable views of business, had a front page article on the controversy over whether mycotoxins produced by molds are harmful, that was critical of scientist-business community connections. They pointed out that some scientific experts in the professional societies, who had issued statements minimizing harmful effects, had not disclosed their links to companies defending lawsuits in this area.

The connection between scientific expertise, the research that is done, and the source of support, has always been an ethical gray area, but the above examples and recent instances of experimental fraud have reinforced the impression that the ethical standards of scientists have deteriorated considerably. In our area of interest, insufficient attention has been paid to the influence the power and communication industries may be having on the research of those assessing EMF safety. At the Third International Standard Setting Seminar (October 2003) in Guilin, China, Prof. Henry Lai of the University of Washington summarized 179 cell phone studies showing that independent researchers were twice as likely to report biological effects due to RF in comparison to those funded by industry. This was very much in line with the earlier JAMA comment on the drug industry. Published reports have started to appear (Hardell et al, 2006; Huss et al, 2007) documenting the correlation of EMF research outcome with the source of support. Recognition of the phenomenon is a first step toward minimizing abuses, and one hopes that this information will eventually be factored into evaluation of the experimental results. I am not overly optimistic, since those who wish their influence to remain hidden can channel support through unaffiliated committees with non-committal names.

Science is a cooperative enterprise in the long run, but in day-to-day practice, there has always been competition among scientists for recognition and support. In EMF research, the atmosphere has become especially adversarial in the selection of participants and subjects to be covered in recent evaluations. Two important examples are the International Committee on Electromagnetic Safety (ICES) and IEEE sponsored symposium on "Reviews of Effects of RF Energy on Human Health" (BEMS Supplement 6, 2003), and the more recent WHO sponsored symposium "Sensitivity of Children to

EMF Exposure" (BEMS Supplement 7, 2005). Both collections of papers appeared in *Bioelectromagnetics*, the journal of the primary research society in this scientific specialty, where publication carries a certain aura of authority in the field. Of course, one expects the highest of ethical standards, and the editor assured everyone that normal reviewing procedures, etc, had been followed. However, all that had come after the scope of the papers had been narrowly defined so that there was no coverage of recent research on the EMF stimulated stress response or stimulation of DNA to initiate protein synthesis. An older mind set pervaded the choice of the topics and the papers. That mind set appeared to be stuck in the belief that non-thermal EMF was biologically inert, that the nucleus was an impregnable structure that unlocked the genetic information in its DNA only at the time of cell division, etc. These two meetings took place only a few years ago, in a world of science where it had already been known for some time that biochemical signals are continuously changing DNA in cell nuclei and mitochondria, turning on protein synthesis, checking and repairing DNA itself, etc. Research on the stress response had even shown that DNA was unusually sensitive to EMF by finding responses in the non-thermal ELF range. One expects to find such papers in symposia organized by the Mobile Manufacturers Forum, but not in *Bioelectromagnetics*.

A science based evaluation process cannot limit its scope of interest so as to ignore a research area that is so central in biology today, and that is obviously affected by EMF. Information on the EMF stimulated stress response and stimulation of DNA to initiate protein synthesis must be an integral part of the evaluation process, and its omission in earlier evaluations compromised the scientific basis of those reviews and distorted their conclusions.

It is ironic that the review in *Bioelectromagnetics* Supplement 6 listed as its first guiding principle that "The RF safety standard should be based on science", essentially a reaffirmation of the IEEE guideline for the revision of C95.1-1991 safety standards. Scientific research is designed to answer questions, and answers do not come from deciding *a priori* that certain types of studies are not relevant or can be ignored because they have not been adequately proven in the eyes of the organizers. Scientific method is not democratic. The word 'proof' in 'scientific proof' is best understood in terms of its older meaning of 'test'. It does not rely on an adversarial 'weight of the evidence', where opposing results and arguments are presented and compared. Answers do not come from keeping a scoreboard of positive versus negative results and merely tallying the numbers to get a score. In scientific proof, number and weight do not count. It is hard to see how the review in *Bioelectromagnetics* Supplement 6 could reconcile its advocacy of science as a guiding principle with its subsequent endorsement of "the weight of evidence approach" to be used in their assessment.

We should be reminded that 'scientific proof' is not symmetric (Popper, 1959). One cannot prove that EMF is harmless no matter how many negative results one presents. One single reproducible (significant) harmful effect would outweigh all the negative results.

The above characteristics of science are generally acknowledged to be valid as abstract

principles, but in EMF research, it has been quite common to list positive and negative findings and thereby imply equal weights. Table 1 is an alphabetical listing by first author of positive and negative findings, with the negative studies indicated as **NO** in bold. There is no scoreboard, since the studies are on many different systems, etc, and not of the same quality. The listing is not meant to be complete or to be scored, but rather to present the variety of biological systems studied in the different EMF ranges. Negative studies play an important role in science, and there is good reason to publish them when they are failures to replicate earlier positive results. This can often lead to important clarifications of the effect, the technique, etc. However, negative studies are being used in another way. Although they cannot prove there is no positive effect, they do have an influence in the unscientific 'weight of evidence approach'. In epidemiology, where it is difficult to compare studies done under different conditions, it is common to make a table of the positive and negative results. The simple listing has the effect of a tally, and the overall score substitutes for an evaluation. In any case, one can write that the evidence is 'not consistent', 'not convincing' or claims are 'unsubstantiated' and therefore 'unproven'. The same is true in experimental studies. Funds are generally not available for an independent study to track down the causes of the differences in results, so the contradictory results are juxtaposed and a draw is implied. This is a relatively cheap but effective way to neutralize or negate a positive study.

VII.   Replication and failures to replicate experimental results

Independent replication of experiments is an essential criterion for acceptance of a result and one of the pillars of scientific proof. However, as we shall see below, it is very difficult to actually replicate a biological experiment. We need only remember the experience with the 'Henhouse' project run by the Office of Naval Research many years ago, when chicken eggs from different suppliers led to different effects of EMFs on chick embryo development.

While scientists generally shun replications, some failures to replicate have been analyzed and explained. The two discussed below had the earmarks of replications, but neither was. In one case, it was clearly shown by Jin et al (2000) that the investigators failed to use the precise cell type population of the original experiment. Jin et al obtained HL60 cells from the two different sources used in the papers with the contradictory results, and showed that the cells had very different growth characteristics, significantly different reactivities and reactions to EMFs. It appears that even different samples of the same cell line in the same laboratory can have different responses to EMFs. The changes that occur in tissue culture over time can result in very different responses to EMFs.

In another example, Utteridge et al (2002) published a paper in *Radiation Research* meant to test the positive results of an earlier study (Repacholi et al, 1997) that had shown a twofold increase in lymphoma in mice exposed to cell phones. They failed to replicate the findings, but even a cursory reading of the paper showed that the study was

poorly designed and executed, and was definitely not a replication. They had used a different exposure regimen and had manually handled the animals, an added stress on the mice. The cancer rate in the control group was three times the rate of the earlier study, possibly due to the handling, making it almost impossible to find any effect of cell phone exposure. There were also unusual inconsistencies in the published data, such as listing the weights of animals that had died months earlier. It is hard to see how the paper passed peer review. The Utteridge study self-destructed, and the results of the Repacholi study are still looked upon as showing a relation between RF and cancer in an animal model. However, there were scientific casualties, the peer review process of the journal and the credibility of its editors.

It may be appropriate to mention that *Radiation Research*, a journal devoted to research with ionizing radiation frequencies, has published studies that almost exclusively show no EMF effects. A quick glance at Table 1 will show that many of the '**NO** effect' listings are published in that journal. It has even gone beyond the frequency range defined in its title and published 'negative' studies in the non-ionizing frequency range. The internet edition of *Microwave News* has an explanation for why this journal repeatedly publishes negative research and appears to have become so politicized on the EMF issue.

It is not unusual for scientists to deviate from an original experimental protocol when repeating an experiment. They generally view the deviations as improvements in technique. Readers who have not worked on that particular system are unlikely to focus on a small difference that does not appear to be significant. Yet, even a small difference may lead to a failed replication. Blank and Soo (2003) showed that EMF accelerated the Belousov-Zhabotinsky (BZ) reaction, which is the catalyzed oxidation of malonic acid. A subsequent study reported no effect of EMF on the BZ reaction (Sontag, 2006), in essence a failed replication. In the second study, the authors did not apply the field at the time the reactants were mixed, as in the original, but only after the reaction was well under way for about seven minutes. This time difference was critical for a reaction that responds to EMF. Other reactions had responded to EMF (Blank and Soo, 2001b; Blank, 2005) only when the field was applied at time zero, when the intrinsic chemical forces were relatively weak. The effect of EMF was even shown to vary inversely with the opposing chemical forces of an enzyme (Blank, 2005). After seven minutes, the BZ reaction was running at full speed and the applied ELF fields were not strong enough to overcome the built up chemical forces.

The above paragraph points up a critical factor often overlooked in EMF experiments. EMF is only one of the factors that can affect the rate of a biochemical reaction, and a relatively weak one in the ELF range. It appears that when an EMF accelerates charge movements associated with a reaction, the applied field competes with intrinsic forces, and the ability to see an effect of the applied EMF depends on minimizing the other forces in the system. It is obvious that an important strategy to minimize unwanted biological effects due to EMF is to maintain intrinsic forces at optimal (healthy) levels.

In the above mentioned experiments with the Na,K-ATPase (Blank, 2005), it was found

that the effect of an applied electric or magnetic field varied inversely with the activity of the enzyme, which could be changed by changing ion concentrations, temperature, inhibitors, or by the normal aging of the preparation. The effect of intrinsic activity was also observed in other systems, electron transfer from cytochrome C to cytochrome oxidase (Blank and Soo, 1998), and in the effect of temperature on the oxidation of malonic acid (Blank and Soo, 2003). Since the effect of EMF in an experiment can vary depending on the other forces acting in the system, it is important to make sure that all relevant parameters are identified and controlled. Replication of biological experiments must ensure a comparable level of intrinsic biological activity before a perturbing EMF is applied. This is especially difficult with enzyme preparations as they age.

In studies of stress protein synthesis, many factors must be considered, but the choice of cells is particularly important. Not all cells respond to EMF, and the results of many experiments have suggested ideas about critical properties that are apt to determine the response and also affect the ability to replicate an experimental result.

A quick look at Table 1 shows that tissue culture cells are more likely to show 'NO effect'. That is not really surprising. Cells in tissue culture have changed significantly to enable them to live indefinitely in the unnatural conditions of a flask in a laboratory, and the changes could have made them unresponsive to EMF. The same is true of the changes in cancer cells, although some (e.g., MCF7) have responded to EMF (e.g., Liburdy et al, 1993), and in one cell line, HL60, some samples respond to EMF and others do not (Jin et al, 2000). On the other hand, the study by Czyz et al (2004) found that p53-deficient embryonic stem cells showed an increased EMF response, but the wild type did not. It is obviously difficult to make generalizations about the necessary conditions for a response to EMF when there are so many variations, and cells can undergo changes in tissue culture.

Some insight into differences between cells has been obtained from a broad study of genotoxic effects in different kinds of cells (Ivancsits et al, 2005). They found no effects with lymphocytes, monocytes and skeletal muscle cells, but did find effects with fibroblasts, melanocytes and rat granulosa cells. Other studies (e.g., Lantow et al, 2006b; Simko et al, 2006) have also found that the blood elements, such as lymphocytes and monocytes are natural cells that have not responded. From an evolutionary point of view, it may be that mobile cells can easily move away from a stress and there is little selective advantage to develop the stress response. The lack of response by skeletal muscle cells is easier to explain (Blank, 1995). It is known that cells containing fast muscle fibers do not synthesize hsp70, while those with slow fibers do. This evolutionary development protects cells from over-reacting to the high temperatures reached in fast muscles during activity.

Other natural cells listed in Table 1, such as epithelial, endothelial and epidermal cells, fibroblasts, yeast, E coli, developing chick eggs, the cells of *Drosophila*, *Sciara* and C elegans, have all been shown to respond. While experiments with non-responding cells have provided little information, studies of the differences between responding and non-

responding cells may be the best experimental strategy for studying the stress response mechanism. Proteomics appears to be an excellent tool for answering many of the questions about the molecular mechanisms that are activated (Leszczynski et al, 2004).

In studies of stress protein synthesis, the time course of a response must be determined. There is generally a rapid induction and a slower falloff of response, but the kinetics can be affected by many other conditions of the experiment. It is, therefore, important to look for stress proteins when they are apt to be present, and not before they have been synthesized or after the response has decayed. This may be the explanation for the inability of Cleary et al, (1997) to observe stress proteins twenty-four hours after exposure. Some additional cautions to be aware of in contemplating or evaluating a study. For example, different stresses elicit different responses, so it is important to determine which of the ~20 different stress proteins are synthesized. The most frequently studied stress proteins are hsp70 and hsp27, but others may be involved and undetected. The exposure history of a cell population must be known, since there are differences in the responses to an initial stimulus and subsequent ones. The need to provide shielding for cells becomes far more complicated when they respond to RF as well as ELF fields and one must insure no pre-exposure.

Obviously, many experiments must be done to determine the optimal conditions for the study of a particular system. This does not shift the burden of proof to those unable to find an effect, but it adds weight to the cautions generally voiced in papers that state their failure to observe stress proteins 'under our experimental conditions'. Those words mean just that, and not that stress proteins were absent.

An experiment on EMF stimulation of cell growth that has almost disappeared from the EMF literature is the work of Robert Liburdy (Liburdy et al, 1993). He reported that weak 60Hz fields can interfere with the ability to inhibit growth in MCF7 breast cancer cells. This finding has been replicated six times, but the original experiment and its replications have been ignored by many health oriented scientists (Liburdy, 2003), including the recent WHO review (BEMS Supplement 7, 2005). Even breast cancer researchers (e.g., Loberg et al, 1999), who have not been directly involved in the EMF debate, appear to be totally unaware of results showing the ability of weak 60Hz fields to affect cancer cell growth. It is shocking when an EMF research review by a presumably scientifically neutral WHO fails to even mention any of the papers that offers insight into the mechanism of a devastating disease that is so prevalent in the population (Blank and Goodman, 2006). Let us not forget the asymmetry in scientific proof (Popper, 1959), where a single reproducible harmful effect would outweigh all the negative results. The many replications of the Liburdy experiment have given us a crucial finding regarding the question of EMF risk, and they cannot be ignored.

VIII.   A critical look at a recent review of the stress response

The earlier discussion of non-scientific influences in the design and presentation of the results of EMF research serves as an introduction to a critical look at the recent review on

RF and the stress response by Cotgreave (2005) 'with contributions of the Forschunggemeinschaft Funk'. I agree with the major conclusion of the review, the need for more research on the stress response with better controls. However, Cotgreave was highly selective in his omission of papers on ELF and stress proteins. Given that there are many relevant ELF papers reporting effects on stress proteins at non-thermal levels, this omission results in significant under-reporting of what is scientifically established. These obvious and scientifically questionable omissions were used to cast doubt on the ability of RF to have a significant biological effect, at a time when much evidence pointed in the opposite direction.

Cotgreave stated correctly that RF is pleiotropic (produces more than one gene effect) for many regulatory events, in addition to the stress response. That observation comes as no surprise to biologists who know that cellular systems are interconnected and that the complexity of the signaling pathways resembles that of the old interlinked intermediary metabolism charts. It is also no surprise to those familiar with early papers on EMFs, which showed activation of genes such as c-*myc* (Goodman and Shirley-Henderson, 1991; Lin et al, 1994;1996) and c-*fos* (Rao and Henderson, 1996) at about the same time the EMF stress response was first described (Blank et al, 1994; Goodman et al, 1994). The EMF stimulated synthesis of many proteins (Goodman and Henderson, 1988) and the binding of specific transcription factors AP-1, AP-2 and SP-1 were also previously described (Lin et al, 1998).

By highlighting the previously known pleiotropic nature of the EMF response, Cotgreave played down the role of the stress response as a protective mechanism. Had he analyzed the biological implications of the many genes activated, he could have pointed to evidence from proteomics and gene analysis that there is a relevant pattern to the pleiotropism. Kültz (2005) recently summarized the evidence that specific groups of genes are activated along with stress genes across the biological spectrum. It is of particular interest to the EMF discussion that this 'universally conserved proteome' consists largely of genes involved in sensing and repairing damage to DNA and proteins, evidence that the stress response is a reaction to molecular damage across the biological spectrum. The stress response is one of many stimulated by RF, but other parts of the response also show evidence of damage control in reaction to an EMF.

By limiting the scope of his review to effects of RF, Cotgreave overlooked much that is relevant to understanding the effects of EMFs. That was a bit like writing a review on the physiological effects of alcohol and limiting the discussion to scotch whiskey. The EM spectrum is continuous and its divisions arbitrary, so there is no good reason to limit the discussion to RF when living cells are activated and synthesize stress proteins in both RF and ELF ranges (Blank and Goodman, 2004a). Furthermore, emissions from cell phones include both RF and ELF frequencies (Linde and Mild, 1997; Jokela, 2004; Sage et al, 2007). The bulk of the original research on EMFs and the stress response was done using ELF (see review by Goodman and Blank, 1998). ELF studies also led to information about the DNA consensus sequence sensitive to EMFs that differs from the 'heat shock' consensus sequence (Lin et al, 1999). This is a critical piece of molecular evidence showing the difference between thermal and non-thermal responses. Cotgreave described

the heat shock consensus sequence, but not the EMF consensus sequence or the experiments in which such sequences were transferred and retained sensitivity to an EMF (Lin et al, 2001). For any insight into EMF-DNA interaction, it was absolutely essential to describe the molecularly based biological sensitivity to EMFs, inherent in DNA structure, that differs from thermal sensitivity and that can be manipulated.

More importantly, by considering both ELF and RF responses, it becomes obvious that the practice of describing EMF 'dose' in terms of SAR is meaningless for the stress response (Blank and Goodman, 2004a). The research on ELF stimulated stress response has shown unequivocally that SAR at the threshold is many orders of magnitude lower than in the RF range. The separation of thermal and non-thermal mechanisms had already been shown by Mashevich et al (2002), where chromosomal damage observed under RF in lymphocytes was not seen when the cells were exposed to elevated temperatures. The importance of non-thermal mechanisms was also made clear in the experiments of Bohr and Bohr (2000) in a much simpler biochemical system, showing that both denaturation and renaturation of β-lactoglobulin are accelerated by microwave EMF, and by de Pomerai et al (2003), who showed that microwave radiation causes protein aggregation without bulk heating. These as well as the ELF enzyme kinetics studies listed in Table 2 should have indicated that EMFs can cause changes in molecular structure without requiring heating.

Cotgreave overlooked a similarity between electric and magnetic ELF stimulation of DNA and endogenous electric stimulation of protein synthesis. Blank (1995) had reviewed this effect in striated muscle, and recently Laubitz et al (2006) showed that myoelectrical activity in the gut can trigger heat shock response in E coli and Caco-2 cells. The mechanism in striated muscle is well known. Body builders stimulate muscle activity to increase muscle mass, and biologists have known that the electric fields associated with muscle action potentials stimulate the synthesis of muscle proteins. The particular proteins synthesized appear to be related to the frequency of the action potentials, and one can even change the protein composition of a muscle by changing the frequency of the action potentials (Pette and Vrbova, 1992). Under normal physiological conditions, the action potentials along the muscle membrane drive currents across the DNA in nuclei adjacent to the membrane. The estimated magnitude of electric field, ~10V/m, provides a large safety margin in muscle, since fields as low as 3mV/m stimulate biosynthesis in HL60 cells (Blank et al, 1992). The fact that a physiological mechanism links electric stimulation to protein synthesis suggests that EMF can cause stress protein synthesis by a similar mechanism.

As a matter of proper scholarly attribution "heat shock' was first described in Drosophila by Ritossa (1962), and the first description of stress response due to EMF was in back-to-back papers showing similar protein distributions stimulated by temperature and ELF (Blank et al, 1994), and that both stimuli resulted in proteins that reacted with the same specific antibody for the stress protein hsp70 (Goodman et al, 1994). The ability of power frequency fields to alter RNA transcription patterns had been reported even earlier by Goodman et al (1983).

The above discussion acknowledges that Cotgreave's review was a positive contribution that summarized much useful information, but one that failed to properly assess the state of knowledge in EMF stress protein research. He gave the impression that much of the information was tenuous and that the thermal mechanism was the only one to consider. This may be his point of view and that of co-contributor, Forschunggemeinschaft Funk. However, at the very least, he should have incorporated relevant research on stimulation of the stress response by non-thermal EMFs. The ELF data have convinced many to reject the paradigm of thermal effects only. A reader would have learned more about the stress response had the author devoted more space to the ELF papers than to papers on something called 'athermal heating'.

IX.   Rethinking EMF safety in a biology context

Studies of the stress response in different cells under various conditions have enabled us to characterize the molecular mechanisms by which cells respond to EMF and their effects on health risk. That information can now correct assumptions about biological effects of EMF, and establish a scientific basis for new safety standards.

In setting standards, it is essential that basic findings in all relevant research areas are taken into account. Relevance is not subjective. It is determined by whether a study adds to our knowledge of how cells react to EMF, and this criterion determined inclusion of the references in Table 1. The criteria for the references in the IEEE list were not focused on the molecular biology of cellular responses that illuminate disease mechanisms, but were based on such assumptions as arbitrarily defined divisions of the spectrum, on thermal responses only, etc. It is therefore not surprising that many relevant studies were omitted in the IEEE literature review. Fewer than one quarter of the references listed in Table 1 appear in the IEEE list. The result of having omitted many EMF studies, including those on the stress response, is that many research results have not been utilized in setting EMF safety standards. A careful examination of basic assumptions will show that the omissions are crucial and that they indicate an urgent need to reconsider the entire basis for EMF safety standards. Here in bold are the assumptions, followed by the re-evaluations:

> • **Safety standards are set by division of the EM spectrum.** It may come as a surprise to the engineers and physicists who set up the divisions of the EM spectrum, but biology does not recognize EM spectrum divisions.   The same biological reaction can be stimulated in more than one subdivision of the EM spectrum. The arbitrarily defined divisions of the spectrum do not in any way confine the reactions of cells to EMF, and ELF studies do indeed contribute to an understanding of how cells respond to RF. This was discussed in the critique of Cotgreave's (2005) review. This area clearly demands immediate attention. People are getting ELF and RF simultaneously from the same device, and they are being protected from thermal effects only. This ignores the potentially harmful

21

effects from non-thermal ELF and RF discussed next.

•   **EMF standards are based on the assumption that only ionizing radiation causes chemical change.** The stress response in both ELF and RF ranges has shown that non-ionizing radiation also causes chemical change. Several additional examples of EMF stimulated chemical change in the ELF range are listed in Table 2.

•   **EMF standards are based on the assumption that non-ionizing EMF only causes damage by heating (i.e., damage by thermal effects only).** Research on the stress response in the ELF range has shown that a thermal response to a rise in temperature and the non-thermal response to EMF are associated with different DNA segments of the same gene. Both the thermal and the non-thermal mechanisms are natural responses to potential damage. Furthermore, the non-thermal stress response can occur in both the ELF and RF ranges. Other non-thermal effects of EMF have been demonstrated, e.g., acceleration of electron transfer reactions and DNA strand breaks.

•   **Safety limits in the non-ionizing range are in terms of rate of heating (SAR).** The above described effects occur below the thermal safety limits in the non-ionizing range, so the safety limits provide no protection against non-thermal damage. Safety limits must include non-thermal effects.

X.   Summary

It is generally agreed that EMF safety standards should be based on science, yet recent EMF research has shown that a basic assumption used to determine EMF safety is not valid. The safety standard assumes that EMF causes biological damage only by heating, but cell damage occurs in the absence of heating and well below the safety limits. This has been shown in the many studies, including the cellular stress response where cells synthesize stress proteins in reaction to potentially harmful stimuli in the environment, including EMF. The stress response to both the power (ELF) and radio (RF) frequency ranges shows the inadequacy of the thermal (SAR) standard.

The same mechanism is stimulated in both ranges, but in the ELF range, where no heating occurs, the energy input rate is over a billion times lower than in the RF range.

The stress response is a natural defense mechanism activated by molecular damage caused by environmental forces. The response involves activation of DNA, i.e., stimulating stress genes as well as genes that sense and repair damage to DNA and proteins. Scientific research has identified specific segments of DNA that respond to EMF and it has been possible to move these specific segments of DNA and transfer the sensitivity to EMF. At high EMF intensities, the interaction with DNA can lead to DNA strand breaks that could result in mutation, an initiating step in the development of cancer.

Scientific research has shown that ELF/RF interact with DNA to stimulate protein synthesis, and at higher intensities to cause DNA damage. The biological thresholds (field strength, duration) are well below current safety limits. To be in line with EMF research, a biological standard must replace the thermal (SAR) standard, which is fundamentally flawed. EMF research also indicates a need for protection against the cumulative biological effects stimulated by EMF across the EM spectrum.

XI.   References

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Table 1. Studies of EMF Stimulation of DNA and Protein Synthesis
(page 1)

Table 1 summarizes both ELF and RF studies (mainly frequencies 50Hz, 60Hz, 900MHz, 1.8GHz) relevant to stimulation of DNA and stress protein synthesis in many different cells.

Study/Journal	Frequency	Cells/effect on hsps
Balcer-Kubicek et al, 1996 Radiation Res	60Hz	HL60 **NO** synthesis of myc
Blank et al, 1994 Bioelectrochem Bioenerg	60Hz	*Sciara* salivary glands [temperature, EMF, cause same new proteins]
Capri et al, 2004 Int J Radiat Biol	1800MHz	monocytes **NO** effect on apoptosis, hsp70
Caraglia et al, 2005 J Cell Physiol	1.95GHz	epidermoid cancer cells Induces apoptosis, hsp70
Chauhan et al, 2006 Radiation Res	1.9GHz	human lymphoblastoma (TK6) **NO** hsp response
Chauhan et al, 2006 Int J Radiat Biol	1.9GHz	two human immune cell-lines HL60,MM6 **NO** hsp response
Cleary et al, 1997 Bioelectromagnetics	27MHz	HeLa, CHO (also at 2450MHz mammalian cells **NO** hsp after 2 hr exposure, 24 hr to measurement
Chow and Tung, 2000 FEBS Letters	50Hz	E. coli strain XL-1 BLUE + plasmid pUCB DNA repair improved
Czyz et al, 2004 Bioelectromagnetics	modulated 1.71GHz	p53-deficient embryonic stem cells hsp70 expression, but not in wild type

Table 1. Studies of EMF Stimulation of DNA and Protein Synthesis
(page 2)

Daniells et al, 1998   750MHz   C elegans
Mutat Res   induced hsp16

Dawe et al, 2005   750MHz   C elegans (same lab as above paper)
Bioelectromagnetics   hsp 16 may be due to temperature rise

Di Carlo et al, 2002   60Hz   chick embryo
J Cell Biochem   repeated EMF causes lower hsp response

Diem et al, 2005.   1800MHz   fibroblasts, GFSH-R-17 granulosa cells
Mutation Res   non-thermal DNA breakage

Fritze et al, 1997   900MHz   rat brain
Neuroscience   blood brain barrier leakage at high SAR

Goodman et al, 1983   pulsed 60Hz   *Sciara* larvae
Science   induce cellular transcription

Goodman et al, 1994   60Hz   *Sciara* larvae
Bioelectrochem Bioenerg   increased hsp70 transcripts

Harvey et al, 2000   864.3MHz   human mast cell line, HMC-1
Cell Biol Int   effects on protein kinase C , stress genes

Hirose et al, 2006a   2.1425GHz   Human IMR-90 fibroblasts
Bioelectromagnetics   **NO** effect on gene expression of p53

Hirose et al, 2006b   2.1425GHz   human glioblastoma A172, IMR-90
Bioelectromagnetics   fibroblasts
                                                       NO effect on apoptosis,
                                                       phosphorylation of hsp27

Ivancsits et al, 2005   intermittent 50Hz   **NO** effect lymphocyte, monocyte,
Mutation Res   muscle: DNA damage: fibroblast,
                                                       melanocyte, rat granulose

Jin et al, 1997   60Hz   HL60 cells from two sources
Bioelectrochem Bioenerg   *myc* expression in one population, not in other

Kwee et al, 2001   960MHz   human epithelial amnion (AMA) cells
Electro- and Magnetobiology   hsp70 increased

Table 1. Studies of EMF Stimulation of DNA and Protein Synthesis
(page 3)

Lacy-Hulbert et al, 1995 Radiation Res	50Hz	HL60 **NO** synthesis of myc or β-actin
Lai & Singh, 1997a Bioelectromagnetics	60Hz	rat brain cells melatonin blocks DNA strand breaks
Lai & Singh, 2005 Electromag Biol Med	1800MHz	rat brain cells noise blocks DNA strand breaks
Lantow et al, 2006a Radiation Res	1800MHz	human Mono Mac 6 and K562 cells **NO** hsp response
Lantow et al, 2006b Radiat Environ Biophys	1800MHz	primary human monocytes, lymphocytes **NO** hsp response
Lantow et al, 2006c Radiation Res	1800MHz	human Mono Mac 6 and K562 cells **NO** effect on apoptosis or necrosis
Laszlo et al, 2005 Radiation Res	835MHz	cultured mammalian cells **NO** 'effect within sensitivity of assay'
Laubitz et al, 2006 Experimental Physiol	muscle generated ELF	E coli, Caco-2 cells induce hsp70, protect vs apoptosis
Lee JS et al, 2005 Int J Radiat Biol	849, 1763 MHz	hsp70.1-deficient mice **NO** hsp induction
Lee S et al, 2005 FEBS Lett	2.45GHz	cultured human cells gene regulation: apoptosis 88, cell cycle99
Leszczynski et al, 2002 Differentiation	900MHz	human endothelial cells activate hsp27/p38MAPK stress pathway
Liburdy et al, 1993 J Pineal Res	60Hz	ER$^+$ MCF7 breast cancer cells block melatonin's oncostatic action
Lim et al, 2005 Radiation Res	900MHz	human leukocytes. **NO** effect on hsp
Lin et al, 1994 J Cell Biochem	60Hz	human HL60 cells EMF region of the c-*myc* promoter

Table 1. Studies of EMF Stimulation of DNA and Protein Synthesis
(page 4)

Lin et al, 1996	60Hz	human HL60 cells
Bioelectrochem Bioenerg		changes in c-myc transcript levels
Lin et al, 1999	60Hz	human HL60 cells
J Cell Biochem		EMF consensus sequence in HSP70 promoter
Lin et al, 2001	60Hz	human HL60 cells
J Cell Biochem		EMF consensus sequence response elements
Lixia et al, 2006	1.8GHz	human lens epithelial cells
Mutat Res		increased hsp70 protein
Maes et al, 2006 [Epub]	900MHz	peripheral blood lymphocytes
Mutagenesis		**NO** effect on DNA damage
Malagoli et al, 2004	50Hz	mussel immunocyte
Comp Biochem Physiol		activate p38 MAP kinase,
		induce hsp70, hsp90
Mashevich et al, 2003	830MHz	human peripheral blood lymphocytes
Bioelectromagnetics		chromosomal instability
McNamee et al, 2002	1.9Ghz	human leukocytes
Radiat Res		**NO** effect on DNA damage, micronuclei
Miyakawa et al, 2001	60Hz	C elegans
Bioelectromagnetics		induction of hsp16
Nylund & Leszczynski,2004	900MHZ	human endothelial cell line EA.hy926
Proteomics		effects on cytoskeletal proteins
Nylund & Leszczynski,2006	900MHZ	human endothelial cell line EA.hy926
Proteomics		response genome- and proteome-dependent
Oktem et al, 2005.	900MHz	rats (oxidative kidney damage)
Arch Med Res		oxidative damage protected by melatonin
Ozguner et al, 2005	900MHz	rats (oxidative myocardial damage)
Toxicol Ind Health		protection by caffeic acid phenethyl ester

Table 1. Studies of EMF Stimulation of DNA and Protein Synthesis
(page 5)

Penafiel et al, 1997   840MHz (AM, FM)   mouse L929 cells (ornithine
Bioelectromagnetics   decarboxylase activity)
frequency dependent AM effect,
no FM effect

Phillips et al, 1998   813, 836MHz   Molt-4 T-lymphoblastoid cells
Bioelectrochem Bioenerg   DNA damage (and ability to repair)
varied with SAR

Saffer & Thurston, 1995   60Hz   HL60, Daudi cells
Radiation Res   **NO** synthesis of myc

Sanchez et al, 2006   900MHz   human skin cells
FEBS J   slight but significant increase in hsp70

Sarimov et al, 2004   895, 915MHz   transformed human lymphocytes
IEEE Trans Plasma Sci   affect chromatin conformation

Shallom et al, 2002   915MHz   chick embryos
J Cell Biochem   induces hsp70, protects against hypoxia

Shi et al, 2003.   60Hz   human keratinocytes
Environ health Perspect   **NO** phosphorylation, expression of hsp27

Simko et al, 2006   900MHz   human Mono Mac 6 cells
Toxicol Lett   **NO** hsp reponse

Vanderwaal et al, 2006   900MHz   cultured HeLa, S3 and EA Hy296 cells
\Int J Hyperthermia   **NO** hsp27 phosphorylation increases

Velizarov et al, 1999   960MHz   human epithelial cells
Bioelectrochem Bioenerg   cell proliferation

Wang et al, 2006   2450MHz   human glioma A172 cells
Bioelectromagnetics   **NO** hsp70, hsp27

Weisbrot et al, 2003   900MHz   *Drosophila*
J Cell Biochem   hsp708, affects development, reproduction

Winker et al, 2005   intermittent 50Hz   human diploid fibroblasts
Mutation Res   micronuclei, chromosomal damage

Table 2   **Biological Thresholds in the ELF Range**

Biological System	Threshold*	Reference
Enzyme reaction rates		
Na,K-ATPase	.2-.3µT	Blank & Soo, 1996
cytochrome oxidase	.5-.6µT	Blank & Soo, 1998
ornithine decarboxylase	~2µT	Mullins et al, 1999
Oxidation-reduction rate		
Belousov-Zhabotinsky	<.5µT	Blank & Soo, 2001b
Biosynthesis of stress proteins		
HL60, Sciara, yeast,	<.8µT	Goodman et al, 1994
breast (HTB124, MCF7)	<.8µT	Lin et al, 1998
chick embryo (anoxia)	~2µT	DiCarlo et al, 2000
Disease related block melatonin inhibition		
of breast carcinoma	.2<1.2µT	Liburdy et al, 1993
leukemia epidemiology	.3-.4µT	Ahlbom et al, 2000
		Greenland et al, 2000

*The estimated values are for departures from the baseline, although Mullins et al (1999) and DiCarlo et al (2000) generally give inflection points in the dose-response curves. The leukemia epidemiology values are not experimental and are listed for comparison.

SECTION 8

EVIDENCE FOR EFFECTS ON THE IMMUNE SYSTEM

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Table of Contents

I. Basic concepts and components of the immune system

The human immune system is part of a general defense barrier towards our surrounding environment. We live in a biological system, the world, dominated by various microorganisms, including microbes and viruses, many of which can cause harm. The immune system serves as the primary line of defense against invasion by such microbes. As we are, practically speaking, built as a tube, the outer surface - the skin - and the innermost surface - the gastrointestinal tract - are the major borders between us and the rest of the universe. These borders must be guarded and protected since any damage to them could be fatal.

The skin and the mucous membranes are part of the innate or non-adaptive immune system. However, if these barriers are broken (e.g. after cutting a finger), then microbes, including potential pathogens (i.e. harmful microbes) can enter the body and then begin to multiply rapidly in the warm, moist, nutrient-rich environment. The cut may not be as physical, brutal and abrupt as a knife cut, it could also very well be an internal leakage, such as the one found after microwave exposure of the fragile blood-brain-barrier (cf. Persson et al, 1997). Such a leakage could indeed be fatal, causing nerve cell damage and consecutive cellular death (cf. Salford et al, 2003).

One of the first cell types to be encountered by a foreign organism after a cut in the skin is the phagocytic white blood cells which will congregate within minutes and begin to attack the invading foreign microbes. Following this, the next cell type to be found in the area of such a local infection will be the so-called neutrophils. They are also phagocytic and use pattern-regonizing surface receptor molecules to detect structures commonly found on the surface of bacteria. As a result, these bacteria - as well as other forms of particulate materia - will be ingested and degraded by the neutrophils. Various other protein components of serum, including the complement components may bind to the invader organisms and facilitate their phagocytosis, thereby further limiting the source of infection/disease. Other small molecules, the interferons, mediate an early response to viral infection by the innate system.

The innate immune system is often sufficient to destroy invading microbes. If it fails to clear an infection, it will rapidly activate the adaptive or acquired immune response, which - as a consequence - takes over.The molecular messenger connection between the innate and the adaptive systems are molecules known as cytokines (actually, the interferons are part of this molecular family).

The first cells in this cellular orchestra to be activated are the T and B lymphocytes. These cells are normally at rest and are only recruited at need, i.e. when encountering a foreign (=non-self) entity referred to as an antigen. The T and B lymphocytes, together with a wide spectrum of other cell types, have antigen receptors or antigen-recognizing molecules on their surface. Among them you find the classical antibodies (=B cell antigen receptors), T cell antigen receptors as well as the specific protein products of special genetic regions (=the major histocompatibility complexes). The genes of humans are referred to as human leukocyte antigen (HLA) genes and their protein products as HLA molecules. The antibodies - apart from being B cell surface receptors - are also found as soluble antigen-recognizing molecules in the blood

(immunoglobulins). The adaptive immune response is very highly effective but rather slow; it can take 7-10 days to mobilize completely. It has a very effective pathogen (non-self) recognition mechanism, a molecular memory and can improve it's production of pathogen-recognition molecules during the response.

A particularly interesting set of cells are the various dendritic cells of the skin. In the outermost portion, the epidermis, you find both dendritic melanocytes, the cells responsible for the pigment-production, as well as the Langerhans cells with their antigen-presenting capacity. In the deeper layer, the dermis, you find corresponding cells, as well as the basophilic mast cells, often showing a distinct dendritic appearance using proper markers such as chymase, tryptase or histamine. All these cells are the classical reactors to external radiation, such as radioactivity, X-rays and UV light. For that reason, our demonstration (Johansson et al, 1994) of a high-to-very high number of somatostatin-immunoreactive dendritic cells in the skin of persons with the functional impairment electrohypersensitivity is of the greatest importance. Also, the alterations found in the mast cell population of normal healthy volunteers exposed in front of ordinary house-hold TVs and computer screens (Johansson et al, 2001) are intriguing, as are the significantly increased number of serotonin-positive mast cells in the skin ($p<0.05$) and neuropeptide tyrosine (NPY)-containing nerve fibers in the thyroid ($p<0.01$) of rats exposed to extremely low-frequency electromagnetic fields (ELF-EMF) compared to controls, indicating a direct EMF effect on skin and thyroid vasculature (Rajkovic et al, 2005a,b, 2006; for further details and refs., see below). In the gastrointestinal tract, you will find corrsponding types of cells guardening our interior lining towards the universe.

In essence, the immune system is a very complex one, built up of a large number of cell types (B and T lymphocytes, macrophages, natural killer cells, mast cells, Langerhans cells, etc.) with certain basic defense strategies. It has evolved during an enormously long time-span and is constructed to deal with it's known enemies, including bacteria. Among the known enemies are, of course, not modern electromagnetic fields, such as power-frequent electric and magnetic fields, radiowaves, TV signals, mobile phone or Wi-Fi microwaves, radar signals, X-rays or radioactivity. They have been introduced during the last 100 years, in many cases during the very last decades. They are an entirely new form of exposure and could pose to be a biological "terrorist army" against which there are no working defence walls. They do penetrate the body from outside and in. Some of them have already been proven to be of fatal nature, and today no-one would consider having a radioactive wrist watch with glowing digits (as you could in the 1950s), having your children's shoes fitted in a strong X-ray machine (as you could in the 1940s), keeping radium in open trays on your desk (as scientists could in the 1930s), or X-raying each other at your garden party (as physicians did in the 1920s). That was, of course, just plain madness. However, the persons doing so and selling these gadgets were not misinformed or less intelligent, not at all. The knowledge at the time was just lacking as was a competent risk analysis behaviour coupled to a parallel analysis of true public need.

II. Hypersensitivity reactions

The immune system can react in an excessive manner and it can cause damage to the local tissue as well as generally to the entire body. Such events are called

hypersensitivity reactions and they occur in response to three different types of antigens: a) infectious agents, b) environmental disturbances, and c) self-antigens. The second one is related to the impact of the new electromagnetic fields of today's modern world. Hypersensitivity can occur in response to innocuous environmental antigens - one example of this is allergy. For example, in hay fever, grass pollens themselves are incapable of causing damage; it is the immune response to the pollen that causes harm.

II A. Hypersensitivity to environmental substances

For environmental substances to trigger hypersensitivity reactions, they must be fairly small in order to gain access to the immune system. Dust triggers off a range of responses because they are able to enter the lower extremities of the respiratory tract, an area that is rich in adaptive immune-response cells. These dusts can mimic parasites and may stimulate an antibody response. If the dominant antibody is IgE, they may subsequently trigger immediate hypersensitivity, which is manifest as allergies such as asthma or rhinitis, If the dust stimulates IgG antibodies it may trigger off a different kind of hypersensitivity, e.g. farmer's lung.

Smaller molecules sometimes diffuse into the skin and these may act as haptens, triggering a delayed hypersensitivity reaction. This is the basis of contact dermatitis caused by nickel.

Drugs administered orally, by injection or onto the surface of the body can elicit hypersensitivity reactions mediated by IgE or IgG antibodies or by T cells. Immunologically mediated hypersensitivity reactions to drugs are very common and even very tiny doses of drugs can trigger life-threatening reactions. These are well classified as idiosyncratic adverse drug reactions.

In this respect, of course electromagnetic fields could be said to fulfil the most important demands: they can penetrate the entire body and if they are small.

II B. Hypersensitivity to self antigens

Some degree of immune response to self antigens is normal and is present in most people. When these become exaggerated or when tolerance to further antigens breaks down, hypersensitivity reactions can occur and manifest themselves as an autoimmune disease, many of which that are truly serious and may even end fatally.

II C. Types of hypersensitivity reactions

The hypersensitivity classification system was first described by Coombs and Gell. The system classifies the different types of hypersensitivity reaction by the types of immune responses involved. Each type of hypersensitivity reaction produces characteristic clinical diseases whether the trigger is an enviromental, infectious or self-antigen. For example, in type III hypersensitivity the clinical result is similar whether the antigen is streptococcus, a drug or an autoantigen such as DNA.

Hypersensitivity reactions are reliant on the adaptive immune system. Prior exposure to antigen is required to prime the adaptive immune response to produce IgE (type I),

IgG (type II and III) or T cells (type IV). Because prior exposure is required, hypersensitivity reactions do not take place when an individual is first exposed to antigen. In each type of hypersensitivity reaction the damage is caused by different adaptive and innate systems, each of which with their respective role in clearing infections.

Type I

Type I hypersensitivity is mediated through the degranulation of mast cells and eosinophils. The effects are felt within minutes of exposure and this type of hypersensitivity is sometimes referred to as immediate hypersensitivity and is also known as allergy. Among such reactions are hay fever and the classical skin prick test that can be used to reveal such reaction patterns. -The mast cell is a common denominator in the functional impairment electrohypersensitivity (earlier referred to as "electrical allergy").

Type II

Type II hypersensitivity is caused by IgG reacting with antigen present on the surface of cells. The bound immunoglobulin then interacts with complement or with Fc receptors on macrophages. These innate mechanisms then damage the target cells using processes that may take several hours, as in the case of drug-induced hemolysis.

Type III

Immunoglobulin is also responsible for the type III hypersensitivity. In this case, immune complexes of antigen and antibody form and either cause damage at the site of production or circulate and cause damage elsewhere. Immune complexes take some time to form and to initiate tissue damage. Among the cells types involved are neutrophils. Post-streptococcal glomerulonephritis is a good example of immune complex disease.

Type IV

The slowest form of hypersensitivity is that mediated by T cells (type IV hypersensitivity). This can take 2-3 days to develop and is referred to as delayed hypersensitivity. Macrophages are frequently involved. A well-known example of such delayed reactions is contact dermatitis.

III. The old and new electromagnetic environment

"Electromagnetic radiation" covers a broad range of frequencies (over 20 orders of magnitude), from low frequencies in electricity supplies, radiowaves and microwaves, infrared and visible light, to x-rays and cosmic rays.

III A. Definitions and sources

Electric fields are created by differences in voltage: the higher the voltage, the stronger will be the resultant field. Magnetic fields are created when electric current flows: the greater the current, the stronger the magnetic field. An electric field will exist even when there is no current flowing. If current does flow, the strength of the magnetic field will vary with power consumption but the electric field strength will be constant.

III B. Natural sources of electromagnetic fields
Electromagnetic fields are present everywhere in our environment but are invisible to the human eye. Electric fields are produced by the local build-up of electric charges in the atmosphere associated with thunderstorms. The earth's magnetic field causes a compass needle to orient in a North-South direction and is used by birds and fish for navigation.

III C. Human-made sources of electromagnetic fields
Besides natural sources the electromagnetic spectrum also includes fields generated by human-made sources: X-rays are employed to diagnose a broken limb after a sport accident. The electricity that comes out of every power socket has associated low frequency electromagnetic fields. And various kinds of higher frequency radiowaves are used to transmit information – whether via TV antennas, radio stations or mobile phone base stations.

III D. What makes the various forms of electromagnetic fields so different?
One of the main characteristics which defines an electromagnetic field (EMF) is its frequency or its corresponding wavelength. Fields of different frequencies interact with the body in different ways. One can imagine electromagnetic waves as series of very regular waves that travel at an enormous speed, the speed of light. The frequency simply describes the number of oscillations or cycles per second, while the term wavelength describes the distance between one wave and the next. Hence wavelength and frequency are inseparably intertwined: the higher the frequency the shorter the wavelength.

III E. A few basic facts
Field strength: An electromagnetic field consist of an electrical part and a magnetic part. The electrical part is produced by a voltage gradient and is measured in volts/metre. The magnetic part is generated by any flow of current and is measured in Tesla. For example, standing under a power line would expose you to an electrical voltage gradient due to the difference between the voltage of the line (set by the power company) and earth. You would also be exposed to a *magnetic* field proportional to the current actually flowing through the line, which depends on consumer demand. Both types of field give biological effects, but the magnetic field may be more damaging since it penetrates living tissue more easily. Magnetic fields as low as around 2 milligauss (mG) or 0.2 microTesla (a millionth of a Tesla) can produce biological effects. For comparison, using a mobile (cell) phone or a PDA exposes you to magnetic pulses that peak at several tens of microTesla (Jokela et al, 2004; Sage et al, 2007), which is well over the minimum needed to give harmful effects. Because mobile phones and other wireless gadgets are held close to the body and are used frequently, these devices are potentially the most dangerous sources of electromagnetic radiation that the average person possesses.

Frequency: The fields must vary with time, e.g. those from alternating currents, if they are to have biological effects. Extremely low frequencies (ELF) represent power-lines and domestic appliances, and here, just now in June 2007, the WHO again has pointed them out as an area for general caution since they are believed to be one of the causes for children's leukemia. Pulsed or amplitude modulated, at a biologically active lower frequency (i.e. when the radio signal strength rises and falls in time with

the lower frequency), high-frequencies are the hallmark of mobile phones, WiFi systems, PDAs, etc.

III F. Electromagnetic fields at low frequencies

Electric fields exist whenever a positive or negative electrical charge is present. They exert forces on other charges within the field. The strength of the electric field is measured in volts per metre (V/m). Any electrical wire that is charged will produce an associated electric field. This field exists even when there is no current flowing. The higher the voltage, the stronger the electric field at a given distance from the wire. Electric fields are strongest close to a charge or charged conductor, and their strength rapidly diminishes with distance from it. Conductors such as metal shield them very effectively. Other materials, such as building materials and trees, provide some shielding capability. Therefore, the electric fields from power lines outside the house are reduced by walls, buildings, and trees. When power lines are buried in the ground, the electric fields at the surface are hardly detectable.

Plugging a wire into an outlet creates electric fields in the air surrounding the appliance. The higher the voltage the stronger the field produced. Since the voltage can exist even when no current is flowing, the appliance does not have to be turned on for an electric field to exist in the room surrounding it.

Magnetic fields arise from the motion of electric charges. The strength of the magnetic field is measured in amperes per meter (A/m); more commonly in electromagnetic field research, scientists specify a related quantity, the flux density (in microtesla, µT) instead. In contrast to electric fields, a magnetic field is only produced once a device is switched on and current flows. The higher the current, the greater the strength of the magnetic field.

Like electric fields, magnetic fields are strongest close to their origin and rapidly decrease at greater distances from the source. Magnetic fields are not blocked by common materials such as the walls of buildings.

III G. How do static fields differ from time-varying fields?

A static field does not vary over time. A direct current (DC) is an electric current flowing in one direction only. In any battery-powered appliance the current flows from the battery to the appliance and then back to the battery. It will create a static magnetic field. The earth's magnetic field is also a static field. So is the magnetic field around a bar magnet which can be visualized by observing the pattern that is formed when iron filings are sprinkled around it.

In contrast, time-varying electromagnetic fields are produced by alternating currents (AC). Alternating currents reverse their direction at regular intervals. In most European countries electricity changes direction with a frequency of 50 cycles per second or 50 Hertz. Equally, the associated electromagnetic field changes its orientation 50 times every second. North American electricity has a frequency of 60 Hertz.

What are the main sources of low, intermediate and high frequency fields? The time-varying electromagnetic fields produced by electrical appliances are an example of extremely low frequency (ELF) fields. ELF fields generally have frequencies up to

300 Hz. Other technologies produce intermediate frequency (IF) fields with frequencies from 300 Hz to 10 MHz and radiofrequency (RF) fields with frequencies of 10 MHz to 300 GHz. The effects of electromagnetic fields on the human body depend not only on their field level but on their frequency and energy. Our electricity power supply and all appliances using electricity are the main sources of ELF fields; computer screens, anti-theft devices and security systems are the main sources of IF fields; and radio, television, radar and cellular telephone antennas, and microwave ovens are the main sources of RF fields. These fields induce currents within the human body, which if sufficient can produce a range of effects such as heating and electrical shock, depending on their amplitude and frequency range. (However, to produce such effects, the fields outside the body would have to be very strong, far stronger than present in normal environments.)

There are four phenomena that emerge from the use of electricity: ground currents; "electromagnetic smog" from communications equipment; magnetic fields from power lines and specialized equipments; and radiofrequencies on power lines or so-called "dirty electricity." They may all be potential environmental toxins and this is an area of research that must be further pursued.

Electromagnetic fields at high frequencies
Mobile telephones, television and radio transmitters and radar produce RF fields. These fields are used to transmit information over long distances and form the basis of telecommunications as well as radio and television broadcasting all over the world. Microwaves are RF fields at high frequencies in the GHz range. In microwaves ovens, we use them to quickly heat food at 2.45 GHz (or 2,450 MHz).

Communications and radar antennae expose those who live or work near these installations to their emissions. The radiation travels through buildings, and can also be conducted along electrical wires or metal plumbing. Wireless communications create levels within buildings that are orders of magnitude higher than natural background levels.

At radio frequencies, electric and magnetic fields are closely interrelated and we typically measure their levels as power densities in watts per square metre (W/m^2).

IV. The immune system and the impairment electrohypersensitivity

An increasing number of studies has clearly shown various biological and medical effects at the cellular level of electromagnetic fields, including power-frequency and radiofrequency/microwave exposures at low-intensity levels. Such electromagnetic fields are present in everyday life, at the workplace, in ~~your home~~ in homes and at places of leisure. Such bioeffects and health impacts are substantially documented in the scientific literature, and are directly relevant to public health.

Direct effects on the immune system were first reported in relation to people with symptoms of electrohypersensitivity. Subjective and objective skin- and mucosa-related symptoms, such as itch, smarting, pain, heat sensation, redness, papules,

pustles, etc., after exposure to visual display terminals (VDTs), mobile phones, DECT telephones, WI-FI equipments, as well as other electromagnetic devices were reported. Frequently, symptoms from internal organ systems, such as the heart and the central nervous system were reported.

A working definition of EHS from Bergqvist et al. (1997) is:

"a phenomenon where individuals experience adverse health effects while using or being in the vicinity of devices emanating electric, magnetic or electromagnetic fields (EMFs)".

Stenberg (2004) distinguishes between two groups: those who experience facial skin symptoms in connection with VDT work (sensory sensations of the facial skin including stinging, itching, burning, erythema, rosacea) while EHS symptoms include these and also fatigue, headache, sleeplessness, dizziness, cardiac and cognitive problems.

Hillert (2004) reports that symptoms of EHS may include facial skin complaints, eye irritation, runny or stuffy nose, impaired sense of smell, hoarse dry throat, coughing, sense of pressure in ear(s), fatigue, headache, heaviness in the head, nausea/dizziness, and difficulties in concentrating.

Cox (2004) reported on a study of electrical hypersensitivity in the United Kingdom. Symptoms reported by mobile phone users included headaches (85%), dizziness (27%), fatigue (24%), nausea (15%), itching (15%), redness (9%), burning 61%), and cognitive problems (42%). For those individuals reporting EHS symptoms in the UK population, the percentage of patients with symptoms from cell phone masts was 18%, DECT cordless phones (36%), landline phones (6%), VDTs (27%), television (12%) and fluorescent lights (18%).

Fox et al (2004) reported that a questionnaire survey of EHS individuals revealed symptoms of nausea, muzziness/disorientation.

Levallois et al. (2002) reported on their study of prevalence of self-perceived hypersensitivity to electromagnetic fields in California. They found that about 3% of the population reports to be electrohypersensitive. About 0.5% of the population has reported the necessity to change jobs or to remain unemployed due to the severity of their electrohypersensitivity symptoms. Underestimation of these percentages is discussed, since the population surveyed was found through contact with either an occupational clinic or a support group, and electrohypersensitive people very frequently cannot due normal outings (go out, travel, meet in buildings with EMF exposures, etc). The study concludes that while there was no clinical confirmation of the reported symptoms of electrohypersensitivity, the perception is of public health importance in California, and perhaps North America. The results were based on a telephone survey among a sample of 2,072 Californians. Being "allergic or very sensitive" to getting near electrical devices was reported by 68 subjects resulting in an adjusted prevalence of 3.2% (95% confidence interval: 2.8, 3.7). Twenty-seven subjects (1.3%) reported sensitivity to electrical devices but no sensitivity to chemicals. Alleging that a doctor had diagnosed "environmental illness or multiple chemical sensitivity" was the strongest predictor of reporting being hypersensitive to

EMF in this population (adjusted prevalence odds ratio = 5.8, 95 % confidence interval: 2.6 - 12.8. This study confirms the presence of this self-reported disorder in North America.

A recent German survey suggests that the prevalence of subjects who attribute health complaints to EMF exposures is not negligible. In a sample of 2,500 interviewees, 8% specifically attributed health complaints to exposures from mobile phone base station antennas or the use of mobile or cordless phones [Institut für angewandte Sozialwissenschaft (infas), 2004]. In Sweden, 3.1% of the population claimed to be hypersensitive to EMF. Considerable variation across countries, regions within countries, and surveys in the same regions has been noted before. In 1997, a European expert group reported that electrical hypersensitivity had a higher prevalence in Sweden, Germany, and Denmark than in the United Kingdom, Austria, and France [European group of experts, 1997]. All these data suggest that the true number is still uncertain and the topic merits further research (cf. Schuz et al, 2006).

Roosli et al. (2004a, 2004b) estimates that the proportion of individuals in Switzerland with EHS symptoms is about 5%, where the exposures of concern are cited to be powerlines, handheld phones, television and computer exposures rather than base stations (cell towers). He reported that about half the Swiss population is concerned about health effects from EMF exposures in general.

V. Scientific studies of electrohypersensitivity, as well as effects of electromagnetic fields on humans

Lyskov et al. (2004) reported that EHS individuals exhibited sensitivity to VDTs, fluorescent lights and television, all of which produce flickering light. EHS individuals that were given provocation tests with flickering light exhibited a higher critical flicker frequency (CFF) than normal, and their visual evoked potential (VEP) was significantly higher than in controls. Follow-up studies, individuals with EHS demonstrated increased CFF, increased VEP, increased heart rate, decreased heart rate variability (HRV) and increased electrodermal (EDA) reaction to sound stimuli. These results indicate an imbalance in the autonomic nervous system and a lack of normal circadian rhythms in these EHS individuals. However, it may also just show that they feel ill.

Mueller and Schierz (2004) reported that soundness of sleep and well-being in the morning but not sleep quality were affected by exposure in EHS individuals to overnight EMF exposures. An effect was reported where EHS individuals shifted their position in the bed during sleep to the non-exposed (or probably less exposed) side of the bed.

Vecchio et al (2007) have reported that EMF from mobile phones affects the synchronization of cerebral rhythms. Their findings suggest that prolonged exposure to mobile phone emissions affect cortical activity and the speed of neural synchronization by interhemispherical functional coupling of EEG rhythms. This may be evidence that such exposure can affect the way in which the brain is able to process information, by interfering with the synchronization rhythms between the

halves of the brain, and by disregulating the normal alpha wave 2 (about 8-10 Hz) and alpha 3 (10-12 Hz) bands.

Markova et al. (2005) reported that non-thermal microwave exposure from Global System for Mobile Communication (GSM) mobile telephones at lower levels than the ICNIRP safety standards affect 53BP1 and γ-H2AX foci and chromatin conformation in human lymphocytes. They investigated effects of microwave radiation of GSM at different carrier frequencies on human lymphocytes from healthy persons and from persons reporting hypersensitivity to electromagnetic fields (EMFs). They measured the changes in chromatin conformation, which are indicative of stress response and genotoxic effects, by the method of anomalous viscosity time dependence, and analyzed tumor suppressor p53-binding protein 1 (53BP1) and phosphorylated histone H2AX (γ-H2AX), which have been shown to colocalize in distinct foci with DNA double-strand breaks (DSBs), using immunofluorescence confocal laser microscopy. The authors reported that microwave exposure from GSM mobile telephones affect chromatin conformation and 53BP1/γ-H2AX foci similar to heat shock. For the first time, they reported that effects of microwave radiation from mobile telephones on human lymphocytes are dependent on carrier frequency. On average, the same response was observed in lymphocytes from hypersensitive and healthy subjects. These effects occurred at non-thermal microwave exposure levels from mobile telephones. These levels are presently permissible under safety standards of the International Commission for Non-Ionizing Radiation Protection (ICNIRP).

Recent evidence has indicated activation of stress-induced pathways in cultivated cells in response to microwaves (Leszczynski et al, 2002). Their article indicated that mobile telephone microwaves activate a variety of cellular signal transduction pathways, among them the hsp27/p38MAPK stress response pathway (Leszczynski et al, 2002). Whether activation of stress response pathways relates to apoptosis, blood-brain barrier permeability, or increased cancer in humans remains to be investigated. Further work reported gene and protein expression changes in human endothelial cell lines with microwave 900 MHz mobile phone exposure (Leszczynski and Nylund, 2006).

Persons claiming adverse skin reactions after having been exposed to computer screens or mobile phones very well could be reacting in a highly specific way and with a completely correct avoidance reaction, especially if the provocative agent was radiation and/or chemical emissions -- just as would happen if you had been exposed to e.g. sun rays, X-rays, radioactivity or chemical odors. The working hypothesis, thus, early became that they react in a cellularly correct way to the electromagnetic radiation, maybe in concert with chemical emissions such as plastic components, flame retardants, etc., something later focussed upon by professor Denis L. Henshaw and his collaborators at the Bristol University (cf. Fews et al, 1999a,b). This is also covered in great depth by the author Gunni Nordström in her latest book (2004).

Very early immune cell alterations were observed when exposing two EHS individuals to a TV monitor (Johansson et al, 1994). In this people were placed in front of, in front of an ordinary TV set (an open provocation study). Subjects who regarded themselves as suffering from skin problems due to work at video display terminals were tested. Employing immunohistochemistry, in combination with a wide range of antisera directed towards cellular and neurochemical markers, we observed

and reported a high-to-very high number of somatostatin-immunoreactive dendritic cells as well as histamine-positive mast cells in skin biopsies from the anterior neck taken before the start of the provocation. At the end of the provocation the high number of mast cells was unchanged, however, all the somatostatin-positive cells had seemingly disappeared. The reason for this latter finding may be discussed in terms of loss of immunoreactivity, increase of breakdown, etc. The high number of mast cells present may explain the clinical symptoms of itch, pain, edema and erythema.

In facial skin samples of electrohypersensitive persons, the most common finding is a profound increase of mast cells as monitored by various mast cell markers, such as histamine, chymase and tryptase (Johansson and Liu, 1995). From these studies, it is clear that the number of mast cells in the upper dermis is increased in the electrohypersensitivity group. A different pattern of mast cell distribution also occurred in the electrohypersensitivity group, namely, the normally empty zone between the dermo-epidermal junction and mid-to-upper dermis disappeared in the electrohypersensitivity group and, instead, this zone had a high density of mast cell infiltration. These cells also seemed to have a tendency to migrate towards the epidermis (=epidermiotrophism) and many of them emptied their granular content (=degranulation) in the dermal papillary layer. Furthermore, more degranulated mast cells could be seen in the dermal reticular layer in the electrohypersensitivity group, especially in those cases which had the mast cell epidermiotrophism phenomenon described above. Finally, in the electrohypersensitivity group, the cytoplasmic granules were more densely distributed and more strongly stained than in the control group, and, generally, the size of the infiltrating mast cells was found to be larger in the electrohypersensitivity group as well. It should be noted, that increases of similar nature later on were demonstrated in an experimental situation employing normal healthy volunteers in front of visual display units, including ordinary house-hold television sets (cf. Johansson et al, 2001).

Mast cells, when activated, release a spectrum of mediators, among them histamine, which is involved in a variety of biological effects with clinical relevance, e.g., allergic hypersensitivity, itch, edema, local erythema, and many types of dermatoses. From the results of the above studies, it is clear that electromagnetic fields affect the mast cell, and also the dendritic cell, population, and may degranulate these cells.

The release of inflammatory substances, such as histamine, from mast cells in the skin results in a local erythema, edema, and sensation of itch and pain, and the release of somatostatin from the dendritic cells may give rise to subjective sensations of ongoing inflammation and sensitivity to ordinary light. These are, as mentioned, the common symptoms reported from persons suffering from electrohypersensitivity/screen dermatitis. Mast cells occur in the brain (Zhuang et al, 1999) and their presence may, under the influence of electromagnetic field and/or radiofrequency radiation exposure lead to chronic inflammatory response by the mast cell degranulation.

Mast cells are also present in the heart tissue and their localization is of particular relevance to their function. Data from studies made on interactions of electromagnetic fields with the cardiac function have demonstrated that changes are present in the heart after exposure to electromagnetic fields. Some electrically sensitive people have symptoms similar to heart attacks after exposure to electromagnetic fields.

We have also compared facial skin from electrohypersensitive persons with corresponding material from normal healthy volunteers (Johansson et al, 1996). The aim of the study was to evaluate possible markers to be used for future double-blind or blind provocation investigations. Differences were found for the biological markers calcitonin gene-related peptide (CGRP), somatostatin (SOM), vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine amide (PHI), neuropeptide tyrosine (NPY), protein S-100 (S-100), neuron-specific enolase (NSE), protein gene product (PGP) 9.5 and phenylethanolamine N-methyltransferase (PNMT). The overall impression in the blind-coded material was such that it turned out easy to blindly separate the two groups from each other. However, no single marker was 100% able to pin-point the difference, although some were quite powerful in doing so (CGRP, SOM, S-100). In our on-going investigations, we have also found alterations of the Merkel cell number in the facial skin of electrohypersensitive persons (Yoshimura et al, 2006). However, it has to be pointed out that we cannot, based upon those results, draw any definitive conclusions about the cause of the changes observed. Blind or double-blind provocations in a controlled environment (Johansson et al, 2001) are necessary to elucidate the underlying causes for the changes reported in this particular investigation.

Gangi and Johansson (1997, 2000) have proposed models for how mast cells and substances secreted from them (e.g., histamine, heparin, and serotonin) could explain sensitivity to electromagnetic fields similar to those used to explain UV- and ionizing irradiation-related damages. We discuss an increasing number of persons who report cutaneous problems as well as symptoms from certain internal organs, such as the central nervous system and the heart, when being close to electric equipment. Many of these respondents are users of video display terminals, and have both subjective and objective skin- and mucosa-related symptoms, such as pain, itch, heat sensation, erythema, papules, and pustules. The central nervous system-derived symptoms are, e.g., dizziness, tiredness, and headache, erythema, itch, heat sensation, edema, and pain which are also common symptoms of sunburn (UV dermatitis). Alterations have been observed in cell populations of the skin of electrohypersensitive persons similar to those observed in the skin damaged due to ultraviolet light or ionizing radiation.

Gangi and Johansson (1997, 2000), have proposed a theoretical mechanism to explain how mast cells and substances secreted from them could cause sensitivity to electromagnetic fields. The mechanism derives from known facts in the fields of UV- and ionizing irradiation-related damage. Alterations seen after power-frequency or microwave electromagnetic field-exposures that result in electrohypersensitivity symptoms may be understood by comparison to to ionizing radiation damage according to the type of immune function responses seen in both.

The working hypothesis is that electrohypersensitivity is a kind of irradiation damage, since the observed cellular changes are very much the same as the ones documented in tissue subjected to UV-light or ionizing radiation (see references below).

Mast cells are located in close proximity to neurons in the peripheral and central nervous systems, suggesting a functional role in normal and aberrant neurodegenerative states. They also possess many of the features of neurons, in terms of monoaminergic systems, responsiveness to neurotrophins and neuropeptides and the ability to synthesise and release bioactive neurotrophic factors. Mast cells are able

to secrete an array of potent mediators which may orchestrate neuroinflammation and affect the integrity of the blood-brain barrier. The «cross-talk» between mast cells, lymphocytes, neurons and glia constitutes a neuroimmune axis which is implicated in a range of neurodegenerative diseases with an inflammatory and/or autoimmune component, such as multiple sclerosis and Alzheimer's disease.

Mast cells are involved in numerous activities ranging from control of the vasculature, to tissue injury and repair, allergic inflammation and host defences. They synthesize and secrete a variety of mediators, activating and modulating the functions of nearby cells and initiating complex physiological changes. Interestingly, NO produced by mast cells and/or other cells in the microenvironment appears to regulate these diverse roles. Some of the pathways central to the production of NO by mast cells and many of the tightly controlled regulatory mechanisms involved have been identified. Several cofactors and regulatory elements are involved in NO production, and these act at transcriptional and post-translational sites. Their involvement in NO production and the possibility that these pathways are critically important in mast cell functions should be investigated. The effects of NO on mast cell functions such as adhesion, activation and mediator secretion ought to be examined with a focus on molecular mechanisms by which NO modifies intracellular signalling pathways dependent or independent of cGMP and soluble guanylate cyclase. Metabolic products of NO including peroxynitrite and other reactive species may be the critical elements that affect the actions of NO on mast cell functions. Further understanding of the actions of NO on mast cell activities may uncover novel strategies to modulate inflammatory conditions.

It is important to remember that mastocytosis - an abnormal accumulation of mast cells in one or more organ system - can occur secondarily to other causes, such as inflammation and some kinds of leukemia. The increase in EHS being described here is more accurately thought of as "primary" mastocytosis, meaning that the increased number of mast cells occurs independently of any other cause. However, because of the increased number of mast cells in primary mastocytosis, conditions such as osteoporosis and inflammation may arise as a result of the activity of those mast cells. The manner in which primary mastocytosis can be distinguished from secondary mastocytosis and other conditions should be addressed.

Research of mast cells and mastocytosis has made impressive progress over the past decade toward understanding what is different about mast cells in patients who have mastocytosis compared with mast cells in people who do not. A group of 23 researchers from Europe and the United States met in Vienna in September, 2000, and, after lengthy discussions, arrived at a consensus as to what criteria will accurately diagnose mastocytosis, and how to classify the various sub-types. Their conclusions are reported in a series of articles in the July, 2001, issue of Leukemia Research. Unfortunately, nothing was mentioned about mast cells and EMF effects.

Patients with mastocytosis may or may not have constitutional symptoms, including weight loss, pain, nausea, headache, malaise, or fatigue. These symptoms may be due to uncontrolled proliferation of mast cells or involvement of distinct organs, such as the stomach and intestines, or bone or bone marrow. Constitutional symptoms also can result from high levels of mast cell mediators in the blood stream. The severity of symptoms varies from mild to life-threatening.

The study of biopsy tissue in patients with suspected mastocytosis requires the use of appropriate stains. Tryptase is the stain of choice, as toluidine blue and Giemsa stains are more likely to be affected by tissue processing and may not always produce reliable results.

In skin, accumulation of groups of mast cells combined with the presence of urticaria pigmentosa or mastocytoma is diagnostic of cutaneous mastocytosis. In some cases, it may be difficult to establish a diagnosis. The absence of skin lesions does not rule out the diagnosis of mastocytosis.

The abnormalities that may be seen in mastocytosis mast cells are elongated shape, oval nuclei that are not in the center of the mast cell, and fewer than usual granules inside the mast cells, with those present being in groups rather than scattered. If two or more of these features are found, the cells are referred to as atypical mast cells. Sometimes the nucleus of atypical mast cells will have "lobes."

When the diagnosis of mastocytosis has not previously been established, specialized analyses may be required to differentiate between mastocytosis and other non-mast cell disorders of the blood-forming system, such as leukemias and myeloproliferative disorders. In some of these other disorders, the diseased cells contain and release low amounts of tryptase. Additional blood cell studies and chromosome analysis may be necessary to make a clear diagnosis in such cases.

Holmboe and Johansson (2005) reported on testing for the presence of increased levels of IgE or signs of a positive Phadiatop Combi (which is a screening test for allergies towards certain articles of food, pollen, insects, and other animals) which both would be indicators of an immune system alert. Twenty-two people (5 men, 17 women) participated in the study. Skin and nervous system effects were the primary symptoms reported by participants in the study. The most frequently reported symptoms were skin redness, eczema and sweating, loss of memory, concentration difficulties, sleep disturbances, dizziness, muscular and joint-related pain, and muscular and joint-related weakness. Headache, faintness, nasal stuffiness, and fatigue were also common. In addition, 19 of the people had disturbances of the gastrointestinal tract. All the people with the impairment electrohypersensitivity had tinnitus.

No connection between IgE blood levels and symptoms were found. All the people who reported electrohypersensitivity had normal values (<122 kU/l). Only 3 people had a positive Phadiatop Combi. Such increases could be used in the diagnosis of electrohypersensitivity, but they were not found to be useful indicators.

Animal Studies
In addition to the studies in humans, series of animal experiments were performed in collaboration with the Department of Biology, Faculty of Sciences, Novi Sad, Serbia and Montenegro), and the Karolinska Institute, Stockholm, Sweden (Rajkovic et al, 2005a,b, 2006).
The aim of these was to investigate the influence of extremely low-frequency electromagnetic fields (ELF-EMFs) on mast cells, parafollicular cells, and nerve fibers in rat skin and thyroid gland, as seen using light and transmission electron

microscopy. The experiments were performed on 2-month-old Wistar male rats exposed for 4 h a day, 5 or 7 days a week for 1 month to power-frequent (50 Hz) EMFs (100-300 µT, 54-160 V/m). After sacrifice, samples of skin and thyroid were processed for indirect immunohistochemistry or toluidine blue staining and were then analyzed using the methods of stereology. Antibody markers to serotonin, substance P, calcitonin gene-related peptide (CGRP), and protein gene product 9.5 (PGP) were applied to skin sections and PGP, CGRP, and neuropeptide Y (NPY) markers to the thyroid. A significantly increased number of serotonin-positive mast cells in the skin ($p<0.05$) and NPY-containing nerve fibers in the thyroid ($p<0.01$) of rats exposed to ELF-EMF was found compared to controls, indicating a direct EMF effect on skin and thyroid vasculature.

After ultrastructural examination, a predominance of microfollicles with less colloid content and dilated blood capillaries was found in the EMF group. Stereological counting showed a statistically significant increase of the volume density of follicular epithelium, interfollicular tissue and blood capillaries as well as the thyroid activation index, as compared to the controls. The volume density of colloid significantly decreased. Ultrastructural analysis of thyroid follicular cells in the EMF group revealed the frequent finding of several colloid droplets within the same thyrocyte with the occasional presence of large-diameter droplets. Alterations in lysosomes, granular endoplasmic reticulum and cell nuclei compared to the control group were also observed. Taken together, the results of this study show the stimulative effect of power-frequency EMFs on thyroid gland at both the light microscope and the ultrastructural level.

The-animal results reported in these studies can not be explained away as psychosomatic in origin because they were conducted on animals, not humans.

In summary, both human and animal studies report large immunohistological changes in mast cells, and other measures of immune disfunction and disregulation due to exposures to ELF and RF at environmental levels associated with new electrical and wireless technologies.

It iss evident from our preliminary experimental data that various biological alterations are present in the electrohypersensitive persons claiming to suffer from exposure to electromagnetic fields. The alterations are themselves enough to fully explain the EHS symptoms, and the involvement of the immune system is evident. In view of recent epidemiological studies, pointing to a correlation between long-term exposure from power-frequent magnetic fields or microwaves and cancer, our data ought to be taken seriously and to be further analyzed.

Thus, it is of paramount importance to continue the investigation of persons with the impairment electrohypersensitivity. We would favour studies of electromagnetic fields' interaction with mast cell release of histamine and other biologically active substances, studies of lymphocyte viability as well as studies of the newly described serotonin-containing melanocytes. Also, continued analysis of the intraepidermal nerve fibers and their relations to these mast cells and serotonin-containing melanocytes are very important. Finally, not to be forgotten, a general investigation - of persons with the impairment electrohypersensitivity versus normal healthy volunteers - regarding the above markers as well as other markers for cell traffic,

proliferation and inflammation is very much needed. Such scientific work may lay a firm foundation for necessary adjustment of accessibility, thus helping and supporting all persons with the functional impairment electrohypersensitivity.

VI. Direct effects of EMFs on the immune system

Childhood leukemia was early connected to power-frequent magnetic fields already in the pioneering work by Wertheimer and Leeper (1979), and more recently Scandinavian scientists have identified an increased risk for acoustic neuroma (i.e., a benign tumor of the eighth cranial nerve) in cell phone users, as well as a slightly increased risk of malignant brain tumors such as astrocytoma and meningioma on the same side of the brain as the cell phone was habitually held (Hardell et al, 1999, 2004, 2005; Lonn et al, 2004). In addition, a clear association between adult cancers and FM radio broadcasting radiation has been noticed, both in time and location (Hallberg and Johansson, 2002b, 2004a, 2005a). Initial studies on facial nevi indicates that nowadays also young children can have a substantial amount of these. If it can be shown that radiofrequency radiation is not correlated with childhood cancers the current focus on low-frequency electromagnetic fields can continue. If there is also a radiofrequency and/or microwave correlation then this must be considered in future research as well as in today's preventive work.

Anane and coworkers (2003) studied the effects of acute exposure to GSM-900 microwaves (900 MHz, 217 Hz pulse modulation) on the clinical parameters of the acute experimental allergic encephalomyelitis (EAE) model in rats in two independent experiments: rats were either habituated or nonhabituated to the exposure restrainers. EAE was induced with a mixture of myelin basic protein and Mycobacterium tuberculosis. Female Lewis rats were divided into cage control, sham exposed, and two groups exposed either at 1.5 or 6.0 W/kg local specific absorption rate (SAR averaged over the brain) using a loop antenna placed over their heads. No effect of a 21-day exposure (2 h/day) on the onset, duration, and termination of the EAE crisis was seen.

The object of the study by Boscol et al. (2001) was to investigate the immune system of 19 women with a mean age of 35 years, for at least 2 years (mean = 13 years) exposed to electromagnetic fields induced by radiotelevision broadcasting stations in their residential area. In September 1999, the EMFs (with range 500 KHz-3 GHz) in the balconies of the homes of the women were (mean +/- S.D.) 4.3 +/- 1.4 V/m. Forty-seven women of similar age, smoking habits and atopy composed the control group, with a nearby resident EMF exposure of < 1.8 V/m. Blood lead and urinary trans-trans muconic acid (a metabolite of benzene), markers of exposure to urban traffic, were higher in the control women. The EMF exposed group showed a statistically significant reduction of blood NK CD16+-CD56+, cytotoxic CD3(-)-CD8+, B and NK activated CD3(-)-HLA-DR+ and CD3(-)-CD25+ lymphocytes. 'In vitro' production of IL-2 and interferon-gamma (INF-gamma) by peripheral blood mononuclear cells (PBMC) of the EMF exposed group, incubated either with or without phytohaemoagglutinin (PHA), was significantly lower; the 'in vitro' production of IL-2 was significantly correlated with blood CD16+-CD56+ lymphocytes. The stimulation index (S.I.) of blastogenesis (ratio between cell proliferation with and without PHA) of PBMC of EMF exposed women was lower than that of the control subjects. The S.I. of blastogenesis of the EMF exposed group

(but not blood NK lymphocytes and the 'in vitro' production of IL-2 and INF-gamma by PBMC) was significantly correlated with the EMF levels. Blood lead and urinary trans-trans muconic acid were barely correlated with immune parameters: the urinary metabolite of benzene of the control group was only correlated with CD16+-CD56+ cells indicating a slight effect of traffic on the immune system. In conclusion, this study demonstrates that high-frequency EMFs reduce cytotoxic activity in the peripheral blood of women without a dose-response effect. Such an effect could, of course, only be considered as very serious, since this could hamper the immune system in it's daily struggle against various organisms/agents.

On the other hand, Chagnaud and Veyret in 1999 could not demonstrate an effect of low-level pulsed microwaves on the integrity of the immune system. They investigated the effects of GSM-modulated microwaves on lymphocyte sub-populations of Sprague-Dawley rats and their normal mitogenic responses using flow cytometry analysis and a colorimetric method. No alterations were found in the surface phenotype of splenic lymphocytes or in their mitogenic activity.

 Cleary et al. (1990) reported a biphasic, dose-dependent effect of microwave radiation on lymphycyte proliferation with non-thermal exposures. -Whole human blood was exposed or sham-exposed in vitro for 2 h to 27 or 2,450 MHz radio-frequency electromagnetic (RF) radiation under isothermal conditions (i.e., 37 +/- 0.2 degrees C). Immediately after exposure, mononuclear cells were separated from blood by Ficoll density-gradient centrifugation and cultured for 3 days at 37 degrees C with or without mitogenic stimulation by phytohemagglutinin (PHA). Lymphocyte proliferation was assayed at the end of the culture period by 6 h of pulse-labeling with 3H-thymidine (3H-TdR). Exposure to radiation at either frequency at specific absorption rates (SARs) below 50 W/kg resulted in a dose-dependent, statistically significant increase of 3H-TdR uptake in PHA-activated or unstimulated lymphocytes. Exposure at 50 W/kg or higher suppressed 3H-TdR uptake relative to that of sham-exposed cells. There were no detectable effects of RF radiation on lymphocyte morphology or viability. Notwithstanding the characteristic temperature dependence of lymphocyte activation in vitro, the isothermal exposure conditions of this study warrant the conclusion that the biphasic, dose-dependent effects of the radiation on lymphocyte proliferation were not dependent on heating.

Cleary et al. (1996) subsequently published ~~yet~~ another paper reporting a biphasic response of lymphycytes to radiofrequency/microwave radiation where higher SARs resulted in decreased cell proliferation and lower SARs result in increased cell proliferation, dependent on the mitotic state of the cells. -Previous in vitro studies had provided evidence that RF electromagnetic radiation modulates proliferation of human glioma, lymphocytes, and other cell types. The mechanism of such RF radiation cell proliferation modulation, as well as mechanisms for effects on other cell physiologic endpoints, however, were not well understood. To obtain insight regarding interaction mechanisms, they investigated effects of RF radiation exposure on interleukin 2 (IL-2) -dependent proliferation of cytolytic T lymphocytes (CTLL-2). After exposure to RF radiation in the presence or absence of IL-2 cells were cultured at various physiological concentrations of IL-2. Treatment effects on CTLL-2 proliferation were determined by tritiated thymidine incorporation immediately or 24 h after exposure. Exposure to 2,450 MHz RF radiation at specific absorption rates (SARs) of greater than 25 W/kg (induced E-field strength 98.4 V/m) induced a

consistent, statistically significant reduction in CTLL-2 proliferation, especially at low IL-2 concentrations. At lower SARs, 2,450 MHz exposure increased CTLL-2 proliferation immediately after exposure but reduced 24 h post-exposure proliferation. RF radiation effects depended on the mitotic state of the cells at the time of exposure.

In 1992, Czerska et al. studied the effects of continuous and pulsed 2,450-MHz radiation on spontaneous lymphoblastoid transformation of human lymphocytes in vitro. Normal human lymphocytes were isolated from the peripheral blood of healthy donors. One-ml samples containing one million cells in chromosome medium 1A were exposed for 5 days to conventional heating or to continuous wave (CW) or pulsed wave (PW) 2,450-MHz radiation at non-heating (37 degrees C) and various heating levels (temperature increases of 0.5, 1.0, 1.5, and 2 degrees C). The pulsed exposures involved 1-microsecond pulses at pulse repetition frequencies from 100 to 1,000 pulses per second at the same average SAR levels as the CW exposures. Actual average SARs ranged to 12.3 W/kg. Following termination of the incubation period, spontaneous lymphoblastoid transformation was determined with an image analysis system. The results were compared among each of the experimental conditions and with sham-exposed cultures. At non-heating levels, CW exposure did not affect transformation. At heating levels both conventional and CW heating enhanced transformation to the same extent and correlate with the increases in incubation temperature. PW exposure enhanced transformation at non-heating levels. This finding is significant (p<0.002). At heating levels PW exposure enhanced transformation to a greater extent than did conventional or CW heating. This finding is significant at the 0.02 level. It was concluded that PW 2,450-MHz radiation acts differently on the process of lymphoblastoid transformation in vitro compared with CW 2,450-MHz radiation at the same average SARs.

In 2003, Dabrowski et al. exposed samples of mononuclear cells isolated from peripheral blood of healthy donors (n = 16) to 1,300 MHz pulse-modulated microwaves at 330 pps with 5 µs pulse width. The samples were exposed in an anechoic chamber at the average value of power density of S = 10 W/m^2 (1 mW/cm^2). The average specific absorption rate (SAR) was measured in rectangular waveguide and the value of SAR = 0.18 W/kg was recorded. Subsequently, the exposed and control cells were assessed in the microculture system for several parameters characterizing their proliferative and immunoregulatory properties. Although the irradiation decreased the spontaneous incorporation of 3H-thymidine, the proliferative response of lymphocytes to phytohemagglutinin (PHA) and to Con A as well as the T-cell suppressive activity (SAT index) and the saturation of IL-2 receptors did not change. Nevertheless, the lymphocyte production of interleukin (IL)-10 increased (p< 0.001) and the concentration of IFNγ remained unchanged or slightly decreased in the culture supernatants. Concomitantly, the microwave irradiation modulated the monokine production by monocytes. The production of IL-1β increased significantly (p< 0.01), the concentration of its antagonist (IL-1ra) dropped by half (p< 0.01) and the tumor necrosis factor (TNF-α) concentration remained unchanged. These changes of monokine proportion (IL-1 β vs. IL-1ra) resulted in significant increase of the value of LM index (p<0.01), which reflects the activation of monocyte immunogenic function. The results indicate that pulse-modulated microwaves represent the potential of immunotropic influence, stimulating preferentially the immunogenic and proinflammatory activity of monocytes at relatively low levels of exposure,

Following these findings of G_o phase peripheral blood mononulclear cells (PBMC) exposed to low-level (SAR = 0.18 W/kg) pulse-modulated 1300 MHz microwave,s and subsequently cultured, demonstrating changed immune activity (as of above), in 2006 Stankiewicz and coworkers investigated whether cultured immune cells induced into the active phases of cell cycle (G_1, S) and then exposed to microwaves will also be sensitive to electromagnetic fields. An anechoic chamber containing a microplate with cultured cells and an antenna emitting microwaves (900 MHz simulated GSM signal, 27 V/m, SAR 0.024 W/kg) was placed inside an ASSAB incubator. The microcultures of PBMC exposed to microwaves demonstrated significantly higher response to mitogens and higher immunogenic activity of monocytes (LM index) than control cultures. The LM index, described in detail elsewhere (Dabrowski et al, 2001), represents the monokine influence on lymphocyte mitogenic response. The results suggest that immune activity of responding lymphocytes and monocytes can be additionally intensified by 900 MHz microwaves. The above described effects of an immune system activity-intensifying effect of 900 MHz microwaves are, of course, a very important warning signal as well as a very important piece of the explanatory jigsaw puzzle regarding, for instance, the functional impairment electrohypersensitivity. In the latter, affected persons very often describe "influenza-like" sensations in their body. Maybe the mobile phones, as well as other high-frequency devices, have aroused the immune system to a too high an activation level?

In an attempt to understand how non-atopic and atopic fertile women with uniform exposure to toxic compounds produced by traffic - immunologically react to high or low frequency electromagnetic fields (ELMF), Del Signore et al. (2000) performed a preliminary study. Women were divided in group A (non-atopic, non-exposed to ELMF); B (atopic, non-exposed to ELMF); C (non-atopic, exposed to ELMF); D (atopic, exposed to ELMF). In vitro cell proliferation of peripheral blood mononuclear cells (PBMC) of atopic women (groups B and D) stimulated by phytohaemoglutinin (PHA) was reduced. The ELMF exposed women (groups C and D) showed lower levels of blood NK CD16(+)-CD56+ lymphocyte subpopulations and of "in vitro" production of interferon-gamma (both spontaneously and in presence of PHA) by PBMC, suggesting that ELMF reduces blood cytotoxic activity. Serum IgE of the atopic women exposed to ELMF (group D) was higher than that of the other groups. Linear discriminant analysis including serum zinc and copper (essential enzymes for immune functions), blood lead and urinary transtrans muconic acid, a metabolite of benzene (markers of exposure to traffic) and key parameters of immune functions (CD16(+)-CD56+ lymphocyte subset, serum IgE, interferon-gamma produced by PBMC in presence of PHA, stimulation index of blastogenesis) showed absence of significant difference between groups A and C and a marked separation of groups B and D. This datum suggests that ELMF have a greater influence on atopic women exposed to traffic than on non-atopic ones, again pointing out differing reaction capacities in the human population – maybe dependent on varying immune functions based on variations in genetic make-up.

A more general reaction pattern was found by Dmoch and Moszczynski (1998) who assessed immunoglobulin concentrations and T-lymphocyte subsets in workers of TV re-transmission and satellite communication centres. An increase in IgG and IgA

concentrations, an increased count of lymphocytes and T8 lymphocytes, an decreased count of NK cells and a lower value of T-helper/T-suppressor ratio were found.

Elekes et al. (1996) found a very interesting sex-difference. The effect of continuous (CW; 2.45 GHz carrier frequency) or amplitude-modulated (AM; 50 Hz square wave) microwave radiation on the immune response was tested. CW exposures (6 days, 3 h/day) induced elevations of the number of antibody-producing cells in the spleen of male Balb/c mice (+37%). AM microwave exposure induced elevation of the spleen index (+15%) and antibody-producing cell number (+55%) in the spleen of male mice. No changes were observed in female mice. It is concluded that both types of exposure conditions induced moderate elevation of antibody production only in male mice.

Irradiation with electromagnetic waves (8.15-18 GHz, 1 Hz within, 1 microW/cm2) in vivo increases the cytotoxic activity of natural killer cells of rat spleen (Fesenko et al, 1999a). In mice exposed for 24-72 h, the activity of natural killer cells increased by 130-150%, the increased level of activity persisting within 24 h after the cessation of treatment. Microwave irradiation of animals in vivo for 3.5 and 5 h, and a short exposure of splenic cells in vitro did not affect the activity of natural killer cells.

Whole body microwave sinusoidal irradiation of male NMRI mice with 8.15-18 GHz (1 Hz within) at a power density of 1 microW/cm2 caused a significant enhancement of TNF production in peritoneal macrophages and splenic T lymphocytes (Fesenko et al, 1999b). Microwave radiation affected T cells, facilitating their capacity to proliferate in response to mitogenic stimulation. The exposure duration necessary for the stimulation of cellular immunity ranged from 5 h to 3 days. Chronic irradiation of mice for 7 days produced the decreasing of TNF production in peritoneal macrophages. The exposure of mice for 24 h increased the TNF production and immune proliferative response, and these stimulatory effects persisted over 3 days after the termination of exposure. Microwave treatment increased the endogenously produced TNF more effectively than did lipopolysaccharide, one of the most potential stimuli of synthesis of this cytokine. Microwaves, thus, indeed can be a factor interfering with the process of cell immunity!

Gapeev et al. (1996) reported that low-intensity electromagnetic radiation of extremely high frequency in the near field of modified the acitivity of mouse peritoneal neutrophils in a quasi-reasonance fashion., He compared the effect of radiation from various types of antennae, including one which created a uniform spatial distribution of specific absorbed rating in the frequency range used and wide-band matching with the object both in near field and far field zones of the radiator. The authors extremely high frequency in near field zone but not the far field zone of the channel radiator modified the activity of mouse peritoneal neutrophils on a quasi-resonance manner. The interaction of electromagnetic radiation with the biological object has been revealed in the narrow-band frequencies of 41.8-42.05 GHz and consists in inhibition of luminol-dependent chemiluminescence of neutrophils activated by opsonized zymosan. It is not found any frequency dependence of the electromagnetic radiation effects in the far field zone of the radiator. The results obtained suggest, that the quasi-resonance dependence of the biological effect on the frequency of the electromagnetic radiation in the near field zone is conditioned by structure and nature of the electromagnetic radiation in this zone.

In 2003, Gatta et al. studied the effects of in vivo exposure to GSM-modulated 900 MHz radiation on mouse peripheral lymphocytes. The aim of this study was to evaluate whether daily whole-body exposure to 900 MHz GSM-modulated radiation could affect spleen lymphocytes. C57BL/6 mice were exposed 2 h/day for 1, 2 or 4 weeks in a TEM cell to an SAR of 1 or 2 W/kg. Untreated and sham-exposed groups were also examined. At the end of the exposure, mice were killed humanely and spleen cells were collected. The number of spleen cells, the percentages of B and T cells, and the distribution of T-cell subpopulations (CD4 and CD8) were not altered by the exposure. T and B cells were also stimulated ex vivo using specific monoclonal antibodies or LPS to induce cell proliferation, cytokine production and expression of activation markers. The results did not show relevant differences in either T or B lymphocytes from mice exposed to an SAR of 1 or 2 W/kg and sham-exposed mice with few exceptions. After 1 week of exposure to 1 or 2 W/kg, an increase in IFN-gamma (Ifng) production was observed that was not evident when the exposure was prolonged to 2 or 4 weeks. This suggests that the immune system might have adapted (!) to RF radiation as it does with other stressing agents. All together, from their in vivo data, they made the conclusion that it indicated that the T- and B-cell compartments were not substantially affected by exposure to RF radiation and that a clinically relevant effect of RF radiation on the immune system is unlikely to occur. Another explanation could be that the cells were unable to deal with the exposure and the obvious follow-up question then will be: What happened with the immune cells after months and years of exposure?

On the other hand, Kolomytseva et al. (2002), in their whole-body exposure experiment designed to study the dynamics of leukocyte number and functional activity of peripheral blood neutrophils under whole-body exposure of healthy mice to low-intensity extremely-high-frequency electromagnetic radiation (EHF EMR, 42.0 GHz, 0.15 mW/cm2, 20 min daily), showed that such a whole-body exposure of healthy mice to low-intensity EHF EMR has a profound effect on the indices of nonspecific immunity. It was shown that the phagocytic activity of peripheral blood neutrophils was suppressed by about 50% (p<0.01 as compared with the sham-exposed control) in 2-3 h after the single exposure to EHF EMR. The effect persisted for 1 day after the exposure, and then the phagocytic activity of neutrophils returned to the norm within 3 days. A significant modification of the leukocyte blood profile in mice exposed to EHF EMR for 5 days was observed after the cessation of exposures: the number of leukocytes increased by 44% (p<0.05 as compared with sham-exposed animals), mostly due to an increase in the lymphocyte content. The supposition was made that EHF EMR effects can be mediated via the metabolic systems of arachidonic acid and the stimulation of adenylate cyclase activity, with subsequent increase in the intracellular cAMP level.

The modification of indices of the humoral immune response to thymus-dependent antigen (sheep erythrocytes) after a whole-body exposure of healthy mice to low-intensity extremely-high-frequency electromagnetic radiation was reported by Lushnikov et al. in 2001. Male NMRI mice were exposed in the far-field zone of horn antenna at a frequency of 42.0 GHz and energy flux density of 0.15 mW/cm2 under different regimes: once for 20 min, for 20 min daily during 5 and 20 successive days before immunization, and for 20 min daily during 5 successive days after immunization throughout the development of the humoral immune response. The intensity of the humoral immune response was estimated on day 5 after immunization

by the number of antibody-forming cells of the spleen and antibody titers. Changes in cellularity of the spleen, thymus and red bone marrow were also assessed. The indices of humoral immunity and cellularity of lymphoid organs changed insignificantly after acute exposure and series of 5 exposures before and after immunization of the animals. However, after repeated exposures for 20 days before immunization, a statistically significant reduction of thymic cellularity by 17.5% ($p<0.05$) and a decrease in cellularity of the spleen by 14.5% ($p<0.05$) were revealed. The results show that low-intensity extremely-high-frequency electromagnetic radiation with the frequency and energy flux density used does not influence the humoral immune response intensity in healthy mice but influences immunogenesis under multiple repeated exposures.

The immunoglobulins' concentrations and T lymphocyte subsets during occupational exposures to microwave radiation were assessed in 1999 by Moszczynski et al. In the workers of retransmission TV center and center of satellite communications on increased IgG and IgA concentration and decreased count of lymphocytes and T8 cells was found. However, in the radar operators IgM concentration was elevated and a decrease in the total T8 cell count was observed. The different behaviour of examined immunological parameters indicate that the effect of microwave radiation on immune system depends on character of an exposure. Disorders in the immunoglobulins' concentrations and in the T8 cell count did not cause any reported clinical consequences.

Experiments have also been conducted to elucidate the effects of chronic low power-level microwave radiation on the immunological systems of rabbits (Nageswari et al, 1991). Fourteen male Belgian white rabbits were exposed to microwave radiation at 5 mW/cm2, 2.1 GHz, 3 h daily, 6 days/week for 3 months in two batches of 7 each in specially designed miniature anechoic chambers. Seven rabbits were subjected to sham exposure for identical duration. The microwave energy was provided through S band standard gain horns connected to a 4K3SJ2 Klystron power amplifier. The first batch of animals were assessed for T lymphocyte-mediated cellular immune response mechanisms and the second batch of animals for B lymphocyte-mediated humoral immune response mechanisms. The peripheral blood samples collected monthly during microwave/sham exposure and during follow-up (5/14 days after termination of exposures, in the second batch animals only) were analysed for T lymphocyte numbers and their mitogen responsiveness to ConA and PHA. Significant suppression of T lymphocyte numbers was noted in the microwave group at 2 months (p less than 0.01) and during follow-up (p less than 0.01). The first batch animals were initially sensitised with BCG and challenged with tuberculin (0.03 ml) at the termination of microwave irradiation/sham exposure and the increase in foot pad thickness (delta mm), which is a measure of T cell-mediated immunity (delayed type hypersensitivity response, DTH) was noted in both the groups. The microwave group revealed a more robust response than the control group (delta % +12.4 vs. +7.54).

Nakamura et al. (1997) reported on the effect of microwaves on pregnant rats. The authors reported that microwaves at the power of 10 mW/cm2 produced activation of the hypothalamic-pituitary-adrenal axis and increased oestradiol in both virgin and pregnant rats, suggesting that microwaves greatly stress pregnant organisms. Earlier data had indicated that these microwaves produce various detrimental changes based on actions of heat or non-specific stress, although the effects of microwaves on

pregnant organisms was not uniform. This study was therefore designed to clarify the effect of exposure to microwaves during pregnancy on endocrine and immune functions. Natural killer cell activity and natural killer cell subsets in the spleen were measured, as well as some endocrine indicators in blood--corticosterone and adrenocorticotrophic hormone (ACTH) as indices of the hypothalamic-pituitary-adrenal axis--beta-endorphin, oestradiol, and progesterone in six female virgin rats and six pregnant rats (nine to 11 days gestation) exposed to microwaves at 10 mW/cm2 incident power density at 2,450 MHz for 90 minutes. The same measurements were performed in control rats (six virgin and six pregnant rats). Skin temperature in virgin and pregnant rats increased immediately after exposure to microwaves. Although splenic activity of natural killer cells and any of the subset populations identified by the monoclonal antibodies CD16 and CD57 did not differ in virgin rats with or without exposure to microwaves, pregnant rats exposed to microwaves showed a significant reduction of splenic activity of natural killer cells and CD16+CD57-. Although corticosterone and ACTH increased, and oestradiol decreased in exposed virgin and pregnant rats, microwaves produced significant increases in beta-endorphin and progesterone only in pregnant rats.

Nakamura et al. (1998) evaluated the involvement of opioid systems in reduced natural killer cell activity (NKCA) in pregnant rats exposed to microwaves at a relatively low level (2 mW/cm2 incident power density at 2,450 MHz for 90 min). They assayed beta-endorphin (betaEP) in blood, pituitary lobes, and placenta as well as splenic NKCA in virgin and/or pregnant rats. Although microwaves elevated colonic temperatures by 0.8 degrees C for virgin and 0.9 degrees C for pregnant rats, and betaEP in blood and anterior pituitary lobes (AP) significantly, it did not change blood corticosterone as an index of hypothalamic-pituitary adrenal axis. There were significant interactions between pregnancy and microwave exposure on splenic NKCA, betaEP in both blood and AP, and blood progesterone. Intra-peritoneal administration of opioid receptor antagonist naloxone prior to microwave exposure increased NKCA, blood, and placental betaEP in pregnant rats. Alterations in splenic NKCA, betaEP and progesterone in pregnant rats exposed to microwaves may be due to both thermal and non-thermal actions. These results suggest that NKCA reduced by microwaves during pregnancy is mediated by the pituitary opioid system.

To further clarify the effects of microwaves on pregnancy, Nakamura et al. (2000) investigated rats exposed to continuous-wave (CW) microwave at 2 mW/cm(2) incident power density at 2,450 MHz for 90 min.. The effects on uterine or uteroplacental blood flow and endocrine and biochemical mediators, including corticosterone, estradiol, prostaglandin E(2) (PGE(2)), and prostaglandin F(2)alpha (PGF(2)alpha) were measured, Colonic temperature in virgin and pregnant rats was not significantly altered by microwave treatment. Microwaves decreased uteroplacental blood flow and increased progesterone and PGF(2)alpha in pregnant, but not in virgin rats. Intraperitoneal (i.p.) administration of angiotensin II, a uteroplacental vasodilator, before microwave exposure prevented the reduction in uteroplacental blood flow and the increased progesterone and PGF(2)alpha in pregnant rats. Increased corticosterone and decreased estradiol during microwave exposure were observed independent of pregnancy and pretreatment with angiotensin II. These results suggest that microwaves (CW, 2 mW/cm(2), 2,450 MHz) produce uteroplacental circulatory disturbances and ovarian and placental dysfunction during pregnancy, probably through non-thermal actions. The uteroplacental disturbances

appear to be due to actions of PGF(2)alpha and may pose some risk for pregnancy. Reported pregnancy losses in women (Lee, 2001; Li, 2001) and infertility (Magras and Xenos, 1997) might be related to these laboratory findings.

Nasta et al. (2006), very recently examined the effects of in vivo exposure to a GSM-modulated 900 MHz RF field on B-cell peripheral differentiation and antibody production in mice. Their results show that exposure to a whole-body average specific absorption rate (SAR) of 2 W/kg, 2 h/day for 4 consecutive weeks does not affect the frequencies of differentiating transitional 1 (T1) and T2 B cells or those of mature follicular B and marginal zone B cells in the spleen. IgM and IgG serum levels are also not significantly different among exposed, sham-exposed and control mice. B cells from these mice, challenged in vitro with LPS, produce comparable amounts of IgM and IgG. Moreover, exposure of immunized mice to RF fields does not change the antigen-specific antibody serum level. Interestingly, not only the production of antigen-specific IgM but also that of IgG (which requires T-B-cell interaction) is not affected by RF-field exposure. This indicates that the exposure does not alter an ongoing in vivo antigen-specific immune response. In conclusion, the results of Nasta et al. (2006) do not indicate any effects of GSM-modulated RF radiation on the B-cell peripheral compartment and antibody production.

Whole-body microwave sinusoidal irradiation of male NMRI mice, exposure of macrophages in vitro, and preliminary irradiation of culture medium with 8.15-18 GHz (1 Hz within) at a power density of 1 microW/cm2 caused a significant enhancement of tumor necrosis factor production in peritoneal macrophages (Novoselova et al, 1998). The role of microwaves as a factor interfering with the process of cell immunity must, thus, be seriously considered. Furthermore the effect of 8.15-18 GHz (1 Hz within) microwave radiation at a power density of 1 microW/cm2 on the tumor necrosis factor (TNF) production and immune response was tested by Novoselova et al. (1999). A single 5 h whole-body exposure induced a significant increase in TNF production in peritoneal macrophages and splenic T cells. The mitogenic response in T lymphocytes increased after microwave exposure. The activation of cellular immunity was observed within 3 days after exposure. The diet containing lipid-soluble nutrients (beta-carotene, alpha-tocopherol and ubiquinone Q9) increased the activity of macrophages and T cells from irradiated mice.

Obukhan (1998) has performed cytologic investigations designed to study bone marrow, peripheral blood, spleen, and thymus of albino rats irradiated by an electromagnetic field, 2,375, 2,450, and 3,000 MHz. Structural and functional changes in populations of megakaryocytes, immunocompetent cells as well as of undifferentiated cells, and of other types of cells that are dependent on the intensity of irradiation.

The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with x-rays was recently investigated in vitro using several assays on human lymphocytes by Stronati and colleagues (2006). The chosen specific absorption rate (SAR) values are near the upper limit of actual energy absorption in localized tissue when persons use some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and

established mutagen. Blood specimens from 14 donors were exposed continuously for 24 h to a Global System for Mobile Communications (GSM) basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in vitro cell cycling. By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did not modify any measured effects of the x-radiation. In conclusion, this study has used several standard in vitro tests for chromosomal and DNA damage in Go human lymphocytes exposed in vitro to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic per se or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed.

Tuschl et al. (1999) recorded a considerable excess of recommended exposure limits in the vicinity of shortwave diathermy devices used for medical treatment of patients. Different kinds of field probes were used to measure electric and magnetic field strength and the whole body exposure of medical personnel operating shortwave, decimeter wave and microwave units was calculated. To investigate the influence of chronic exposure on the immune system of operators, blood was sampled from physiotherapists working at the above mentioned devices. Eighteen exposed and thirteen control persons, matched by sex and age, were examined. Total leucocyte and lymphocyte counts were performed and leucocytic subpopulations determined by flow cytometry and monoclonal antibodies against surface antigens. In addition, to quantify subpopulations of immunocompetent cells, the activity of lymphocytes was measured. Lymphocytes were stimulated by mitogen phytohemagglutinin and their proliferation measured by a flow cytometric method. No statistically significant differences between the control and exposed persons were found. In both study groups all immune parameters were within normal ranges.

Despite the important role of the immune system in defending the body against infections and cancer, only few investigations on possible effects of radiofrequency (RF) radiation on function of human immune cells have been undertaken. One of these is the investigation by Tuschl et al. in 2005 where they assessed whether GSM modulated RF fields have adverse effects on the functional competence of human immune cells. Within the frame of the multidisciplinary project "Biological effects of high frequency electromagnetic fields (EMF)" sponsored by the National Occupation Hazard Insurance Association (AUVA) in vitro investigations were carried out on human blood cells. Exposure was performed at GSM Basic 1950 MHz, an SAR of 1 mW/g in an intermittent mode (5 min "ON", 10 min "OFF") and a maximum Delta T of 0.06 degrees C for the duration of 8 h. The following immune parameters were evaluated: (1) the intracellular production of interleukin-2 (IL-2) and interferon (INF) gamma in lymphocytes, and IL-1 and tumor necrosis factor (TNF)-alpha in monocytes were evaluated with monoclonal antibodies. (2) The activity of immune-relevant genes (IL 1-alpha and beta, IL-2, IL-2-receptor, IL-4, macrophage colony stimulating factor (MCSF)-receptor, TNF-alpha, TNF-alpha-receptor) and

housekeeping genes was analyzed with real time PCR. (3) The cytotoxicity of lymphokine activated killer cells (LAK cells) against a tumor cell line was determined in a flow cytometric test. For each parameter, blood samples of at least 15 donors were evaluated. No statistically significant effects of exposure were found and there is no indication that emissions from mobile phones are associated with adverse effects on the human immune system.

Irradiation by pulsed microwaves (9.4 GHz, 1 microsecond pulses at 1,000/s), both with and without concurrent amplitude modulation (AM) by a sinusoid at discrete frequencies between 14 and 41 MHz, was assessed for effects on the immune system of Balb/C mice (Veyret et al, 1991). The mice were immunized either by sheep red blood cells (SRBC) or by glutaric-anhydride conjugated bovine serum albumin (GA-BSA), then exposed to the microwaves at a low rms power density (30 microW/cm2; whole-body-averaged SAR approximately 0.015 W/kg). Sham exposure or microwave irradiation took place during each of five contiguous days, 10 h/day. The antibody response was evaluated by the plaque-forming cell assay (SRBC experiment) or by the titration of IgM and IgG antibodies (GA-BSA experiment). In the absence of AM, the pulsed field did not greatly alter immune responsiveness. In contrast, exposure to the field under the combined-modulation condition resulted in significant, AM-frequency-dependent augmentation or weakening of immune responses.

Finally, in addition, classical allergy reactions, such as chromate allergy, has been studied by Seishima et al. (2003). The background for the study was an earlier case report about a patient with allergic contact dermatitis caused by hexavalent chromium plating on a cellular phone. The new study described the clinical characteristics and results of patch tests (closed patch tests and photopatch tests were performed using metal standard antigens) in 8 patients with contact dermatitis possibly caused by handling a cellular phone. The 8 patients were 4 males and 4 females aged from 14 to 54 years. They each noticed skin eruptions after 9-25 days of using a cellular phone. All patients had erythema, and 7 had papules on the hemilateral auricle or in the preauricular region. Three of 8 patients had a history of metal allergy. Chromate, aluminium and acrylnitrile-butadiene-styrene copolymer were used as plating on the cellular phones used by these patients. The patch test was positive for 0.5, 0.1 and 0.05% potassium dichromate in all 8 patients. The photopatch test showed the same results. One patient was positive for 2% cobalt chloride and one for 5% nickel sulfate. Based on these data, it is important to consider the possibility of contact dermatitis due to a cellular phone, possibly caused by chromate, when the patients have erythema and papules on the hemilateral auricle or in the preauricular region.

VII. Electromagnetic fields and health

Since the formation of life on Earth, as we know it, more than 3.5 billion years ago, the only real source of radiation, apart from Earth's static geomagnetic field, has been the sun. All living organisms that have evolved and not been able to cope with it are either gone or have adapted to it in one of several ways. Living under-ground, only being active during night, living in the deeper waters (1 meter or deeper) in oceans and lakes, under the foliage of jungle-trees, or - as all day-active organisms have – developed a skin (or, for plants, a cortex) containing a pigment (animals and plants have very similar ones) that will shield some heat and some sunshine…but not very

much. Any fair-skinned Irish or Scandinavian person learns very early to avoid even the rather bleak sun up-north, because – if not – you will easily get a nasty sunburn. Later on, that sunburn will develop into a postinflammatory hyperpigmentation, with it's cosmetic values, however, well before it you will get a strong alarm signal in the form of a redness of the skin.

When considering other frequencies, the pigment does not furnish any protection at all, something mankind has found out during the last 100 years. Cosmic rays, radioactivity, X-rays, UVC, UVB and now even UVA are considered, together with radar-type microwaves to be very, or even extremely, dangerous to your health. You are translucent to exposures such as power-frequent magnetic fields as well as mobile phone and WI-FI microwaves, but this does not mean that they are without possible effect, through thermal or non-thermal mechanisms.

Is it possible that we can adapt our biology to altered exposure conditions in less than 100 years, or do we have to have thousands of years for such an adaptation? And, in the meantime, what kind of safety standards must we adopt if the current public safety limits are not sufficiently protective of public health?

The World Health Organization (WHO) has acknowledged the condition of electrohypersensitivity, and published a 2006 research agenda for radio-frequency fields (see Addendum to Chapter 12 on the Swedish Government response to persons with Electrosensitivity). The WHO recommends that people reporting sensitivities receive a comprehensive health evaluation. It states: "Some studies suggest that certain physiological responses of EHS individuals tend to be outside the normal range. In particular, hyperactivity in the central nervous system and imbalance in the autonomic nervous system need to be followed up in clinical investigations and the results for the individuals taken as input for possible treatment." Studies of individuals with sensitivities ought to consider sufficient acclimatization of subjects as recommended for chemical sensitivities, as well as recognition of individuals' wavelength-specific sensitivities. Reduction of electromagnetic radiation may ameliorate symptoms in people with chronic fatigue.

Off-gassing of electrical equipment may also contribute to sensitivities. Different sorts of technology (e.g. various medical equipment, analogue or digital telephones; flat screen monitors and laptop computers or larger older monitors) may vary significantly in strength, frequency and pattern of electromagnetic fields. One challenging question for science is to find out if, for instance, 50- or 60-Hz ELF pure sine wave, square waves or sawtooth waveform, ELF-dirty (e.g. radiofrequencies on power lines), ELF-modulated radiofrequency fields, continuous wave radiofrequency radiation and particularly pulsed radiofrequency signals are more or less bioactive, e.g. as neurotoxic and/or carcinogenic environmental exposure parameters. (see Chapter 8 on Disruption by Modulation).

VIII. Conclusions

• Both human and animal studies report large immunological changes with exposure to environmental levels of electromagnetic fields (EMFs). Some of these exposure levels are equivalent to those of e.g. wireless technologies in daily life.

• Measurable physiological changes (mast cells increases, for example) that are bedrock indicators of allergic response and inflammatory conditions are stimulated by EMF exposures.

• Chronic exposure to such factors that increase allergic and inflammatory responses on a continuing basis may be harmful to health.

• It is possible that chronic provocation by exposure to EMF can lead to immune dysfunction, chronic allergic responses, inflammatory responses and ill health if they occur on a continuing basis over time. This is an important area for future research.

 • Specific findings from studies on exposures to various types of modern equipment and/or EMFs report over-reaction of the immune system; morphological alterations of immune cells; profound increases in mast cells in the upper skin layers, increased degranulation of mast cells and larger size of mast cells in electrohypersensitive individuals; presence of biological markers for inflammation that are sensitive to EMF exposure at non-thermal levels; changes in lymphocyte viability; decreased count of NK cells; decreased count of T lymphocytes; negative effects on pregnancy (uteroplacental circulatory disturbances and placental dysfunction with possible risks to pregnancy); suppressed or impaired immune function; and inflammatory responses which can ultimately result in cellular, tissue and organ damage.

• Electrical hypersensitivity is reported by individuals in the United States, Sweden, Switzerland, Germany. Denmark and many other countries of the world. Estimates range from 3% to perhaps 10% of populations, and appears to be a growing condition of ill-health leading to lost work and productivity.

• The WHO and IEEE literature surveys do not include all of the relevant papers cited here, leading to the conclusion that evidence has been ignored in the current WHO ELF Health Criteria Monograph; and the proposed new IEEE C95.1 RF public exposure limits (April 2006).

• The current international public safety limits for EMFs do not appear to be sufficiently protective of public health at all, based on the studies of immune function. New, biologically-based public standards are warranted that take into account low-intensity effects on immune function and health that are reported in the scientific

IX.   Acknowledgements

X. References

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Appendix 8-A   Some legal aspects of the functional impairment electrohypersensitivity in Sweden

In Sweden, electrohypersensitivity (EHS) is an officially fully recognized functional impairment (i.e., it is not regarded as a disease). Survey studies show that somewhere between 230,000 - 290,000 Swedish men and women, out of a population of 9,000,000 people, report a variety of symtoms when being in contact with electromagnetic field (EMF)-sources.

The electrohypersensitive persons have their own handicap organisation; The Swedish Association for the ElectroSensitive; http://www.feb.se (the website has an English version). This organisation is included in the Swedish Disability Federation (Handikappförbundens SamarbetsOrgan; HSO). HSO is the unison voice of the Swedish disability associations towards the government, the parliament and national authorities and is a cooperative body that today consists of 43 national disability organisations (where The Swedish Association for the ElectroSensitive is 1 of these 43 organisations) with all together about 500,000 individual members. You can read more on http://www.hso.se (the site has an English short version). The Swedish Association for the ElectroSensitive gets a governmental subsidy   as a handicap organization according to SFS 2000:7 §2 (SFS = The Swedish   Governmental Statute-Book).   EHS persons' right to get disablement allowances has been settled in The Swedish   Supreme Administrative Court, i.a. in the judgement "dom 2003-01-29, mål nr. 6684-2001".

Swedish municipalities, of course, have to follow the UN 22 Standard Rules on the equalization of opportunities for persons with disabilities ("Standardregler för att tillförsäkra människor med funktionsnedsättning delaktighet och jämlikhet"; about the UN 22 Standard Rules, see website: http://www.un.org/esa/socdev/enable/dissre00.htm). All persons with disabilities shall, thus, be given the assistance and service they have the right to according to the Swedish Act concerning Support and Service for Persons with Certain Functional Impairments (LSS-lagen) and the Swedish Social Services Act (Socialtjänstlagen). Persons with disabilities, thus, have many different rights and can get different kinds of support. The purpose of those rights and the support is to give every person the chance to live like everyone else. Everyone who lives in the Swedish municipalities should be able to lead a normal life and the municipalities must have correct knowledge and be able to reach the persons who need support and service. Persons with disabilities shall be able to get extra support so that they can live, work, study, or do things they enjoy in their free time. The municipalities are responsible for making sure that everyone gets enough support. Everyone shall show respect and remember that such men and women may need different kinds of support.

In Sweden, impairments are viewed from the point of the environment. No human being is in itself impaired, there are instead shortcomings in the environment that cause the impairment (as the lack of ramps for the person in a wheelchair or rooms electrosanitized for the person with electrohypersensitivity). This environment-related impairment view, furthermore, means that even though one does not have a scientifically-based complete explanation for the impairment electrohypersensitivity, and in contrast to disagreements in the scientific society, the person with

electrohypersensitivity shall always be met in a respectful way and with all necessary support with the goal to eliminate the impairment. This implies that the person with electrohypersensitivity shall have the opportunity to live and work in an electrosanitized environment.

This view can fully be motivated in relation to the present national and international handicap laws and regulations, including the UN 22 Standard Rules and the Swedish action plan for persons with impairments (prop. 1999/2000:79 "Den nationella handlingplanen för handikappolitiken - Från patient till medborgare"). Also the Human Rights Act in the EU fully applies.

A person is disabled when the environment contains some sort of impediments. It means that in that moment a man or woman in a wheelchair can not come onto the bus, a train, or into a restaurant, this person has a disability, he or she is disabled. When the bus, the train or the restaurant are adjusted for a wheelchair, the person do not suffer from his disability and are consequently not disabled. An electrohypersensitive person suffers when the environment is not properly adapted according to their personal needs. Strategies to enable a person with this disability to attend common rooms such as libraries, churches and so on, are for instance to switch off the high-frequency fluorescent lamps and instead use ordinary light bulbs. Another example is the possibility to switch off - the whole or parts of - the assistive listening systems (persons with electrohypersensitivity are often very sensitive to assistive listening systems).

In the Stockholm municipality - were I live and work as a scientist with the responsibility to investigate comprehensive issues for persons with electrohypersensitivity - such persons have the possibility to get their home sanitized for EMFs. It means for example that ordinary electricity cables are changed to special cables. Furthermore, the electric stove can be changed to a gas stove and walls, roof and floors can be covered with special wallpaper or paint with a special shelter to stop EMFs from the outside (from neighbours and mobile telephony base stations). Even the windows can be covered with a thin aluminum foil as an efficient measure to restrain EMFs to get into the room/home. If these alterations turn out not to be optimal they have the possibility to rent small cottages in the countryside that the Stockholm municipality owns. These areas have lower levels of irradiation than others. The Stockholm municipality also intend to build a village with houses that are specially designed for persons who are electrohypersensitive. This village will be located in a low-lewel irradiation area. [One of my graduate students, Eva-Rut Lindberg, has in her thesis project studied the "construction of buildings for persons with the impairment electrohypersensitivity". The doctoral thesis will be presented during the Autumn.]

Persons with electrohypersensitivity also have a general (legal) right to be supported by their employer so that they can work despite of this impairment. For instance, they can get special equipment such as computers that are of low-emission type, that high-frequency fluorescent lamps are changed to ordinary light bulbs, no wireless DECT telephones in their rooms, and so on.

Some hospitals in Sweden (e.g. in Umeå, Skellefteå and Karlskoga) also have built special rooms with very low EMFs so that persons who are hypersensitive can get

medical care. Another example is the possibility for persons who are electrohypersensitive to get a specially designed car so that the person can transport himself/herself between his/her home and their workplace.

Recently, some politicians in the Stockholm municipality even proposed to the politicians responsible for the subway in the Stockholm City that a part of every trainset should be free from mobile phones; that the commuters have to switch of the phones in these selected parts to enable persons with electrohypersensitivity to travel with the subway (compare this with persons who have an allergy for animal fur whereupon people consequently is prohibited to have animals, such as dogs or cats, in selected parts of the trainset).

In addition, when the impairment electrohypersensitivity is discussed it is also of paramount importance that more general knowledge is needed with the aim to better adapt the society to the specific needs of the persons with this impairment. The Swedish "Miljöbalk" (the Environmental Code) contains an excellent prudence avoidance principle which, of course, most be brought into action also here, together with respect and willingness to listen to the persons with electrohypersensitivity.

Naturally, all initiatives for scientific studies of the impairment electrohypersensitivity must be characterized and marked by this respect and willingness to listen, and the investigations shall have the sole aim to help the persons with this particular impairment. Rule 13 in the UN 22 Standard Rules clearly says that scientific investigations of impairments shall, in an unbiased way - and without any prejudice - focus on cause, occurrence and nature and with the sole and explicit purpose to help and support the person with the impairment.

A unique conference recently was held in Stockholm in May, 2006. The theme for the conference was "The right for persons with the impairment electrohypersensitivity to live in a fully accessible society". The conference was organized by the Stockholm City municipality and the Stockholm County Council and dealt with the most recent measures to make Stockholm fully accessible for persons with the impairment electrohypersensitivity. Among such measures are to offer home equipment adjustments, ban mobile phones from certain underground cars as well as certain public bus seats, and through electrosanitized hospital wards. The conference was documented on film.

SECTION 9

EVIDENCE FOR EFFECTS ON

NEUROLOGY AND BEHAVIOR

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Table of Contents

I. Introduction

This chapter is a brief review of recent studies on the effects of radiofrequency radiation (RFR) on neuronal functions and their implication on learning and memory in animal studies, effects on electrical activity of the brain and relation to cognitive functions, and finally a section on the effects of cell phone radiation on the auditory system. There is also a set of studies reporting subjective experience in humans exposed to RFR. This includes reports of fatigue, headache, dizziness, and sleep disturbance, etc.

The close proximity of a cellular telephone antenna to the user's head leads to the deposition of a relatively large amount of radiofrequency energy in the head. The relatively fixed position of the antenna to the head causes a repeated irradiation of a more or less fixed amount of body tissue, including the brain at a relatively high intensity to ambient levels. The question is whether such exposure affects neural functions and behavior.

II. Chemical and cellular changes

Several studies have investigated the effect of RFR on the cholinergic system because of its involvement in learning and wakefulness and animals. Testylier et al. [2002] reported modification of the hippocampal cholinergic system in rats during and after exposure to low-intensity RFR. Bartier et al. [2005] reported that RFR exposure induced structural and biochemical changes in AchE, the enzyme involved in acetylcholine metabolism. Vorobyov et al. [2004] reported that repeated exposure to low-level extremely low frequency-modulated RFR affected baseline and scopolamine-modified EEG in freely moving rats. However, recently Crouzier et al [2007] found no significant change in acetylcholine-induced EEG effect in rats exposed for 24 hours to a 1.8 MHz GSM signal at 1.2 and 9 W/cm^2.

There are several studies on the inhibitory and excitatory neurotransmitters. A decrease in GABA, an inhibitory transmitter, content in the cerebellum was reported by Mausset et al. [2001] after exposure to RFR at 4 W/kg. The same researchers [Maussset-Bonnefont et al., 2004] also reported changes in affinity and concentration of NMDA and GABA receptors in the rat brain after an acute exposure at 6 W/kg. Changes in GABA receptors has also been reported by Wang et al. [2005], and reduced excitatory synaptic activity and number of excitatory synapses in cultured rat hippocampal neurons have been reported by Xu et al. [2006] after RFR exposure. Related to the findings of changes in GABA in the brain is that RFR has been shown to facilitate seizure in rats given subconvulsive doses of picotoxin, a drug that blocks the GABA system [Lopez Martin et al., 2006]. This finding raises the concern that humans with epileptic disorder could be more susceptible to RFR exposure.

Not much has been done on single cell in the brain after RFR exposure. Beason and Semm [2002] reported changes in the amount of neuronal activity by brain cells of birds exposed to GSM signal. Both increase and decrease in firing were observed. Salford et al. [2003] reported cellular damage and death in the brain of rat after acute exposure to GSM signals. Tsurita et al. [2000] reported no significant morphological change in the cerebellum of rats exposed for 2-4

weeks to 1439-MHz TDMA field at 0.25 W/kg. More recently, Joubert et al. [2006, 2007] found no apoptosis in rat cortical neurons exposed to GSM signals in vitro.

III. Learning in Animals

Few animal learning studies have been carried out. All of them reported no significant effect of exposure to cell phone radiation on learning. Bornhausen and Scheingrahen [2000] found no significant change in operant behavior in rats prenatally exposed to a 900-MHz RFR. Sienkiewicz et al. [2000] reported no significant effect on performance in an 8-arm radial maze in mice exposed to a 900-MHz RFR pulsed at 217 Hz at a whole body SAR of 0.05 W/Kg. Dubreuil et al. [2002, 2003] found no significant change in radial maze performance and open-field behavior in rats exposed head only for 45 min to a 217-Hz modulated 900-MHz field at SARs of 1 and 3.5 W/kg. Yamaguichi et al. [2003] reported a change in T-maze performance in the rat only after exposure to a high whole body SAR of 25 W/kg.

IV. Electrophysiology

Studies on EEG and brain evoked-potentials in humans exposed to cellular phone radiation predominantly showed positive effects. The following is a summary of the findings in chronological order. (There are seven related papers published before 1999).

Von Klitzing et al. [1995] were the first to report that cell phone radiation affected EEG alpha activity during and after exposure to cell phone radiation.

Mann and Roschke [1996] reported that cell phone radiation modified REM sleep EEG and shortened sleep onset latency.

Rosche et al. [1997] found no significant change in spectral power of EEG in subjected exposure to cell phone radiation for 3.5 minutes.

Eulitz et al. [1998] reported that cell phone radiation affected brain activity when subjects were processing task-relevant target stimuli and not for irrelevant standard stimuli.

Freude et al. [1998] found that preparatory slow brain potential was significantly affected by cellular phone radiation in certain regions of the brain when the subjects were performing a cognitive complex visual task. The same effects were not observed when subjects were perfoming a simple task.

Urban et al. [1998] reported no significant change in visual evoked potentials after 5 minutes of exposure to cell phone radiation.

Wagner et al. [1998, 2000] reported that cell phone radiation had no significant effect on sleep EEG.

Borbely et al. [1999] reported that the exposure induced sleep and also modified sleep EEG during the non-rapid eye movement (NREM) stage.

Hladky et al. [1999] reported that cell phone use did not affect visual evoked potential.

Freude et al. [2000] confirmed their previous report that cellular phone radiation affected slow brain potentials when subjects are performing a complex task. However, they also reported that the exposure did not significantly affect the subjects in performing the behavioral task. Huber et al. [2000] reported that exposure for 30 minutes to a 900-MHz field at 1 W/kg peak SAR during waking modified EEG during subsequent sleep.

Hietanen et al. [2000] found no abnormal EEG effect, except at the delta band, in subjects exposed for 30 minutes to 900- and 1800-MHz fields under awake, closed-eye condition.

Krause et al. [2000a] reported that cell phone radiation did not affect resting EEG but modified brain activity in subjects performing an auditory memory task.

Krause et al. [2000b] reported that cell phone radiation affected EEG oscillatory activity during a cognitive test. The visual memory task had three different working memory load conditions. The effect was found to be dependent on memory load.

Lebedeva et al. [2000] reported that cell phone radiation affected EEG.

Jech et al. [2001] reported that exposure to cell phone radiation affected visual event-related potentials in narcolepsy patient performing a visual task.

Lebedeva et al. [2001] reported that cell phone radiation affected sleep EEG.

Huber et al [2002] reported that exposure to pulsed modulated RFR prior to sleep affected EEG during sleep. However, effect was not seen with unmodulated field. They also found that the pulsed field altered regional blood flow in the brain of awake subjects.

Croft et al. [2002] reported that radiation from cellular phone altered resting EEG and induced changes differentially at different spectral frequencies as a function of exposure duration.

D'Costa et al. [2003] found EEG effect affected by the radiation within the alpha and beta bands of EEG spectrum.

Huber et al. [2003] reported EEG effect during NREM sleep and the effect was not dependent on the side of the head irradiated. They concluded that the effect involves subcortical areas of the brain that project to both sides of the brain. Dosimetry study shows that the SAR in those area during cell phone use is relatively very low, e.g., 0.1 W/kg at the thalamus. Recently, Aalta et al. [2006], using PET scan imaging, reported a local decrease in regional cerebral blood flow under the antenna in the inferior temporal cortex, but an increase was found in the prefrontal cortex.

Kramarenko et al. [2003] reported abnormal EEG slow waves in awake subjects exposed to cell phone radiation.

Marino et al. [2003] reported an increased randomness of EEG in rabbits.

Hamblin et al. [2004] reported changes in event-related auditory evoked potential in subjects exposed to cellular phone radiation when performing an auditory task. They also found an increase in reaction time in the subjects, but no change in accuracy in the performance.

Hinrich and Heinze [2004] reported a change in early task-specific component of event-related magnetic field in the brain of exposed subjects during a verbal memory encoding task.

Krause et al. [2004] repeated the experiment with auditory memory task [Krause et al., 2000b] and found different effects.

Papageorgiou et al. [2004] reported that cell phone radiation affected male and female EEG differently.

Vorobyov et al. [2004] reported that repeated exposure to modulated microwaves affected baseline and scopolamine-modified EEG in freely moving rats.

Curcio et al. [2005] reported that EEG spectral power affected in the alpha band and the effect was greater when the field was on during EEG recording than when applied before recording.

Hamblin et al. [2005] stated that they could not replicate their previous results on auditory evoked potentials.

Huber et al. [2005] found altered cerebral blood flow in humans exposed to pulsed modulated cell phone radiation. They concluded that, "This finding supports our previous observation that pulse modulation of RF EMF is necessary to induce changes in the waking and sleep

EEG, and substantiates the notion that pulse modulation is crucial for RF EMF-induced alterations in brain physiology."

Loughran et al. [2005] reported that exposure to cell phone radiation prior to sleep promoted REM sleep and modified sleep in the first NREM sleep period.

Ferreri et al. [2006] tested excitability of each brain hemisphere by transcranial magnetic stimulation and found that, after 45 minutes of exposure to cellular phone radiation, intracortical excitability was significantly modified with a reduction of inhibition and enhancement in facilitation.

Krause et al. [2006] reported that cell phone radiation affected brain oscillatory activity in children doing an auditory memory task.

Papageorgiou et al. [2006] reported that the radiation emitted by cell phone affects pre-attentive working memory information processing as reflected by changes in P50 evoked potential.

Yuasa et al. [2006] reported no significant effect of cell phone radiation on human somatosensory evoked potentials after 30 minutes of exposure.

Krause et al. [2007] reported effects on brain oscillatory responses during memory task performance. But, they concluded that "The effects on the EEG were, however, varying, unsystematic and inconsistent with previous reports. We conclude that the effects of EMF on brain oscillatory responses may be subtle, variable and difficult to replicate for unknown reasons."

Vecchio et al. [2007] reported that exposure to GSM signal for 45 min modified interhemispheric EEG coherence in cerebral cortical areas.

Hung et al. [2007] reported that after 30 min of exposure to talk-mode mobile phone radiation, sleep latency was markedly and significantly delayed beyond listen and sham modes in healthy human subjects. This condition effect over time was also quite evident in 1-4Hz EEG frontal power, which is a frequency range particularly sensitive to sleep onset.

There is little doubt that electromagnetic fields emitted by cell phones and cell phone use affect electrical activity in the brain. The effect also seems to depend on the mental load of the subject during exposure, e.g., on the complexity of the task that a subject is carrying out. Based on the observation that the two sides of the brain responded similarly to unilateral exposure, Huber et al. [2003] deduced that the EEG effect originated from subcortical areas of the brain. Dosimetry calculation indicates that the SAR in such areas could be as low as 0.1 W/kg.

However, the behavioral consequences of these neuroelectrophysiological changes are not always predictable. In several studies (e.g., Freude et al., 2000; Hamblin et al, 2004), cell phone radiation-induced EEG changes were not accompanied by a change in psychological task performance of the subjects. The brain has the flexibility to accomplish the same task by different means and neural pathways. Does cell phone radiation alter information-processing functions in the brain as reported previously with RFR exposure [Wang and Lai, 2000]? In the next section, we will look at the effects of cell phone radiation exposure on cognitive functions in humans.

V. Cognitive functions

Again, findings are listed below in chronological order.

Preece et al. [1999] were the first to report an increase in responsiveness, strongly in the analogue and less in the digital cell phone signal, in choice reaction time.

Cao et al. [2000] showed that the average reaction time in cell phone users was significantly longer than that in control group in psychological tests. The time of use was negatively associated with corrected reaction number.

Koivisto et al. [2000a, b] reported a facilitation of reaction in reaction time tasks during cell phone radiation exposure. In a working memory test, exposure speeded up response times when the memory load was three items but no significant effect was observed with lower loads.

Jech et al. [2001] reported that cell phone radiation may suppress the excessive sleepiness and improve performance while solving a monotonous cognitive task requiring sustained attention and vigilance in narcolepsy patients.

Lee et al. [2001] reported a facilitation effect of cell phone radiation in attention functions.

Edelstyn and Oldershaw [2002] found in subjects given 6 psychological tests a significant difference in three tests after 5 min of exposure. In all cases, performance was facilitated following cell phone radiation exposure.

Haarala et al. [2003] found no significant effect of cell phone radiation on the reaction time and response accuracy of subjects performed in 9 cognitive tasks.

Lee et al. [2003] reported that the facilitation effect of cell phone radiation on attention functions is dose (exposure duration)-dependent.

Smythe and Costall [2003] using a word learning task, found that male subjects made significantly less error than unexposed subject. However, the effect was not found in female subjects. (Papageorgiou et al. [2004] also reported that cell phone radiation affected male and female EEG differently.)

Curcio et al. [2004] found in subjects tested on four performance tasks, an improvement of both simple- and choice-reaction times. Performance needed a minimum of 25 min of EMF exposure to show significant changes.

Haarala et al. [2004] reported that they could not replicate their previous results [Koivisto ret al., 2000a] on the effect of cell phone radiation on short-term memory.

Maier et al. [2004] found that subjects exposed to GSM signal showed worse results in their auditory discrimination performance as compared with control conditions.

Basset et al. [2005] reported no significant effect of daily cell phone use on a battery of neuropsychological tests screening: information processing, attention capacity, memory function, and executive function. The authors concluded that "…our results indicate that daily MP use has no effect on cognitive function after a 13-h rest period."

Haarala et al [2005] reported that 10-14 year old children's cognitive functions were not affected by cell phone radiation exposure.

Preece et al. [2005] concluded that, "this study on 18 children did not replicate our earlier finding in adults that exposure to microwave radiation was associated with a reduction in reaction time." They speculated that the reason for the failure to replicate was because a less powerful signal was used in this study.

Schmid et al. [2005] reported no significant effect of cell phone radiation on visual perception.

Eliyaku et al. [2006] reported in subjects given 4 cognitive tasks that exposure of the left side of the brain slowed down the left-hand response time in three of the four tasks.

Keetley et al. [2006] tested 120 subjects on 8 neuropsychological tests and concluded that cell phone emissions "improve the speed of processing of information held in working memory."

7

Russo et al. [2006] reported that GSM or CW signal did not significantly affect a series of cognitive tasks including a simple reaction task, a vigilance task, and a subtraction task.

Terao et al. [2006] found no significant effect of cell phone use on the performance of visuo-motor reaction time task in subjects after 30 minutes of exposure.

Haarala et al. [2007] concluded that 'the current results indicate that normal mobile phones have no discernible effect on human cognitive function as measured by behavioral tests.'

Terao et al. [2007] reported no significant effect of a 30-min exposure to mobile phone radiation on the performance of various saccade tasks (visually-guided, gap, and memory-guide), suggesting that the cortical processing for saccades and attention is not affected by the exposure.

Cinel et al. [2007] reported that acute exposure to mobile phone RF EMF did not affect performance in the order threshold task.

Thus, a majority of the studies (13/23) showed that exposure to cell phone could affect cognitive functions and affect performance in various behavioral tasks. Interestingly, most of these studies showed a facilitation and improvement in performance. Only the studies of Cao et al. [2000], Maier et al. [2004] and Eliyaku et al. [2006] reported a performance deficit. (It may be significant to point out that of the 10 studies that reported no significant effect, 6 of them were funded by the cell phone industry and one [Terao et al., 2006] received partial funding from the industry.)

VI. Auditory effect

Since the cell phone antenna is close to the ear during use, a number of studies have been carried out to investigate the effect of cell phone radiation on the auditory system and its functions. Kellenyi et al. [1999] reported a hearing deficiency in the high frequency range in subjects after 15 minutes of exposure to cell phone radiation. Mild hearing loss was reported by Garcia Callejo et al. [2005], Kerckhanjanarong et al [2005] and Oktay and Dasdag [2006] in cell phone users. However, these changes may not be related to exposure to electromagnetic fields. Recently, Davidson and Lutman [2007] reported no chronic effects of cell phone usage on hearing, tinnitus and balance in a student population.

Auditory-evoked responses in the brain have been studied. Kellenyi et al. [1999], in addition to hearing deficiency, also reported a change in auditory brainstem response in their subjects. However, no significant effect on brainstem and cochlear auditory responses were found by Arai et al.[2003], Aran et al. [2004], and Sievert et al. [2005]. However, Maby et al. [2004, 2005, 2006] reported that GSM electromagnetic fields modified human auditory cortical activity recorded at the scalp.

Another popular phenomenon studied in this aspect is the distorted product otoacoustic emission, a measure of cochlear hair cell functions. Grisanti et al. [1998] first reported a change in this measurement after cell phone use. Subsequent studies by various researchers using different exposure times and schedules failed to find any significant effect of cell phone radiation [Aren et al. 2004; Galloni et al., 2005 a,b; Janssen et al., 2005; Kizilay et al, 2003; Marino et al., 2000; Monnery et al., 2004; Mora et al., 2006; Ozturan et al., 2002; Parazzini et al., 2005; Uloziene et al., 2005].

There have been reports suggesting that people who claimed to be hypersensitive to EMF have higher incidence of tinnitus [Cox, 2004: Fox, 2004; Holmboe and Johansson, 2005]. However, data from the physiological studies described above do not indicate that EMF exposure could cause tinnitus.

VII. Human subjective effects

Abdel-Rassoul G, El-Fateh OA, Salem MA, Michael A, Farahat F, El-Batanouny M, Salem E. Neurobehavioral effects among inhabitants around mobile phone base stations. Neurotoxicology. 28:434-440, 2007.

Al-Khlaiwi T, Meo SA. Association of mobile phone radiation with fatigue, headache, dizziness, tension and sleep disturbance in Saudi population. Saudi Med J. 25(6):732-736, 2004.

Balik HH, Turgut-Balik D, Balikci K, Ozcan IC. Some ocular symptoms and sensations experienced by long term users of mobile phones. Pathol Biol (Paris). 53(2):88-91, 2005. Balikci K, Cem Ozcan I, Turgut-Balik D, Balik HH. A survey study on some neurological symptoms and sensations experienced by long term users of mobile phones. Pathol Biol (Paris). 53(1):30-34, 2005.

Bergamaschi A, Magrini A, Ales G, Coppetta L, Somma G. Are thyroid dysfunctions related to stress or microwave exposure (900 MHz)? Int J Immunopathol Pharmacol. 17(2 Suppl):31-36, 2004.

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Wilen J, Sandstrom M, Hansson Mild K. Subjective symptoms among mobile phone users-A consequence of absorption of radiofrequency fields? Bioelectromagnetics 24(3):152-159, 2003.
Wilen J, Johansson A, Kalezic N, Lyskov E, Sandstrom M. Psychophysiological tests and provocation of subjects with mobile phone related symptoms. Bioelectromagnetics 27:204-214, 2006.

The possible existence of physical symptoms from exposure to RFR from various sources including cell phones, cell towers and wireless systems has been a topic of significant public concern and debate. This is an issue that will require additional attention. Symptoms that have been reported include: sleep disruption and insomnia, fatigue, headache, memory loss and confusion, tinnitus, spatial disorientation and dizziness. However, none of these effects has been studied under controlled laboratory conditions. Thus, whether they are causally related to RFR exposure is unknown.

VIII. Summary and Discussion

A. Research data are available suggesting effects of RFR exposure on neurological and behavioral functions. Particularly, effects on neurophysiological and cognitive functions are quite well established. Interestingly, most of the human studies showed an enhancement of cognitive function after exposure to RFR, whereas animals studied showed a deficit. However, research on electrophysiology also indicates that effects are dependent on the mental load of the subjects during exposure. Is this because the test-tasks used in the animal studies are more complex or the nervous system of non-human animals can be easier overloaded? These point to an important question on whether RFR-induced cognitive facilitation still occurs in real life situation when a person has to process and execute several behavioral functions simultaneously. Generally speaking, when effects were observed, RFR disrupted behavior in animals, such as in the cases of behaviors to adapt to changes in the environment and learning. This is especially true when the task involved complex responses. In no case has an improvement in behavior been reported in animals after RFR exposure. It is puzzling that only disruptions in behavior by RFR exposure are reported in non-human animals. In the studies on EEG, both excitation and depression have been reported after exposure to RFR. If these measurements can be considered as indications of electrophysiological and behavioral arousal and depression, improvement in behavior should occur under certain conditions of RFR exposure. This is now reported in humans exposed to cell phone radiation.

B. On the other hand, one should be very careful in extrapolating neurological/behavioral data from non-human in vivo experiments to the situation of cell phone use in humans. The structure and anatomy of animal brains are quite different from those of the human brain. Homologous structures may not be analogous in functions. Differences in head shape also dictate that different brain structures would be affected under similar RF exposure conditions. Thus, neurological data from human studies should be more reliable indicators of cell phone effects.

C. Another consideration is that most of the studies carried out so far are short-term exposure experiments, whereas cell phone use causes long-term repeated exposure of the brain. Depending on the responses studied in neurological/behavioral experiments, several outcomes have been reported after long term exposure: (1) an effect was observed only after prolonged (or repeated)

exposure, but not after one period of exposure; (2) an effect disappeared after prolonged exposure suggesting habituation; and (3) different effects were observed after different durations of exposure. All of these different responses reported can be explained as being due to the different characteristics of the dependent variable studied. These responses fit the pattern of general responses to a 'stressor'. Indeed, it has been proposed that RFR is a 'stressor' (e.g., see http://www.wave-guide.org/library/lai.html). Chronic stress could have dire consequences on the health of a living organism. However, it is difficult to prove that an entity is a stressor, since the criteria of stress are not well defined and the caveat of stress is so generalized that it has little predictive power on an animal's response.

D.   From the data available, in general, it is not apparent that pulsed RFR is more potent than continuous-wave RFR in affecting behavior in animals. Even though different frequencies and exposure conditions were used in different studies and hardly any dose-response study was carried out, there is no consistent pattern that the SARs of pulsed RFR reported to cause an effect are lower than those of continuous-RFR. This is an important consideration on the possible neurological effects of exposure to RFR during cell phone use, since cell phones emit wave of various forms and characteristics.

E.   Thermal effect cannot be discounted in the effects reported in most of the neurological/behavioral experiments described above. Even in cases when no significant change in body or local tissue temperature was detected, thermal effect cannot be excluded. An animal can maintain its body temperature by actively dissipating the heat load from the radiation. Activation of thermoregulatory mechanisms can lead to neurochemical, physiological, and behavioral changes. However, several points raised by some experiments suggest that the answer is not a simple one. They are: (a) 'Heating controls' do not produce the same effect of RFR; (b) Window effects are reported; (c) Modulated or pulsed RFR is more effective in causing an effect or elicits a different effect when compared with continuous-wave radiation of the same frequency.

F. It is also interesting to point out that in most of the behavioral experiments, effects were observed after the termination of RFR exposure. In some experiments, tests were made days after exposure. This suggests a persistent change in the nervous system after exposure to RFR.

G. In many instances, neurological and behavioral effects were observed at a SAR less than 4 W/kg. This directly contradicts the basic assumption of the IEEE guideline criterion.

H. A question that one might ask is whether different absorption patterns in the brain or body could elicit different biological responses in an animal. If this is positive, possible outcomes from the study of bioelectromagnetics research are: (a) a response will be elicited by some exposure conditions and not by others, and (b) different response patterns are elicited by different exposure conditions, even though the average dose rates in the conditions are equal. These data indicate that energy distribution in the body and other properties of the radiation can be important factors in determining the outcome of the biological effects of RFR.

I. Even though the pattern or duration of RFR exposure is well-defined, the response of the biological system studied will still be unpredictable if we lack sufficient knowledge of the

response system. In most experiments on the neurological effects of RFR, the underlying mechanism of the dependent variable was not fully understood. The purpose of most of the studies was to identify and characterize possible effects of RFR rather than the underlying mechanisms responsible for the effects. Understanding the underlying mechanism is an important criterion in understanding an effect.

J. Another important consideration in the study of the central nervous system should be mentioned here. It is well known that the functions of the central nervous system can be affected by activity in the peripheral nervous system. This is especially important in the in vivo experiments when the whole body is exposed. However, in most experiments studying the effects of RFR on the central nervous system, the possibility of contribution from the peripheral nervous system was not excluded in the experimental design. Therefore, caution should be taken in concluding that a neurological effect resulted solely from the action of RFR on the central nervous system.

K. In conclusion, the questions on the neurological effects (and biological effects, in general) of RFR and the discrepancies in research results in the literature can be resolved by (a) a careful and thorough examination of the effects of the different radiation parameters, and (b) a better understanding of the underlying mechanisms involved in the responses studied. With these considerations, it is very unlikely that the neurological effects of RFR can be accounted for by a single unifying neural mechanism.

L. Finally, does disturbance in behavior have any relevance to health? The consequence of a behavioral deficit is situation dependent and may not be direct. It probably does not matter if a person is playing chess and RFR in his environment causes him to make a couple of bad moves. However, the consequence would be much more serious if a person is flying an airplane and his response sequences are disrupted by RFR radiation.

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Appendix 9-A

NEUROLOGICAL EFFECTS OF RADIOFREQUENCY ELECTROMAGNETIC RADIATION in "Advances in Electromagnetic Fields in Living Systems, Vol. 1," J.C. Lin (ed.), Plenum Press, New York. (1994) pp. 27-88

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INTRODUCTION

Many reports in the literature have suggested the effect of exposure to radiofrequency electromagnetic radiation (RFR) (10 kHz-300,000 MHz) on the functions of the nervous system. Such effects are of great concern to researchers in bioelectromagnetics, since the nervous system coordinates and controls an organism's responses to the environment through autonomic and voluntary muscular movements and neurohumoral functions. As it was suggested in the early stages of bioelectromagnetics research, behavioral changes could be the most sensitive effects of RFR exposure. At the summary of session B of the proceedings of an international symposium held in Warsaw, Poland, in 1973, it was stated that "The reaction of the central nervous system to microwaves may serve as an early indicator of disturbances in regulatory functions of many systems" [Czerski et al., 1974].

Studies on the effects of RFR on the nervous system involve many aspects: morphology, electrophysiology, neurochemistry, neuropsychopharmacology, and psychology. An obvious effect of RFR on an organism is an increase in temperature in the tissue, which will trigger physiological and behavioral thermal regulatory responses. These responses involve neural activities both in the central and peripheral nervous systems. The effects of RFR on thermoregulation have been extensively studied and reviewed in the literature [Adair, 1983; Stern, 1980]. The topic of thermoregulation will not be reviewed in this chapter. Since this paper deals mainly with the effects of RFR on the central nervous system, the effect on neuroendocrine functions also will not be reviewed here. It is, however, an important area of research since disturbances in neuroendocrine functions are related to stress, alteration in immunological responses, and tumor development [Cotman et al., 1987; Dunn, 1989; Plotnikoff et al., 1991]. Excellent reviews of research on this topic have been written by Lu et al.[1980] and Michaelson and Lin [1987].

In order to give a concise review of the literature on the effects of RFR on neural functions, we have to first understand the normal functions of the nervous system.

PRINCIPLES OF NEURAL FUNCTIONS

The nervous system is functionally composed of nerve cells (neurons) and supporting cells known as glia. In higher animal species, it is divided into the central and peripheral nervous systems. The central nervous system consists of the brain and the spinal cord and is enveloped in a set of membranes known as the meninges. The outer surface as well as the inner structures of

the central nervous system are bathed in the cerebrospinal fluid (CSF) that fills the ventricles of the brain and the space at the core of the spinal cord.

The brain is generally subdivided into regions (areas) based on embryological origins. The anterior portion of the neural tube, the embryonic tissue from which the nervous system is developed, has three regions of expansion: the forebrain, midbrain, and hindbrain. From the forebrain, the cerebral hemispheres and the diencephalon will develop. The diencephalon consists of the thalamus, epithalamus, subthalamus, and hypothalamus. The midbrain remains mostly unchanged from the original structure of the neural tube; however, two pairs of structures, the superior and inferior colliculi, develop on its dorsal surface. These are parts of the visual and auditory systems, respectively. The hindbrain develops into the medulla, pons, and cerebellum.

The thalamus of the diencephalon is divided into various groups of cells (nuclei). Some of these nuclei are relays conveying sensory information from the environment to specific regions of the cerebral cortex, such as the lateral and medial geniculate nuclei that relay visual and auditory information, respectively, from the eyes and ears to the cerebral cortex. Other nuclei have more diffuse innervations to the cerebral cortex. The hypothalamus is involved in many physiological regulatory functions such as thermoregulation and control of secretion of hormones.

The cerebral hemispheres consist of the limbic system (including the olfactory bulbs, septal nucleus, amygdala, and hippocampus), the basal ganglia (striatum), and the cerebral cortex. The limbic system serves many behavioral functions such as emotion and memory. The striatum is primarily involved in motor controls and coordination. The cerebral cortex especially in the higher animal species is divided into regions by major sulci: frontal, parietal, temporal, and occipital cortex, etc. The function of some regions can be traced to the projection they receive from the thalamus, e.g., the occipital cortex (visual cortex) processes visual information it receives from the lateral geniculate nucleus of the thalamus and the temporal cortex (auditory cortex) receives auditory information from the medial geniculate nucleus. There are other cortical areas, however, known as secondary sensory areas and 'association' cortex that receive no specific thalamic innervations. One example of the association cortical areas is the prefrontal cortex, which is supposed to subserve higher behavioral functions, e.g., cognition.

The basic design of the central nervous system is similar among species in the phylogenetic scale; however, there are differences in the details of structure among species. Most of the brain regions mentioned in the above sections have been studied in bioelectromagnetics research to a various extent.

On the neurochemical level, neurons with similar biochemical characteristics are usually grouped together to form a nucleus or ganglion. Information is transmitted by electrochemical means via fibers (axons) protruding from the neuron. In addition to making local innervations to other neurons within the nucleus, nerve fibers from the neurons in a nucleus are also grouped into bundles (pathways) that connect one part of the brain to another. Information is generally passed from one neuron to another via the release of chemicals. These chemicals are called neurotransmitters or neuromodulators depending upon their functions. Many neurotransmitters have been identified in the central nervous system. Some are small molecules such as acetyl-choline, norepinephrine, dopamine, serotonin, and γ–amino-butyric acid (GABA), whereas the others are polypeptides and proteins such as the endogenous opioids, substance-P, etc. Effects of RFR on most of these neurotransmitters have been investigated. Nerve fibers in a pathway usually release the same neurotransmitter. The anatomy of some of these neurotransmitter

pathways are well studied such as those of dopamine, norepinephrine, serotonin, and acetylcholine.

After a neurotransmitter is released, it passes a space gap (synapse) between two adjacent cells and reacts with a molecule known as "receptor" at the cell membrane of the receiving (postsynaptic) cell. Such a reaction is usually described as analogous to the action of the key and lock. A particular neurotransmitter can only bind to its specific receptor to exert an effect. Binding of the neurotransmitter to a receptor triggers a series of reactions that affect the postsynaptic cell. Properties of the receptors can be studied by the receptor-ligand binding technique. Using this method the concentration and the binding affinity to the neurotransmitter of the receptors in a neural tissue sample can be determined.

Pharmacologically, one can affect neural functions by altering the events of synaptic transmission by the administration of a drug. Drugs can be used to decrease or increase the release of neurotransmitters or affect the activity of the receptors. Many drugs exert their effects by binding to neurotransmitter receptors. Drugs which have actions at the receptors similar to those of the natural neurotransmitters are called agonists, whereas drugs which block the receptors (thus blocking the action of the endogenous neurotransmitters) are known as antagonists. The property of antagonists provides a powerful conceptual tool in the study of the functions of the nervous system. Neural functions depend on the release of a particular type of neurotransmitter. If a certain physiological or behavioral function is blocked by administration of a certain antagonist to an animal, one could infer that the particular neurotransmitter blocked by the antagonist is involved in the function. In addition, since neurons of the same chemical characteristics are grouped together into pathways in the nervous system, from the information obtained from the pharmacological study, one can speculate on the brain areas affected by a certain treatment such as RFR.

The activity in the synapses is dynamic. In many instances as a compensatory response to changes in transmission in the synapses, the properties (concentration and/or affinity) of the receptors change. Generally, as a result of repeated or prolonged increase in release of a neurotransmitter, the receptors of that neurotransmitter in the postsynaptic cells decrease in number or reduce their binding affinity to the neurotransmitter. The reverse is also true, i.e., increase in concentration or binding affinity of the receptors occurs after prolonged or repeated episodes of decreased synaptic transmission. Such changes could have important implications on an animal's functional state. The changes in neurotransmitter receptors enable an animal to adapt to the repeated perturbation of function. On the other hand, since changes in receptor properties can last for a long time (days to weeks), an animal's normal physiological and behavioral functions will be altered by such changes.

The central nervous system of all vertebrates is enveloped in a functional entity known as the blood-brain barrier, due to the presence of high-resistance tight junctions between endothelial cells in the capillaries of the brain and spinal cord. The blood-brain barrier is impermeable to hydrophilic (polar) and large molecules and serves as a protective barrier for the central nervous system against foreign and toxic substances. Many studies have been carried out to investigate whether RFR exposure affects the permeability of the blood-brain barrier.

Drugs can be designed that cannot pass through the blood-brain barrier and, thus, they can only affect the peripheral nervous system. Using similar antagonists that can and cannot pass through the blood-brain barrier, one can determine whether an effect of an entity such as RFR is mediated by the central or peripheral nervous system. On the other hand, drugs can be directly

injected into the central nervous system (thus, by-passing the blood-brain barrier) to investigate the roles of neural mechanisms inside the brain on a certain physiological or behavioral function.

Changes in neurochemical functions lead to changes in behavior in an animal. Research has been carried out to investigate the effects of RFR exposure on spontaneous and learned behaviors. Motor activity is the most often studied spontaneous behavior. Alteration in motor activity of an animal is generally considered as an indication of behavioral arousal. For learned behavior, conditioned responses were mostly studied in bioelectromagnetics research. The behavior of an animal is constantly being modified by conditioning processes, which connect behavioral responses with events (stimuli) in the environment. Two types of conditioning processes have been identified and they are known as classical and operant conditioning. In classical conditioning, a 'neutral' stimulus that does not naturally elicit a certain response is repeatedly being presented in sequence with a stimulus that does elicit that response. After repeated pairing, presentation of the neutral stimulus (now the conditioned stimulus) will elicit the response (now the conditioned response). Interestingly, the behavioral control probability of the conditioned stimulus is shared by similar stimuli, i.e., presentation of a stimulus similar to the conditioned stimulus can also elicit the conditioned response. The strength and probability of occurrence of the conditioned response depends on the degree of similarity between the two stimuli. This is known as "stimulus generalization."

A paradigm of classical conditioning used in bioelectromagnetics research is the "conditioned suppression" procedure. Generally, in this conditioning process, an aversive stimulus (such as electric shock, loud noise) follows a warning signal. After repeated pairing, the presentation of the warning signal alone can stop or decrease the on-going behavior of the animal. The animal usually "freezes" for several minutes and shows emotional responses like defecation and urination. Again, stimulus generalization to the warning signal can occur.

Operant (or instrumental) conditioning involves a change in the frequency or probability of a behavior by its consequences. Consequences which increase the rate of the behavior are known as "reinforcers". Presentation of a "positive reinforcer", e.g., availability of food to a hungry animal, increases the behavior leading to it. On the other hand, removal of a "negative reinforcer", e.g., an electric shock, also leads to an increase of the behavior preceding it. Presentation of an aversive stimulus will decrease the probability of the behavior leading to it. In addition, removal of a positive reinforcer contingent upon a response will also decrease the probability of further response. Thus, both positive and negative reinforcers increase the probability of a response leading to them, and punishment (presentation of an aversive stimulus or withdrawal of a positive reinforcer) decreases the occurrence of a response. The terms used to describe a consequence are defined by the experimental procedures. The same stimulus can be used as a "negative reinforcer" to increase a behavior or as a punisher to decrease the behavior.

An interesting aspect of behavioral conditioning is the schedule on which an animal is reinforced (schedule-controlled behavior). An animal can be reinforced for every response it emits; however, it can also be reinforced intermittently upon responding. Intermittent reinforcement schedules generally consist of the following: reinforcement is presented after a fixed number of responses (fixed ratio), a fixed period of time (fixed interval), or a variable number of responses (variable ratio) or interval of time (variable interval) around an average value. The intermittent reinforcement schedules have a profound effect on the rate and pattern of responding. The variable schedules generally produce a steadier responding rate than the fixed schedules. A post-reinforcement pulse is associated with the fixed schedules when the rate of responding decreases immediately after a reinforcement and then increases steadily. Ratio

schedules generally produce a higher responding rate than interval schedules. Another simple reinforcement schedule commonly used in bioelectromagnetics research is the differential reinforcement of a low rate of responding (DRL). In this schedule, a reinforcement only follows a response separated from the preceding response by a specific time interval. If the animal responds within that time, the timer will be reset and the animal has to wait for another period of time before it can elicit a reinforceable response. The DRL schedule, dependent of the time interval set, produces a steady but low rate of responding. Compound schedules, consisting of two or more of the above schedule types, can also be used in conditioning experiments to control behavior. A multiple schedule is one in which each component is accompanied by a discriminatory stimulus, e.g., a white light when a fixed interval schedule is on and a green light when a variable interval schedule is on. The multiple schedule paradigm is widely used in pharmacological research to compare the effect of a drug on the patterns of response under different schedules in the same individual. A mixed schedule is a multiple schedule with no discriminative stimulus associated with each schedule component. Thus, a multiple schedule produces descrete patterns of responding depending on the currently active schedule, whereas a mixed schedule produces a response pattern that is a blend of all the different components. A tandem schedule consists of a sequence of schedules. Completion of one schedule leads to access to the next schedule, with no reinforcement presented until the entire sequence of schedules is completed. A chained schedule is a tandem schedule with each component accompanied by a discriminatory stimulus. Other more complicated combinations of schedules can be used in conditioning experiments. These compound schedules pose increased difficulties in an animal's ability to respond and make the performance more sensitive to the disturbance of experimental manipulations such as RFR.

In operant discrimination learning, an animal learns to elicit a certain response in the presence of a particular environmental stimulus, e.g., light, and is rewarded after the response, whereas no reinforcement is available in the absence of the stimulus or in the presence of another stimulus, e.g., tone. In this case, generalization to similar stimuli can also occur.

Another popular paradigm used in the research on the behavioral effects of RFR is escape and avoidance learning. In escape responding an animal elicits a response immediately when an aversive stimulus, e.g., electric foot-shock, is presented in order to escape from it or to turn it off. In avoidance learning an animal has to make a certain response to prevent the onset of an aversive stimulus. The avoidance can be a signalled avoidance-escape paradigm in which a stimulus precedes the aversive stimulus. On the other hand, the aversive stimulus can be nonsignalled. In this case the animal has to respond continuously to postpone the onset of the aversive stimulus, otherwise it will be presented at regular intervals. This paradigm is also known as "continuous-avoidance." It was speculated that avoidance learning was reinforced by reduction of a conditioned fear reaction [Mowrer, 1939; Solomon and Wynne, 1954]. In escape-avoidance learning both classical and operant conditioning processes are involved.

Use of reinforcement-schedules can generate orderly and reproducible behavioral patterns in animals, and thus, allows a systematic study of the effect of an independent variable, such as RFR. However, the underlying mechanisms by which different schedules affect behavior are poorly understood. The significance of studying schedule-controlled behavior has been discussed by Jenkins [1970] and Reynolds [1968]. In addition, de Lorge [1985] has written a concise and informative review and comments on the use of schedule-controlled behavior in the study of the behavioral effects of RFR.

In the following review on the effects of RFR on the central nervous system the concepts described above on the functions of the nervous system will apply.

EFFECTS OF RADIOFREQUENCY RADIATION ON THE MORPHOLOGY OF THE CENTRAL NERVOUS SYSTEM

Cellular Morphology

Radiofrequency radiation-induced morphological changes of the central nervous system are not expected except under relatively high intensity or prolonged exposure to the radiation. Such changes are not a necessary condition for alteration in neural functions after exposure to RFR. Early Russian studies [Gordon, 1970; Tolgskaya and Gordon, 1973] reported morphological changes in the brain of rats after 40 min of exposure to 3000- or 10000-MHz RFR at power densities varying from 40-100 mW/cm^2 (rectal temperature increased to 42-45 ^{o}C). Changes included hemorrhage, edema, and vacuolation formation in neurons. In these studies, changes in neuronal morphology were also reported in the rat brain after repeated exposure to RFR of lower power densities (3000 MHz, thirty-five 30-min sessions, <10 mW/cm^2, SAR 2 W/kg). Changes included neuronal cytoplasmic vacuolation, swelling and beading of axons, and a decrease in the number of dendritic spines. Albert and DeSantis [1975] also reported swollen neurons with dense cytoplasm and decreased rough endoplasmic reticulum and polyribosomes, indicative of decreased protein synthesis, in the hypothalamus and subthalamic region of the brain of hamsters exposed for 30 min to 24 h to continuous-wave 2450-MHz RFR at 50 mW/cm^2 (SAR 15 W/kg). No observable effect was seen in the thalamus, hippocampus, cerebellum, pons, and spinal cord. Recovery was seen at 6-10 days postexposure. In the same study, vacuolation of neurons was also reported in the hypothalamus of hamsters exposed to 2450-MHz RFR at 24 mW/cm^2 (SAR 7.5 W/kg) for 22 days (14 h/day). Similar effects of acute exposure were observed in a second study [Albert and DeSantis, 1976] when hamsters were exposed for 30-120 min to continuous-wave 1700-MHz RFR at either 10 (SAR 3 W/kg) or 25 mW/cm^2 (SAR 7.5 W/kg). The effects persisted even at 15 days postexposure.

Baranski [1972] reported edema and heat lesions in the brain of guinea pigs exposed in a single 3-h session to 3000-MHz RFR at a power density of 25 mW/cm^2 (SAR 3.75 W/kg). After repeated exposure (3 h/day for 30 days) to similar radiation, myelin degeneration and glial cell proliferation were reported in the brains of exposed guinea pigs (3.5 mW/cm^2, SAR 0.53 W/kg) and rabbits (5 mW/cm^2, SAR 0.75 W/kg). Pulsed (400 pps) RFR produced more pronounced effects in the guinea pigs than continuous-wave radiation of the same power density. Switzer and Mitchell [1977] also reported an increase in myelin figures (degeneration) of neurons in the brain of rats at 6 weeks after repeated (5 h/day, 5 day/week for 22 weeks) exposure to continuous-wave 2450-MHz RFR (SAR 2.3 W/kg). In another study [McKee et al., 1980], Chinese hamsters were exposed to continuous-wave 1700-MHz RFR at 10 or 25 mW/cm^2 (SARs 5 and 12.5 W/kg) for 30-120 min. Abnormal neurons were reported in the hypothalamus, hippocampus, and cerebral cortex of the animals after exposure. In addition, platelet aggregation and occlusion of some blood vessels in the brain were also reported.

Two studies investigated the effects of perinatal exposure to RFR on the development of Purkinje cells in the cerebellum. In the first study [Albert et al., 1981a], pregnant squirrel

monkeys were exposed to continuous-wave 2450-MHz RFR (3 h/day, 5 days/week) at a power density of 10 mW/cm^2 (SAR 3.4 W/kg) and the offspring were similarly exposed for 9.5 months after birth. No significant change was observed in the number of Purkinje cells in the uvula areas of the cerebellum of the exposed animals compared to that of controls. In the second study, Albert et al. [1981b] studied the effects of prenatal, postnatal, and pre- and postnatal-RFR exposure on Purkinje cells in the cerebellum of the rat. In the prenatal exposure experiment, pregnant rats were exposed from 17-21 days of gestation to continuous-wave 2450-MHz RFR at 10 mW/cm^2 (SAR 2W/kg) for 21 h/day. The offspring were studied at 40 days postexposure. A decrease (-26%) in the concentration of Purkinje cells was observed in the cerebellum of the prenatally RFR-exposed rats. In the pre- and postnatal-exposure experiment, pregnant rats were exposed 4 h/day between the 16-21 days of gestation and their offspring were exposed for 90 days to continuous-wave 100-MHz RFR at 46 mW/cm^2 (SAR 2.77 W/kg). Cerebellum morphology was studied at 14 months postexposure. A 13% decrease in Purkinje cell concentration was observed in the RFR-exposed rats. The changes observed in the pre- and perinatally-exposed rats seemed to be permanent, since the animals were studied more than a month postexposure. In the postnatal exposure experiment, 6-day old rat pups were exposed 7 h/day for 5 days to 2450-MHz RFR at 10 mW/cm^2 and their cerebella were studied immediately or at 40 days after exposure. A 25% decrease in Purkinje cell concentration was found in the cerebellum of rats studied immediately after exposure, whereas no significant effect was observed in the cerebellum at 40 days postexposure. Thus, the postnatal exposure effect was reversible. The authors suggested that RFR may affect the proliferative activity and migrational process of Purkinje cells during cerebellar development. In a further study [Albert and Sherif, 1988], 1- or 6-day old rat pups were exposed to continuous-wave 2450-MHz RFR for 5 days (7 h/day, 10 mW/cm^2, SAR 2W/kg). Animals were killed one day after the exposure and morphology of their cerebellum was studied. The authors reported two times the number of deeply stained cells with dense nucleus in the external granular layer of the cerebellum. Examination with an electron microscope showed that the dense nuclei were filled with clumped chromatin. Extension and disintegration of nucleus, ruptured nuclear membrane, and vacuolization of the cytoplasm were observed in these cells. Some cells in the external granular layer normally die during development of the cerebellum; therefore, these data showed that postnatal RFR exposure increased the normal cell death. In the same study, disorderly arrays of rough endoplasmic reticulum were observed in the Purkinje cells of the exposed animals indicating an altered metabolic state in these cells.

Blood-Brain Barrier

Intensive research effort was undertaken to investigate whether RFR affected the permeability of the blood-brain barrier [Albert, 1979b; Justesen, 1980]. The blood-brain barrier blocks the entry of large and hydrophilic molecules in the general blood circulation from entering the central nervous system. Its permeability was shown to be affected by various treatments, e.g., electroconvulsive shock [Bolwig, 1988]. Variable results on the effects of RFR on blood-brain barrier permeability have been reported. A reason for this could be due to the difficulties in measuring and quantifying the effect [Blasberg, 1979].

Frey et al. [1975] reported an increase in fluorescein in brain slices of rats injected with the dye and exposed for 30 min to continuous-wave 1200-MHz RFR (2.4 mW/cm^2, SAR 1.0 W/kg) as compared with control animals. The dye was found mostly in the lateral and third ventricles of

the brain. A similar but more pronounced effect was observed when the animals were exposed to pulsed 1200-MHz RFR at an average power density of 0.2 mW/cm^2. These data were interpreted as an indication of an increase in permeability of the blood-brain barrier, since fluorescein injected systemically does not normally permeate into the brain. On the other hand, Merritt et al. [1978] did not observe a significant change in the permeability of fluorescein-albumin into the brain of rats exposed to a similar dose-rate of RFR (1200 MHz, either continuous-wave or pulsed, 30 min, 2-75 mW/cm^2); however, an increase in permeability was observed, if the body temperature of the animal was raised to 40 ^{o}C either by RFR or convective heating. In addition, no significant change in permeability of mannitol and inulin to the brain was reported in this experiment after RFR exposure.

Chang et al. [1982] studied in the dog the penetration of ^{131}I-labelled albumin into the brain. The head of the dog was irradiated with 1000-MHz continuous-wave RFR at 2, 4, 10, 30, 50, or 200 mW/cm^2 and the tracer was injected intravenously. Radioactivity in the blood and cerebrospinal fluid (CSF) was determined at regular time intervals postinjection. An increase in the ratio of radioactivity in the CSF versus that in the blood was considered as an indication of entry of the labelled albumin that normally does not cross the blood-brain barrier. At 30 mW/cm^2, 4 of the 11 dogs studied showed a significant increase in the ratio compared to that of sham-exposed animals, whereas no significant difference was seen at the other power densities. The authors suggested a possible 'power window' effect.

Lin and Lin [1980] reported no significant change in the permeability of sodium fluorescein and Evan's blue into the brain of rats with focal exposure at the head for 20 min to pulsed 2450-MHz RFR at 0.5-1000 mW/cm^2 (local SARs 0.04-80 W/kg), but an increase was reported after similar exposure of the head at an SAR of 240 W/kg [Lin and Lin, 1982]. The brain temperature under the latter exposure condition was 43 ^{o}C. In a further study, by the same laboratory, Goldman et al. [1984] used ^{86}Rb as the tracer to study the permeability of the blood-brain barrier after RFR exposure. The tracer was injected intravenously to rats after 5, 10, or 20 min of exposure to 2450-MHz pulsed RFR (10 µs pulses, 500 pps) at an average power density of 3 W/cm^2 (SAR 240 W/kg) on the left side of the head. Brain temperature was increased to 43 ^{o}C. The ^{86}Rb uptake in the left hemisphere of the brain was studied. Increase in uptake was detected in the hypothalamus, striatum, midbrain, dorsal hippocampus, and occipital and parietal cortex at 5 min postexposure. Increased uptake of the tracer in the cerebellum and superior colliculus was also observed at 20 min after exposure. That increase in brain temperature played a critical role in the effect of RFR on the permeability of the blood-brain barrier was further supported in an experiment by Neilly and Lin [1986]. They showed that ethanol, infused into the femoral vein, reduced the RFR-induced (3150 MHz, 30 W/cm^2 rms for 15 min on the left hemisphere of the brain) increase in penetration of Evan's blue into the brain of rats. Ethanol attenuated the RFR-induced increase in brain temperature.

Several studies used horseradish peroxidase as an indicator of blood-brain barrier permeability. An increase in horseradish peroxidase in the brain after systemic administration could be due to an increase in pinocytosis of the epithelial cells in the capillary of the brain, in addition to or instead of an increase in the leakiness of the blood-brain barrier. Pinocytosis can actively transport the peroxidase from the general blood circulation into the brain. An increase in the concentration of horseradish peroxidase was found in the brain of the Chinese hamster after 2 h of irradiation to continuous-wave 2450-MHz RFR at 10 mW/cm^2 (SAR 2.5 W/kg) [Albert, 1977]. The increase was more concentrated in the thalamus, hypothalamus, medulla, and cerebellum, and less in the cerebral cortex and hippocampus [Albert and Kerns, 1981]. Increases

in horseradish peroxidase permeability were also observed in the brains of rats and Chinese hamsters exposed for 2 h to continuous-wave 2800-MHz RFR at 10 mW/cm^2 (SAR 0.9 W/kg for the rat and 1.9 W/kg for the Chinese hamster). Fewer brain areas were observed with horseradish peroxidase at 1 h postexposure and complete recovery was seen at 2 h [Albert, 1979a]. Sutton and Carroll [1979] also reported an increase in permeability of horseradish peroxidase to the brain of the rat, when the brain temperature was raised to 40-45 ^{o}C by focal heating of the head with continuous-wave 2450-MHz RFR. In addition, cooling the body of the animals before exposure could counteract this effect of the radiation. These results again point to the conclusion that the hyperthermic effect of the RFR can disrupt the blood-brain barrier.

Oscar and Hawkins [1977] reported increased permeability of radioactive mannitol and inulin, and no significant change in dextran permeability into the brain of rats exposed for 20 min to continuous-wave or pulsed 1300-MHz RFR at a power density of 1 mW/cm^2 (SAR 0.4 W/kg). Effect of the pulsed radiation was more prominent. A 'power window' effect was also reported in this study. Preston et al. [1979] exposed rats to continuous-wave 2450-MHz RFR for 30 min at different power densities (0.1-30 mW/cm^2, SARs 0.02-6 W/kg) and observed no significant change in radioactive mannitol distribution in various regions of the brain. In that paper, they suggested that an increase in regional blood flow in the brain could explain the results of Oscar and Hawkins [1977]. In further experiments Preston and Prefontaine [1980] reported no significant change in the permeability of radioactive sucrose to the brain of rats exposed with the whole body to continuous-wave 2450-MHz RFR for 30 min at 1 or 10 mW/cm^2 (SARs 0.2 and 2.0 W/kg) or with the head for 25 min at different power densities. Gruenau et al. [1982] also reported no significant change on the penetration of ^{14}C-sucrose into the brain of rats after 30 min of exposure to pulsed (2 μs pulses, 500 pps) or continuous-wave 2800-MHz RFR of various intensities (1-15 mW/cm^2 for the pulsed radiation, 10 and 40 mW/cm^2 for the continuous-wave radiation). Ward et al. [1982] irradiated rats with 2450-MHz RFR for 30 min at different power densities (0-30 mW/cm^2, SAR 0-6 W/kg) and studied entry of ^{3}H-inulin and ^{14}C-sucrose into different areas of the brain. Ambient temperature of exposure was at either 22, 30, or 40 ^{o}C. They reported no significant increase in penetration of both compounds into the brain due to RFR exposure; however, they reported an increase in ^{14}C-sucrose entry into the hypothalamus when the ambient temperature of exposure was at 40 ^{o}C. The increase was suggested to be due to the hyperthermia induced in the animals under such exposure conditions. In a further study, Ward and Ali [1985] exposed rats to 1700-MHz continuous-wave or pulsed (0.5 μs pulses, 1000 pps) RFR for 30 min with the radiation concentrated at the head of the animal (SAR 0.1 W/kg). They reported no significant change in permeability into the brain of ^{3}H-inulin and ^{14}C-sucrose after the exposure.

Oscar et al. [1981] did observe increased blood flow in various regions of the rat brain after 5 to 60 min of exposure to pulsed 2800-MHz (2 μs pulses, 500 pps) RFR at 1 or 15 mW/cm^2 (SARs 0.2 and 3 W/kg). At 1 mW/cm^2, increased blood flow (measured at ~6 min after exposure) was observed in 16 of the 20 brain areas studied with the largest increase in the pineal gland, hypothalamus, and temporal cortex. After exposure to the radiation at 15 mW/cm^2, the largest increases in blood flow were detected in the pineal gland, inferior colliculus, medial geniculate nucleus, and temporal cortex (the last three areas are parts of the auditory system). It is interesting that patterns of changes involving different brain areas are reported in different studies [Albert and Kerns, 1981; Goldman et al., 1984; Oscar et al., 1981]. One wonders if this is due to the different patterns of energy distribution in the brain leading to different patterns of

increases in local cerebral blood flow, since different exposure conditions were used in these experiments.

Williams et al. [1984a-d] carried out a series of experiments to study the effect of RFR exposure on blood-brain barrier permeability to hydrophilic molecules. Unrestrained, conscious rats were used in these studies. The effects of exposure to continuous-wave 2450-MHz RFR at 20 or 65 mW/cm^2 (SAR 4 or 13 W/kg) for 30, 90, or 180 min were compared with those of ambient heating (42 ^{o}C)-induced hyperthermia and urea infusion, on sodium fluorescein, horseradish peroxidase, and ^{14}C-sucrose permeability into different areas of the brain. In general, they found that hyperosmolar urea was the most effective and ambient heating was as effective as hyperthermic RFR in increasing the tracer concentrations in the brain. However, significant increase of plasma concentrations of sodium fluorescein and ^{14}C-sucrose were also observed in the heat- and RFR-exposed animals, which might result from a decrease in renal function due to hyperthermia. Increase in tracer concentrations in the brain could be due to the increase in plasma concentrations. The authors concluded that RFR did not significantly affect the penetration of the tracers into the brain (via the blood-brain barrier). In the case of horseradish peroxidase, a reduced uptake into the brain was actually observed. The authors speculated that there was a decrease in pinocytotic activity in cerebral micro-vessels after exposure for 30 to 90 min to the radiation at 65 mW/cm^2.

A series of experiments was carried out to study the effect of RFR on the passage of drugs into the central nervous system. Drug molecules that are less lipid soluble are less permeable through the blood-brain barrier. Thus, their actions are confined mainly to the peripheral nervous system after systemic administration. The actions of methylatropine, a peripheral cholinergic antagonist, methylnaltrexone, a peripheral opiate antagonist, and domperidone, a peripheral dopamine antagonist on RFR-exposed rats were studied by Quock et al. [1986a,b; 1987]. After 10 min of irradiation of mice to continuous-wave 2450-MHz RFR at 20 mW/cm^2 (SAR 53 W/kg), they observed antagonism of the apomorphine (a dopamine agonist)-induced stereotypic climbing behavior by domperidone, the analgesic effect of morphine (an opiate) by methylnaltrexone, and the central effects of oxotremorine and pilocarpine (both cholinergic agonists) by methylatropine. The behavioral and physiological responses studied are due to the action of the agonists in the central nervous system and are normally not blocked by the peripheral antagonists used in these studies. Since the enhanced antagonist effects of the peripheral drugs cannot be due to an increase in cerebral blood flow after exposure to the RFR, Quock et al. [1986a] speculated that the effect may be due to the breakdown of capillary endothelial tight-junction or an increase in pinocytosis in the blood-brain barrier.

Neubauer et al. [1990] studied the penetration of rhodamine-ferritin complex into the blood-brain barrier of the rat. The compound was administered systemically to the animals and then the animals were irradiated with pulsed 2450-MHz RFR (10 μs pulses, 100 pps) for 15, 30, 60 or 120 min at an average power density of 5 or 10 mW/cm^2 (SAR of 2 W/kg). Capillary endothelial cells from the cerebral cortex of the rats were isolated immediately after exposure, and the presence of rhodamine-ferritin complex in the cells was determined by the fluorescence technique. An approximately two fold increase in the complex was found in the cells of animals after 30 min or more of exposure to the 10 mW/cm^2 radiation. No significant effect was observed at 5 mW/cm^2. Furthermore, pretreating the animals before exposure with the microtubular function inhibitor colchicine blocked the effect of the RFR. These data indicate an increase in pinocytotic activity in the cells forming the blood-brain barrier. In a more recent study [Lange and Sedmak, 1991], using a similar exposure system, a dose- (power density)

dependent increase in the entry of Japanese encephalitis virus into the brain and lethality was reported in mice after 10 min of RFR exposure (power densities 10-50 mW/cm^2, SARs 24-98 W/kg). The blood-brain barrier is a natural barrier against the penetration of this virus to the brain. The authors also speculated that the high-intensity RFR caused an increase in pinocytosis of the capillary endothelial cells in the central nervous system and the viruses were carried inside by this process.

It is apparent that in the majority of the studies a high intensity of RFR is required to alter the permeability of the blood-brain barrier. Change in brain or body temperature seems to be a necessary condition for the effect to occur. In addition, permeability alteration could be due to a passive change in 'leakiness' or an increase in pinocytosis in the blood-brain barrier.

ELECTROPHYSIOLOGICAL EFFECTS OF RADIOFREQUENCY RADIATION

Electrophysiology of Neurons

Wachtel et al. [1975] and Seaman and Wachtel [1978] described a series of experiments investigating the effect of RFR (1500 and 2400 MHz) on neurons from the isolated abdominal ganglion of the marine gastropod, Aphysia. Two types of cells generating regular action potential spikes or bursts were studied. A majority of cells (87%) showed a decrease in the rate of the spontaneous activity when they were irradiated with RFR. 'Temperature' controls were run and in certain neurons convective warming produced an opposite effect (increased rate of activity) to that produced by RFR (decreased activity). Chou and Guy [1978] exposed temperature-controlled samples of isolated frog sciatic nerves, cat saphenous nerve, and rabbit vagus nerve to 2450-MHz RFR. They reported no significant change in the characteristics of the compound action potentials in these nerve preparations during exposure to either continuous-wave (SARs 0.3-1500 W/kg) or pulsed (peak SARs 0.3-220 W/kg) radiation. No direct field stimulation of neural activity was observed.

Arber and Lin [1985] recorded from Helix aspersa neurons irradiated with continuous-wave 2450-MHz RFR (60 min at 12.9 W/kg) at different ambient temperatures. The irradiation induced a decrease in spontaneous firing at medium temperatures of 8 and 21 ^{o}C, but not at 28 ^{o}C. However, when the neurons were irradiated with noise-amplitude-modulated 2450-MHz RFR (20% AM, 2 Hz-20 kHz) at SARs of 6.8 and 14.4 W/kg, increased membrane resistance and spontaneous activity were observed.

Evoked Potentials

Several studies investigated the effects of RFR on evoked potentials in different brain areas. The evoked potential is the electrical activity in a specific location within the central nervous system responding to stimulation of the peripheral nervous system. Johnson and Guy [1972] recorded the evoked potential in the thalamus of cats in response to stimulation of the contralateral forepaw. The animals were exposed to continuous-wave 918-MHz RFR for 15 min at power densities of 1-40 mW/cm^2 at the head. A power density-dependent decrease in latency of some of the late components, but not the initial response of the thalamic evoked potential was observed. These data were interpreted that RFR affected the multisynaptic neural pathway,

which relates neural information from the skin to the thalamus and is responsible for the late components of the evoked potential. Interestingly, warming the body of the animals decreased the latency of both the initial and late components of the evoked potential.

Taylor and Ashleman [1975] recorded spinal cord ventral root responses to electrical stimulation of the ipsilateral gastrocnemius nerve in cats, using a polyethylene suction electrode. The spinal cord was irradiated with continuous-wave 2450-MHz RFR at an incident power of 7.5 W. Decreases in latency and amplitude of the reflex response were observed during exposure (3 min) and responses returned to normal immediately after exposure. They also reported that raising the temperature of the spinal cord produced electrophysiological effects similar to those of RFR.

Electrophysiology of Auditory Effect of Pulsed RFR

Electrophysiological methods have also been used to study the pulsed RFR-induced auditory effects in animals. The effect was first systemically studied in humans by Frey [1961] and has been reviewed by Chou et al. [1982a] and Lin [1978]. Evoked potential responses were recorded in the eighth cranial nerve, medial geniculate nucleus, and the primary auditory cortex (three components of the auditory system) in cats exposed to pulsed 2450-MHz RFR. These evoked responses were eliminated after damaging the cochlea [Taylor and Ashleman, 1974]. Guy et al. [1975] studied the threshold of evoked responses in the medial geniculate nucleus in the cat in response to pulsed RFR while background noise (50-15000 Hz, 60-80 dB) was used to interfere with the response. They reported that background noise did not significantly affect the threshold to the RFR response, but caused a large increase in threshold to sound stimulus applied to the ear. The authors speculated that RFR interacts with the high frequency component of the auditory response system. In the study, evoked potentials in brain sites other than those of the auditory system were also recorded during pulsed RFR stimulation.

Chou et al. [1975] confirmed the peripheral site of the auditory effect generation. They recorded cochlear microphonics in the guinea pig inner ear during stimulation with 918-MHz pulsed RFR. The response was similar in characteristics to the cochlear microphonics generated by a click. These data were further supplemented by the finding that the middle-ear was not involved in the pulsed RFR-induced auditory responses, since destruction of the middle ear did not abolish the RFR-induced evoked potential in the brainstem [Chou and Galambos, 1979].

Experiments [Chou and Guy, 1979b] studying the threshold of RFR auditory effect in guinea pigs using the brainstem auditory evoked responses showed that the threshold for pulses with pulse width less than 30 μs was related to the incident energy per pulse, and for larger duration pulses it was related to the peak power. In another study Chou et al. [1985b] measured the intensity-response relationship of brainstem auditory evoked response in rats exposed to 2450-MHz pulsed RFR (10 pps) of different intensities and pulse widths (1-10 μs) in a circularly polarized waveguide. They also confirmed in the rat that the response is dependent on the energy per pulse and independent of the pulse width (up to 10 μs in this experiment).

Lebovitz and Seaman [1977a,b] recorded responses from single auditory neurons in the auditory nerve of the cat in response to 915-MHz pulsed RFR. Responses are similar to those elicited by acoustic stimuli. Seaman and Lebovitz [1987; 1989] also recorded in the cat the responses of single neurons in the cochlear nucleus, a relay nucleus in the auditory system, to pulsed 915-MHz RFR applied to the head of the animal. The threshold of response to RFR pulses was determined and found to be low (SAR response threshold determined at the midline

of the brain stem, where the cochlear nucleus is located, was 11.1 mW/g/pulse corresponding to a specific absorption threshold of 0.6 μJ/g/pulse.)

Electroencephalographic Recording

Various experiments studied the effects of acute and chronic RFR exposures on electroencephalograph (EEG). Measurement of electrical activity from the brain using external electrodes provides a non-invasive means of studying brain activity. Electroencephalograph is the summation of neural activities in the brain and provides a gross indicator of brain functions. It is generated by cell activity in the cerebral cortex around the area of recording, but it is modulated by subcortical input, e.g., from the thalamus. Sophisticated techniques and methods are available in the recording and analysis of EEG that provide useful knowledge on brain functions [da Silva, 1991].

In the early studies on the effects of RFR on EEG, metal electrodes were used in recording that distorted the field and possibly led to artifactual results [Johnson and Guy, 1972]. Saline filled glass electrodes [Johnson and Guy, 1972] and carbon loaded Teflon electrodes [Chou and Guy, 1979a] were used in later experiments to record the electrical activity in the brain of animals during RFR exposure. The carbon loaded Teflon electrode has conductivity similar to tissue and, thus, minimizes field perturbation. It can be used for chronic EEG and evoked potential measurements in RFR studies.

Baranski and Edelwejn [1968] reported that acute pulsed RFR (20 mW/cm^2) had little effect on the EEG pattern of rabbits that were given phenobarbital; however, after chronic exposure (7 mW/cm^2, 200 h), desynchronization (arousal) was seen in the EEG after phenobarbital administration, whereas synchronization (sedation) was observed in the controls [Baranski and Edelwejn, 1974]. Goldstein and Sisko [1974] also reported periods of alternating EEG desynchronization and synchronization in rabbits anesthetized with pentobarbital and then subjected to 5 min of continuous-wave 9300-MHz RFR (0.7-2.8 mW/cm^2). Duration of desynchronization correlated with the power density of the irradiation. Servantie et al. [1975] reported that rats exposed for 10 days to 3000-MHz pulsed (1 μs pulses, 500-600 pps) RFR at 5 mW/cm^2 produced an EEG frequency in the occipital cortex (as revealed by spectral analysis) synchronous to the pulse frequency of the radiation. The effect persisted a few hours after the termination of exposure. The authors proposed that the pulsed RFR synchronized the firing pattern of cortical neurons.

Dumansky and Shandala [1974] reported in the rat and rabbit that changes in EEG rhythm occurred after chronic RFR exposure (120 days, 8 h/day) using a range of power densities. The authors interpreted their results as an initial increase in excitability of the brain after RFR exposure followed by inhibition (cortical synchronization and slow wave) after prolonged exposure. Shandala et al. [1979] exposed rabbits to 2375-MHz RFR (0.01-0.5 mW/cm^2) 7 h/day for 3 months. Metallic electrodes were implanted in various regions of the brain (both subcortical and cortical areas) for electrical recording during the exposure period and postexposure. After 1 month of exposure at 0.1 mW/cm^2, the authors observed in the sensory-motor and visual cortex an increase in alpha-rhythm, an EEG pattern indicative of relaxed and resting states of an animal. An increase in activity in the thalamus and hypothalamus was also observed later. Similar effects were also seen in animals exposed to the RFR at 0.05 mW/cm^2; however, rats exposed to a power density of 0.5 mW/cm^2 showed an increase in delta waves of high amplitude in the cerebral cortex after 2 weeks of exposure, suggesting a suppressive effect on EEG activity.

Bawin et al. [1973] exposed cats to 147-MHz RFR amplitude-modulated at 8 and 16 Hz at 1 mW/cm^2. They reported changes in both spontaneous and conditioned EEG patterns. Interestingly, the effects were not observed at lower or higher frequencies of modulation. Takashima et al. [1979] also studied the EEG patterns in rabbits exposed to RFR fields (1-30 MHz) amplitude-modulated at either 15 or 60 Hz. Acute exposure (2-3 h, field strength 60-500 V$_{rms}$/m) elicited no observable effect. Chronic exposure (2 h/day for 4-6 weeks at 90-500 V$_{rms}$/m) produced abnormal patterns including high amplitude spindles, bursts, and suppression of normal activity (shift to pattern of lower frequencies) when recorded within a few hours after exposure.

In an experiment by Chou and Guy [1979a], no significant change in electrical activity from the hypothalamus was detected in rabbits exposed to 2450-MHz RFR at 100 mW/cm^2 (SAR at electrode ~25 W/kg). In a chronic exposure experiment, Chou et al. [1982b] exposed rabbits to continuous-wave 2450-MHz RFR at 1.5 mW/cm^2 (2 h/day, 5 days/week for 90 days). Electroencephalograph and evoked potentials were measured at the sensory-motor and occipital cortex at various times during the exposure period. They reported large variations in the data and a tendency toward a decreased response amplitude in the latter part of the experiment, i.e., after a longer period of exposure.

In a more recent study, Chizhenkova [1988] recorded in the unanesthetized rabbits slow wave EEG in the motor and visual cortex, evoked potential in the visual cortex to light flashes, and single unit activity in the visual cortex during and after exposure to continuous-wave RFR (wavelength = 12.5 cm, 40 mW/cm^2, 1 min exposure to the head) using glass electrodes. She reported that RFR increased the incident of slow wave and spindles in the EEG, which are characteristics of slow wave sleep in animals. However, the radiation facilitated light-evoked responses in the visual cortex. Cells in the visual cortex also showed changes in firing rates (increase or decrease depending on the neuron studied). Driving responses of visual cortical neurons to light flashes, i.e., responses to sequence of light flashes of increasing frequency, were also enhanced by the RFR exposure. The author interpreted the data as showing a decrease in the threshold of visual evoked potential and an increase in excitability of visual cortical cells as a result of RFR exposure.

NEUROCHEMICAL EFFECTS OF RADIOFREQUENCY RADIATION

Neurochemical studies of RFR include those on the concentrations and functions of neurotransmitters, receptor properties, energy metabolism, and calcium efflux from brain tissues.

Changes in Neurotransmitter Functions

In most studies on the effects of RFR on neurotransmitter functions, only the concentration of neurotransmitters (usually measured as amount/gm wet weight of brain tissue) was measured in the brains of animals after irradiation. Data on change in concentration alone tells little about the nature of the effect, since it could result from different causes. For example, a decrease in the concentration could be due to an enhanced release or a decrease in synthesis of the neurotransmitter as the result of RFR exposure. For a more informative study, the turnover rate

of a neurotransmitter should be investigated. This involves the measurement of the rate of decrease in concentration of the neurotransmitter when its synthesis is blocked and/or the rate of accumulation of the metabolites of the neurotransmitter. More recently, the rate of release of a neurotransmitter from a local brain region can be studied by the microdialysis technique.

Snyder [1971] reported a significant increase in the concentrations of serotonin and its metabolite, 5-hydroxyindolacetic acid, in the brain of rats after 1 h of exposure to continuous-wave 3000-MHz RFR at 40 mW/cm^2 (SAR 8 W/kg). However, decreases in both neuro-chemicals were observed in the brain of rats exposed 8 h/day for 7 days at 10 mW/cm^2. Thus, these results indicated an increase in the synthesis and turnover of brain serotonin after acute exposure and a decrease after prolonged exposure to RFR. Furthermore, warming the animals by placing them in an incubator heated at 34 ^{O}C had no significant effect on the turnover rate of serotonin in the brain.

Catravas et al. [1976] also reported an increase in diencephalon serotonin concentration and activity of tryptophan hydroxylase, the synthesis enzyme for serotonin, in the rat after 8 daily (8 h/day) exposures to RFR at 10 mW/cm^2. No significant changes in activity of monoamine oxidase, the degradation enzyme of serotonin, was observed in the brain of the irradiated rats.

Zeman et al. [1973] investigated the effects of exposure to pulsed 2860-MHz RFR on γ-amino-butyric acid (GABA) in the rat brain. No significant difference was observed in GABA concentration nor the activity of its synthesis enzyme, L-glutamate decarboxylase, in the brains of chronic (10 mW/cm^2, 8 h/day for 3-5 days, or 4 h/day, 5 days/week for 4 or 8 weeks) or acutely exposed (40 mW/cm^2 for 20 min, or 80 mW/cm^2 for 5 min) rats compared with those of the sham-exposed animals.

Rats exposed to continuous-wave 1600-MHz RFR at 30 mW/cm^2 for 10 min were reported to have altered concentrations of catecholamines (norepinephrine and dopamine) and serotonin in specific regions of the brain [Merritt et al., 1976]. Norepinephrine was decreased only in the hypothalamus, whereas decrease in serotonin was seen in the hippocampus and decreases in dopamine were observed in the striatum and hypothalamus. These effects were suggested to be caused by an uneven distribution of RFR in different regions of the brain. In a further study, rats exposed to similar radiation (20 or 80 mW/cm^2) were found to have a reduction of norepinephrine concentration in the basal hypothalamus, whereas no significant changes in dopamine and serotonin concentrations were observed even though the brain temperature increased up to 5 ^{O}C [Merritt et al., 1977]. In another study [Grin, 1974], rats were exposed to 2375-MHz RFR at power densities of 50 and 500 μW/cm^2 for 30 days (7 h/day). At 50 μW/cm^2, brain epinephrine was increased on the 20th day of exposure, but returned to normal by day 30. There were slight increases in norepinephrine and dopamine concentrations throughout the exposure period. At 500 μW/cm^2, concentrations of all three neurotransmitters were increased at day 5, but declined continually after further exposure.

Various studies have been carried out to investigate the neurochemical effects of RFR irradiation on acetylcholine in the brain. A decrease in whole brain concentration of acetyl-choline, suggesting an increased release of the neurotransmitter, has been reported in mice exposed to a single 2450-MHz RFR pulse, which deposited 18.7 J in the brain and increased the brain temperature by 2 to 4 ^{O}C [Modak et al., 1981]. Several studies investigated the effect on acetylcholinesterase (AChE), the degradation enzyme for acetylcholine. Acute (30 min) exposure to 9700-MHz RFR was reported to inhibit the membrane-bound AChE activity in a vagal-heart preparation [Young, 1980]. This effect was attributed to a release of bound calcium from the postjunctional membrane. In another study [Baranski, 1972], acute exposure to pulsed RFR

36

(~3000 MHz) at 25 mW/cm^2 caused a decrease in AChE activity in the guinea pig brain. The effect was most pronounced at the diencephalon and mesencephalon (midbrain). After three months (3 h/day) of exposure at a power density of 3.5 mW/cm^2, an increase in brain AChE was observed. Surprisingly, when rabbits were subjected to the same chronic exposure treatment, a decrease in AChE activity was seen. On the other hand, two groups of investigators [Galvin et al., 1981; Miller et al., 1984] showed independently that 2450-MHz RFR exposure at a wide range of SARs did not significantly affect the activity of isolated AChE in vitro. More recently, Dutta et al. [1992] reported an increase in AChE activity in neuroblastoma cells in culture after 30 min of exposure to 147-MHz RFR amplitude-modulated at 16 Hz at SARs of 0.05 and 0.02 W/kg, but not at 0.005, 0.01, or 0.1 W/kg. The authors suggested a 'power window' effect. It is not known whether the effect was a response to the radiofrequency or the 16-Hz component of the radiation. Acetylcholinesterase is a very effective enzyme. A large decrease in its activity will be needed before any change in cholinergic functions can be observed.

D'Inzeo et al. [1988] reported an experiment that showed the direct action of RFR on acetylcholine-related ion channels in cultured chick embryo myotube cells. The acetylcholine-induced opening and closing of a single channel in the membrane of these cells were studied by the patch-clamp technique. Changes in membrane current of the whole cell in response to acetylcholine was also studied. The channels were probably the nicotinic cholinergic receptor channels, which are ligand-gated channels. The cell culture was exposed to continuous-wave 10750-MHz RFR with the power density at the cell surface estimated to be a few μW/cm^2. (Power density of the incident field at the surface of the culture medium was 50 μW/cm^2.) Recordings were made during exposure. The authors reported a decrease in acetylcholine-activated single channel opening, whereas the duration of channel opening and the conductance of the channels were not significantly affected by the radiation. Since these latter two parameters are temperature-dependent, the effect observed was suggested as not related to the thermal effects of RFR. The whole cell membrane current also showed an increase in the recovery rates (desensitization) during irradiation. Thus, RFR decreased the opening probability of the acetylcholine channel and increased the rate of desensitization of the acetylcholine receptors. Opening and desensitization of the nicotinic channels are known to involve different molecular mechanisms.

Lai et al. [1987b,c] performed experiments to investigate the effects of RFR exposure on the cholinergic systems in the brain of the rat. Activity of the two main cholinergic pathways, septo-hippocampal and basalis-cortical pathways, were studied. The former pathway has the cell bodies in the septum and their axons innervate the hippocampus. The latter pathway includes neurons in the nucleus basalis and innervates several cortical areas including the frontal cortex. These two cholinergic pathways are involved in many behavioral functions such as learning, memory, and arousal [Steriade and Biesold, 1990]. Degeneration of these pathways occurs in Alzheimers disease [Price et al., 1985]. In some studies, cholinergic activities in the striatum and hypothalamus were also investigated. Cholinergic activity in the brain tissue was monitored by measuring sodium-dependent high-affinity choline uptake (HACU) from brain tissues. Sodium-dependent high-affinity choline is the rate limiting step in the synthesis of acetylcholine and has widely been used as an index of cholinergic activity in neural tissue [Atweh et al., 1975].

We found that after 45 min of acute exposure to pulsed 2450-MHz RFR (2 μs pulses, 500 pps, 1 mW/cm^2, average whole body SAR 0.6 W/kg), HACU was decreased in the hippocampus and frontal cortex, whereas no significant effect was observed in the striatum, hypothalamus, and inferior colliculus [Lai et al., 1987b]. Interestingly, the effect of RFR on HACU in the

hippocampus was blocked by pretreatment of the animals with the opiate-antagonists naloxone and naltrexone, suggesting involvement of endogenous opioids in the effect. Endogenous opioids are a group of peptides synthesized by the nervous system and have pharmacological properties like opiates. They are involved in a variety of physiological functions such as stress reactions, temperature-regulation, motivational behaviors, etc. Our further research showed that the effects of RFR on central cholinergic activity could be classically conditioned to cues in the exposure environment [Lai et al., 1987c]. These effects of RFR on cholinergic functions are similar to those reported in animals after exposure to stressors [Finkelstein et al., 1985; Lai, 1987; Lai et al., 1986c].

When different power densities of RFR were used, a dose-response relationship could be established from each brain region [Lai et al., 1989a]. Data were analyzed by probit analysis, which enables a statistical comparison of the dose-response functions of the different brain regions. It was found that a higher dose-rate was required to elicit a change in HACU in the striatum, whereas the responses of the frontal cortex and hippocampus were similar. Thus, under the same irradiation conditions, different brain regions could have different sensitivities to RFR.

In further experiments to investigate the contributory effect of different parameters of RFR exposure, we found that the radiation caused a duration-dependent biphasic effect on cholinergic activity in the brain. After 20 instead of 45 min of RFR exposure as in earlier experiments, an increase in HACU was observed in the frontal cortex, hippocampus, and hypothalamus of the rat [Lai et al., 1989b], and these effects could be blocked by pretreatment with the opiate antagonist naltrexone, suggesting the effects are also mediated by endogenous opioids.

Experiments [Lai et al., 1988] were then carried out to compare the effects of exposure in two different systems that produced different energy absorption patterns in the body of the exposed animal. Rats were exposed to pulsed (2 μs pulses, 500 pps) or continuous-wave 2450-MHz RFR in the circular waveguide and the miniature anechoic chamber exposure systems designed by Guy [Guy, 1979; Guy et al., 1979] with the whole body average SAR kept at a constant level of 0.6 W/kg. In the circular waveguide rats were exposed to circularly polarized RFR from the side of the body. In the miniature anechoic chamber rats were exposed dorsally with plane-polarized RFR. The circular waveguide produced a more localized energy absorption pattern than the miniature anechoic chamber. Detailed dosimetry studies in the body and brain of rats exposed in these two exposure systems had been carried out [Chou et al., 1984, 1985a]. After 45 min of exposure to the RFR, a decrease in HACU was observed in the frontal cortex in all exposure conditions studied (circular waveguide vs miniature anechoic chamber, pulsed vs continuous-wave). However, regardless of the exposure system used, HACU in the hippocampus decreased only after exposure to pulsed, but not continuous-wave RFR. Striatal HACU was decreased after exposure to either pulsed or continuous-wave RFR in the miniature anechoic chamber, but no significant effect was observed when the animal was exposed in the circular waveguide. No significant effect on HACU was found in the hypothalamus under all the exposure conditions studied. Thus, each brain region responded differently to RFR exposure depending on the parameters. Effects on the frontal cortex were independent of the exposure system or use of pulsed or continuous- wave RFR. The hippocampus only responded to pulsed but not to continuous-wave RFR. Response of the striatum depended on the exposure system used. The neurochemical changes were correlated with the dosimetry data of Chou et al. [1985a] on the local SARs in different brain areas of rats exposed to RFR in these two exposure systems. The dosimetry data showed that the septum, where the cell bodies of the hippocampal cholinergic pathway are located, had the lowest local SAR among eight brain areas measured in

both exposure systems; however, the hippocampus cholinergic pathway responded to pulsed, but not to continuous-wave RFR. Dosimetry data from the frontal cortex showed a wide range of local SARs in the frontal cortex (0.11-1.85 W/kg per mW/cm$^{2)}$) depending on the exposure system. Yet, exposure in both systems produced similar neurochemical responses in the frontal cortex (30-40% decrease in HACU). More interestingly, in the striatum the local SAR was approximately five times higher when the animals were exposed in the circular waveguide than in the miniature anechoic chamber; however, the striatal cholinergic system responded when the animal was exposed in the miniature anechoic chamber, but not in the circular waveguide. Since the cholinergic innervations in the striatum are mostly from interneurons inside the brain structure, these data would argue against a direct action of RFR on striatal cholinergic neurons causing a decrease in HACU, e.g., a local heating by the radiation. Unless different brain areas have different sensitivities to the direct effect of RFR, we could conclude that the effects of RFR on HACU in the brain areas studied in our experiments originated from other sites in the brain or body.

Neurotransmitter Receptors

Further experiments were conducted to investigate the effects of repeated RFR exposure on the cholinergic systems in the brain. Muscarinic cholinergic receptors were studied using the receptor-binding technique with ^{3}H-quinuclidinyl benzilate (QNB) as the ligand. These receptors are known to change their properties after repeated perturbation of the cholinergic system and that such changes can affect an animal's normal physiological functions [Overstreet and Yamamura, 1979]. After ten daily sessions of RFR exposure (2450 MHz at an average whole body SAR of 0.6 W/kg), the concentration of muscarinic cholinergic receptors changed in the brain [Lai et al., 1989b]. Moreover, the direction of change depended on the acute effect of the RFR. When animals were given daily sessions of 20-min exposure, which increased cholinergic activity in the brain, a decrease in the concentration of the receptors was observed in the frontal cortex and hippocampus. On the other hand, when animals were subjected to daily 45-min exposure sessions that decreased cholinergic activity in the brain, an increase in the concentration of muscarinic cholinergic receptors in the hippocampus resulted after repeated exposure and no significant effect was observed in the frontal cortex. These data pointed to an important conclusion that the long term biological consequence of repeated RFR-exposure depended on the parameters of exposure. Further experiments showed that changes in cholinergic receptors in the brain after repeated RFR exposure also depended on endogenous opioids, because the effects could be blocked by pretreatment before each session of daily exposure with the narcotic antagonist naltrexone [Lai et al., 1991]. Interestingly, changes in neurotransmitter receptor concentration also have been reported in animals after a single episode of exposure to RFR [Gandhi and Ross, 1987]. In the experiment rats were irradiated with 700-MHz RFR at 15 mW/cm^2 to produce a rise in body temperature of 2.5 ^{o}C (~10 min) and in some animals the temperature was allowed to return to normal (~50 min). Alpha-adrenergic and muscarinic cholinergic receptors were assayed in different regions of the brain using ^{3}H-clonidine and ^{3}H-QNB as ligands, respectively. No significant change in binding was observed for both receptors studied at the time when the body temperature reached a 2.5 ^{o}C increase. Decreases in ^{3}H-clonidine binding in the cerebral cortex, hypothalamus, striatum, and hypothalamus, and an increase in ^{3}H-QNB binding in the hypothalamus were observed when the brains were studied at the time the body temperature returned to the base line level. The authors

speculated that the receptor changes were thermoregulatory responses to the hyperthermia. It is not uncommon that the concentration of neurotransmitter receptors in the brain changes after a single exposure to drug or perturbation, e.g., stress [Estevez et al., 1984; Mizukawa et al., 1989].

Data from the above experiments and those described in the previous section indicate that the parameters of irradiation are important determinants of the outcome of the biological effect. Different durations of acute exposure lead to different biological effects and, consequently, the effects of repeated exposure depends upon the duration of each exposure session. On the other hand, the waveform of the irradiation was an important factor. This was seen in the differential effects that occurred after exposure to pulsed vs continuous-wave RFR, plane vs circularly polarized waves, and the pattern of energy absorption in the body of the animal. These data raised the question whether the whole body SAR could be used as the sole factor in considering the biological effects of RFR. Other exposure factors also should be considered.

A series of experiments were carried out to investigate the neural mechanisms mediating the effects of low-level RFR on the cholinergic systems of the rat brain. Our experiments [Lai et al., 1987b, 1989b] showed that some of the neurological effects of RFR are mediated by endogenous opioids in the brain. Since there are three types of endogenous opioid receptors, μ, δ, and κ, in the brain [Mansour et al., 1987; Katoh et al., 1990], the types of opioid receptors mediating the effects of RFR were studied in a further experiment [Lai et al., 1992b]. We found that RFR-induced decrease in HACU in the hippocampus could be blocked by injection of specific μ, δ, and κ opioid-antagonists into the lateral cerebroventricle of rats before exposure to RFR (2450 MHz, 45 min at an average whole body SAR of 0.6 W/kg). Supporting the previous finding that the RFR-induced decrease in HACU in the frontal cortex was not mediated by endogenous opioids [Lai et al., 1987b], all types of opioid receptor antagonists tested were not effective in blocking the effect in the frontal cortex.

More recent research showed that the effects of RFR on both frontal cortical and hippocampal cholinergic systems could be blocked by pretreatment with an intracerebro-ventricular injection of the corticotropin-releasing factor (CRF) antagonist α−helical-CRF$_{9\text{-}41}$ [Lai et al., 1990]. Corticotropin-releasing factor is a hormone that has been implicated in mediating stress responses in animals [Fisher, 1989]. From the above results and data from our other research [Lai and Carino, 1990a], the following sequence of events in the brain was proposed [Lai, 1992] to be triggered by RFR:
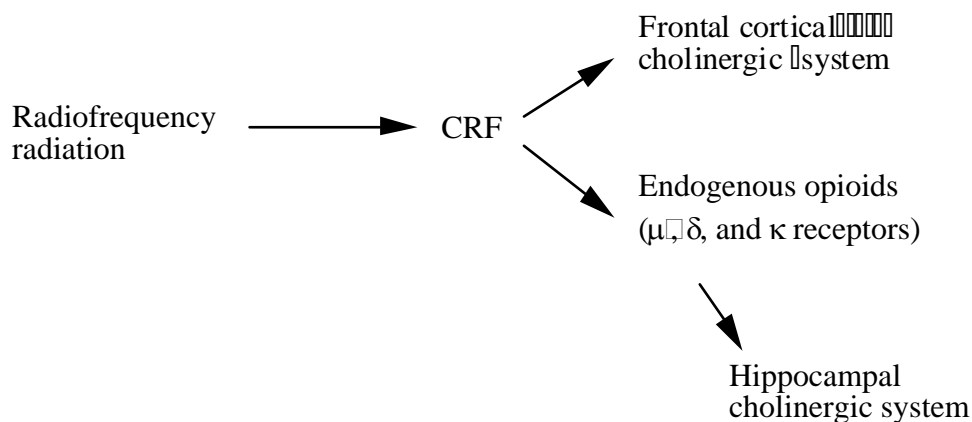
Radiofrequency radiation (2450-MHz, 45 min exposure at an average whole body SAR of 0.6 W/kg) activates CRF, which in turn caused a decrease in the activity of the cholinergic innervations in the frontal cortex and hippocampus of the rat. In addition, the effect of CRF on the hippocampal cholinergic system was mediated by endogenous opioids via μ, δ, and κ receptors. Since these effects can be blocked by direct injection of antagonists into the ventricle of the brain, the neural mechanisms involved are located inside the central nervous system.

A series of experiments were performed to study the effects of RFR on benzodiazepine receptors in the brain. Benzodiazepine receptors have been suggested to be involved in anxiety and stress responses in animals [Polc, 1988] and have been shown to change after acute or repeated exposure to various stressors [Braestrup et al., 1979; Medina et al., 1983a, b]. Exposure to RFR has been previously shown to affect the behavioral actions of benzodiazepines [Johnson et al., 1980; Thomas et al., 1979]. After an acute (45 min) exposure to 2450-MHz RFR (average whole body SAR 0.6 W/kg), increase in the concentration of benzodiazepine receptors occurred in the cerebral cortex of the rat, but no significant effect was observed in the hippocampus and cerebellum. Furthermore, the response of the cerebral cortex adapted after repeated RFR exposure (ten 45-min sessions) [Lai et al.,1992a].

Metabolism of Neural Tissues

With the changes in neurotransmitter functions after exposure to RFR, it would not be surprising to observe changes in second messenger activity in neural tissues that mediate the the reaction between a neurotransmitter and its receptors on the cell membrane. Studies in this area are sparse. Gandhi and Ross [1989] reported that exposure of rat cerebral cortex synaptosomes to 2800-MHz RFR at power densities greater than 10 mW/cm^2 (SAR, 1 mW/gm per mW/cm^2) increased ^{32}Pi incorporation into phosphoinositides, thereby suggesting an increase in inositol metabolism. These phospholipids play an important role in membrane functions and act as second messengers in the transmission of neural information between neurons.

Several studies have investigated the effects of RFR exposure on energy metabolism in the rat brain. Sanders and associates studied the components of the mitochrondrial electron-transport system that generates high energy molecules for cellular functions. The compounds nicotinamide adenosine dinucleotide (NAD), adenosine triphosphate (ATP), and creatine phosphate (CP) were measured in the cerebral cortex of rats exposed to RFR.

Sanders et al. [1980] exposed the head of rats to 591-MHz continuous-wave RFR at 5.0 or 13.8 mW/cm^2 for 0.5-5 min (local SAR at the cortex of the brain was estimated to be between 0.026 and 0.16 W/kg per mW/cm^2). Decreases in ATP and CP and an increase in NADH (the reduced form of NAD) concentration were observed in the cerebral cortex. These changes were found at both power densities of exposure. Furthermore, the authors reported no significant change in cerebral cortical temperature at these power densities. They concluded that the radiation decreased the activity of the mitochrondrial electron-transport system.

In another study [Sanders and Joines, 1984] the effects of hyperthermia and hyperthermia plus RFR were studied. The authors reported brain temperature-dependent decreases in ATP and CP concentrations in the brain. Radiofrequency radiation (591 MHz, continuous- wave, at 13.8 mW/cm^2, for 0.5-5 min) caused a further decline in the concentration of the compounds in addition to the temperature effect.

Sanders et al. [1984] further tested the effect of different frequencies of radiation (200, 591 and 2450 MHz) on the mitochrondrial electron-transport system. The effect on the concentration of NADH was found to be frequency dependent. An intensity-dependent increase in NADH level was observed in the cerebral cortex when irradiated with the 200-MHz and 591-MHz radiations. No significant effect was seen with the 2450-MHz radiation. In their paper, Sanders et al. [1984] made an interesting deduction. Under normal conditions, the concentration of ATP in a cell is maintained by conversion of CP into ATP by the enzyme creatine phosphate kinase. Thus, the concentration of ATP is generally more stable than that of CP, and the concentration of ATP does not decline unless the CP concentration has reached 60% of normal. In the case of the RFR, the concentration of ATP dropped as fast as the CP level. Thus, they speculated that the radiation may have inhibited creatine phosphate kinase activity in the brain tissue.

In a further study [Sanders et al., 1985], the effects of continuous-wave, sinusoidally amplitude-modulated, and pulsed 591-MHz RFR were compared after five min of exposure at power densities of 10 and 20 mW/cm^2 (SARs at the cerebral cortex were 1.8 and 3.6 W/kg). Different modulation frequencies (4-32 Hz) were used in the amplitude-modulation mode. There was no significant difference in the effect on the NADH level across the modulation frequency. Furthermore, pulsed radiations of 250 and 500 pps (5 µs pulses) were compared with power densities ranging from 0.5-13.8 mW/cm^2. The 500 pps radiation was found to be significantly more effective in increasing the concentration of NADH in the cerebral cortex than the 250 pps radiation. Since changes in these experiments occurred when the tissue (cerebral cortex) temperature was normal, the authors speculated that they were not due to hyperthermia, but to a direct inhibition of the electron-transport functions in the mitochrondria by RFR-induced dipole molecular oscillation in divalent metal containing enzymes or electron transport sites.

Another experiment related to brain metabolism after RFR exposure was performed by Wilson et al. [1980]. They studied the uptake of ^{14}C-2-deoxy-D-glucose (2-DG) in the auditory system of the rat after exposure to either pulsed 2450 MHz (20 µs pulses, 10 pps, average power density 2.5 mW/cm^2) or continuous-wave 918-MHz (2.5-10 mW/cm^2) RFR for 45 min. One middle ear of the rats was destroyed before the experiment. Neurons that have increased activity (metabolism) will pick up an increased amount of 2-DG, which will accumulate in the cell body, since it is not a normal substrate for cellular functions. Location in the brain of these neurons can then be identified histologically by the autoradiographic technique. The authors reported a symmetrical (in both brain hemispheres) increase in 2-DG uptake in the inferior colliculus, medial geniculate nucleus, and various other nuclei in the auditory system after exposure. Asymmetric (contralateral to the intact middle ear) uptake was seen in the auditory system of rats exposed to auditory stimuli. Further experiment showed that unilateral destruction of the cochlea before the experiment produced asymmetric 2-DG uptake in the brain after exposure to the RFR. These data confirmed the findings of Chou et al. [1975] and Chou and Galambos [1979] that the cochlea and not the middle ear contributes to the auditory perception of pulsed RFR. However, it is surprising that both continuous-wave and pulsed RFRs produced similar patterns of 2-DG uptake in the auditory system and only pulsed RFR elicited auditory sensation.

Calcium Efflux

Another important topic of research on the neurochemical effects of electromagnetic radiation is the efflux of calcium ions from brain tissue. Calcium ions play important roles in the functions of the nervous system, such as the release of neurotransmitters and the actions of some

neurotransmitter receptors. Thus, changes in calcium ion concentration could lead to alterations in neural functions.

Bawin et al. [1975] reported an increase in efflux of calcium ions from chick brain tissue after 20 min of exposure to a 147-MHz RFR (1 to 2 mW/cm^2). The effect occurred when the radiation was sinusoidally amplitude-modulated at 6, 9, 11, 16, or 20 Hz, but not at modulation frequencies of 0, 0.5, 3, 25, or 35 Hz. The effect was later also observed with 450-MHz radiation amplitude-modulated at 16 Hz, at a power density of 0.75 mW/cm^2. Bicarbonate and pH of the medium were found to be important factors in the effect [Bawin et al., 1978].

In vitro increase in calcium efflux from the chick brain was further confirmed by Blackman et al. [1979, 1985, 1980a,b] using amplitude-modulated 147-MHz and 50-MHz RFR. They also reported both modulation-frequency windows and power windows in the effect. These data would argue against a role of temperature. The existence of a power-density window on calcium efflux was also reported by Sheppard et al. [1979] using a 16-Hz amplitude-modulated 450-MHz field. An increase in calcium ion efflux was observed in the chick brain irradiated at 0.1 and 1.0 mW/cm^2, but not at 0.05, 2.0, or 5.0 mW/cm^2.

Two other papers reported no significant change in calcium efflux from the rat brain irradiated with RFR. Shelton and Merritt [1981] exposed rat brains to 1000-MHz RFR pulse-modulated with square waves (16 and 32 Hz, power density 0.5-15 mW/cm^2). They observed no change in calcium efflux from the tissue. Merritt et al. [1982] exposed rat brains with either 1000-MHz pulsed radiation modulated at 16 Hz at 1 or 10 mW/cm^2 (SARs 0.29 and 2.9 W/kg), or to a pulse-modulated 2450-MHz RFR at 1 mW/cm^2 (SAR 0.3 W/kg). No significant change in calcium efflux was observed in this experiment. These researchers also exposed animals, in vivo, injected with radioactive calcium to pulsed 2060-MHz RFR at different combinations of intensities and pulse repetition rates. No significant change in radioactive calcium content was found in the brains of the animals after 20 min of exposure. It is not known whether the discrepancies between these data and the findings of Bawin et al. [1975, 1978] and Blackman et al. [1979] were due to the use of square-wave instead of sinusoidally modulated radiation or due to the different species of animals studied. Electromagnetic field-induced increases in calcium efflux have also been reported in tissues obtained from different species of animals. Adey et al. [1982] observed an increase in calcium efflux from the brain of conscious cats paralyzed with gallamine and exposed for 60 min to a 450-MHz field (amplitude modulated at 16 Hz at 3.0 mW/cm^2, SAR 0.20 W/kg). Lin-Liu and Adey [1982] also reported increased calcium efflux from synaptosomes prepared from the rat cerebral cortex when irradiated with a 450-MHz RFR amplitude-modulated at various frequencies (0.16-60 Hz). Again, modulation at 16 Hz was found to be the most effective. More recently, Dutta et al. [1984] reported radiation-induced increases in calcium efflux from cultured cells of neural origins. Increases were found in human neuroblastoma cells irradiated with 915-MHz RFR (SARs 0.01-5.0 W/kg) amplitude-modulated at different frequencies (3-30 Hz). A modulation frequency window was reported. Interestingly, at certain power densities, an increase in calcium efflux was also seen with unmodulated radiation. A later paper [Dutta et al., 1989] reported increased calcium efflux from human neuroblastoma cells exposed to 147-MHz RFR amplitude-modulated at 16 Hz. A power window (SAR between 0.05-0.005 W/kg) was observed. When the radiation at 0.05 W/kg was studied, peak effects were observed at modulation frequencies between 13-16 Hz and 57.5-60 Hz. In addition, the authors also reported increased calcium efflux in another cell line, the Chinese hamster-mouse hybrid neuroblastoma cells. Effect was observed when these cells were irradiated with a 147-MHz radiation amplitude-modulated at 16 Hz (SAR 0.05 W/kg).

In more recent studies, Blackman explored the effects of different exposure conditions [Blackman et al., 1988, 1989, 1991]. Multiple power windows of calcium efflux from chick brains were reported. Within the power densities studied in this experiment (0.75-14.7 mW/cm^2, SAR 0.36 mW/kg per mW/cm^2) narrow ranges of power density with positive effect were separated by gaps of no significant effect. The temperature in which the experiment was run was also reported to be an important factor of the efflux effect. A hypothetical model involving the dynamic properties of cell membrane has been proposed to account for these effects [Blackman et al., 1989].

In addition to calcium ion, changes in other trace metal ions in the central nervous system have also been reported after RFR exposure. Stavinoha et al. [1976] reported an increase in zinc concentration in the cerebral cortex of rats exposed to 19-MHz RFR. Increases in the concentration of iron in the cerebral cortex, hippocampus, striatum, hypothalamus, midbrain, medulla, and cerebellum; manganese in the cerebral cortex and medulla; and copper in the cerebral cortex were reported in the rat after 10 min of exposure to 1600-MHz RFR at 80 mW/cm^2 (SAR 48 W/kg) [Chamness et al., 1976]. The significance of these changes is not known. The effects could be as a result of hyperthermia, because the colonic temperature of the animals increased by as much as 4.5 $^{\circ}$C after exposure.

RADIOFREQUENCY RADIATION AND THE ACTIONS OF PSYCHOACTIVE DRUGS

The actions of psychoactive drugs depend on the functions of the neurotransmitter systems in the brain. Changes in neurotransmitter functions after RFR exposure will inevitably lead to changes in the actions of psychoactive drugs administered to the animal. On the other hand, if there is no change in the pharmacokinetics of drugs after RFR exposure, observed changes in psychoactive drug actions would imply RFR-induced changes in neurotransmitter functions in the animal. Pharmacological studies of RFR effects provide an important insight into the neural mechanisms affected by exposure to RFR.

Psychoactive drugs of various types have been tested in animals after exposure to RFR. Since an effect of RFR is to increase the body temperature of an animal, special attention has been given to study the effects of psychoactive drugs on the thermal effect of RFR. Jauchem [1985] has reviewed the effects of drugs on thermal responses to RFR. Radiofrequency radiation of high power densities was used in these studies.

Some psychoactive drugs have a profound effect on thermoregulation and, thus, alter the body temperature of an animal upon administration. The effect could be due to direct drug action on the thermoregulatory mechanism within the central nervous system or effects on autonomic functions such as respiration, cardiovascular and muscular systems, which lead to changes in body temperature. Several studies have investigated the neuroleptic (anti-psychotic) drug, chlorpromazine. Michaelson et al. [1961] reported that chlorpromazine enhanced the thermal effect of RFR in dogs (2800 MHz, pulsed, 165 mW/cm^2). Drug-treated animals had a faster rate of body temperature increase and a higher peak temperature when irradiated with RFR. Similar effects were seen with pentobarbital and morphine sulfate. On the other hand, Jauchem et al. [1983, 1985] reported that chlorpromazine attenuated the thermal effect of RFR in ketamine anesthetized rats. The drug slowed the rate of rise in colonic temperature (from 38.5-39.5 $^{\circ}$C) and facilitated the return to base line temperature after exposure to RFR (2800-MHz, 14

W/kg); however, when the body temperature was allowed to rise to a lethal level, chlorpromazine potentiated the effect of RFR. Interestingly, haloperidol, another neuroleptic drug, was found to have no significant effect on RFR-induced change in colonic temperature. In another study [Lobanova, 1974b], the hyperthermic effect of RFR (40 mW/cm^2) was found to be attenuated by pretreatment with chlorpromazine or acetylcholine and enhanced by epinephrine and atropine (a cholinergic antagonist). This suggests a role of acetylcholine in modifying RFR-induced hyperthermia. Indeed, Ashani et al. [1980] reported that acute RFR exposure (10 min at 10 mW/cm^2) enhanced the hypothermic effects of AChE inhibitors. On the other hand, Jauchem et al. [1983, 1984] observed no significant effect of atropine and propranolol (an adrenergic antagonist) on the hyperthermia produced in ketamine anesthesized rats exposed to 2800-MHz RFR (SAR 14 W/kg).

Several studies investigated the effects of RFR on the actions of barbituates. Barbituates are sedative-hypnotic compounds, which produce narcosis (sleep states and loss of consciousness), synchronization of EEG, and poikilothermia (i.e., loss of body temperature regulatory functions). Baranski and Edelwejn [1974] reported that acute exposure to pulsed RFR (20 mW/cm^2) had little effect on the EEG pattern of rabbits given phenobarbital; however, after 200 h of exposure (at 7 mW/cm^2), desynchronization rather than synchronization of the EEG pattern was seen after phenobarbital administration. Rabbits anesthetized with pentobarbital and subjected to 5 min of RFR (0.7-2.8 mW/cm^2) showed periods of alternating EEG arousal (desynchronization) and sedation (synchronization) and periods of behavioral arousal. The duration of EEG arousal seemed to correlate with the power density of RFR [Goldstein and Sisko, 1974].

Wangemann and Cleary [1976] reported that short term RFR exposure (5-50 mW/cm^2) decreased the duration of pentobarbital induced loss of righting reflex in the rabbit. The investigators speculated that the effect was due to the thermal effect of RFR, which decreased the concentration of pentobarbital in the central nervous system. Supporting this, Bruce-Wolfe and Justesen [1985] reported that warming an animal with RFR while under anesthesia could attenuate the effects of pentobarbital. Mice exposed to continuous-wave 2450-MHz RFR at 25 and 50 mW/cm^2 also showed a power density-dependent reduction in the duration of hexobarbital-induced anesthesia [Blackwell, 1980]. On the other hand, Benson et al. [1983] reported decreased onset-time and prolonged duration of phenobarbital-induced narcosis in mice after exposure to RFR (10 mW/cm^2, 10 min). They showed that the effect was caused by an increase in deposition of phenobarbital in the brain. We [Lai et al., 1984a] have shown that after 45 min of exposure to pulsed 2450-MHz RFR (2 μs pulses, 500 pps, whole-body average SAR 0.6 W/kg), the pentobarbital-induced narcosis and hypothermia in the rat were enhanced. We also found that exposure of rats in two different orientations (with the head of the rat facing or away from the source of the RFR) had different effects on the pentobarbital-induced hypothermia, even though the average whole body SAR was similar under the two conditions. These data suggest that the pattern of localized SAR in the body of the animal might be an important determinant of the outcome of the effect.

When the body temperature of an animal is raised above a certain level, convulsions result. Various psychoactive drugs were studied in an attempt to alter the convulsive effect of RFR. Studies have also been carried out to investigate whether RFR exposure altered the potency of convulsants. It was reported that the susceptibility of rats to the convulsive effect of RFR (14 mW/cm^2, 2 h) was decreased by chloral hydrate, sodium pentobarbital, and bemegride, and enhanced by chlorpromazine, epinephrine, atropine, acetylcholine, nicotine, and monoamine

oxidase inhibitors, but was not significantly affected by serotonin [Lobanova, 1974a]. Some of these results can be explained by the pharmacological properties of the drug tested. Pentobarbital and chloral hydrate are hypnotic agents and are known to have anticonvulsant effects. Chlorpromazine, nicotine, and monoamine oxidase inhibitors can lower the seizure threshold or induce convulsions depending on their dosages. Atropine, a cholinergic antagonist, has been shown to enhance the seizure threshold. It is puzzling that bemegride decreased RFR induced seizures, since it is a nervous system stimulant with similar pharmacological actions as the convulsant pentylenetetrazol.

Exposure to pulsed RFR (7 and 20 mW/cm^2) was reported to affect the effects of the convulsants, pentylenetetrazol and strychnine, on EEG activity [Baranski and Edelwejn, 1974]. Another study showed that low-level RFR altered the sensitivity of animals to the seizure inducing effect of pentylenetetrazol [Servantie et al., 1974]. Rats and mice were subjected to 8-36 days of pulsed RFR (3000 MHz, 0.9-1.2 μs pulses, 525 pps, 5 mW/cm^2). No significant change in susceptibility to the drug was seen after eight days of exposure; however, a decrease in susceptibility was observed after 15 days, and an increase in susceptibility was observed after 20, 27, and 36 days of irradiation. Mice became more susceptible to the convulsive effect of pentylenetetrazol and more animals died from convulsions. Thus, the sensitivity of the nervous system to the convulsive action of the drug changed as a function of the duration of exposure. In another study, Pappas et al. [1983] showed in the rat that acute (30 min) exposure to 2700-MHz pulsed RFR at 5, 10, 15, and 20 mW/cm^2 (SARs 0.75, 1.5, 2.25, and 3.0 W/kg, respectively) produced no significant interaction effect on pentylenetetrazol induced seizure or the efficacy of chlordiazepoxide (an anticonvulsant) to block the seizure.

Drugs affecting cholinergic functions in the nervous system have also been studied. Chronic RFR-exposed rats (10-15 days) were found to be less susceptible to the paralytic effect of curare-like drugs, which block nicotinic cholinergic transmission. A similar effect was observed on muscle preparations from the irradiated rats. Presumably, the cholinergic transmission in the neuromuscular junction was affected by RFR. Ashani et al. [1980] reported that acute pulsed RFR (10 min, 10 mW/cm^2) enhanced the hypothermic effects of an inhibitor of AChE (the degradation enzyme of acetylcholine). The site of this effect was determined to be located inside the central nervous system. Monahan [1988] also reported that RFR (2450 MHz, continuous-wave, whole body SARs 0.5-2.0 W/kg) affected the actions of scopolamine, a cholinergic antagonist, and physostigmine, a cholinergic agonist, on motor activity of mice in a maze. The data suggested enhancement of cholinergic activity after RFR irradiation.

Several studies investigated the actions of benzodiazepines, a group of drugs used for anticonvulsion, sedation-hypnosis, and antianxiety purposes. Two of the most commonly used benzodiazepines for the treatment of anxiety disorders are chlordiazepoxide (Librium) and diazepam (Valium). Low-level pulsed RFR (1 mW/cm^2, whole body SAR 0.2 W/kg) potentiated the effect of chlordiazepoxide on bar-pressing behavior of rats working on a DRL-schedule for food reinforcement; however, the same authors also reported no interaction effects between RFR and diazepam on bar pressing [Thomas et al., 1979, 1980].

Increase in brain benzodiazepine receptors in the brain after RFR exposure [Lai et al, 1992a] could explain the former effect. A possible explanation for the discrepancy of the results observed with chlordiazepoxide and diazepam was that diazepam has a higher potency than chlordiazepoxide. The potency of diazepam that was effective in attenuation of experimental conflict, an animal model of anxiety, was about four times that of chlordiazepoxide [Lippa et al., 1978], and the in vitro relative affinity of diazepam with benzodiazepine receptors was 30-65

46

times that of chlordiazepoxide [Braestrup and Squires, 1978; Mohler and Okada, 1977]. The ranges of diazepam and chlordiazepoxide used in the Thomas studies [Thomas et al., 1979, 1980] were 0.5-20 and 1-40 mg/kg, respectively. Thus, the doses of diazepam studied might be equivalent or higher in potency than the highest dose of chlordiazepoxide used. This supposition was supported by the observation in the Thomas studies that the effects of the two drugs were different. The dose-response curve of chlordiazepoxide on the DRL-schedule operant responses showed a dose-dependent inverted-U function, i.e., potentiation at medium dose, attenuation at higher dose, and only the portion of the response-curve that showed potentiation was affected by RFR [Thomas et al., 1979]. In the study of Thomas et al. [1980] on diazepam, only attenuation of DRL-responses was observed. Thus, the dose range of diazepam used in the study was at the attenuation portion of the dose-response function, which is not affected by RFR. These dose-dependent potentiation and attenuation effects of benzodiazepines on the operant response may involve different neural mechanisms. Radiofrequency radiation may only affect and enhance the potentiating and not the attenuating effect of benzodiazepines, which is possible because our research [Lai et al., 1992a] showed that the effect of RFR on benzodiazepine receptors is brain-region selective. Thus, the data of Thomas et al. [1979, 1980] on the interaction of RFR irradiation on benzodiazepine actions could be explained by a selective increase in benzodiazepine receptors in different regions of the brain. Another possibility is that RFR affects only the subtype of benzodiazepine receptors related to antianxiety effect and not another subtype related to the sedative-hypnotic action of the drugs. In the dose-response curve of benzodiazepine on DRL-schedule maintained behavior, the potentiation portion may be due to the former receptor subtypes and the attenuation portion the latter subtype. There is ample evidence suggesting that different subtypes of benzodiazepine receptors subserve antianxiety and sedative effects [Polc, 1988].

In addition to the above studies on the effect of RFR on benzodiazepines, Monahan and Henton [1979] trained mice to avoid or escape from 2450-MHz RFR (45 W/kg) under an avoidance paradigm. They reported that pretreatment of the animals with chlordiazepoxide decreased the avoidance response and increased the escape responses, which led to an increase in the animal's cumulative exposure to RFR after the drug treatment. The authors speculated that RFR potentiated the effect of chlordiazepoxide and caused a decrement in the avoidance response. It is also interesting that in the procedure the presence of RFR was signalled simultaneously with a tone and the animal could elicit an avoidance response, which resets the timer and delays the further presentation of RFR. Thus, the procedure had both signalled and continuous avoidance components. However, the data indicate that the effect was more like a continuous avoidance paradigm. Generally, anxiolyltic agents like benzodiazepines decrease both avoidance and escape behavior in a signalled-avoidance paradigm, but they can selectively decrease the avoidance response and leave the escape responding intact under a continuous avoidance paradigm.

Johnson et al. [1980] reported that repeated exposure (twenty-one 45-min sessions) to RFR (2450 MHz, pulsed, average whole body SAR 0.6 W/kg) reduced the sedative hypnotic effect, but increased the feeding behavior induced by diazepam. Hjeresen et al. [1987] reported that the attenuation effect of a single (45 min) RFR exposure (2450 MHz, CW, average whole body SAR 0.3 W/kg) on ethanol-induced hypothermia was blocked by treating the rat with the benzodiazepine antagonist, RO 15-1778. The data indicated that benzodiazepine receptors in the brain might mediate the effects of RFR on ethanol-hypothermia. In a more recent study, Quock et al. [1990] investigated the influence of RFR exposure on the effect of chlordiazepoxide on the

stair-case test for mouse, a test for both the sedative and antianxiety effects of benzodiazepines. They reported that acute exposure (5 min at a whole body average SAR of 36 W/kg) caused a significant reduction of the sedative, but not the antianxiety effect of chlordiazepoxide. The effect was probably related to hyperthermia. Some of the above effects of RFR on benzodiazepine actions can be explained by our finding [Lai et al., 1992a] that acute RFR exposure increased benzodiazepine receptors in selective regions of the brain and that adaptation occurred after repeated exposure.

On the other hand, central benzodiazepine receptors can also affect seizure susceptibility in animals. Benzodiazepines are widely used as anticonvulsants. Exposure to RFR has been shown to affect seizure and convulsion susceptibility in animals. For example, Stverak et al. [1974] reported that chronic exposure to pulsed RFR attenuated audiogenic seizures in seizure-sensitive rats. Servantie et al. [1974] showed that mice chronically exposed to pulsed RFR initially showed a decrease and then an increase in susceptibility to the convulsant pentylenetetrazol. However, Pappas et al. [1983] showed no significant interaction effect of RFR on pentylenetetrazol-induced seizures nor the efficacy of chlordiazepoxide to block the seizure in rats. A more thorough study of the different parameters of RFR exposure on benzodiazepine receptors in the brain may explain these findings. Benzodiazepine receptors are very dynamic and can undergo rapid changes in properties in response to environmental stimuli [Braestrup et al., 1979; Lai and Carino, 1990b; Medina et al., 1983a,b; Soubrie et al., 1980; Weizman et al., 1989]. However, the direction of change and extent of effect depend on the stimulus and experimental conditions.

We conducted experiments to study the effect of acute RFR exposure on the actions of various psychoactive drugs [Lai et al., 1983; 1984a,b]. We found that acute (45 min) exposure to pulsed 2450-MHz RFR (2 μs pulses, 500 pps, 1 mW/cm^2, whole body average SAR 0.6 W/kg) enhanced apomorphine-hypothermia and stereotypy, morphine-catalepsy, and pentobarbital-hypothermia and narcosis, but it attenuated amphetamine-hyperthermia and ethanol-hypothermia. These psychoactive drugs are lipid-soluble and readily enter the central nervous system and the effects observed are not unidirectional, i.e., depending on the drug studied, increase or decrease in action was observed after RFR exposure. Therefore, these effects cannot be explained as a change in entry of the drugs into the brain, e.g., change in blood-brain barrier permeability or alteration in drug metabolism as a result of RFR exposure. Our finding that acute low-level RFR attenuated ethanol-hypothermia in the rat was replicated by Hjeresen et al. [1988] at a lower whole body average SAR of 0.3 W/kg. Blood ethanol level measurements indicated that the effect was not due to changes in metabolism or disposition of ethanol in the body. Results from further experiments [Hjeresen et al., 1989] suggested that the β-adrenergic mechanism in the brain might be involved in the attenuation effect of RFR on ethanol-induced hypothermia in the rat.

We further found that the effects of RFR on amphetamine-hyperthermia [Lai et al., 1986b] and ethanol-hypothermia could be classically conditioned to cues in the exposure environment after repeated exposure. Another interesting finding in our research was that some of the effects of RFR on the actions of the psychoactive drugs could be blocked by pretreating the rats with narcotic antagonists before exposure, suggesting the involvement of endogenous opioids [Lai et al., 1986b]. The hypothesis that low-level RFR activates endogenous opioids in the brain was further supported by an experiment showing that the withdrawal syndromes in morphine-dependent rats could be attenuated by RFR exposure [Lai et al., 1986a]. This hypothesis can

explain most of the RFR-psychoactive drug interaction effects reported in our studies [see Table I in Lai et al., 1987a].

In another study [Lai et al., 1984b], water-deprived rats were allowed to drink a 10% sucrose solution from a bottle in the waveguide. Exposure to pulsed 2450-MHz RFR (2 μs pulses, 500 pps, 1 mW/cm^2, SAR 0.6 W/kg) did not significantly affect the consumption of the sucrose solution. However, when the sucrose solution was substituted by a 10% sucrose-15% ethanol solution, the rats drank ~25% more when they were exposed to the RFR than when they were sham exposed. The hypothesis that RFR activates endogenous opioids in the brain can also explain the increased ethanol consumption during RFR exposure. Recent studies have shown that activation of opioid mechanisms in the central nervous system can induce voluntary ethanol drinking in the rat [Nichols et al., 1991; Reid et al., 1991; Wild and Reid, 1990].

Frey and Wesler [1983] studied the effect of low-level RFR (1200 MHz, pulsed, 0.2 mW/cm^2, 15 min) on central dopaminergic functions. Radiofrequency radiation was found to attenuate the effect to both a high dose (1 mg/kg, IP) and a low dose (0.1 mg/kg, IP) of apomorphine on the latency of the tail-flick responses in the rat. The tail-flick test is a measure of pain perception in animals. These data are difficult to explain, since high dose and low dose of apomorphine affect predominantly the post- and presynaptic-dopamine receptors, respectively. These two types of dopamine receptors have opposite effects on dopamine transmission and functions. Other experiments indicating an effect of RFR on dopamine function in the brain are those of Michaelson et al. [1961] and Jauchem et al. [1983, 1985] showing the effect of chlorpromazine on RFR-induced hyperthermia, and our experiment showing an enhancement of apomorphine-hypothermia by RFR [Lai et al., 1983]. Chlorpromazine and apomorphine are dopamine antagonist and agonist, respectively. On the other hand, Thomas et al. [1980] reported no significant interaction effect between chlorpromazine and pulsed RFR (2800 MHz, 2 μs pulses, 500 pps, 1 mW/cm^2, SAR 0.2 W/kg) on rats responding on a fixed interval reinforcement schedule for food reward. However, Thomas and Maitland [1979] reported that exposure to pulsed 2450-MHz RFR (2 μs pulses, 500 pps, 1 mW/cm^2, SAR 0.2 W/kg) potentiated the effect of d-amphetamine on rats responding on a DRL-schedule of reinforcement. Amphetamine is an agonist of both dopamine and norepinephrine functions in the brain.

Two studies imply RFR affects serotonergic activity in the brain. Galloway and Waxler [1977] reported interaction between RFR and a serotonergic drug. Rhesus monkeys trained on a color-matching task were irradiated with continuous-wave 2450-MHz RFR at different dose rates. The animals were also treated with the serotonergic drug fenfluramine, which inhibits granule reuptake and storage of serotonin in nerve terminals and causes a long-lasting depletion of serotonin in the brain. Radiofrequency radiation alone had no significant effect on performance, whereas fenfluramine alone decreased the response accuracy and response rate in performing the task. Exposure to RFR plus the drug treatment produced a synergistic effect. A severe disruption of responding was observed. The authors speculated that RFR may act like fenfluramine, i.e., decreases serotonergic functions in the brain. This may be related to the finding of Frey [1977] who reported that RFR exposure decreased tail pinch- induced aggressive behavior in the rat. Fenfluramine and other drug treatments that decrease serotonergic functions in the brain were shown to suppress aggressive behavior elicited by electric foot-shock in rats [Panksepp et al., 1973].

Results from one of our experiments also indicated an increase in serotonergic activity in the brain of rats exposed to RFR. We [Lai et al., 1984c] observed an increase in body temperature (~1.0 ^{o}C) in the rat after acute (45 min) exposure to pulsed 2450-MHz RFR (2 μs

pulses, 500 pps, 1 mW/cm^2, SAR 0.6 W/kg). This hyperthermic effect was blocked by pretreating the rats before exposure with the serotonin antagonists, cinanserin, cyproheptadine, and metergoline, but not by the peripheral serotonin antagonist, xylamidine, implying that the effect is mediated by serotonergic mechanism inside the central nervous system.

The findings that RFR can affect (potentiate or attenuate) the actions of psychoactive drugs could have important implication in considering the possible hazardous effects of the radiation. Most of the drugs studied, such as the benzodiazepines and neuroleptics, are widely used for therapeutic purposes. On the other hand, drugs can enhance the biological effects of RFR. Example are the studies of Kues and Monahan [1992] and Kues et al. [1990; 1992] showing synergistic effects of drugs on corneal endothelium damages and retinal degeneration in the monkey induced by repeated exposure to RFR. They found that application of the drugs timolol and pilocarpine to the eye before RFR exposure could lower the threshold of the RFR effect by 10 folds (from 10 to 1 mW/cm^2). Timolol and pilocarpine are commonly used in the treatment of glaucoma.

PSYCHOLOGICAL EFFECTS OF RADIOFREQUENCY RADIATION

A necessary consequence of change in neurological activity is a change in behavior. If RFR alters electrophysiological and neurochemical functions of the nervous system, changes in behavior will result. Effects of RFR on both spontaneous and learned behaviors have been investigated.

Spontaneous Behaviors

The effects of RFR on motor activity were the subjects of various studies. Changes in motor activity are generally regarded as indications of changes in the arousal state of an animal. Hunt et al. [1975] reported increased motor activity in rats after 30 min of exposure to 2450-MHz RFR (SAR of 6.3 W/kg) and decreased swimming speed in cold (24 ^{o}C) water. However, Roberti [1975] reported no significant change in locomotor activity in rats after long term (185-408 h) exposure to RFR at different frequencies and intensities (SARs 0.15-83 W/kg). Modak et al. [1981] reported a decrease in motor activity in rats exposed to a single pulse (15 or 25 ms) of 2450-MHz RFR, which increased the brain temperature by 2-4 ^{o}C.

Mitchell et al. [1977] reported an increase in motor activity on a small platform of rats exposed to 2450-MHz RFR (average SAR 2.3 W/kg, 5 hr/day, 5 days/week for 22 weeks). Motor activity of the RFR exposed rats increased during the first week of exposure and stayed higher than controls throughout the period of the experiment. Moe et al. [1976] reported a decrease in motor activity of rats exposed to RFR (918 MHz, SARs 3.6-4.2 W/kg) during the dark period of the light-dark cycle in a chronic exposure experiment (10 h/night for 3 weeks). Lovely et al. [1977] repeated the experiment using a lower intensity (2.5 mW/cm^{2}, SARs 0.9-1.0 W/kg, 10 h/night, 13 weeks) and found no significant change in motor activity in the exposed rats. Frey [1977] subjected rats to 1300-MHz pulsed RFR (0.5 ms pulses, 1000 pps, average power density of 0.65 or 0.2 mW/cm^{2}, peak power densities 1.3 and 0.4 mW/cm^{2}). He reported a decrease in tail pinch-induced aggressive behavior in RFR-exposed rats. Increased latency, decrease in duration, and episodes of fighting after tail pinching were observed between two rats being irradiated with RFR. Decrease in motor coordination on a motor-rod was also reported in pulsed RFR-exposed (1300 and 1500 MHz, 0.5 ms pulses, 1000 pps) rats. The effect occurred at peak power densities between 0.4 and 2.8 mW/cm^{2}.

Rudnev et al. [1978] studied the behavior of rats exposed to 2375-MHz RFR at 0.5 mW/cm^{2} (SAR 0.1 W/kg), 7 h/day for 1 month. They reported decreases in food intake, balancing time in a treadmill and inclined rod, and motor activity in an open-field after 20 days of exposure. Interestingly, the open-field activity was found to be increased even at 3 months postexposure. In a long-term exposure study [Johnson et al., 1983], rats were exposed to pulsed 2450-MHz RFR (10 μs pulses, 800 pps) from 8 weeks to 25 months of age (22 h/day). The average whole body SAR varied as the weight of the rats increased and was between 0.4-0.15 W/kg. Open field activity was measured in 3-min sessions with an electronic open-field apparatus once every 6 weeks during the first 15 months and at 12 week intervals in the final 10 weeks of exposure. They reported a significantly lower open field activity only at the first test session and a rise in the blood corticosterone level was also observed at that time. The authors speculated that RFR might be minimally stressful to the rats.

D'Andrea et al. [1979, 1980] reported decreased motor activity on a stabilimetric platform and no significant change in running wheel activity measured overnight in rats exposed to 2450-MHz RFR (5 mW/cm^2, SAR 1.2 W/kg). However, an increase in both measurements was observed in rats exposed to 915-MHz RFR (5 mW/cm^2, SAR 2.5 W/kg). These changes in locomotor activity could be due to the thermal effect of RFR.

In a more recent experiment, Mitchell et al. [1988] studied several behavioral responses in rats after 7 h of exposure to continuous-wave 2450-MHz RFR (10 mW/cm^2, average SAR 2.7 W/kg). Decreases in motor activity and responsiveness (startle) to loud noise (8 kHz, 100 dB) were observed immediately after exposure. The rats were then trained to perform a passive avoidance task and tested for retention of the learning one week later. There was no significant difference in retention between the RFR-exposed and sham-exposed animals. The authors concluded that RFR altered responsiveness to novel environmental stimuli in the rat.

Two studies investigated the effects of pre- and postnatal-RFR on behavior. Kaplan et al. [1982] exposed groups of pregnant squirrel monkeys starting at the second trimester of pregnancy to 2450-MHz RFR at SARs of 0, 0.034, 0.34, and 3.4 W/kg (3 h/day, 5 days/week). The motor activity of the monkeys was observed at different times during the third trimester. No significant difference was observed among the different exposure groups. After birth, some dams and neonates were exposed for 6 months at the same prenatal conditions and then the offspring were exposed for another 6 months. Behavior of the mothers and offspring was observed and scored each week for the first 24 weeks postpartum. The authors observed no significant difference in maternal behavior or the general activity of the offspring among the different exposure groups. Visual-evoked EEG changes in the occipital region of the skull of the offspring were also studied at 6, 9, and 12 months of age. No significant effect of perinatal RFR-exposure was reported.

In another study [Galvin et al., 1986], rats were exposed to 2450-MHz RFR (10 mW/cm^2, 3 h/day) either prenatally (days 5-20 of gestation, whole body SAR estimated to be 2-4 W/kg) or perinatally (prenatally and on days 2-20 postnatally, whole body SARs 16.5-5.5 W/kg). Several behaviors including motor behavior, startle to acoustic and air-puff stimuli, fore- and hind-limb grip strength, negative geotaxis, reaction to thermal stimulation, and swimming endurance were studied in the rats at various times postnatally. They reported a decrease in swimming endurance (time remaining afloat in 20 ^{o}C water with a weight clipped to the tail) in 30-day old perinatally-exposed rats. The air-puff startle response was enhanced in magnitude in the prenatally exposed rats at 30 days, but decreased at 100 days of age. The authors concluded that perinatal exposure to RFR altered the endurance and gross motor activity in the rat. It would be interesting to study the neurochemistry or brain morphology of these animals. As described in a previous section, Albert et al. [1981a,b] and Albert and Sherif [1988] observed morphological changes in the cerebellum of rats subjected to RFR exposure perinatally at lower SAR (2-3 W/kg). It is well known that interference of cerebellar maturation can affect an animal's motor development [Altman, 1975].

O'Connor [1988] exposed pregnant rats to continuous-wave 2450-MHz (27-30 mW/cm^2) RFR between day 1 to day 18 or 19 of gestation (6 h/day). Their offspring were studied at different ages. She reported no significant effect of prenatal RFR exposure on visual cliff test, open field behavior, climbing behavior on an inclined plane, and avoidance behavior in a shuttlebox. The exposed animals showed altered sensitivity to thermally related tests evidenced by preference for the cooler section of a temperature-gradient alley way, longer latency to develop thermally induced seizure, and formed smaller huddle groups at 5 days of age.

Learned Behaviors

Many studies have investigated the effect of RFR exposure on learned behavior. King et al. [1971] used RFR as the cue in a conditioned suppression experiment. In conditioned suppression an animal is first trained to elicit a certain response (e.g., bar-press for food). Once a steady rate of response is attained, a stimulus (e.g., a tone) will signify the on-coming of a negative reinforcement (e.g., electric foot shock). The animal will soon learn the significance of the stimulus and a decrease in responding (conditioned suppression) will occur after the presentation of the stimulus. In the experiment of King et al. [1971], rats were trained to respond at a fixed-ratio schedule for sugar water reward. In a 2-h session, either a tone or RFR would be presented and occasionally followed by an electric foot shock. Radiofrequency radiation of 2450 MHz, modulated at 12 and 60 Hz and at SARs of 0.6, 1.2, 2.4, 4.8, and 6.4 W/kg were used as the conditioned stimulus. With training, consistent conditioned suppression was observed with RFR at 2.4 W/kg and higher.

Several studies used RFR as a noxious stimulus, i.e., a negative reinforcer, to induce or maintain conditioned behavior. In an earlier paper, Monahan and Ho [1976] speculated that mice exposed to RFR tended to change their body orientation in order to reduce the SAR in the body, suggesting that they were avoiding the radiation. To support the point that RFR is a noxious stimulus, Monahan and Henton [1977b] demonstrated that mice can be trained to elicit an operant response in order to escape or avoid RFR (2450-MHz, 40 W/kg).

In a series of experiments, Frey and his associates [Frey and Feld, 1975; Frey et al., 1975] demonstrated that rats spent less time in the unshielded compartment of a shuttlebox, when the box was exposed to 1200-MHz pulsed RFR (0.5 μs pulses, 1000 pps, average power density 0.2 mW/cm^2, peak power density 2.1 mW/cm^2) than during sham exposure. When a continuous-wave RFR (1200-MHz, 2.4 mW/cm^2) was used, rats showed no significant preference to remain in the shielded or unshielded side of the box. The authors also reported that rats exposed to the pulsed RFR were more active. Hjeresen et al. [1979] replicated this finding using pulsed 2880-MHz RFR (2.3 μs pulses, 100 pps, average power density 9.5 mW/cm^2) and showed that the preference to remain in the shielded side of a shuttlebox during RFR exposure could be generalized to a 37.5-kHz tone. Masking the radiation-induced auditory effect with a 10-20 kHz noise also prevented the development of shuttlebox-side preference during pulsed RFR exposure. These data suggest that the pulsed RFR-induced side preference is due to the auditory effect. In the studies of Frey et al. [1975] and Hjeresen et al. [1979] increase in motor activity was also reported when the animals were exposed to the pulsed RFR. Interestingly, this pulsed RFR-induced increase in motor activity was not affected by noise masking. Thus, the RFR avoidance and enhancement in motor activity by pulsed RFR may involve different neural mechanisms. Related to the above experiments is that the auditory effect of pulsed RFR can be used as a cue to modify an animal's behavior. Johnson et al. [1976] trained rats to respond (making nose pokes) on a fixed ratio reinforcement schedule for food pellets in the presence of a tone (7.5 kHz, 10 pps, 3 μs pulses). Reinforced period was alternated with periods of no reward when no tone was presented. Rats, after learning this response, responded when the tone was replaced by pulsed RFR (918 MHz, 10 μs pulses, 10 pps, energy per pulse 150 μJ/cm^2) during both reinforced and unrewarded periods. Apparently, the response to the tone had generalized to the pulsed RFR.

In another experiment, Carroll et al. [1980] showed that rats did not learn to go to a 'safe' area in the exposure cage in order to avoid exposure to RFR (918-MHz, pulse modulated at 60 Hz, SAR 60 W/kg), whereas the animals learned readily to escape from electric foot shock by going to the 'safe' area. In a further study, Levinson et al. [1982] showed that rats could learn to enter a 'safe' area, when the RFR (918-MHz, 60 W/kg) was paired with a light stimulus. Entering the area would turn off both the radiation and light. They also showed that rats could learn to escape by entering the 'safe' area when RFR was presented alone, but learned at a lower rate than when the RFR was paired with the light.

Several studies investigated the effect of RFR on conditioned taste aversion. It was discovered that consumption of food or drink of novel taste followed by a treatment which produced illness, e.g., X-irradiation or poison, an animal will learn to associate the taste with the illness and will later avoid the food or drink. Different from the traditional conditioning process, where conditioning occurs only when the response is followed immediately by the reinforcement, taste aversion conditioning can occur even if the illness is induced 12 h after the taste experience. Another characteristic of conditioned taste aversion is that the conditioning is very selective. An animal can learn to associate the taste with the illness, but not the place where the food or drink was taken, i.e., it will avoid the taste, but not the place where the food or drink was consumed. This phenomenon is known as 'belongingness', i.e., association (conditioning) between some stimulus pairs is easier than others [Garcia and Koelling, 1966; Garcia et al., 1966]. Thus, RFR has to produce the 'proper' type of adverse effect in the animal in order for conditioned taste aversion to occur.

Monahan and Henton [1977a] irradiated rats for 15 min with 915-MHz RFR of various intensities (up to a SAR of ~17 W/kg) after 15 min of access to 10% sucrose solution as a substitute for the normal drinking water. When the animals were offered the sucrose solution 24 h later, no conditioned taste aversion was observed. They drank the same amount of sucrose solution as the previous day. Conditioned taste aversion was also studied by Moe et al. [1976] and Lovely et al. [1977] in experiments of similar design in which rats were exposed chronically to 918-MHz RFR at 10 mW/cm^2 (SAR 3.9 W/kg) and 2.5 mW/cm^2 (SAR 1.0 W/kg), respectively. Rats were provided with 0.1% saccharin drinking solution during the whole period of exposure in the Moe et al. [1976] study and between the 9th to 13th week of exposure in the Lovely et al. [1977] study. They observed no significant difference in the consumption of saccharin solution, nor a preference for either water or saccharin solution between the RFR-exposed and sham-exposed animals. Thus, no taste aversion developed. Perhaps, RFR does not produce an intensive sickness or the proper type of 'belongingless' for the conditioning to occur. However, in another study, Lovely and Guy [1975] reported that rats that were exposed to continuous-wave 918-MHz RFR for 10 min at >25 mW/cm^2 (SAR ~22.5 W/kg) and then allowed to drink saccharin solution, showed a significant reduction in saccharin consumption when tested 24 h later. No significant effect was found in rats exposed to RFR at 5 or 20 mW/cm^2.

In addition to using RFR as an aversive stimulus, it has also been used as a positive reinforcer. Marr et al. [1988] reported that rhesus monkeys could be trained to press a lever on a fixed ratio schedule to obtain 2 sec-pulses of RFR (6500 MHz, 50 mW/cm^2, estimated SAR 12 W/kg) when the monkeys were placed in a cold environment (0 ^{o}C).

A study by Bermant et al. [1979] investigated the thermal effect of RFR using the classical conditioning paradigm. They reported that after repeated pairing of a 30 sec tone with RFR (2450 MHz, 10 sec at SAR 420 W/kg or 30 sec at SAR 220 W/kg), the tone when presented

alone could elicit a conditioned hyperthermia from the rat. An effect which may be relevant to the finding of this experiment is that drug-induced changes in body temperature (hyperthermia or hypothermia) in animals can also be classically conditioned [Cunningham et al., 1984].

We have conducted experiments to investigate whether the effects of low-level RFR on psychoactive drug actions and central cholinergic activity can be classically conditioned to cues in the exposure environment. Classical conditioning of drug effects with environmental cues as the conditioned stimulus have been reported and such conditioned responses have been suggested to play a role in drug response, abuse, tolerance, and withdrawal [Le et al., 1979; Siegel, 1977, Siegel et al., 1982, Wikler, 1973a; Woods et al., 1969]. We found that the effects of RFR on amphetamine-induced hyperthermia and cholinergic activity in the brain can be classically conditioned to environmental cues [Lai et al., 1986b, 1987c].

In earlier experiments, we reported that acute (45 min) exposure to 2450-MHz RFR at average whole body SAR of 0.6 W/kg attenuated amphetamine-induced hyperthermia [Lai et al., 1983] and decreased HACU in the frontal cortex and hippocampus [Lai et al., 1987b] in the rat. In the conditioning experiments, rats were exposed to 2450-MHz pulsed RFR (2 μs pulses, 500 pps, 1.0 mW/cm^2, SAR 0.6 W/kg) in ten daily 45-min sessions. On day 11, animals were sham-exposed for 45 min and either amphetamine-induced hyperthermia or high-affinity choline uptake (HACU) in the frontal cortex and hippocampus was studied immediately after exposure. In this paradigm the RFR was the unconditioned stimulus and cues in the exposure environment were the neutral stimuli, which after repeated pairing with the unconditioned stimulus became the conditioned stimulus. Thus on the 11th day when the animals were sham-exposed, the conditioned stimulus (cues in the environment) alone would elicit a conditioned response in the animals. In the case of amphetamine-induced hyperthermia [Lai et al., 1986b], we observed a potentiation of the hyperthermia in the rats after the sham exposure. Thus, the conditioned response (potentiation) was opposite to the unconditioned response (attenuation) to RFR. This is known as 'paradoxical conditioning' and is seen in many instances of classical conditioning [cf. Mackintosh, 1974]. In addition, we found in the same experiment that, similar to the unconditioned response, the conditioned response could be blocked by the drug naloxone, implying the involvement of endogenous opioids. In the case of RFR-induced changes in cholinergic activity in the brain, we [Lai et al., 1987c] found that conditioned effects also occurred in the brain of the rat after the session of sham exposure on day 11. An increase in HACU in the hippocampus (paradoxical conditioning) and a decrease in the frontal cortex were observed. In addition, we found that the effect of RFR on hippocampal HACU habituated after 10 sessions of exposure, i.e., no significant change in HACU in the hippocampus was observed in animals exposed to the RFR on day 11. On the other hand, the effect of RFR on frontal cortical HACU did not habituate after the repeated exposure.

An explanation for the paradoxical conditioning phenomenon was given by Wikler [1973b] and Eikelboom and Stewart [1982]. The direction of the conditioned response (same as or opposite to the unconditioned response) depends on the site of action of the unconditioned stimulus, whether it is on the afferent or efferent side of the affected neural feedback system. Thus, in order to further understand the neural mechanisms of the conditioned effects, the site of action of RFR on the central nervous system has to be identified.

Little work has been done to investigate the effects of RFR on memory functions. We [Lai et al., 1989b] studied the effect of acute (20 or 45 min) RFR exposure (2450-MHz, 1 mW/cm^2, SAR 0.6W/kg) on the rats' performance in a radial-arm maze, which measures spatial learning and memory functions. The maze consists of a central circular hub with arms radiating out like

the spokes of a wheel. In this task, food-deprived animals are trained to explore the arms of the maze to obtain food reinforcement at the end of each arm. In each session they have to enter each arm once and a reentry is considered as an error. This task requires the so called 'working memory', i.e., the rat has to remember the arms it has already entered during the course of a session. Working memory requires the functions of the cholinergic innervations in the frontal cortex and hippocampus [Dekker et al., 1991; Levin, 1988]. Both have been shown to be affected by acute RFR exposure [Lai et al., 1987b]. We [Lai et al., 1989b] found that acute (45 min) exposure to RFR before each session of maze running significantly retarded the rats' abilities to perform in the maze. They made significantly more errors than the sham-exposed rats. This result agrees with the neurochemical finding that 45 min of RFR exposure decreased the activity of the cholinergic systems in the frontal cortex and hippocampus of the rats [Lai et al., 1987b]. However, 20 min of RFR exposure, which increased cholinergic activity in the brain, did not significantly affect maze performance. Apparently, increase in cholinergic activity cannot further improve the performance, since the neural systems involved in the memory function may be working at optimal levels under normal conditions. In a recent experiment [Lai et al., 1993], we have shown that the microwave-induced working memory deficit in the radial-arm maze was reversed by pretreating the rats before exposure with the cholinergic agonist physostigmine or the opiate antagonist naltrexone, whereas pretreatment with the peripheral opiate antagonist naloxone methiodide showed no reversal of effect. These data indicate that both cholinergic and endogenous opioid neurotransmitter sysatems inside the central nervous system are involved in the microwave-induced spatial memory deficit.

Several studies have investigated the effect of RFR on discrimination learning and responding. Hunt et al. [1975] trained rats to bar press for saccharin water rewards in the presence (5 sec duration) of a flashing light and not to respond in the presence of a tone (unrewarded). After 30 min of exposure to 2450-MHz RFR, modulated at 20 Hz and at SAR of 6.5 or 11.0 W/kg, rats made more misses at the presence of the light, but there were no significant changes in the incidences of bar-pressing errors when the tone was on. The effect was more prominent at the higher dose rate. Galloway [1975] trained rhesus monkeys on two behavioral tasks to obtain food reward. One was a discrimination task in which the monkey had to respond appropriately depending on which of the two stimuli was presented. The other task was a repeated acquisition task in which a new sequence of responses had to be learned everyday. After training, the animals were irradiated with continuous-wave 2450-MHz RFR applied to the head prior to each subsequent behavioral session. The integral dose rates varied from 5-25 W. Some of these dose rates caused convulsions in the monkeys. The radiation was shown to exert no significant effect on the discrimination task, whereas a dose-dependent deficit in performance was observed in the repeated acquisition task. Cunitz et al., [1979] trained two rhesus monkeys to move a lever in different directions depending on the lighting conditions in the exposure cage in order to obtain food reinforcement on a fixed ratio schedule. After the animals' performance had reached a steady and consistent level, they were irradiated at the head with continuous-wave 383-MHz RFR at different intensities in subsequent sessions. Radiation started 60 min before and during a session of responding. The authors reported a decrease in the rate of correct responding when the SAR at the head reached 22-23 W/kg. In another study, Scholl and Allen [1979] exposed rhesus monkeys to continuous-wave 1200-MHz RFR at SARs of 0.8-1.6 W/kg and observed no significant effect of the radiation on a visual tracking task.

de Lorge [1976] trained rhesus moneys on an auditory vigilance (observing-response) task. The task required continuous sensory-motor activities in which the monkeys had to coordinate

their motor responses according to the stimulus cues presented. In the task the monkeys had to press the right lever that produced either a 1070-Hz tone for 0.5 sec or a 2740-Hz tone. The 1070-Hz tone signalled an unrewarded situation. Pressing a left lever when the 2740-Hz tone was on would produce a food reward. Presentation of the higher frequency tone was on a variable interval schedule. After the monkeys had learned to perform the task at a steady level, they were irradiated with 2450-MHz RFR of different intensities. Decreased performance and increased latency time in pressing the left lever were observed when the power density at the head was at 72 mW/cm^2. The deficits could be due to an increase in colonic temperature after exposure to the high intensity RFR.

de Lorge [1979] trained squirrel monkeys to respond to another observing-response task using visual cues. After learning the task, the animals were exposed to 2450-MHz RFR (sinusoidally modulated at 120 Hz) for 30 or 60 min at different power densities (10-75 mW/cm^2) in subsequent sessions. Their performances were disrupted at power densities >50 mW/cm^2. The disruption was power density-dependent and occurred when the rectal temperatures increased more than 1 ^oC. In a more recent experiment, de Lorge [1984] studied rhesus monkeys trained on the auditory vigilance task and the effects of exposure to RFRs of different frequencies (225, 1300, and 5800 MHz). Reduction in performance was observed at different power density thresholds for the frequencies studied: 8.1 mW/cm^2 (SAR 3.2 W/kg) for 225 MHz, 57 mW/cm^2 (SAR 7.4 W/kg) for 1300 MHz, and 140 mW/cm^2 (SAR 4.3 W/kg) for 5800 MHz. de Lorge concluded that the behavioral disruption under different frequencies of exposure was more correlated with change in body temperature. Disruption occurred when the colonic temperature of the animal had increased by 1 ^oC.

Many studies have investigated the effects of RFR on reinforcement schedule-controlled behavior. Sanza and de Lorge [1977] trained rats on a fixed interval schedule for food pellets. After 60 min of exposure to 2450-MHz RFR (modulated at 120 Hz) at 37.5 mW/cm^2, a decrease in response with an abrupt onset was observed. This effect was more pronounced in rats with a high base line of response rate on the fixed interval schedule. No significant effect on response was observed at power densities of 8.8 and 18.4 mW/cm^2.

D'Andrea et al. [1976] trained rats to bar-press for food at a variable interval schedule. After a constant responding rate was attained, the animals were irradiated with continuous- wave RFRs of 360, 480, or 500 MHz. Bar-press rates were decreased only when the rats were exposed to the 500-MHz radiation at a SAR of approximately 10 W/kg. The animals also showed significant signs of heat stress. In a subsequent study [D'Andrea et al., 1977] RFRs of different frequencies and intensities were studied on their effect on bar-pressing rate on a variable interval schedule. It was found that the latency time of stoppage to respond after the radiation was turned on correlated with the rate of rise in body temperature of the animal. These experiments definitely demonstrated the thermal effect of RFR on operant behavior.

Gage [1979a] trained rats on a variable interval schedule for food reinforcement. Different groups of rats were exposed overnight (15 h) to continuous-wave 2450-MHz RFR at either 5, 10, or 15 mW/cm^2. Responses were tested immediately after exposure. No significant difference in performance was found between the RFR- and sham-exposed rats when exposure was done at an ambient temperature of 22 ^oC. However, a power density- dependent reduction in response rate and increase in response duration was found in the RFR-exposed rats when the irradiation was carried out at 28 ^oC. At the higher ambient temperature, heat dissipation from the body was less efficient and the exposed rats had higher body temperatures postexposure.

Lebovitz [1980] also studied the effects of pulsed 1300-MHz (1 µs pulses, 600 pps) RFR on rats bar-pressing on a fixed interval schedule for food reinforcement. Both food reinforced bar presses and unrewarded bar presses during the intervals were studied. No significant effect was detected in both types of response at SAR of 1.5 W/kg. However, at 6 W/kg, there was a slight reduction in rewarded bar presses and a large reduction in unrewarded bar presses. The authors concluded that the unrewarded behavior was more susceptible to the effect of RFR than the rewarded behavior. Another related experiment was reported by Sagan and Medici [1979] in which water-deprived chicks were given access to water on fixed intervals irrespective of their responses. During the time between water presentations the chicks showed an increase in motor activity known as 'interim behavior'. Exposure to 450-MHz RFR amplitude-modulated at 3 and 16 Hz at power densities of either 1 or 5 mW/cm^2 during session had no significant effect on the 'interim behavior'.

Effects of RFR on complex operant response sequence and reinforcement schedules were studied in various experiments. de Lorge and Ezell [1980] tested rats on a vigilance behavioral task during exposure to pulsed 5620-MHz RFR and then to pulsed 1280-MHz RFR. In this task, rats had to discriminate two tones in order to press one of two bars appropriately for food reinforcement. Behavioral decrement was observed at an SAR of 2.5 W/kg with the 1280-MHz radiation, but at 4.9 W/kg with the 5620-MHz radiation. Gage [1979b] trained rats to alternate responses between 2 levers at 11-30 times for a food reinforcement. Decrement in response rates was observed after 15 h of exposure to continuous-wave 2450-MHz RFR at 10, 15, and 20 mW/cm^2 (0.3 W/kg per mW/cm^2).

Thomas et al. [1975] trained rats to bar press on two bars: a fixed ratio of 20 on the right bar (20 bar presses produced a food pellet reward) and differential reinforcement of low rate (DRL) on the left bar (bar presses had to be separated by at least 18 sec and no more than 24 sec to produce a reward). There was a time-out period between schedules, i.e., no reinforcement available for responding. Animals were tested 5-10 min after 30 min of exposure to either continuous-wave 2450-MHz, pulsed 2860-MHz (1 µs pulses, 500 pps) or pulsed 9600-MHz (1 µs pulses, 500 pps) RFR at various power densities. An increase in DRL response rate was observed with 2450-MHz radiation >7.5 mW/cm^2 (SAR 2.0 W/kg), 2860-MHz RFR >10 mW/cm^2 (2.7 W/kg), and 9600-MHz RFR >5 mW/cm^2 (SAR 1.5 W/kg). A decrease in the rate of response at the fixed ratio schedule was seen in all three frequencies when the power density was greater than 5 mW/cm^2. In addition, an increase in response rate was observed during time-out periods under irradiation of the three frequencies of RFR at greater than 5 mW/cm^2.

In another study, Thomas et al. [1976] trained rats to bar press on a tandem schedule using 2 bars. Pressing the right bar for at least 8 times before pressing the left bar would give a food pellet reward. A power density-dependent decrease in the percentage of making 8 or more consecutive responses on the right bar before pressing the left bar was observed in the animals after 30 min of exposure to pulsed 2450-MHz RFR (1 µs pulses, 500 pps) at power densities of 5, 10, and 15 mW/cm^2.

Schrot et al [1980] also trained rats to learn a new daily sequence of pressing of three bars for food reinforcement. An increased number of errors and decreased learning rates were observed in the animals after 30 min of exposure to pulsed 2800-MHz RFR (2 µs pulses, 500 pps) at average power densities of 5 and 10 mW/cm^2 (SARs 0.7 and 1.7 W/kg, respectively). No significant effect on performance was observed at power densities of 0.25, 0.5, and 1 mW/cm^2.

Several studies investigated the effects of chronic RFR exposure on schedule controlled-behavior. Mitchell et al. [1977] trained rats to respond on a mixed schedule of reinforcement

(FR-5 EXT-15 sec), in which 5 responses would give a reward and then a 15 sec lapse time (extinction period) was required before a new response would be rewarded. In addition, the schedule of reinforcement was effective when a lamp was on, while no reinforcement was given when the lamp was off. Rats were then exposed to 2450-MHz RFR (average SAR 2.3 W/kg) for 22 weeks (5 h/day, 5 days/week) and tested at different times during the exposure period. The RFR-exposed rats showed higher responses during the extinction period, indicating poorer discrimination of the response cues. In another also pretrained task, rats had to press a bar to postpone the onset of unsignalled electric foot-shocks (unsignalled avoidance paradigm). No significant difference in performance of this task was observed between the RFR- and sham-exposed animals.

Two series of well-designed experiments were run by D'Andrea et al. [1986a,b] to investigate the effects of chronic RFR exposure on behavior. In one experiment, rats were exposed for 14 weeks (7 h/day, 7 days/week) to continuous-wave 2450-MHz RFR at 2.5 mW/cm^2 (SAR 0.7 W/kg). Decrease in the threshold of electric foot shock detection (i.e., increase in sensitivity) was observed in the irradiated rats during the exposure period. Increased open-field exploratory behavior was observed in the rats at 30 days postexposure. After exposure, the rats were trained to bar press on an interresponse time criterion (IRT). In this schedule, the animals had to respond within 12 to 18 sec after the previous response in order to receive a food reward. Radiofrequency radiation exposed rats emitted more responses during the training period. When the training was completed, the RFR-exposed rats had lower efficiency in bar-pressing to obtain food pellets, i.e., they made more inappropriate responses and received fewer food pellets than the sham-exposed rats during a session. In a signalled two-way active avoidance shuttlebox test, the RFR-exposed rats showed less avoidance response than the sham-exposed rats during training; however, no significant difference in responses in the shuttlebox test was detected at 60 days after exposure between the RFR- and sham-exposed animals. In another series of experiments, rats were exposed to 2450-MHz RFR at 0.5 mW/cm^2 (SAR 0.14 W/kg) for 90 days (7 h/day, 7 days/week). Open-field behavior, shuttlebox performance, and IRT schedule-controlled bar-pressing behavior for food pellets were studied at the end of the exposure period. A small deficit in shuttlebox performance and increased rate of bar-pressing were observed in the RFR exposed rats. Summarizing the data from these two series of experiments [D'Andrea et al., 1986a,b], D'Andrea and his co-workers concluded that the threshold for the behavioral and physiological effects of chronic RFR exposure in the rats studied in their experiments occurred between the power densities of 0.5 mW/cm^2 (SAR 0.14 W/kg) and 2.5 mW/cm^2 (SAR 0.7 W/kg).

D'Andrea et al. [1989] recently studied the behavioral effects of high peak power RFR pulses of 1360-MHz. Rhesus monkeys performing on a complicated reinforcement-schedule involving time-related behavioral tasks (inter-response time, time discrimination, and fixed interval responses) were exposed to high peak power RFR (131.8 W/cm^2 rms, pulse repetition rate 2-32 Hz). No significant disturbance in performance was observed in the monkeys.

Akyel et al. [1991] also studied the effects of exposure to high peak power RFR pulses on behavior. In their experiment, rats pretrained to bar-press for food reinforcement on either fixed ratio, variable interval, or DRL schedule were exposed for 10 min to 1250-MHz pulses. Each pulse (10 μs width) generated a whole body specific absorption of 2.1 J/kg, which corresponds to a whole body average SAR of 0.21 mW/kg. The pulse rate was adjusted to produce different total doses (0.5-14 kJ/kg). Only at the highest dose (14 kJ/kg), stoppage of responding was observed after exposure, when the colonic temperature was increased by ~2.5 $^\circ$C. Responding

resumed when colonic temperature returned to within 1.1 °C above the preexposure level. When responding resumed, the response rates on the fixed ratio and variable interval schedules were below the preexposure base line level. Responses on the DRL schedule were too variable to allow a conclusion to be drawn. The authors concluded that the effect of the high peak power RFR pulses on schedule-controlled behavior was due to hyperthermia.

Behavior conditioning using different reinforcement schedules generates stable base line responses with reproducible patterns and rates. The behavior can be maintained over a long period of time (hrs) and across different experimental sessions. Thus, schedule-controlled behavior provides a powerful means for the study of RFR-behavior interaction in animals. On the other hand, the behavior involves complex stimulus-response interactions. It is difficult to conclude from the effects of RFR on schedule-controlled behavior the underlying neural mechanisms involved.

In a sense, these studies of RFR are similar to those of psychoactive drugs. A large volume of literature is available on the latter topic. A review of the literature on the effects of psychoactive drugs on schedule-controlled behavior reveals the complexity of the interaction and the limitation in data interpretation. In general, the effects of psychoactive drugs on schedule-controlled behavior is dose-dependent. In many cases, especially in behavior maintained by positive reinforcement, an inverted-U-function has been reported, i.e., the behavior is increased at low doses and decreased at high doses of the drug. In addition, the way that a certain drug affects schedule-controlled behavior depends on three main factors: (a) the base line level and pattern of responding of the animal: a general rule is that drugs tend to decrease the rate when the base line responding rate is high and vice versa. This is known as rate-dependency and is true with psychomotor stimulants, major and minor tranquilizers, sedative-hypnotics, and narcotics; (b) the schedule of reinforcement: in addition to its effect on the base line responding rate, a reinforcement schedule can have other specific effects on responses. For example, amphetamine has different effects on responses maintained on DRL schedule and punishment-suppressed responding schedule, even though both schedules generate a similar low response rate; and (c) the stimulus-control involved in the study: e.g., responses maintained by electric shock are more resistant to drug effects than responses maintained by positive reinforcers. On the other hand, some drugs have differential effects on signalled-avoidance versus continuous avoidance responding.

Thus, to fully understand the effect of RFR, the parameters of the radiation (different dose rates, frequency, duration of exposure, etc.), different reinforcement-schedules, and conditioning procedures have to be carefully studied and considered. However, there is evidence that the above determining factors on schedule-controlled behavior may also hold in the case of RFR. Exposure to RFR caused a decrease in response rate when a variable interval schedule that produces a steady rate of responding was used [D'Andrea et al., 1976; 1977; Gage, 1979a], and an increase in responding when the DRL-schedule of reinforcement was used [Thomas et al., 1975]. This may reflect the rate-dependency effect. On the other hand, stimulus control as a determinant of response outcome was seen in the study of Lebovitz [1980] when unrewarded responses were disrupted more by RFR than rewarded responses, and the study of Hunt et al. [1975] that showed the reverse relationship. In the former experiment a fixed interval schedule was used, whereas in the latter a discrimination paradigm was studied.

Another related point is that most psychoactive drugs affect body temperature. Stimulants cause hyperthermia, barbiturates cause hypothermia, and narcotics have a biphasic effect on body temperature (hyperthermia at low doses and hypothermia at high doses). It is not

uncommon to observe a change of 2-3 ^{O}C within 30 min after a drug is administered. However, in reviewing the literature, there is no general correlation between the effects of the drugs on body temperature and schedule-controlled behavior. Thus, body temperature may not be an important factor in an animal's responding under schedule-controlled behavior, at least in the case of psychoactive drugs. On the contrary, some of the experiments described above strongly suggest the role of hyperthermia on the RFR effect on the behavior. Perhaps, a sudden and large increase in body temperature as in the case of RFR can have a major effect on responding.

Generally speaking, when effects were observed, RFR disrupted operant behavior in animals such as in the cases of discrimination responding [de Lorge and Ezell, 1980; Hunt et al., 1975; Mitchell et al., 1977], learning [Lai, 1989b; Schrot et al., 1980], and avoidance [D'Andrea et al., 1986a,b]. This is especially true when the task involved complex schedules and response sequence. In no case has an improvement in operant behavior been reported after RFR exposure. It is interesting that only disruptions in behavior by RFR exposure are reported. In the studies on EEG, both excitation (desynchronization) and depression (synchronization) have been reported after exposure to RFR [Bawin et al., 1979; Chizhenkova, 1988; Chou et al., 1982b; Dumansky and Shandala, 1976; Goldstein and Sisko, 1974; Dumansky and Shandala, 1976; Takeshima et al., 1979]. Motor activity has also been reported to increase [D'Andrea et al., 1979, 1980; Hunt et al., 1975; Mitchell et al., 1977; Rudnev et al., 1978] and decrease [Johnson et al., 1983; Mitchell et al., 1988; Moe et al., 1976; Rudnev et al., 1978] after RFR exposure. If these measurements can be considered as indications of electrophysiological and behavioral arousal and depression, improvement in operant behavior should occur under certain conditions of RFR exposure. This is especially true with avoidance behavior. Psychomotor stimulants that cause EEG desynchronization and motor activation improve avoidance behavior, whereas tranquilizers that have opposite effects on EEG and motor activity decrease avoidance behavior.

GENERAL DISCUSSION

After reviewing the studies on the effects of RFR on the central nervous system, one obvious question comes to my mind: "What is the mechanism responsible for the effects reported?" In most cases, especially the in vivo studies in which high intensities of irradiation were used resulting in an increase in body temperature, thermal effect is most likely the answer. Even in cases when no significant change in body temperature was detected, thermal effect cannot be excluded. An animal can maintain its body temperature by actively dissipating the heat load from the radiation. Activation of thermoregulatory mechanisms can lead to neuro-chemical, physiological, and behavioral changes. Temperature can be better controlled during in vitro studies. Uneven heating of the sample can still generate temperature gradients, which may affect the normal responses of the specimen studied. However, several points raised by some experiments suggest that the answer is not a simple one. They are: (a) 'Heating controls' do not produce the same effect of RFR [D'Inzeo et al., 1988; Seaman and Wachtel, 1978; Synder, 1971; Johnson and Guy, 1971; Wachtel et al., 1975]; (b) Window effects are reported [Bawin et al., 1975, 1979; Blackman et al., 1979, 1980a,b, 1989; Chang et al., 1982; Dutta et al., 1984, 1989, 1992; Lin-Liu and Adey, l982; Oscar and Hawkins, 1977; Sheppard et al., 1979]; (c) Modulated or pulsed RFR is more effective in causing an effect or elicits a different effect when compared with continuous-wave radiation of the same frequency [Arber and Lin, 1985; Baranski, 1972; Frey et al., 1973, 1975; Oscar and Hawkins, 1977; Sanders et al., 1983]; (d) Different

frequencies of RFR produce different effects [D'Andrea et al., 1979, 1985; de Lorge and Ezell, 1980; Sanders et al., 1984; Thomas et al., 1975]; and (e) Different exposure orientations or systems of exposure produce different effects at the same average whole body SAR [Lai et al., 1984a, 1988].

I think most of these effects can be explained by the following factors:

1. The physical properties of RFR absorption in the body and the mechanisms by which RFR affects biological functions were not fully understood. In addition, use of different exposure conditions make it difficult to compare the results from different experiments.

2. Characteristics of the response system, i.e., the dependent variable, were not fully understood. In many cases, the underlying mechanism of the response system studied was not known.

3. Dose-response relationship was not established in many instances and conclusions were drawn from a single RFR intensity or exposure duration.

It is well known that the distribution of RFR in an exposed object depends on many factors such as frequency, orientation of exposure, dielectric constant of the tissue, etc. D'Andrea et al. [1987] and McRee and Davis [1984] pointed out the uneven distribution of energy absorbed in the body of an exposed animal with the existence of 'hot spots'. In experiments studying the central nervous system, Williams et al. [1984d] also reported a temperature gradient in the brain of rats exposed to RFR. Structures located in the center of the brain, such as the hypothalamus and medulla, had higher temperatures than peripheral locations, such as the cerebral cortex. In a study by Chou et al. [1985a], comparisons were made of the local SARs in eight brain sites of rats exposed under seven exposure conditions, including exposure in a circular waveguide with the head or tail of an animal facing the radiation source, near field and far field exposures with either E- or H-field parallel to the long-axis of the body, and dorsal exposure in a miniature anechoic chamber with E- or H-field parallel to the long axis of the body. Statistical analysis of the data showed that a) there was a significant difference in local SARs in the eight brain regions measured under each exposure condition, and b) the pattern of energy absorption in different regions of the brain depended on the exposure condition. However, it must be pointed out that in another study [Ward et al., 1986], no temperature 'hot spots' were detected in the brains of rat carcasses and anesthetized rats after irradiation with 2450-MHz RFR. Temperature increases in various regions of the brain were found to be uniform and dependent on the power density of the radiation.

A question that one might ask is whether different absorption patterns in the brain or body could elicit different biological responses in the animal. If this is positive, possible outcomes from the study of bioelectromagnetics research are: (1) a response will be elicited by some exposure conditions and not by others, and (2) different response patterns are elicited by different exposure conditions, even though the average dose rates in the conditions are equal. We [Lai et al., 1984a] reported a difference in responses to the hypothermic effects of pentobarbital depending on whether the rat was exposed with its head facing toward or away from the source of radiation in the waveguide with the average whole body SAR under both conditions remaining the same; however, the patterns of energy absorption in the body and the brain differed in the two exposure conditions. Studies of HACU activity in the different regions of the brain [Lai et al., 1988] also showed that different responses could be triggered using different exposure systems or different waveforms of RFR (continuous-wave or pulsed) with the average whole body SAR held constant under each exposure condition. These data indicate that the energy distribution in the body and other properties of the radiation can be important factors in determining the

outcome of the biological effects of RFR. A series of studies by Frei et al. [1989a,b] also demonstrated some interesting results on this issue. The effects of high intensity 2450- and 2800-MHz RFRs on heart rate, blood pressure, and respiratory rate in ketamine-anesthetized rats were studied. Both frequencies produced increases in heart rate and blood pressure and no significant difference was observed whether continuous-wave or pulsed radiation was used. A difference was observed, however, when the animals were exposed with their bodies parallel to the H- or E-field. In the case of 2450-MHz RFR, the E-orientation exposure produced greater increases in heart rate and blood pressure than the H-orientation exposure; whereas no significant difference in the effects between the two exposure orientations was observed with the 2800-MHz radiation. The authors speculated that the differences could be attributed to the higher subcutaneous temperature and faster rise in colonic temperature in the E-orientation when the rats were exposed at 2450 MHz than at 2800 MHz. Once again, this points out that subtle differences in exposure parameters could lead to different responses. Therefore, due to the peculiar pattern of energy deposition and heating by RFR, it may be impossible to replicate the thermal effect of RFR by general heating, i.e., use of temperature controls.

The fact that dosimetry data were based on stationary models that usually show discrete patterns of energy absorption, further complicate the matter. In animal studies, unless the animal is restrained, the energy absorption pattern changes during the exposure period depending on the position and the orientation of the animal. A possible solution would be to perform long-term exposure experiments, thus, the absorption pattern on the average would be made more uniform.

Another important consideration regarding the biological effects of RFR is the duration or number of exposure episodes. This is demonstrated by the results of some of the studies on the neurological effects of RFR. Depending on the responses studied in the experiments, several outcomes could result: an effect was observed only after prolonged (or repeated) exposure, but not after acute exposure [Baranski, 1972; Baranski and Edelwejn, 1968, 1974; Mitchell et al., 1977; Takashima et al., 1979], an effect disappeared after prolonged exposure suggesting habituation [Johnson et al., 1983; Lai et al., 1987c, 1992a], and different effects were observed after different durations of exposure [Baranski, 1972; Dumanski and Shandala, 1974; Grin, 1974; Lai et al., 1989a, 1989b; Servantie et al., 1974; Snyder, 1971]. All of these different responses reported can be explained as being due to the different characteristics of the dependent variable studied. An interesting question related to this is whether or not intensity and duration of exposure interact, e.g., can exposure to a low intensity over a long duration produce the same effect as exposure to a high intensity radiation for a shorter period?

Thus, even though the pattern or duration of RFR exposure is well-defined, the response of the biological system studied will still be unpredictable if we lack sufficient knowledge of the response system. In most experiments on the neurological effects of RFR, the underlying mechanism of the dependent variable was not fully understood. The purpose of most of the studies was to identify and characterize possible effects of RFR rather than the underlying mechanisms responsible for the effects. This lack of knowledge of the response system studied is not uncommon in biological research. In this regard, it may be appropriate to compare the biological and neurological effects of RFR with those of ethanol. Both entities exert non-specific effects on multiple organs in the body. Their effects are nonspecific, because both ethanol and RFR are not acting on specific receptors. The biological effects of ethanol could be a general action on cell membrane fluidity.

In reviewing the literature on the neurological effects of ethanol, one notices some similarity with those of RFR. In both cases, a wide variety of neurological processes were

reported to be affected after exposure, but without a known mechanism. On the other hand, inconsistent data were commonly found. For example, in the case of the effects of ethanol on dopamine receptors in the brain, an increase [Hruska, 1988; Lai et al., 1980], a decrease [Lucchi et al., 1988; Syvalahti et al., 1988], and no significant change [Muller, 1980; Tabakoff and Hoffman, 1979] in receptor concentration have been reported by different investigators. Such inconsistencies have existed since the late 70's and there has been no satisfactory explanation for them. Similar research findings of increase, decrease, and no significant change in the concentration of muscarinic cholinergic receptors in the cerebral cortex of animals treated with ethanol have also been reported in the literature [Kuriyama and Ohkuma, 1990]. Dosage and route of ethanol treatment, the frequency of administration, and the species of animal studied, etc., could all attribute to variations in the findings [Keane and Leonard, 1989]. As we have discussed earlier, such considerations on the parameters of treatment also apply to the study of the biological effects of RFR. These are further complicated by the special properties of the radiation, such as waveform and modulation. In addition, RFR effects could have rapid onset and offset when the source was turned on and off, whereas the biological effect of ethanol depends on the rates of absorption and metabolism.

Thus, an understanding of the response characteristics of the dependent variables to different parameters of RFR, such as power density, frequency, waveform, etc., is important. Lack of knowledge about such characteristics may explain some of the discrepancies in bioelectromagnetics research results in the literature. Non-linear response characteristics are frequently observed in biological systems, because different mechanisms are involved in producing a response. For example, in the case of apomorphine-induced locomotor activity, a low dose of apomorphine (e.g., 0.1 mg/kg) decreases locomotor activity, whereas a higher dosage (e.g., 1.0 mg/kg) of the drug causes a profound enhancement. A dose in between may cause an insignificant effect. An explanation for this phenomenon is that a low dose of apomorphine activates selectively presynaptic dopamine receptors in the brain, which decreases dopamine release from its terminals and, thus, a decrease in motor activity. At a high dose, apomorphine stimulates the postsynaptic dopamine receptors, leading to an increase in motor activity.

Another common response-characteristic is the inverted-U function. In this situation, a response is only seen at a certain dose range and not at higher or lower dosages. An example of an inverted-U dose-response function is the effect of benzodiazepines on schedule controlled operant behavior. There is not a good explanation for the occurrence of this function. One possible explanation might be that at least two mechanisms, a facilitatory and an inhibitory function, are involved in the response. At a lower dose range of the drug, for example, the facilitatory mechanism predominates and leads to enhancement of the response, whereas, as the dosage increases an inhibitory mechanism is activated, leading to a decline in response. Thus, it is essential that the dose-response function be determined.

The inverted-U response-characteristic can be the basis of some of the 'window' effects reported in bioelectromagnetics research. Thus, with the above considerations, it is not surprising that RFR can cause enhancement, decrement, and no significant effect on a particular response depending upon the exposure conditions. Blackman et al. [1991] stated on the effect of temperature on calcium ion efflux from brain tissue that, "... either outcome (*inhibition or enhancement in release of calcium ions*), or a null result, is possible, depending on the temperature of tissue sample before and during exposure". However, it must be pointed out that

the inverted-U function is not sufficient to account for the 'multiple window' effect reported in one of Blackman's studies [Blackman et al., 1989].

Another important consideration in the study of the central nervous system should be mentioned here. It is well known that the functions of the central nervous system can be affected by activity in the peripheral nervous system. Thirty years ago, McAfee [1961, 1963] pointed out that the thermal effect of RFR on the peripheral nervous system can lead to changes in central nervous system functions and behavior in the exposed animal. This is especially important in the in vivo experiments when the whole body is exposed. However, in most experiments studying the effects of RFR on the central nervous system, the possibility of contribution from the peripheral nervous system was not excluded in the experimental design. Therefore, caution should be taken in concluding that a neurological effect resulted solely from the action of RFR on the central nervous system.

An interesting question arose, whether or not RFR could produce 'non-thermal' biological effects. Many have speculated whether RFR can directly affect the activity of excitable tissues. Schwan [1971, 1977] pointed out that it would take a very high intensity of RFR to directly affect the electrical activity of a cell. On the other hand, Wachtel et al. [1975] have speculated that an RFR-induced polarized current in the membrane of a neuron could lead to changes in activity. Adey [1988] has suggested that cooperative processes in the cell membrane might be reactive to the low energy of oscillating electromagnetic field, leading to a change in membrane potential. Pickard and Barsoum [1988] recorded from cells of the Characeae plant exposed to 0.1-5 MHz pulsed RFR and observed a slow and fast component of change in membrane potential. The slow component was temperature dependent and the fast component was suggested to be produced by rectification of the oscillating electric field induced by RFR on the cell membrane. However, the effect disappeared when the frequency of radiation reached ~10 MHz.

An extreme example of the direct interaction of electromagnetic radiation with a specific biological molecule triggering a neurological effect is the rhodopsin molecules in the rod photoreceper cells that transduce light energy into neural signals. In 1943, a psychophysical experiment by Hecht et al. [1942] suggested that a single photon could activate a rod cell. The molecular biology of rhodopsin is now well understood. It is now known that a single photon can activate a single molecule of rhodopsin. A photon of the visible spectrum turns 11-cis retinol, a moiety of the rhodopsin molecule, to an all-trans form. This triggers a cascade of molecular activities involving specific G-protein, the conversion of cyclic-GMP to 5'-GMP, and eventually closing the sodium-ion channels on the cell membrane of the rod cell. This cascade action leads to a powerful amplification of the photon signal. It was estimated that one photon can affect several hundred C-GMP molecules. Such change is enough to hyperpolarize a rod cell and lead to signal transmission through its synapse [Liebman et al., 1987; Stryer, 1987]. Can a similar molecular sensitive to RFR exist? The problem is that RFR energy is several orders of magnitude (~10^6) lower than that of a photon at the visual spectrum. It is difficult to visualize a similar molecular mechanism sensitive enough to detect RFR.

Another consideration is that the ambient level of RFR is very low in the natural environment and could not have generated enough selection pressure for the evolutionary development of such a molecular mechanism. On the other hand, there may be some reason for the development of a molecular mechanism for the detection of static or low frequency electric or magnetic fields. An example is the electroreception mechanism of two Australian monotremes, the platypus, *Ornithorhynchus anatinus*, and the echidna, *Tachyglossus aculeatus* [Gregory et al.,

1989a,b; Iggo et al., 1992; Scheich et al., 1986]. Apparently, receptors sensitive to low-level electric fields exist in the snout and bill of these animals, respectively. Electrophysiological recordings from the platypus show that receptors in the bill can be sensitive to a static or sinusoidally changing (12-300 Hz) electric field of 4-20 mV/cm, and cells in the cerebral cortex can respond to a threshold field of 300 µV/cm. Moreover, behavioral experiments showed that the platypus can detect electric fields as small as 50 µV/cm. In the echidna snout, receptors can respond to fields of 1.8-73 mV/cm. These neural mechanisms enable the animals to detect muscular movements of their prey, termites and shrimps. It would be interesting to understand the transduction mechanism in the electroreceptors in these animals. However, it remains to be seen whether RFR can generate a static or ELF field in tissue and that a similar electroreceptor mechanism exists in other mammals.

Another possible explanation suggested for the neurological effects of RFR is stress. This hypothesis has been proposed by Justesen et al. [1973] and Lu et al. [1980] and based on high intensity of exposure. We have also proposed recently that low-level RFR may be a 'stressor' [Lai et al., 1987a]. Our speculation is based on the similarity of the neurological effects of known stressors (e.g., body-restraint, extreme ambient temperature) and those of RFR (see Table 1 in Lai et al., 1987a). Our recent experiments suggesting that low-level RFR activates both endogenous opioids and corticotropin-releasing factor in the brain further support this hypothesis. Both neurochemicals are known to play important roles in an animal's responses to stressors [Amir et al., 1980; Fisher, 1989]. However, it is difficult to prove that an entity is a stressor, since the criteria of stress are not well defined and the caveat of stress is so generalized that it has little predictive power on an animal's response.

In conclusion, I believe the questions on the biological effects of RFR and the discrepancies in research results in the literature can be resolved by (a) a careful and thorough examination of the effects of the different radiation parameters, and (b) a better understanding of the underlying mechanisms involved in the responses studied. With these considerations, it is very unlikely that the neurological effects of RFR can be accounted for by a single unifying neural mechanism.

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Appendix 9-B -   **Memory and Behavior**

Presention: The Biological Effects, Health
Consequences and Standards for Pulsed Radiofrequency Field.
International Commission on Nonionizing Radiation
Protection and the World Health Organization, Ettoll
Majorare, Centre for Scientific Culture, Italy, 1999.

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The nervous system is very sensitive to environmental disturbance. In the proceedings of an international symposium on the "Biological Effects and Health Hazard of Microwave Radiation" hold in Warsaw, Poland in 1973, it was stated in a summary section that 'the reaction of the central nervous system to microwaves may serve as an early indicator of disturbances in regulatory functions of many systems' [Czerski et al., 1974].

Disturbance to the nervous system leads to behavioral changes. On the other hand, alteration in behavior would imply a change in function of the nervous system. Studies on the effect of radiofrequency radiation (RFR) on behavior have been carried out since the beginning of Bioelectromagnetics research. Some of these studies are briefly reviewed below.

It has been speculated that a pulsed RFR is more potent than its continuous-wave (CW) counterpart in causing biological effects [e.g., Barenski, 1972; Frey et al., 1975; Oscar and Hawkins, 1977]. To evaluate this, it is necessary to compare the effects of pulsed RFR with those of CW radiation. Thus, studies on both CW and pulsed (and frequency-modulated) RFRs are included in this review. Comparing the effects of CW and pulsed RFR can actually be related to the popular debate on the distinction between 'thermal' and 'non-thermal/athermal' effect. If an effect is elicited by a pulsed RFR but not by a CW RFR of the same frequency and intensity under the same exposure conditions, it may imply the existence of 'non-thermal/athermal' effect.

Behavior is generally divided into two main categories: spontaneous and learned. Effects of RFR exposure on both types of behavior have been investigated.

Spontaneous Behavior

Spontaneous behaviors are generally considered to be more resistant to disturbance. The most well studied spontaneous behavior in Bioelectromagnetics research is motor (locomotor) activity. Change in motor activity is generally regarded as an indication of change in the arousal state of an animal.

Hunt et al. [1975] reported decreased motor activity in rats after 30 min of exposure to pulsed 2450-MHz RFR (2.5 msec pulses, 120 pps, SAR 6.3 W·kg^{-1}). Mitchell et al. [1988] also

observed a decrease in motor activity in rats after 7 hr of exposure to CW 2450-MHz RFR (10 mW·cm^{-2}, average SAR 2.7 W·kg^{-1}).

Roberti [1975] reported no significant change in locomotor activity in rats after long-term (185-408 h) exposure to RFR of different frequencies (10.7-GHz CW; 3-GHz CW; 3-GHz with 1.3 ms pulses and 770 pps) and various intensities (SAR 0.15-7.5 W·kg^{-1}). Mitchell et al. [1977] reported an increase in motor activity on a small platform of rats exposed to 2450-MHz RFR (CW, average SAR 2.3 W·kg^{-1}, 5 hr/day, 5 days/week for 22 weeks). Motor activity of the RFR exposed rats increased during the first week of exposure and stayed higher than controls throughout the period of the experiment. D'Andrea et al. [1979, 1980] reported decreased motor activity on a stabilimetric platform and no significant change in running wheel activity measured overnight in rats exposed to a 2450-MHz RFR (CW, 5 mW·cm^{-2}, SAR 1.2 W·kg^{-1}, exposed 5 day/week with a total exposure time of 640 hrs, activity was measured every 2-weeks). However, they reported no significant effect in both behaviors in rats similarly exposed to a 915-MHz RFR even at a higher energy absorption rate (CW, 5 mW·cm^{-2}, SAR 2.5 W·kg^{-1}). Moe et al. [1976] reported a decrease in motor activity of rats exposed to 918 MHz RFR (CW, SAR 3.6-4.2 W·kg^{-1}) during the dark period of the light-dark cycle in a chronic exposure experiment (10 hr/night for 3 weeks). Lovely et al. [1977] repeated the experiment using a lower intensity (2.5 mW·cm^{-2}, SAR 0.9 W·kg^{-1}, 10 hr/night, 13 weeks) and found no significant change in motor activity in the exposed rats. Thus, the threshold of response under their exposure conditions is between1 and 4 W·kg^{-1}.

The results from the above studies indicate that it would need a rather high energy absorption rate (>1 W·kg^{-1}) to affect motor activity in animals. However, there are two studies reporting effects on motor activity at relatively low SARs. In a long-term exposure study, Johnson et al. [1983] exposed rats to pulsed 2450-MHz RFR (10 ms pulses, 800 pps) from 8 weeks to 25 months of age (22 hr/day). The average whole body SAR varied as the weight of the rats increased and was between 0.4-0.15 W·kg^{-1}. Open field activity was measured in 3-min sessions with an electronic open-field apparatus once every 6 weeks during the first 15 months and at 12-week intervals in the final 10 weeks of exposure. They reported a significantly lower open field activity only at the first test session, and a rise in the blood corticosterone level was also observed at that time. The authors speculated that RFR might be 'minimally stressful' to the rats. Rudnev et al. [1978] studied the behavior of rats exposed to CW 2375-MHz RFR at 0.5 mW·cm^{-2} (SAR 0.1 W·kg^{-1}), 7 h/day for 1 month. They reported a decrease in balancing time in a treadmill and inclined rod and motor activity in an open-field after 20 days of exposure. The open-field motor activity was found to be increased at 3 months post-exposure. Interestingly, Frey [1977] also reported a decrease in motor coordination on a motor-rod in rats exposed to a 1300-MHz pulsed RFR (0.5 ms pulses, 1000 pps, average power density of 0.65 or 0.2 mW·cm^{-2}).

Another type of spontaneous behavior studied was consummatory behavior. In the Rudnev et al. [1978] study, the authors reported a decrease in food intake in their animals after long-term exposure to CW RFR at 0.1 W·kg^{-1}. Ray and Behari [1990] also reported a decrease in eating and drinking behavior in rats exposed for 60 days (3 hr/day) to a 7.5-GHz RFR (10-KHz square wave modulation) at an SAR of 0.0317 W·kg^{-1} (average power density 0.6 mW·cm^{-2}).

Learned behavior

Several psychological studies have been carried out to investigate whether animals can detect RFR. One of the early studies was that of King et al. [1971] in which RFR was used as

the cue in a conditioned suppression experiment. In conditioned suppression, an animal is first trained to elicit a certain response (e.g., bar-press for food). Once a steady rate of response is attained, a stimulus (e.g., a tone) will be presented to signify the on coming of a negative reinforcement (e.g., electric foot shock). The animal will soon learn the significance of the stimulus and a decrease in responding (conditioned suppression) will occur immediately after the presentation of the stimulus. In the experiment of King et al. [1971], rats were trained to respond at a fixed-ratio schedule for sugar water reward. In a 2-hr session, either a tone or RFR would be presented and occasionally followed by an electric foot shock. Radiofrequency radiation of 2450 MHz, modulated at 12 and 60 Hz and at SARs of 0.6, 1.2, 2.4, 4.8, and 6.4 $W{\cdot}kg^{-1}$ was used as the conditioned stimulus. With training, consistent conditioned suppression was observed with the radiation at 2.4 $W{\cdot}kg^{-1}$ and higher. This indicates that rats can detect RFR at 2.4 $W{\cdot}kg^{-1}$. Monahan and Henton [1977] also demonstrated that mice could be trained to elicit a response in order to escape or avoid RFR (CW, 2450-MHz, 40 $W{\cdot}kg^{-1}$). In another experiment, Carroll et al. [1980] showed that rats did not learn to go to a 'safe' area in the exposure cage in order to escape exposure to RFR (918-MHz, pulse modulated at 60 Hz, SAR 60 $W{\cdot}kg^{-1}$) (i.e., entering the 'safe' area resulted in an immediate reduction of the intensity of the radiation), whereas the animals learned readily to escape from electric foot shock by going to the 'safe' area. In a further study from the same laboratory, Levinson et al. [1982] showed that rats could learn to enter a 'safe' area, when the RFR was paired with a light stimulus. Entering the area would turn off both the radiation and light. They also showed that rats could learn to escape by entering the 'safe' area when RFR was presented alone, but learned at a lower rate than when the RFR was paired with a light. All these studies indicate that animals can detect RFR, probably as a thermal stimulus.

One of the most well established effects of pulsed RFR is the 'auditory effect'. Neurophysiological and psychological experiments indicate that animals can probably perceive microwave pulses as a sound stimulus [Chou et al., 1982a; Lin, 1978]. In a series of experiments, Frey and his associates [Frey and Feld, 1975; Frey et al., 1975] demonstrated that rats spent less time in the unshielded compartment of a shuttlebox, when the box was exposed to 1200-MHz pulsed RFR (0.5-ms pulses, 1000 pps, average power density 0.2 $mW{\cdot}cm^{-2}$, peak power density 2.1 $mW{\cdot}cm^{-2}$) than during sham exposure. When a CW RFR (1200-MHz, 2.4 $mW{\cdot}cm^{-2}$) was used, rats showed no significant preference to remain in the shielded or unshielded side of the box. Hjeresen et al. [1979] replicated this finding using pulsed 2880-MHz RFR (2.3 ms pulses, 100 pps, average power density 9.5 $mW{\cdot}cm^{-2}$) and showed that the preference to remain in the shielded side of a shuttlebox during RFR exposure could be generalized to a 37.5-kHz tone. Masking the 'radiation-induced auditory effect' with a 10-20 kHz noise also prevented shuttlebox-side preference during pulsed RFR exposure. These data indicate that the pulsed RFR-induced 'avoidance' behavior is due to the auditory effect.

The question is why rats avoid pulsed RFR? Is the 'auditory effect' stressful? This question was recently raised by Sienkiewicz [1999]. In an attempt to replicate our radial-arm experiment (Lai et al., 1989), he exposed mice to 900-MHz radiation pulsed at 217 Hz for 45 min a day for 10 days at a whole body SAR of 0.05 $W{\cdot}kg^{-1}$. He didn't observe any significant effect of RFR exposure on maze learning, but reported that 'some of the exposed animals in our experiment appeared to show a stress-like response during testing in the maze. The animals tested immediately after exposure showed a more erratic performance, and were slower to complete the task compared to the animals tested after a short delay following exposure. This pattern of behavior may be consistent with increased levels of stress.' He also reported that

exposed animals showed increased urination and defecation. He speculated that these behavioral effects were caused by the 'auditory effect' of the pulsed RFR.

Many studies investigated the effects of RFR exposure on schedule-controlled behavior. A schedule is the scheme by which an animal is rewarded (reinforced) for carrying out a certain behavior. For example, an animal can be reinforced for every response it makes, or reinforced intermittently upon responding according to a certain schedule (e.g., once every ten responses). Schedules of different complexity are used in psychological research. The advantage of using reinforcement schedules is that they generate in animals an orderly and reproducible behavioral pattern that can be maintained over a long period of time. This allows a systematic study of the effect of RFR. Generally speaking, more complex behaviors are more susceptible to disruption by environmental factors. However, the underlying neural mechanisms by which different schedules affect behavior are poorly understood.

In a study by D'Andrea et al. [1977], RFRs of different frequencies and intensities were studied on their effects on bar-pressing rate on a variable-interval schedule. It was found that the latency time of stoppage to respond after the radiation was turned on correlated with the rate of rise in body temperature of the animal. Lebovitz [1980] also studied the effects of pulsed 1300-MHz RFR (1 ms pulses, 600 pps) on rats bar-pressing on a fixed-ratio schedule for food reinforcement. A 15-minute 'rewarded' period, when bar pressing was rewarded with food, was followed by a 10-min 'unrewarded' period. Both food reinforced bar presses and unrewarded bar presses during the periods were studied. No significant effect was detected in both types of response at SAR of 1.5 $W{\cdot}kg^{-1}$. However, at 6 $W{\cdot}kg^{-1}$, there was a slight reduction in rewarded bar presses and a large reduction in unrewarded bar presses. The authors concluded that the unrewarded behavior was more susceptible to the effect of RFR than the rewarded behavior. However, Hunt et al. [1975] trained rats to bar press for saccharin water rewards in the presence (5- second duration) of a flashing light and not to respond in the presence of a tone. After 30 min of exposure to 2450-MHz RFR (modulated at 20 Hz, SAR of 6.5 or 11.0 $W{\cdot}kg^{-1}$), rats made more misses at the presence of the light, but there were no significant changes in the incidences of bar-pressing error when the tone was on (unrewarded). Gage [1979] trained rats to alternate responses between 2 levers at 11-30 times for a food reinforcement. Decrement in response rates was observed after 15 hrs of exposure to CW 2450-MHz RFR at 10, 15, and 20 $mW{\cdot}cm^{-2}$ (0.3 $W{\cdot}kg^{-1}$ per $mW{\cdot}cm^{-2}$).

Effects of RFR on more complex operant response sequence and reinforcement schedules were studied in various experiments. de Lorge and Ezell [1980] tested rats on an auditory vigilance (observing-response) behavioral task during exposure to pulsed 5620-MHz (0.5 or 2 ms, 662 pps) and 1280-MHz (3 ms, 370 pps) RFR. In this task, rats had to discriminate two tones in order to press one of two bars appropriately for food reinforcement. The task required continuous sensory-motor activities in which the animal had to coordinate its motor responses according to the stimulus cues (tone) presented. Behavioral decrement was observed at a SAR of 3.75 $W{\cdot}kg^{-1}$ with the 1280-MHz radiation, and at 4.9 $W{\cdot}kg^{-1}$ with the 5620-MHz radiation. The authors concluded that '…the rat's observing behavior is disrupted at a lower power density at 1.28 than at 5.62 GHz because of deeper penetration of energy at the lower frequency, and because of frequency-dependent differences in anatomic distribution of the absorbed microwave energy.' In another experiment, de Lorge [1984] studied rhesus monkeys trained on the auditory vigilance (observing-response) task. After the training, the effects of exposure to RFR of different frequencies (225, 1300, and 5800 MHz) were studied [225-MHz-CW; 1300-MHz- 3 ms pulses, 370 pps; 5800-MHz- 0.5 or 2 ms pulses, 662 pps]. Reduction in performance was

observed at different power density thresholds for the frequencies studied: 8.1 mW·cm^{-2} (SAR 3.2 W·kg^{-1}) for 225 MHz, 57 mW·cm^{-2} (SAR 7.4 W·kg^{-1}) for 1300 MHz, and 140 mW·cm^{-2} (SAR 4.3 W·kg^{-1}) for 5800 MHz. de Lorge concluded that the behavioral disruption under different frequencies of exposure was more correlated with change in body temperature. Disruption occurred when the colonic temperature of the animal had increased by 1°C.

Thomas et al. [1975] trained rats to bar press on two bars: a fixed ratio of 20 on the right bar (20 bar presses produced a food pellet reward) and differential reinforcement of low rate (DRL) on the left bar (bar presses had to be separated by at least 18 sec and no more than 24 sec to produce a reward). There was a time-out period between schedules, i.e., no reinforcement available for responding. Animals were tested 5-10 min after 30 min of exposure to either CW 2450-MHz, pulsed 2860-MHz (1 ms pulses, 500 pps) or pulsed 9600-MHz (1 ms pulses, 500 pps) RFR at various power densities. An increase in DRL response rate was observed with 2450-MHz radiation >7.5 mW·cm^{-2} (SAR 2.0 W·kg^{-1}), 2860-MHz RFR >10 mW·cm^{-2} (2.7 W·kg^{-1}), and 9600-MHz RFR >5 mW·cm^{-2} (SAR 1.5 W·kg^{-1}). A decrease in the rate of response at the fixed ratio schedule was seen in all three frequencies when the power density was greater than 5 mW·cm^{-2}. In addition, an increase in response rate was observed during time-out periods under irradiation of the three frequencies of RFR at greater than 5 mW·cm^{-2}. This indicates a disruption of the animals' ability to discriminate the different schedule situations.

Schrot et al. [1980] trained rats to learn a new daily sequence of pressing of three bars for food reinforcement. An increased number of errors and decreased learning rates were observed in the animals after 30 min of exposure to pulsed 2800-MHz RFR (2 ms pulses, 500 pps) at average power densities of 5 and 10 mW·cm^{-2} (SAR 0.7 and 1.7 W·kg^{-1}, respectively). No significant effect on performance was observed at power densities of 0.25, 0.5, and 1 mW·cm^{-2}.

D'Andrea et al. [1989] studied the behavioral effects of high peak power RFR pulses of 1360-MHz. Rhesus monkeys performing on a complicated reinforcement-schedule involving time-related behavioral tasks (inter-response time, time discrimination, and fixed interval responses) were exposed to high peak power RFR (131.8 W·cm^{-2} rms, pulse repetition rate 2-32 Hz). No significant disturbance in performance was observed in the monkeys. Akyel et al. [1991] also studied the effects of exposure to high peak power RFR pulses on behavior. In their experiment, rats pre-trained to bar-press for food reinforcement on either fixed ratio, variable interval, or DRL schedule were exposed for 10 min to 1250-MHz pulses. Each pulse (10 ms width) generated a whole body specific absorption of 2.1 J·kg^{-1}, which corresponds to a whole body average SAR of 0.21 mW·kg^{-1}. The pulse rate was adjusted to produce different total doses (0.5-14 kJ·kg^{-1}). Only at the highest dose (14 kJ·kg^{-1}), stoppage of responding was observed after exposure, when the colonic temperature was increased by ~2.5^{o}C. Responding resumed when colonic temperature returned to within 1.1^{o}C above the pre-exposure level. When responding resumed, the response rates on the fixed ratio and variable interval schedules were below the pre-exposure base line level. Responses on the DRL schedule were too variable to allow a conclusion to be drawn. The authors concluded that the effect of the high peak power RFR pulses on schedule-controlled behavior was due to hyperthermia.

Several studies investigated the effects of long-term RFR exposure on schedule controlled-behavior. Mitchell et al. [1977] trained rats to respond on a mixed schedule of reinforcement (FR-5 EXT-15 sec), in which 5 responses would give a reward and then a 15 sec lapse time (extinction period) was required before a new response would be rewarded. In addition, the schedule of reinforcement was effective when a lamp was on, while no reinforcement was given when the lamp was off. Rats were then exposed to CW 2450-MHz

RFR (average SAR 2.3 W·kg^{-1}) for 22 weeks (5 hr/day, 5 days/week) and tested at different times during the exposure period. The RFR-exposed rats showed higher responses during the extinction period, indicating poorer discrimination of the response cues. Navakatikian and Tomashevskaya [1994] described a complex series of experiments in which they observed disruption of a behavior (active avoidance) by RFR. In the study, rats were first trained to perform the behavior and then exposed to either CW 2450-MHz RFR or pulsed 3000-MHz RFR (400-Hz modulation, pulse duration 2 ms, and simulation of radar rotation of 3, 6, and 29 rotations/min) for 0.5-12 hrs or 15-80 days (7-12 hr/day). Behavioral disruption was observed at a power density as low as 0.1 mW·cm^{-2} (0.027 W·kg^{-1}).

Two series of well-designed experiments were run by D'Andrea and his colleagues to investigate the effects of chronic RFR exposure on behavior. In one experiment [D'Andrea et al., 1986 a], rats were exposed for 14 weeks (7 hr/day, 7 days/week) to CW 2450-MHz RFR at 2.5 mW·cm^{-2} (SAR 0.7 W·kg^{-1}). After exposure, the rats were trained to bar press on an interresponse time criterion (IRT). In this schedule, the animals had to respond within 12 to 18 sec after the previous response in order to receive a food reward. Radiofrequency radiation exposed rats emitted more responses during the training period. When the training was completed, the RFR-exposed rats had lower efficiency in bar-pressing to obtain food pellets, i.e., they made more inappropriate responses and received fewer food pellets than the sham-exposed rats during a session. In a signalled two-way active avoidance shuttlebox test, the RFR-exposed rats showed less avoidance response than the sham-exposed rats during training; however, no significant difference in responses in the shuttlebox test was detected at 60 days after exposure between the RFR- and sham-exposed animals. In this experiment, a decrease in the threshold of electric foot shock detection (i.e., increase in sensitivity) was also observed in the irradiated rats during the exposure period, and an increased open-field exploratory behavior was observed in the rats at 30 days post-exposure. It may be interesting to point out that Frey [1977] also reported a decrease in tail pinch-induced aggressive behavior in RFR-exposed rats. Increased latency, decrease in duration, and episodes of fighting after tail pinching were observed between two rats being irradiated with RFR. This could be due to a decreased sensitivity or perception of pain and the RFR-induced activation of endogenous opioids described below.

In a second experiment [D'Andrea et al., 1986 b], rats were exposed to 2450-MHz RFR at 0.5 mW·cm^{-2} (SAR 0.14 W·kg^{-1}) for 90 days (7 hr/day, 7 days/week). Open-field behavior, shuttlebox performance, and schedule-controlled bar-pressing behavior for food pellets were studied at the end of the exposure period. A small deficit in shuttlebox performance and an increased rate of bar-pressing were observed in the RFR exposed rats. Summarizing the data from these two series of experiments [D'Andrea et al., 1986 a,b], D'Andrea and his co-workers concluded that the threshold for the behavioral and physiological effects of chronic RFR exposure in the rats studied in their experiments occurred between the power densities of 0.5 mW·cm^{-2} (SAR 0.14 W·kg^{-1}) and 2.5 mW·cm^{-2} (SAR 0.7 W·kg^{-1}).

In a further experiment, DeWitt et al. [1987] also reported an effect on an operant task in rats after exposure for 7hr/day for 90 days to CW 2450-MHz RFR at a power density of 0.5 mW·cm^{-2} (0.14 W·kg^{-1}).

Little work has been done to investigate the effects of RFR on memory functions. We [Lai et al., 1989] studied the effect of short-term (45 min) RFR exposure (2450-MHz, 2 msec pulses, 500 pps, 1 mW·cm^{-2}, SAR 0.6 W·kg^{-1}) on the rats' performance in a radial-arm maze, which measures spatial working (short-term) memory function. The maze consists of a central circular hub with arms radiating out like the spokes of a wheel. In this task, food-deprived

animals are trained to explore the arms of the maze to obtain food reinforcement at the end of each arm. In each session they have to enter each arm once and a reentry is considered as an error. This task requires 'working memory', i.e., the rat has to remember the arms it has already entered during the course of a session. We found that short-term (45 min) exposure to RFR before each session of maze running significantly retarded the rats' abilities to perform in the maze. They made significantly more errors than the sham-exposed rats. In a further experiment [Lai et al., 1994], we found that the RFR-induced working memory deficit in the radial-arm maze was reversed by pretreating the rats before exposure with the cholinergic agonist physostigmine or the opiate antagonist naltrexone, whereas pretreatment with the peripheral opiate antagonist naloxone methiodide showed no reversal of effect. These data indicate that both cholinergic and endogenous opioid neurotransmitter systems inside the central nervous system are involved in the RFR-induced spatial working memory deficit. Spatial working memory requires the functions of the cholinergic innervations in the frontal cortex and hippocampus. The behavior result agrees with our previous neurochemical findings that RFR exposure decreased the activity of the cholinergic systems in the frontal cortex and hippocampus of the rats [Lai et al., 1987]. Endogenous opioids [Lai et al., 1992] and the 'stress hormone' corticotropin-releasing factor [Lai et al., 1990] are also involved. Our hypothesis is that radiofrequency radiation activates endogenous opioids in the brain, which in turn cause a decrease in cholinergic activity leading to short-term memory deficit. Related to this that there is a report by Kunjilwar and Behari [1993] showing that long-term exposure (30-35 days, 3 hrs/day, SAR 0.1-0.14 W/kg) to 147-MHz RFR and its sub-harmonics 73.5 and 36.75 MHz, amplitude modulated at 16 and 76 Hz, decreased acetylcholine esterase activity in the rat brain, whereas short-term exposure (60 min) had no significant effect on the enzyme. There is another report by Krylova et al. [1992] indicating that 'cholinergic system plays an important role in the effects of electromagnetic field on memory processes'. There are also two studies suggesting the involvement of endogenous opioids in the effects of RFR on memory functions [Krylov et al., 1993; Mickley and Cobb, 1998].

In a more recent experiment, we [Wang and Lai, 2000] studied spatial long-term memory using the water maze. In this test, rats are trained to learn the location of a submerged platform in a circular water pool. We found that rats exposed to pulsed 2450-MHz RFR (2 ms pulses, 500 pps, 1.2 $W{\cdot}kg^{-1}$, 1 hr) were significantly slower in learning and used a different strategy in locating the position of the platform.

Comments

(1) From the data available, it is not apparent that pulsed RFR is more potent than CW RFR in affecting behavior in animals. Even though different frequencies and exposure conditions were used in different studies and hardly any dose-response study was carried out, there is no consistent pattern that the SARs of pulsed RFR reported to cause an effect are lower than those of CW RFR. For example, the Thomas et al [1975] study showed that the thresholds of effect of CW 2450-MHz (2.0 $W{\cdot}kg^{-1}$) and pulsed 2860-MHz (2.7 $W{\cdot}kg^{-1}$) radiation on DRL bar-pressing response are quite similar.

(2) Thermal effect is definitely a factor in the effects reported in some of the experiments described above. A related point is that most psychoactive drugs also affect body temperature. Stimulants cause hyperthermia, barbiturates cause hypothermia, and narcotics have a biphasic effect on body temperature (hyperthermia at low doses and hypothermia at high doses). It is not uncommon to

observe a change of 2-3°C within 30 min after a drug is administered. However, in reviewing the literature, there is no general correlation between the effects of psychoactive drugs on body temperature and schedule-controlled behavior. Thus, body temperature may not be a major factor in an animal's responding under schedule-controlled behavior, at least in the case of psychoactive drugs. On the contrary, some of the experiments described above strongly suggest the role of hyperthermia on the RFR effect on the behavior. Perhaps, a sudden and large increase in body temperature as in the case of RFR can have a major effect on responding.

(3) Generally speaking, when effects were observed, RFR disrupted schedule-controlled behavior in animals such as in the cases of discrimination responding [de Lorge and Ezell, 1980; Hunt et al., 1975; Mitchell et al., 1977], learning [Schrot et al., 1980], and avoidance [D'Andrea et al., 1986 a,b]. This is especially true when the task involved complex schedules and response sequence. In no case has an improvement in behavior been reported in animals after RFR exposure. It is puzzling that only disruptions in behavior by RFR exposure are reported. In the studies on EEG, both excitation (desynchronization) and depression (synchronization) have been reported after exposure to RFR [Bawin et al., 1973; Chizhenkova, 1988; Chou et al., 1982b; Dumansky and Shandala, 1974; Goldstein and Sisko, 1974; Takeshima et al., 1979]. Motor activity has also been reported to increase [D'Andrea et al., 1979, 1980; Frey et al., 1975; Hjeresen et al., 1979; Mitchell et al., 1977; Rudnev et al., 1978] and decrease [Hunt et al., 1975; Johnson et al., 1983; Mitchell et al., 1988; Moe et al., 1976; Rudnev et al., 1978] after RFR exposure. If these measurements can be considered as indications of electrophysiological and behavioral arousal and depression, improvement in behavior should occur under certain conditions of RFR exposure. This is especially true with avoidance behavior. Psychomotor stimulants that cause EEG desynchronization and motor activation improve avoidance behavior, whereas tranquilizers that have opposite effects on EEG and motor activity decrease avoidance behavior.

(4) It is difficult to conclude from the effects of RFR on schedule-controlled behavior the underlying neural mechanisms involved. In general, the effects of the effect of RFR on schedule-controlled behavior is similar to those of other agents, e.g., psychoactive drugs. For example, the way that a certain drug affects schedule-controlled behavior depends on the base line level of responding. A general rule is that drugs tend to decrease the rate when the base line responding rate is high and vice versa. This is known as rate-dependency. Exposure to RFR caused a decrease in response rate when a variable interval schedule that produces a steady rate of responding was used [D'Andrea et al., 1976; 1977], and an increase in responding when the DRL-schedule of reinforcement, that produces a low base line of responding, was used [Thomas et al., 1975]. This may reflect a rate-dependency effect. The effect of an agent can also depend on the schedule of reinforcement. For example, amphetamine has different effects on responses maintained on DRL schedule and punishment-suppressed responding schedule, even though both schedules generate a similar low response rate. Stimulus control as a determinant of response outcome was seen in the study of Lebovitz [1980] when unrewarded responses were disrupted more by RFR than rewarded responses, and the study of Hunt et al. [1975] that showed the reverse relationship. In the former experiment a fixed interval schedule was used, whereas in the latter a discrimination paradigm was studied.

(5) It is also interesting to point out that in most of the behavioral experiments, effects were observed after the termination of RFR exposure. In some experiments (e.g., Rudnev et al., 1978; D'Andrea et al., 1986 a,b), tests were made days after exposure. This suggests a persistent change in the nervous system after exposure to RFR.

(6) In many instances, effects on learned behavior were observed at a SAR less than 4 W·kg^{-1}. (D'Andrea et al [1986a,b] 0.14 to 0.7 W·kg^{-1}; DeWitt et al. [1987] 0.14 W·kg^{-1}; Gage [1979] 3 W·kg^{-1}; King et al.[1971] 2.4 W·kg^{-1}; Lai et al. [1989] 0.6 W·kg^{-1}; Mitchell et al. [1977] 2.3 W·kg^{-1}; Navakatikian and Tomashevskaya [1994] 0.027 W·kg^{-1}; Schrot et al. [1980] 0.7 W·kg^{-1}; Thomas et al. [1975] 1.5 to 2.7 W·kg^{-1}; Wang and Lai [2000] 1.2 W·kg^{-1}).

(7) Does disturbance in behavior have any relevance to health? The consequence of a behavioral deficit is situation dependent and may not be direct. It probably does not matter if a person is playing chess and RFR in his environment causes him to make a couple of bad moves. However, the consequence would be much more serious if a person is flying an airplane and his response sequences are disrupted by RFR radiation.

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SECTION 10 – Part 1

EVIDENCE FOR BRAIN TUMORS AND ACOUSTIC NEUROMAS

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Table of Contents

I. Introduction

During the recent decade potential health risks from microwave exposure during use of wireless phones has been discussed both in scientific settings but also by the layman. Especially the use of mobile phones has been of concern, to less extent use of cordless desktop phones (digital enhanced cordless telephone; DECT). The Nordic countries were among the first in the world to widely adopt use of such devices, probably due to the mobile phone companies like Ericsson in Sweden and Nokia in Finland.

These countries may be taken as models for the introduction of this new technology on the market. Thus, the analogue mobile phone system (Nordic Mobile Telephony, NMT) using 450 MHz started to operate in Sweden in 1981. First, it was used in cars with external antenna but from 1984 mobile (portable!) phones existed. This system is still used in Sweden but only to a minor extent. The 900 MHz NMT system operated in Sweden between 1986-2000. The GSM phone (Global System for Mobile communication) started in 1991 and is the most used phone type today, although the 3G phone (third generation mobile phone, UMTS) is increasingly used now.

The risk of brain tumors has been of special concern since the brain is the organ mainly exposed during such phone calls. Most studies on this topic have been of the case-control design and no results exist from prospective cohort studies. However, the results have been hampered by too short tumor-induction period in most studies or with limited number of long-term users, i.e. $\geq$ 10 years latency time. As to carcinogenesis short latency period is of limited value to predict long-term health risks. Usually a latency period of at least 10 years is needed for more firm conclusions. It should noted that for several carcinogens longer latency periods are often

required, such as smoking and lung cancer, asbestos and lung cancer, dioxins and certain cancer types etc.

By now a number of studies exist that give results for brain tumour risk and use of mobile phones for subjects with latency period $\geq$ 10 years. Most of these results are based on low numbers but nevertheless may together give a pattern of increased risk. In this review we discuss all studies on this topic that have been published so far. Moreover, we present a meta-analysis of results from studies with at least 10 years latency period. Only the Hardell group in Sweden has published results also for use of cordless phones. Recently the same group published an overview of long-term use of cellular phones and the risk for brain tumors, especially with use for 10 years or more (Hardell et al 2007). In the following a brief summary is given of these results with the addition of two more study published after that review (Klaeboe et al 2007, Schlehofer et al 2007). For further details see Hardell et al (2007).

II. Materials and Methods

The Pub Med database (**www.ncbi.nlm.nih.gov**) was used for an up-dated search of published studies in this area using mobile/cellular/cordless telephone and brain tumour/neoplasm/acoustic neuroma/meningioma/glioma as searching terms. Personal knowledge of published studies was also used in order to get as comprehensive review as possible. Regarding several publication of the same study the most recent one with relevant data was used. We identified 20 studies to be included. Two were cohort studies (one study analysed twice) and 18 were case-control studies. No mortality studies were included. Three studies came from USA, four from Denmark, one from Finland, five from Sweden, two from Germany, one from the UK, one from Japan, one from Norway and two from study groups partly overlapping previously mentioned studies.

III. Results

A. The first Swedish studies

The first study by Hardell et al (1999, 2001) included cases and controls collected during 1994-96 in Sweden. Only living cases were included. Two controls were selected to each case from the Population Registry. The questionnaire was answered by 217 (93 %) cases and 439 (94 %) controls. Overall no association between mobile phone use and brain tumours was found, but when analysing ipsilateral phone use a somewhat increased risk was seen especially for tumours in the temporal, occipital or temporoparietal lobe yielding odds ratio (OR) = 2.4, 95 % confidence interval (CI) = 0.97-6.1 (Hardell et al 2001).

Hardell et al (2006a) made a pooled analysis for benign brain tumours from their two case-control studies. Cases were reported from Cancer Registries and controls were population based. The questionnaire was answered by 1,254 (88 %) cases and 2,162 (89 %) controls. Also use of cordless desktop phones was assessed. Use of cellular phones gave for acoustic neuroma OR = 1.7, 95 % CI 1.2-2.3 increasing to OR = 2.9, 95 % CI = 1.6-5.5 with > 10 year latency period. The corresponding results for cordless phones were OR = 1.5, 95 % CI = 1.04-2.0, and OR = 1.0, 95 % CI 0.3-2.9, respectively. Regarding meningioma cellular phones gave OR = 1.1, 95 % CI = 0.9-1.3, and cordless OR = 1.1, 95 % CI = 0.9-1.4. Using > 10 year latency period ORs increased, for cellular telephones OR = 1.5, 95 % CI = 0.98-2.4, and for cordless phones OR = 1.6, 95 % CI = 0.9-2.8.

The pooled analyses of the two case control studies of malignant brain tumours by Hardell et al (2006b) included 905 (90%) cases and the same control group as for benign tumours was used,

2,162 (89 %) subjects. Overall for low-grade astrocytoma cellular phones gave OR= 1.4, 95 %

CI = 0.9-2.3 and cordless phones OR = 1.4, 95 % CI = 0.9-3.4. The corresponding results for

high-grade astrocytoma were OR = 1.4, 95 % CI = 1.1-1.8, and OR = 1.5, 95 % CI = 1.1-1.9,

respectively. Using > 10 year latency period gave for low-grade astrocytoma and use of cellular

phones OR = 1.5, 95 % CI = 0.6-3.8 (ipsilateral OR = 1.2, 95 % CI = 0.5-5.8), and for cordless

phones OR = 1.6, 95 % CI = 0.5-4.6 (ipsilateral OR = 3.2, 95 % CI = 0.6-16). For high-grade

astrocytoma in the same latency period cellular phones gave OR = 3.1, 95 % CI = 2.0-4.6

(ipsilateral OR = 5.4, 95 % CI = 3.0-9.6), and cordless phones OR = 2.2, 95 % CI = 1.3-3.9

(ipsilateral OR = 4.7, 95 % CI = 1.8-13).

B. Studies from USA

Muscat et al (2000) studied patients with malignant brain tumours from five different hospitals

in USA. Controls were hospital patients. Data from 469 (82 %) cases and 422 (90 %) controls

were available. Overall no association was found, OR for handheld cellular phones was 0.9, 95

% CI = 0.6-1.2, but the mean duration of use was short, only 2.8 years for cases and 2.7 years

for controls. For neuroepithelioma OR = 2.1, 95 % CI = 0.9-4.7, was reported. The study is

inconclusive since no data were available on long-term users (≥ 10 years latency period). Some

support of an association was obtained since of 41 evaluable tumours, 26 occurred at the side of

the head mostly used during calls and 15 on the contralateral side.

Also the study by Inskip et al (2001) from USA had few long-term users of mobile phones, only

11 cases with glioma, 6 with meningioma and 5 with acoustic neuroma with ≥ 5 years regular

use. No subjects had ≥ 10 years use. The study comprised 489 (92 %) hospital cases with

malignant brain tumours, 197 with meningioma and 96 with acoustic neuroma, and 799 (86 %)

hospital-based controls. Overall no significant associations were found. Regarding different

6

types of glioma OR = 1.8, 95 % CI = 0.7-5.1 was found for anaplastic astrocytoma. Duration of

use ≥ 5 years gave for acoustic neuroma OR increased to 1.9, 95 % CI = 0.6-5.9.

In another study by Muscat et al (2002) presented results from a hospital based case-control

study on acoustic neuroma on 90 (100 %) patients and 86 (100 %) controls. Cell phone use 1-2

years gave OR = 0.5, 95 % CI = 0.2-1.3 (n=7 cases), increasing to OR = 1.7, 95 % CI = 0.5-5.1

(n=11 cases), in the group with 3-6 years use. Average use among cases was 4.1 years and

among controls 2.2 years.

C. Danish cohort study

A population based cohort study in Denmark of mobile phone users during 1982 to 1995

included over 700,000 users (Johansen et al 2001). About 200,000 individuals were excluded

since they had company paid mobile phones. Of digital (GSM) subscribers only nine cases had

used the phone for ≥ 3 years duration yielding standardised incidence ratio (SIR) of 1.2, 95 % CI

= 0.6-2.3. No subjects with 10-year use were reported.

This cohort study was updated with follow-up through 2002 for cancer incidence (Schüz et al

2006). There was no truly unexposed group for comparison since a large part of the population

uses wireless phones. Moreover the excluded company subscribers (> 200 000 or 32 %) were

apparently included in the reference population. There was also a very skewed sex distribution

with 85 % men and only 15 % women in the cohort. SIR was significantly decreased to 0.95, 95

% CI = 0.9-0.97 for all cancers indicating a "healthy worker" effect in the study. In the group

with ≥ 10 years since first subscription significantly decreased SIR of 0.7, 95 % CI = 0.4-0.95

was found for brain and nervous system tumours indicating methodological problems in the

study. No latency data were given or laterality of phone use in relation to tumour localisation in

the brain. This study was uninformative regarding long-term health effects from mobile phone use.

D. Finnish study

Auvinen et al (2002) did a register based case-control study on brain and salivary gland tumors in Finland. All cases aged 20-69 years diagnosed in 1996 were included; 398 brain tumour cases and 34 salivary gland tumour cases. The duration of use was short, for analogue users 2-3 years and for digital less than one year. No association was found for salivary gland tumours. For glioma OR = 2.1, 95 % CI = 1.3-3.4 was calculated for use of analogue phones, but no association was found for digital mobile phones. When duration of use of analogue phones was used as a continuous variable an increased risk was found for glioma with OR = 1.2, 95 % CI = 1.1-1.5 per year of use.

E. The Interphone studies

1. Acoustic neuroma

The Swedish part of the Interphone study on acoustic neuroma included exposure data from 148 (93 %) cases and 604 (72 %) population based controls (Lönn et al 2004). Use of digital phones with time $\geq$ 5 years since first use gave OR = 1.2, 95 % CI = 0.7-2.1. No subjects were reported with use of a digital phone $\geq$ 10 years. An association was found for use of analogue phones yielding for $\geq$ 10 years latency period OR = 1.8, 95 % CI = 0.8-4.3 increasing to OR = 3.9, 95 % CI = 1.6-9.5 for ipsilateral use.

In Denmark the Interphone study included 106 (82 %) interviewed cases with acoustic neuroma and 212 (64 %) population-based controls (Christensen et al 2004). Significantly larger tumours were found among cellular phone users, 1.66 cm^3 compared with 1.39 cm^3 among non-users, $p =$

0.03. However OR was not significantly increased but only two cases had use a mobile phone regularly ≥ 10 years.

Schoemaker et al (2005) presented results for acoustic neuroma as part of the Interphone study performed in 6 different regions in the Nordic countries and UK, as previously partly reported (Lönn et al 2004; Christensen et al 2004). The results were based on 678 (82 %) cases and 3,553 (42 %) controls. Lifetime use of mobile phone for ≥ 10 years gave for ipsilateral acoustic neuroma OR = 1.8, 95 % CI = 1.1-3.1, and for contralateral OR = 0.9, 95 % CI = 0.5-1.8.

The study from Japan by Takebayashi et al (2006) included 101 (84 %) acoustic neuroma cases aged 30-69 years and diagnosed during 2000-2004. Using random digit dialling 339 (52 %) controls were interview. No association was found, OR = 0.7, 95% CI = 0.4 – 1.2. No exposure related increase in the risk of acoustic neuroma was observed when the cumulative length of use (<4 years, 4-8 years, >8 years) or cumulative call time (<300 hours, 300-900 hours, >900 hours) was used as an exposure index. The OR was 1.1, 95% CI = 0.6 - 2.1, when the reference date was set to five years before the diagnosis. Further, laterality of mobile phone use was not associated with tumours. No cases with ≥ 10 years latency period were reported.

Use of mobile phones and risk of acoustic neuroma were published from Norway as part of the Interphone study (Klaeboe et al 2007). It included 45 (68 %) acoustic neuroma cases and 358 (69 %) controls. A decreased risk was found with OR = 0.5, 95 % CI = 0.2-1.0. Using different criteria such as duration of regular use, time since first regular use, cumulative use etc 22 additional ORs and CIs were calculated. Time since first regular use for < 6 years gave OR =

1.0, 95 % CI = 0.2-5.7. All 21 other ORs were < 1.0 indicating systematic bias in the study. No

case had a latency period of 10 years.

Schlehofer et al (2007) reported results from the German part of the Interphone study on

sporadic acoustic neuroma. The study was performed during October 2000 and October 2003.

Four study areas were included and cases were aged 30-59 years, but from October 1, 2001

extended to include the age group 60-69 years. They were recruited from hospitals and included

97 (89 %) cases, however, three with trigeminus neuroma. Controls were randomly selected

from population registries and in total 202 (55 %) agreed to participate. No association was

found for regular mobile phone use, OR = 0.7, 95 % CI = 0.4-1.2. Most ORs were < 1.0 and a

decreasing trend of the risk was found for time since first regular use, lifetime number of use

and duration of calls. No case had a latency period > 10 years. However, increased OR was

found for highly exposed in "specified occupational exposure" yielding OR = 1.5, 95 % CI =0.5-

4.2.

> *E. The Interphone studies*
>
> 2. *Glioma, meningioma*

Lönn et al (2005) also studied glioma and meningioma. Data were obtained for 371 (74 %)

glioma and 273 (85 %) meningioma cases. The control group consisted of 674 (71 %) subjects.

No association was found although time since first regular phone use for $\geq$ 10 years gave for

ipsilateral glioma OR = 1.6, 95 % CI = 0.8-3.4 and for contralateral glioma OR = 0.7, 95 % CI =

0.3-1.5.

For ipsilateral meningioma OR = 1.3, 95 % CI = 0.5-3.9 was calculated and for contralateral OR

= 0.5, 95 % CI = 0.1-1.7 using 10 $\geq$ years latency period.

The Danish part of the Interphone study on brain tumours (Christensen et al, 2005) included 252 (71 %) persons with glioma, 175 (74 %) with meningioma and 822 (64 %) controls. For meningioma OR = 0.8, 95 % CI = 0.5-1.3 was calculated and for low-grade glioma OR = 1.1, 95 % CI = 0.6-2.0, and for high-grade glioma OR = 0.6, 95 % CI = 0.4-0.9 were found. Use for $\geq$ 10 years yielded for meningioma OR = 1.0, 95 % CI = 0.3-3.2, low-grade glioma OR = 1.6, 95 % CI = 0.4-6.1 and for high-grade glioma OR = 0.5, 95 % CI = 0.2-1.3. Regarding high-grade glioma 17 ORs were presented and all showed OR < 1.0.

Results from England were based on 966 (51 %) glioma cases and 1,716 (45 %) controls (Hepworth et al 2006). Cases were ascertained from multiple sources including hospital departments and cancer registries. The controls were randomly selected from general practioners' lists. Regular phone use gave OR = 0.9, 95 % CI = 0.8-1.1, increasing to OR = 1.2, 95 % CI = 1.02-1.5 for ipsilateral use but OR = 0.8, 95 % CI = 0.6-0.9 for contralateral use. Ipsilateral use for $\geq$ 10 years produced OR = 1.6, 95 % CI = 0.9-2.8, and contralateral OR = 0.8, 95 % CI = 0.4-1.4.

Schüz et al (2006) carried out a population-based case-control study in three regions of Germany, with incident cases of glioma and meningioma aged 30-69 years during 2000-2003. Controls were randomly drawn from population registries. In total, 366 (80 %) glioma cases, 381 (88 %) meningioma cases, and 1,494 (61 %) controls were interviewed. For glioma OR = 1.0, 95% CI = 0.7 - 1.3 and for meningioma OR = 0.8, 95% CI = 0.6 - 1.1 were obtained. However, among persons who had used cellular phones for $\geq$ 10 years increased risk was found for glioma; OR = 2.2, 95% CI = 0.9 - 5.1 but not for meningioma; OR = 1.1, 95% CI = 0.4 – 3.4. Among women they found OR = 2.0, 95 % CI = 1.1-3.5 for high-grade glioma for "regular" cell-phone use.

Summary results for mobile phone use and risk of glioma in Denmark, and parts of Finland,

Norway, Sweden and United Kingdom have been published (Lahkola et al 2007). Of the

included Interphone studies results had already been published from Sweden (Lönn et al 2005),

Denmark (Christensen et al 2005) and UK (Hepworth et al 2006). The results were based on

2,530 eligible cases but only 1,521 (60%) participated. Regular mobile phone use gave OR =

0.8, 95 % CI = 0.7-0.9, but cumulative hours of use yielded OR = 1.006, 95 % CI = 1.002-1.010

per 100 hours. Ipsilateral mobile phone use for $\geq$ 10 years gave OR = 1.4, 95 % CI = 1.01-1.9, p

trend = 0.04 and contralateral use OR = 1.0, 95 % CI = 0.7-1.4.

Use of mobile phones and risk of glioma and meningioma were published from Norway as part

of the Interphone study (Klaeboe et al 2007). It included 289 (71 %) glioma cases, 207 (69 %)

meningioma cases and 358 (69 %) controls. Significantly decreased OR = 0.6, 95 % CI = 0.4-

0.9 was found for glioma and decreased OR = 0.8, 95 % CI = 0.5-1.1 for meningioma. For

glioma 22 additional ORs were calculated using different exposure criteria as discussed above

and all calculations yielded OR < 1.0, seven significantly so. Also for meningioma most ORs

were < 1.0. Again these results indicate systematic bias in the study.

F. Meta-analysis

A meta-analysis of the risk for acoustic neuroma, glioma and meningioma was performed for

mobile phone use with a latency period of 10 years or more (Hardell et al 2007). For acoustic

neuroma studies by Lönn et al (2004), Christensen et al (2004) Schoemaker et al (2005) and

Hardell et al (2006a) were included, all giving results for at least 10 years latency period or

more. Overall OR = 1.3, 95 % CI = 0.6-2.8 was obtained increasing to OR = 2.4, 95 % CI = 1.1-5.3 for ipsilateral mobile phone use (Lönn et al 2004, Schoemaker et al 2005, Hardell et al 2006). For glioma OR = 1.2, 95 % CI = 0.8-1.9 was calculated (Lönn et al 2005, Christensen et al 2005, Hepworth et al 2006, Schüz et al 2006, Hardell et al 2006b, Lahkola et al 2007). Ipsilateral use yielded OR = 2.0, 95 % CI = 1.2-3.4 (Lönn et al 2005, Hepworth et al 2006, Hardell et al 2006b, Lahkola et al 2007). In total OR = 1.3, 95 % CI = 0.9-1.8 was found for meningioma (Lönn et al 2005, Christensen et al 2005, Schüz et al 2006, Hardell et al 2006a) increasing to OR = 1.7, 95 % CI = 0.99-3.1 for ipsilateral use (Lönn et al 2005, Hardell et al 2006b).

IV. Discussion

This review included 20 studies, two cohort studies and 18 case-control studies. We recently made a review on this topic and more details can be found in that publication (Hardell et al 2007). Only two studies have been published since then. Both were on acoustic neuroma (Klaeboe et al 2007, Schlehofer et al 2007). They were small with no cases with a latency period of at least 10 years. Furthermore, most ORs were < 1.0 indicating serious methodological problems in the studies.

So far most studies have had no or limited information on long-term users. No other studies than from the Hardell group has published results for use of cordless phones (Hardell et al 2006a,b). As we have discussed in our publications it is pertinent to include also such use in this type of studies. Cordless phones are an important source of exposure to microwaves and they are usually used for a longer time period on daily basis as compared with mobile phones. Thus, to exclude such use seems to underestimate the risk for brain tumors from use of wireless phones.

It should be noted that the Hardell group has included also use of cordless phones, and thus in the exposure assessment the "unexposed" cases and controls have not been exposed to either cordless or cellular phones. This is in contrast to the Interphone study where the "unexposed" may have been exposed to cordless phones of unknown amount.

Of the 18 case-control studies 11 gave results for $\geq$ 10 years use or latency period. However, most of the results were based on low numbers. Thus, it is necessary to get an overview if there is a consistent pattern of increased risk with longer latency period and to make a formal meta-analysis of these findings. Since brain tumours are a heterogenic group of tumours it is reasonable to separate the results for malignant and benign tumours, as has been done in the various studies.

The Danish cohort study (Johansen et al, 2001) is not very informative due to limits in study design, analysis and follow-up. Schüz et al. (2006) reported an update of this previous study on mobile phone subscribers in Denmark. Since this report has gained substantial media coverage as "proof" of no brain tumor risk from mobile phone use we will discuss the shortcomings of the study in more detail in the following.

The cohort was established for persons that some time during 1982–1995 were registered cellular telephone users and has now been followed against the Danish Cancer Registry until 2002, seven years more than in the previous study. Previously (Johansen et al, 2001) 9 persons with brain tumors had used GSM phones for > 3 years, and OR =1.2 was reported. Now, data were not provided for type of phone or years of use. Rather the calculation of latency was based on first year of registration.

During early 1980s almost all cellular telephones were used in cars with external antennae. These subjects were unexposed to electromagnetic fields (EMF). No information regarding such use is provided, and one may assume that such participants are now included as exposed although they were not. Over 200 000 (32 %) company subscribers were excluded from the cohort. These are the heaviest users and are billed 4.5 times more than the layman in Sweden. They started use the earliest, but were included in the "non-user" group, i.e., the general Danish population.

SIR among cellular telephone users was 1.21 for temporal glioma (Schüz et al 2006), a region most exposed to EMF, based on 54 persons and not on phone type or time of first use (latency period). No information regarding the ear used and correlation with tumor site was given. The expected numbers were based on the general population. Because a large part of the population uses mobile phones and/or cordless phones, and the latter use was not assessed at all in the study, there is no truly unexposed group for comparison. Risk of cancer was underestimated, e.g., in the group with first use $\geq$ 10 years, the associated risk for brain tumors was low (SIR =0.7, 95 % CI = 0.4- 0.95). Relying on private cellular network subscription as measure of mobile phone use has been questioned (Ahlbom et al 2004, Funch et al 1996).

There seems to be a "healthy worker" effect in the study because of the decreased overall cancer risk (SIR= 0.9, 95 % CI = 0.9-0.95). Of the subscribers 85 % were men and 15 % women. Certainly early mobile phone users are not socioeconomically representative of the whole Danish population, used for comparison. The cohort only included people > 18 years of age. We reported (Hardell et al 2004, 2006a,b) that cellular telephone use beginning before age 20 is associated with a higher risk of brain tumours than use starting after age 20.

The authors do not acknowledge the contribution by the telecom industry as cited in the first publication (Johansen et al 2001), i.e., TelemarkDanmarkMobil and Sonofom. Two of the authors are affiliated with the private International Epidemiology Institute, Rockville, MD, USA, which has contributed financially to the study. Where the International Epidemiology Institute gets its money from is not declared. In the application to the Danish National Mobile Phone Program, which funded part of the study, no mention of the involvement or payment of these two consultants was made, a fact that is now being set under question.

Regarding the case-control studies there seems to be a consistent pattern of an increased risk for acoustic neuroma using a 10-year latency period and considering ipsilateral exposure. It might be a "signal" tumour type for increased brain tumour risk from microwave exposure, since it is located in an anatomical area with high exposure during calls with cellular or cordless phones (Hardell et al, 2003). Christensen et al (2004) found no association using a ≥ 10 year latency period, but the result was based on only 2 cases. Interestingly, the tumours were significantly larger in the total group of regular mobile phone users.

 In our study we found an increased risk also with shorter latency period than 10 years (Hardell et al 2006a). However, it is not known at what stage in the carcinogenesis microwaves act. An effect might exist at different stages both of promoter and initiator type. We conclude that the results on acoustic neuroma are consistent with an association with use of cellular phones using a latency period of ≥ 10 years.

Regarding meningioma no consistent pattern of an association was found, although ipsilateral exposure in the ≥ 10 years latency group increased the risk in the meta-analysis. For a definite

conclusion longer follow-up studies are needed. We conclude that the results are not consistent with an association between use of mobile phones and meningioma.

Malignant brain tumours have been studied in 8 case-control studies. One study was register based and showed an increased risk associated with analogue phone use although the latency period seemed to be short (Auvinen et al 2002). The risk of glioma increased significantly per year of use. Five studies gave results for use of cell phone for 10 years or more. The pattern of an association was consistent in the different studies, except for the Danish study by Christensen et al (2005). In that study all 17 odds ratios for high-grade glioma were < 1.0 indicating systematic bias in assessment of exposure.

Our meta-analysis showed a significantly increased risk for ipsilateral use. We conclude that using ≥ 10 years latency period gives a consistent pattern of an association between use of mobile phones and glioma.

Regarding the Interphone studies the German part (Schüz et al 2006) was commented on by Morgan (2006) and these comments may also apply to the other Interphone studies. Morgan noted that the definition of a "regular" cell-phone user was so minimal that almost all "regular" cell-phone users would not be expected to be at risk, even if cell-phone use was found to create very high risks of glioma and meningioma. As for longer periods of "regular" cell-phone use, Schüz et al (2006) reported that only 14 percent of the glioma cases and 6 percent of the meningioma cases had used a cell phone for 5 years or more. For 10 years or more, the percentages were 3 percent and 1 percent, respectively. The authors replied that even long-term users in the study had barely more than 10 years of regular use and, in the beginning, were not heavy users; hence, they could not draw conclusions on heavy long-term use.

Methodological issues in the Interphone studies have been also discussed by Vrijhed et al (2006a,b). It was concluded that actual use of mobile phones was underestimated in light users and overestimated in heavy users. Random recall bias could lead to large underestimation in the risk of brain tumours associated with mobile phone use. According to the authors there was a selection bias in the Interphone study resulting in under selection of unexposed controls with decreasing risk at low to moderate exposure levels. Some of the Interphone studies had a low response rate, especially among controls giving potential selection bias.

A formal meta-analysis on mobile phone use and intracranial tumors was performed by Lahkola et al (2006). No data were given for $\geq$ 10 year latency period. Overall the risk increased for ipsilateral tumors, OR = 1.3, 95 % CI = 0.99-1.9 whereas no increased risk was found for contralateral tumors, OR = 1.0, 95 % CI = 0.8-1.4.

V. Conclusions

In summary we conclude that our review yielded a consistent pattern of an increased risk for acoustic neuroma and glioma after $\geq$ 10 years mobile phone use. We conclude that current standard for exposure to microwaves during mobile phone use is not safe for long-term brain tumor risk and needs to be revised.

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Table. Summary of 20 studies on the use of cellular telephones and brain tumour risk. For further details, see Hardell et al (2007). Odds ratio (OR), 95 % confidence interval (CI) and standardised incidence ratio (SIR) are given.

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Hardell et al 1999, 2001 Sweden	1994-1996 Case-control	20-80 years	Brain tumours	217	OR 1.0 (0.7-1.4)	Analogue and digital cell phone use
				34	OR 1.1 (0.6-1.8)	Ipsilateral
				16	OR 1.2 (0.6-2.6)	> 10 year latency, analogue cell phone
Muscat et al 2000 USA	1994-1998 Case-control	18-80 years	Brain tumours	17	OR 0.7 (0.4-1.4)	Mean duration of use, 2.8 years
			Neuorepithelioma	35	OR 2.1 (0.9-4.7)	
Johansen et al 2001 Denmark	1982-1995 Cohort	0 to > 65 years	Brain tumours	20	SIR 1.3 (0.8-2.1)	Analogue and digital cell phone use
				9	SIR 1.2 (0.6-2.3)	≥ 3 years duration of digital subscription
Inskip et al 2001 USA	1994-1998 Case-control	≥ 18 years	Acoustic neuroma	5	OR 1.9 (0.6-5.9)	≥ 5 years of cell phone use
			Glioma	11	OR 0.6 (0.3-1.3)	
			Meningioma	6	OR 0.9 (0.3-2.7)	
Muscat et al 2002 USA	1997-1999 Case-control	≥ 18 years	Acoustic neuroma	11	OR 1.7 (0.5-5.1)	3-6 years of cell phone use
Auvinen et al 2002 Finland	1996 Case-control, register based	20-69 years	Glioma	119	OR 1.5 (1.0-2.4)	Analogue and digital cell phone "ever" use
				40	OR 2.1 (1.3-3.4)	Analogue cell phone "ever" used
				11	OR 2.4 (1.2-5.1)	Analogue cell phone use 1-2 years
				11	OR 2.0 (1.0-4.1)	Analogue cell phone use, >2 years
Lönn et al 2004 Sweden Interphone	1999-2002 Case-control	20-69 years	Acoustic neuroma	12	OR 1.8 (0.8-4.3)	≥10 years of cell phone use, result for either side of head
				12	OR 3.9 (1.6-9.5)	≥10 years of cell phone use on same side of head as tumour

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Christensen et al 2004 Denmark Interphone	2000-2002 Case-control	20-69 years	Acoustic neuroma	45	OR 0.9 (0.5-1.6)	Regular use
				2	OR 0.2 (0.04-1.1)	$\geq$ 10 years cell phone use on same side of head as tumour. Significantly larger tumours among cellular phone users 1.66 cm^3 *versus* 1.39 cm^3, p=0.03.
Lönn et al 2005 Sweden Interphone	2000-2002 Case-control	20-69 years	Glioma	214	OR 0.8 (0.6-1.0)	Regular use
				15	OR 1.6 (0.8-3.4)	$\geq$10 years since first "regular" cell phone use on same side of head as tumour
				11	OR 0.7 (0.3-1.5)	$\geq$10 years since first "regular" cell phone use on opposite side of head as tumour.
			Meningioma	118	OR 0.7 (0.5-0.9)	Regular use
				5	OR 1.3 (0.5-3.9)	$\geq$10 years since first "regular" cell phone use on same side of head as tumour
				3	OR 0.5 (0.1-1.7)	$\geq$10 years since first "regular" cell phone use on opposite side of head as tumour.

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Schoemaker et al 2005 Denmark, Finland, Sweden, Norway, Scotland, England, Interphone	1999-2004 Case-control	18-69 years (variable)	Acoustic neuroma	360	OR 0.9 (0.7-1.1)	Regular use
				23	OR **1.8** (1.1-3.1)	≥ 10 lifetime years of cell phone use on same side of head as tumour
				12	OR 0.9 (0.5-1.8)	≥ 10 lifetime years of cell phone use on opposite side of head as tumour
Christensen et al 2005 Denmark Interphone	2000-2002 Case-control	20-69 years	Low-grade glioma	47	OR 1.1 (0.6-2.0)	Regular use
				9	OR 1.6 (0.4-6.1)	≥10 years since first regular use of cell phone
			High-grade glioma	59	OR 0.6 (0.4-0.9)	Regular use
				8	OR 0.5 (0.2-1.3)	≥10 years since first regular use of cell phone 17 odds ratios for high-grade glioma, all < 1.0, indicates systematic bias
			Meningioma	67	OR 0.8 (0.5-1.3)	Regular use
				6	OR 1.0 (0.3-3.2)	≥10 years since first regular use of cell phone
Hepworth et al 2006 UK Interphone	2000-2004 Case-control	18-69 years	Glioma	508	OR 0.9 (0.8-1.1)	Regular use
				NA	OR 1.6 (0.9-2.8)	≥10 years of cell phone use on same side of head as tumour.
				NA	OR 0.8 (0.4-1.4)	>10 years of cell phone use on opposite side of head as tumour.

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Schüz et al 2006 Germany Interphone	2000-2003 Case-control	30-59 years	Glioma	138	OR 1.0 (0.7-1.3)	Regular use
				12	OR 2.2 (0.9-5.1)	≥ 10 years since first regular use of cell phone
				30	OR **2.0** (1.1-3.5)	Female regular use of cell phone
			Meningioma	104	OR 0.8 (0.6-1.1)	Regular use
				5	OR 1.1 (0.4-3.4)	≥ 10 years since first regular use of cell phone

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Hardell et al 2006a Sweden	1997-2003 Case-control	20-80 years	Acoustic neuroma	130	OR **1.7** (1.2-2.3)	> 1 year latency of cell phone use
				20	OR **2.9** (1.6-5.5)	> 10 years latency of cell phone use
				10	OR **3.5** (1.5-7.8)	> 10 years of ipsilateral cell phone use
				4	OR 1.0 (0.3-2.9)	> 10 years latency of cordless phone use
				3	OR 3.1 (0.8-12)	> 10 years latency of ipsilateral cordless phone use
			Meningioma	347	OR 1.1 (0.9-1.3)	> 1 year latency of cell phone use
				38	OR 1.5 (0.98-2.4)	> 10 years latency of cell phone use
				15	OR 2.0 (0.98-3.9)	> 10 years latency of ipsilateral cell phone use
				23	OR 1.6 (0.9-2.8)	> 10 years latency of cordless phone use
				9	OR **3.2** (1.2-8.4)	> 10 years latency of ipsilateral cordless phone use
Hardell et al 2006b Sweden	1997-2003 Case-control	20-80 years	Glioma, high-grade	281	OR **1.4** (1.1-1.8)	> 1 year latency of cell phone use
				71	OR **3.1** (2.0-4.6)	> 10 years latency of cell phone use
				39	OR **5.4** (3.0-9.6)	> 10 years latency of ipsilateral cell phone use
				23	OR **2.2** (1.3-3.9)	> 10 years of cordless phone use
				10	OR **4.7** (1.8-13)	> 10 years latency of ipsilateral cordless phone use
			Glioma, low-grade	65	OR 1.4 (0.9-2.3)	> 1 year latency of cell phone use
				7	OR 1.5 (0.6-3.8)	> 10 years latency of cell phone use
				2	OR 1.2 (0.3-5.8)	> 10 years latency of ipsilateral cell phone use
				5	OR 1.6 (0.5-4.6)	> 10 years latency of cordless phone use
				3	OR 3.2 (0.6-16)	> 10 years latency of ipsilateral cordless phone use

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Takebayashi et al 2006 Tokyo Interphone	2000-2004 Case-control	30-69 years	Acoustic neuroma	51	OR 0.7 (0.4-1.2)	Regular use
				4	OR 0.8 (0.2-2.7)	Length of use > 8 years
				20	OR 0.9 (0.5-1.6)	Ipsilateral use
Schüz et al 2006 Denmark	1982-2002 Cohort	>18 years	Glioma	257	SIR 1.0 (0.9-1.1)	420 095 telephone subscribers
			Meningioma	68	SIR 0.9 (0.7-1.1)	
			Nerve sheat tumors	32	SIR 0.7 (0.5-1.0)	
			Brain and nervous system	28	SIR 0.7 (0.4-0.95)	Latency $\geq$ 10 years
Lahkola et al 2007 Denmark, Norway, Finland, Sweden, UK Interphone	September 2000-February 2004 (differed between countries) Case-control	20-69 years (Nordic countries), 18-59 years (UK)	Glioma	867	OR 0.8 (0.7-0.9)	Regular use
				77	OR 1.4 (1.01-1.9)	Ipsilateral mobile phone use, $\geq$ 10 years since first use, p for trend = 0.04
Klaeboe et al 2007 Norway Interphone	2001-2002 Case-control	19-69 years	Glioma	161	OR 0.6 (0.4-0.9)	Regular use
			Meningioma	111	OR 0.8 (0.5-1.1)	
Schlehofer et al 2007 Germany Interphone	2000-2003 Case-control	30-69 years	Acoustic neuroma	29	OR 0.7 (0.4-1.2)	Regular use

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SECTION 10 – Part 2

EVIDENCE FOR BRAIN TUMORS

(EPIDMIOLOGICAL)

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TABLE OF CONTENTS

I.   INTRODUCTION

Primary central nervous system (CNS) tumors are a heterogeneous group of benign and malignant neoplasms localized in the brain, the spinal cord and their coverings. They differ in histological type, tissue of origin, anatomic site, growth pattern, age distribution, sex ratio, clinical appearance and many other features including molecular neuropathological markers. These features are not independent but little is known about the etiology of these tumors and the reason for the observed epidemiological patterns. The rapidly developing field of molecular neuropathology may provide clues to solve these problems in the future.

Brain tumors, accounting for the majority of CNS tumors, are rare. Annually about 36,000 36000 new cases are diagnosed in the US and about 180,000 180000 world-wide. The age distribution has two peaks: incidence is about 35 cases per million per year below 10 years of age (which is mainly due to tumors originating from mesodermal and embryonic tissues, medulloblastoma and astrocytoma of the juvenile pilocytic type), and after age 15 there is a steady increase of incidence with increasing age reaching its second peak of about 200 cases per million per year at an age around 75 years. The burden of CNS cancers is distinctly higher in children making up around 20% of all childhood malignancies, while in adults less than 2% of all cancers are primary brain cancers.

There are some rare cases of inherited cancer syndromes (e.g. von Hippel-Lindau disease, Li-Fraumeni syndrome) that are related to brain tumor risk, accounting for a small fraction of cases. Except for therapeutic x-rays no environmental or lifestyle life-stile factor has unequivocally been established as risk factor for brain tumors. Non-whites Non whites seem to have lower risk, and incidence tends to be higher with increasing socio-economic status. However, because of the rather advanced age of 75 of peak incidence, such differences may partly be due to differences in life-expectancy. During the last decades some types of brain tumors show a steady increase of a few percent per year, which might to some extent be related to the introduction of computed tomography and other high-resolution neuroimaging methods.

Since the report of Wertheimer and Leeper in 1979 of an increased incidence of brain tumors in children living in homes with an expected higher exposure to power-frequency electric and magnetic fields, exposure to electromagnetic fields have become an area of interest in the study of factors affecting brain tumor risk.

This review focuses on the radio frequency (RF) part of the electromagnetic spectrum (3 kHz to 300 GHz). However, because the epidemiology of mobile phone use is covered in another section, it will be restricted to RF exposure conditions other than microwaves from mobile phone use. Exposure to ELF magnetic fields and childhood brain tumors is covered in the chapter about childhood cancers.

II.   Material and Methods

Published articles of relevant studies restricted to the last 20 years were obtained by searching PubMed using the following terms:
("radio frequency" OR electromagnetic* OR microwaves) AND ("brain cancer" OR brain tumor* OR "CNS cancer" OR CNS tumor* OR glioma* OR meningioma* OR neuroma*) NOT ("power frequency" OR "low frequency") AND epidemiology

The search resulted in 101 hits. After removing reviews and animal or in vitro studies as well as studies of mobile phone use, 8 articles remained. A hand search in review papers (Krewski et al. 2001; Elwood 2003; Ahlbom et al. 2004; Kundi et al. 2004) and reference lists of the articles found in PubMed revealed another 7 papers; hence the final body of evidence consists of 15 studies of exposure to various types of RF fields.

Of the 15 studies 8 were cohort studies, 3 case-control studies and 4 of an ecological type. The majority (11) were occupational studies, two studies investigated children, and one ecological study investigated adults and one study both, adults and children.

III.   Epidemiological studies of RF fields and brain tumors

Table 1 gives an overview of the 15 studies obtained by the literature search with respect to study type, assessment of exposure and outcome, confounders considered and matching variables used, number of cases included and selection method of study participants. Results are summarized in Table 2.

In the following paragraphs each study is briefly discussed with respect to its strengths and weaknesses.

A. Thomas et al. 1987

This case-control study included 435 deaths from brain or CNS tumors and 386 deaths from other causes as controls. Only adult males were included. Basis of data collection on occupational history were interview with next-of-kin. Two methods of classification were used: one method assigned subjects to one of three categories (never exposed to RF/ever exposed to RF in an electrical or electronics job/ever exposed to RF but not in an electrical or electronics job), the other method consisted in a classification of each job by an industrial hygienist hyginiest for presumed exposure to RF, soldering fumes, and lead. Both methods revealed significantly increased brain tumor risks of presumed occupational exposure to RF fields. This increase was due to an association in electronics and electrical jobs with astrocytic tumors as the predominant outcome associated with employment in these categories. In addition a significant increase of brain tumor risk was found for increasing duration of exposure.

Although relying on information of next-of-kin could be a source of misclassification, one strength of this study is it's its relying on occupational history only that could be assumed to be more accurate than recall of exposure to various agents. The two methods of classification led to almost the same results, which lends support to the hypothesis that indeed exposure in electrical and electronics jobs is associated with an increased brain tumor risk. Due to the strong relationship between RF exposure and exposure to lead, solvents or soldering fumes in these jobs, it is not possible to separate effects of these exposures. However, analysis of exposure to lead did not show a consistent relationship with brain tumor risk, indicating that it may not confound the relationship to RF exposure.

Because this study is of dead cases only it is likely over-representing high grade brain tumors that may not all be associated with exposure which leads to an effect dilution. Exposure misclassification, if it is non-differential in cases and controls, also tends to reduce effect estimates.

A weakness of this study is obviously its lack of an exposure indicator other than the occupational category. While there is no doubt that in these jobs some exposure to RF fields occur quite regularly, specific characteristics including frequency ranges, modulation, intensity, duration and distance from the source vary considerably. Overall the study (as well

as two earlier ones outside the search window: Lin et al. 1985 and Milham 1985) are sufficient to formulate a research hypothesis that can be tested in appropriately designed subsequent investigations. Unfortunately such studies have never been conducted.

B. Milham 1988

In this cohort study of 67,829 amateur radio operators holding a license within 1/1979 to 6/1984 in Washington and California 29 brain tumor deaths occurred during the follow up period with 21 expected.

It should be noted that there was a substantial and statistically significant lower number of overall deaths of less than three quarters of deaths expected from country mortality rates. This could be due to both a 'healthy-worker' effect as well as an effect of socio-economic status. In lieu of computing standardized mortality ratios (SMR) it may be instructive to look at the proportional mortality rates in the reference population and the amateur radio operators: 0.6% of all deaths are expected to be due to brain tumors in the reference population while in amateur radio operators twice as many occurred (1.2%). Whether or not this is an indication of an increased brain tumor risk due to RF exposure is difficult to assess. First of all this study is a register only investigation and no information on intensity, frequency and duration of engagement in amateur radio operations are available. In a later analysis the author reported about results using a proxy of intensity and duration of exposure: the license class. In this analysis indications of an increase of risk with increasing license class were obtained.

This study could and should have started off a thorough follow up of amateur radio operators and nested case-control studies to address the problem of potential confounders and to narrow down the conditions that may be responsible for the increased mortality from some cancers. It is another loose end that leaves us without a clear message.

Although no risk factor for brain cancer except therapeutic ionizing radiation is known, there are some indications that risk increases with social class. The reason for this association is unknown but life-style factors may play a role as well as concomitant causes of death that could lead to a spurious reduction of risk in lower class populations because brain tumors have their peak close to life-expectancy.

C. Selvin et al. 1992

The objective of this investigation was not primarily to study the relationship between RF exposure and childhood cancer but to address the general problem of how to assess disease incidence or mortality in relation to a point source. As the point source the Sutro Tower in San Francisco, the only microwaves emitting tower in this county, was chosen. A total of 35 brain tumor deaths occurred among 50,686 white individuals at risk aged less than 21 in the years 1973-88 in an area of approximately 6 km around the tower. The exact location of residence could not be obtained; therefore each case was located in the center of the census tract. Different methods of analysis were applied to assess a potential relationship between distance from the tower and brain tumor risk. Relative risk for brain tumors for a distance less than 3.5 km from Sutro Tower compared to more than 3.5 km was 1.162 and not significant.

The study explored different methodological procedures and has its merits from a methodological point of view. However, it starts from the wrong assumption: that distance to a point source is a valid proxy for intensity of exposure. Under ideal conditions of spherical symmetry of an emission this assumption holds, however, there are almost no real life situations where this assumption is sufficiently close to actual exposure levels. And it is definitely not true for the Sutro Tower. Radiations from the antennae are directed towards the horizon and the complex pattern of emission with main and side lobes results in a complex pattern of RF exposure at ground level. Furthermore, the area is topographically structured with hills and valleys such that areas of high exposure at the vertices are in close proximity to areas of low exposure at the shadowed side downhill.

Studying the relationship between a point source and disease is not only difficult due to the complex relationship between distance and exposure but also because of the fact that humans are not stable at a certain location. This is of greater importance for adults who may commute from and to work places and have generally a greater radius of activity as compared to children. Nevertheless, there is at least a high chance of one long-lasting stable location that is when people sleep in their beds. Therefore, studies in relation to a point source should attempt to assess exposure at the location of the bed. Because the objective of this study was not the assessment of a potential brain tumor risk but the application of methods for the analysis of spatial data, no attempts were made to measure actual exposure.

D. Tynes et al. 1992

In this study information on occupations obtained for all Norwegians every 10 years was used to assess cancer incidence in relation to job titles. In 1960 37,945 male workers were identified that had jobs with possible exposure to EMFs and among these 3,017 with possible RF exposure. Overall 119 brain tumor cases were found in the cancer registry between 1961 and 1985. Of these cases 6 occurred in the subgroup of workers possibly exposed to RF fields. The overall expected number of brain tumor cases was 109 and 12 for the subgroup with possible RF exposure. Hence no increased brain tumor risk could be detected.

Despite the long follow-up period of 25 years with an accumulated number of 65,500 person-years the expected number of brain tumors diagnosed during that period is too low to detect a moderately elevated risk of 1.3 to 1.5.

As mentioned above, all studies solely relying on job titles lead to exposure misclassification and, therefore, to a dilution of risk. For dichotomous exposure variables (exposed/not exposed) and assuming a negligibly small proportion of exposed in the reference population standardized incidence ratios (SIR) are biased by a factor $(1+f*(SIR-1))/SIR$, if f denotes the fraction of true exposed and SIR is the true incidence ratio. Hence a true SIR of 2.0 is reduced to 1.5 if only 50% in the cohort are actually exposed. The observed SIR is further reduced if the assumption of a negligible fraction of exposed in the reference population is wrong. In this case the bias factor given above is further divided by $(1+g*(SIR-1))$, where g is the fraction of exposed in the general population.

While a cohort study that is based on registry data has the advantage of independence from recall errors and selection bias due to possible differential participation, it has the disadvantage that registry data are generally insufficient to provide reliable exposure indicators. While no association with brain tumors could be detected in this study it revealed an increased number of leukemia cases in occupations with possible RF exposure. This could be due to the higher incidence of leukemia or to a stronger association or to different latency periods and various other reasons including chance.

E.   Grayson 1996

In this case-control study nested within approx. 880,000 US Air Force personnel with at least one years of service during the study period of 1970-89 primary malignant brain tumor cases were ascertained by screening hospital discharge records. The study included only males and only as long as they were on Air Force records. From 246 cases detected 16 were dropped due to incomplete or ambiguous data. For each case four controls were randomly selected from the case's risk set matching it exactly on year of birth and race. Controls who were diagnosed with diseases that may be associated with EMF exposure (leukemia, breast cancer, malignant melanoma) were excluded from the risk set.

One strength of this study is the detailed job history filed for each cohort member that could be used for retrospective exposure assessment. Furthermore, Air Force files contained detailed data from personal dosímetry on ionizing radiation for the different posts and jobs. Classification of RF field exposure was based on a detailed job exposure matrix with over 1,950 entries, indexing 552 different job titles. One source of classification was recorded events of exposure to RF fields above 100 W/m2. By this method probable exposure was assigned if for a job such events were recorded in the past as well as for closely related jobs. Possible exposure was assigned for jobs that required operation of RF emitters but without recorded overexposure.

A further strength is the thorough consideration of possible confounders. Because of the possible relationship of brain tumor risk with socio-economic status (SES), military rank was used as a surrogate for SES and included in the analysis as well as ionizing radiation exposure that has previously been shown to increase brain tumor risk.

Exposure to RF fields was associated with a moderate but statistically significant increased risk of OR=1.39. Investigation of duration of exposure was compromised by an ambiguity introduced by the calculation of an exposure score as the product of exposure and months. Nevertheless, for those ever exposed there were indications of an increasing risk with increasing exposure duration.

A weakness of this investigation is its incomplete follow-up of cohort members. This could have resulted in an underestimation of the true risk. Leaving the Air Force could have been

more likely in those exposed to RF fields and developing a brain tumor. Some malignant brain tumors have early signs that could be incompatible with the Air Force job especially if involving operation of RF equipment (like seizures, severe headaches, somnolence, and absences). Because the study did not involve personal contact it is free of other selection biases.

F. Szmigielski 1996

In this military cohort study of cancer morbidity Polish military career personnel was assessed for occupational exposure to RF fields based on service records. The study covered 15 years (1971-85) including approx. 128,000 persons per year. Expected rates for 12 cancer types were calculated based on the age specific morbidity in those classified as unexposed.

For brain and nervous system tumors a significantly increased ratio of observed to expected (OER=1.91) was found. Other malignancies with significantly increased incidence in exposed were: esophageal and stomach cancers, colorectal cancers, melanoma, and leukemia/lymphoma.

One strength of this study is its substantial size with almost 2 million person-years of follow-up. Furthermore, accurate military records on job assignment and on exposure from military safety groups gives a unique opportunity to assess long-term exposure effects based on already filed data.

Some important data are missing because they were military classified information that could not be provided in the paper. This includes the exact number of cases of the different neoplasms. However, from the data presented an observed number of brain tumors of about 46 can be calculated.

The study has been criticized for an alleged bias because more information on risk factors was available for cancer cases. It is true that military medical boards collected data for cases such as life style factors and exposure to possible carcinogens during service, however, at no stage this information entered the analysis. Therefore, this criticism is unfounded. Such information could have been utilized within a nested case-control study applying the same methods of assessment of risk factors for controls as has been done for cases. Because some findings, such as the increased risk for esophagus/stomach cancer, that are rarely reported in relation to

RF exposure warrant further study, such a nested case-control approach is recommended. It could, albeit with some difficulties, even be successfully conducted retrospectively.

    G.   Hocking et al. 1996

In an ecological study cancer incidence and mortality in nine municipalities of northern Sydney during 1972-90 three of which surround three TV towers were assessed. Population size in the three municipalities located within a radius of approximately approx. 4 km around the TV towers amounts to 135,000 while population size in the six municipalities further away was 450,000. High-power transmission commenced in 1956, an additional 100 kW transmission started in 1965 and another 300 kV broadcast in 1980. Carrier frequencies varied between 63 and 533 MHz for TV broadcasting and was around 100 MHz for FM radio broadcast.

During the study period 740 primary malignant brain tumors were diagnosed in adults and 64 in children, 606 deaths due to brain cancer occurred in adults and 30 in children. While incidence of lymphatic leukemia was significantly higher in adults as well as in children inhabiting the three municipalities surrounding the transmission towers compared to the six districts further away, brain tumor incidence was not significantly elevated (RR=0.89 in adults and 1.10 in children).

As has been stated above, distance from a transmitter is a poor proxy for exposure. Some measurements done in the study area obtained levels much lower than those calculated from the emission power and antenna gain. Several factors are responsible for this effect: multiple reflections, attenuation by buildings and vegetation, ground undulations, non-coincidence of maxima for the different signals as well as complex radiation characteristics of the broadcast antennae.

The exact location of the residence of cases could not be provided which reduces the potential of the study to relate incidences to measurements or calculations of RF fields. Authors discussed some potential sources of bias such as migration and other exposures in the different regions. However, the most important disadvantage in such studies is that individual risk factors cannot be adjusted for. Both spurious positive as well as false negative results can be obtained by disregarding such individual variables.

H.   Tynes et al. 1996

In a historical cohort study 2,619 Norwegian female radio and telegraph operators certified between 1920 and 1980 were followed from 1961 through 1991 for entries in the cancer registry. During this period a total of 140 cases of cancer occurred which are about 20% more than expected from the Norwegian population. Among these were 5 brain tumor cases closely matching the number expected.

An excess for breast cancer was found in this study that may be related to a combination of RF field exposure and night work. For other cancers including brain cancer numbers of cases were too low to address exposure risk.

In this very thoroughly conducted study including a nested case-control approach for breast cancer, measurements at historical transmitters on ships, comparison with women at other jobs on sea, brain tumors were not distinctly higher than expected from the reference population. However, because of the limited cohort size a moderately increased risk cannot be excluded.

I.   Dolk et al. 1997a

This ecological small area study of cancer incidence 1974-86 near the Sutton Coldfield TV/radio transmitter at the northern edge of the city of Birmingham (England) was initiated by an unconfirmed report of a 'cluster' of leukemias and lymphomas. The transmitter came into service in 1949. Transmission at 1 megawatt (effective radiated power erp) began in 1964, at 3 MW in 1969, and at 4 MW in 1982. The tower has a height of 240 m with no big hills in the surrounding area. The study area was defined by a circle of 10 km radius centered at the transmitter. The population within this area was about 408,000. All cancers, excluding non-melanoma skin cancer, were considered focusing on hematopoietic and lymphatic cancers, brain and nervous system cancers, eye cancer, and male breast cancer. Childhood cancers were restricted to all cancers and all leukemias.

In the study area a small but significant excess of all cancers was observed in adults. All leukemias and non-Hodgkin's lymphoma were particularly elevated and incidence within 2 to

4 km from the tower was about 30% higher than expected. Brain tumors were only analyzed for distances of within 2 km and the whole study area. Within 2 km an increased OER of 1.29 for all brain tumors and 1.31 for malignant brain tumors was calculated based on 17 and 12 cases, respectively.

Also this investigation suffers from using distance from the tower as proxy for intensity of exposure. The wrong assumption that exposure decreases with increasing distance invalidates the statistical trend test applied. Measurements conducted in the study area revealed the poor relationship with distance but without consequences on the evaluation of the data. Overall the study is consistent with a moderately increased risk of hematopoietic and lymphatic cancers as well as some other cancers including brain cancer in the vicinity of high-power transmitters that, if related to RF fields, must be substantially higher for actual exposure.

The Sutton Coldfield study was later continued (Cooper & Saunders 2001) to cover the period 1987-94. The study revealed, compared to the earlier period, an almost unchanged increase of leukemias and non-Hodgkin's lymphoma in adults and a slight increase in children.

J.   Dolk et al. 1997b

Because the Sutton Coldfield study was triggered by a cluster report and to provide independent test of hypotheses arising from that study, similar methods as applied in the previous study were used to study all high-power TV/radio transmitters ($\geq$ 500 kW ERP) in Great Britain. In adults leukemias, bladder cancer, and skin melanoma, and in children, leukemias and brain tumors were studied. The study period was 1974-86 for England and somewhat shorter in Wales and Scotland.

Although population density around transmitters was not always as high as in the case of the Sutton Coldfield tower, with an average population density of only about one third of that around Sutton Coldfield tower within 2 km from the towers, in the most important range of 2 to 4 km from the transmitters, where in many cases the maximum of radiated RF at ground level is reached, population density was similar. The study of all high-power transmitters essentially corroborated the findings for adult leukemias with an increase of incidence between 10 and 50% in the distance band of 2 to 4 km from the transmitters for the different transmitter types. Most of these increased incidences were statistically significant.

For children only the incidence in the whole study area and within a distance of 2 km was calculated, which is unfortunate because the area close to the towers is sparsely populated and exposure is low. Number of brain tumors in children was slightly above expectation (244 observed and 231 expected).

In contrast to the interpretation by the authors, the study of all high power transmitters essentially replicated and supported the findings of an excess incidence of leukemias in relation to RF emission from TV/radio towers. Because the different heights and radiation characteristics of the transmitters result in different exposure patterns at ground level, the consistent increase in an area that is likely close to the maximum of exposure supports the hypothesis of an association.

K. Lagorio et al. 1997

A mortality study of a cohort of 481 female plastic-ware workers employed between 1962-92 in an Italian plant, 302 of which were engaged in the sealing department with exposure to RF fields, was reported by Lagorio et al. (1997). For RF-sealers 6,772 person-years of follow-up were accumulated and overall 9 deaths occurred, 6 of which were from malignant neoplasms (which are twice as many as expected from comparison with the local reference population). In the 31 years only one brain cancer occurred but only 0.1 were expected.

Although the small size of the cohort and the potential exposure to other agents except RF fields such as solvents and vinyl chloride prohibit far reaching conclusion, much more of such thorough follow-up studies of exposed cohorts are needed to accumulate a body of evidence that can provide a useful basis for analysis.

L. Finkelstein 1998

A preliminary study intended to form the basis for an assessment of cancer risks associated with handheld radar devices was conducted among a cohort of 20,601 male Ontario police officers. The retrospective follow up covered the period of 1964-95. By linkage with the cancer registry and mortality database 650 cases of cancer were detected.

Testicular cancer and melanoma showed an excess incidence while overall cancer incidence was reduces as expected from a working cohort. Overall 16 cases of primary malignant brain tumors occurred which are slightly less than expected.

The author had difficulties to build up a proper cohort because some departments refused to participate and others couldn't spare the time to provide lists of all officers employed during the target period. Furthermore, while cancer sites of primary interest showed actually an increased incidence calling for a nested case-control approach, this study was never conducted due to lack of interest and support of the authorities.

M. Morgan et al. 2000

In an occupational cohort study all US Motorola employees with at least 6 months cumulative employment and at least 1 day of employment in the period 1976-96 were included. A total of 195,775 workers contributing about 2,7 million person-years were available for the study. The cohort was compared to the SSA Master Mortality File and the National Death Index to obtain vital status. Death certificates were obtained by states' vital statistics offices and company records. Exposure was assessed by expert opinion. Four RF exposure groups were defined with increasing level of estimated RF exposure. Only about 5% of the total cohort was classified as highly exposed and more than 70% with only background exposure. Neither private nor occupational mobile phone use was included.

Overall 6,296 deaths occurred in the cohort in 21 years, which were only two thirds of deaths expected from mortality data of the four countries where most Motorola facilities are located. This reduction is too pronounced to be solely due to a healthy worker effect, other factors such as higher SES must have contributed, an interpretation supported by the substantial reduction of mortality from all life-style associated causes of death. Internal comparisons were done for mortality from brain cancer and hematopoietic and lymphatic cancers. Brain tumor mortality was slightly but insignificantly elevated in high and moderately high exposed workers as compared to those with no or low RF exposure.

This study of a huge cohort demonstrates the limitations of such a study design. The majority of the cohort (58%) consisted of retired or terminated workers that may or may not accumulate further RF exposure at other companies. Furthermore, it can be assumed that

Motorola employees were among the first that used mobile phones at the workplace and privately. Neglecting mobile phone use may diminish the gradient of exposures between occupational groups studied. It would have been better to conduct nested case-control studies instead of using internal comparison that may be compromised by mobility bias, exposure misclassification and other sources of bias.

N. Groves et al. 2002

In this military cohort study of 40,581 men followed from the year of graduation (1950-1954) from Navy technical schools through 1997, known as the Korean War Veterans study, groups of sailors with imputed difference in likelihood and amount of exposure to radar waves were compared with respect to mortality. The original study, with a follow up through 1974, (Robinette et al. 1980) reported increased risks of cancer of the hematopoietic and lymphatic system, of the lung and digestive system for the high exposure group but was handicapped by the lack of information on date of birth of the cohort members. For the extended follow up study many missing birth dates were found in the Veterans Administration Master Index. Nevertheless, birth date remained unknown for over 8% of the cohort. Based on expert opinion low RF exposure was assigned to job classifications of radioman, radarman, and aviation electrician's mate, high exposure stratum included men with job classifications of electronics technician, aviation electronics technician, and fire control technician.

By matching against the Social Security Administration's Death Master File and the National Death Index 8,393 deceased subjects were identified through 1997. This number is substantially and significantly lower as expected from the male white US population. A healthy soldier effect may have been responsible for a lower mortality rate in the 1950ies but cannot explain the reduced mortality after 40 years. It has not been reported how long the cohort members stayed in service nor were life-style factors investigated; however, of more than 40% of the cohort no social security number could be obtained suggesting possible under-estimation of deaths.

Comparison of high- with low-exposure groups revealed significantly lower mortality from life-style associated causes of death (lung cancer, vascular diseases, diabetes mellitus, chronic obstructive pulmonary disease, liver cirrhosis) and significantly higher mortality from all leukemias and external causes of death. Increased mortality from leukemias was found in all high exposure groups but the most pronounced increase was observed in aviation electronics

technicians. Brain cancer was less frequent in all high exposure groups compared to the low exposure category.

The long period of follow up of this large cohort with start of follow up almost at the same time (1950-54) and at a time when exposure commenced is a great advantage of this investigation. However, there are a number of shortcomings: follow up was possibly incomplete by unknown social security number of a substantial proportion of the cohort; almost half of all deaths in the first 20 years were from external causes which could have obscured an effect of exposure; duration and intensity of exposure is unknown as well as potential exposure after leaving the Navy; classification into low and high exposure groups may introduce substantial misclassification. In the earlier report, inspection of Navy records for a sample from the high exposure group revealed that 24% had no exposure to radar waves at all.

Concerning brain tumors, assuming an effect of radar exposure on growth rate, exposure during the Korean War and no exposure afterwards would be expected to result in only a slightly increased risk during a period of about 10 years after the war. Sailors were about 20 to 25 years at that time. The fraction with an already initiated brain tumor during this age range is estimated to be less than 3 in 100,000 per year. Increase of growth rate even if substantial cannot result in an effect observable in a cohort of that size. If radar exposure increases the likelihood of malignant transformation this could increase the incidence during a time window of 10 to 20 years after the exposure period. Results of the Israeli study of x-ray treated tinea capitis (Sadetzki et al. 2005) suggest an even longer latency, however, risk decreased with increasing age at first exposure to x-rays. In addition, for malignant brain tumors there is a less pronounced relationship to ionizing radiation, and a higher risk was observed for meningioma that were not investigated in the Korean War Veterans study. Taking the data on ionizing radiation as a guiding principle for brain tumor initiation, radar exposure of sailors during their twenties might result in an increase of brain tumor mortality of about 10 to 15%, i.e. a maximum of 8 additional cases among 20,000. Considering the biases of the study such a low risk is easily obscured. Hence neither tumor promotion nor initiation may be detected in this study even if there is an increased risk. Because of the mentioned limitation to a certain time window with possibly increased incidence due to exposures during service in the Korean War, it would have been instructive to compute Kaplan-Meier estimates for cumulative brain tumor mortality.

N. Berg et al. 2006

In the German part of the Interphone study special attention was paid to occupational history and exposure to RF fields at workplaces. Incident meningioma (n=381, response rate 88%) and glioma cases (n=366, response rate 80%) aged 30-69 years were selected from four neurological clinics. Overall 1,535 (participation rate 63%) were randomly selected from population registries matched to the cases by sex, age, and region. Most cases were interviewed during their stay in hospitals, controls were interviewed at home. The interview contained several screening questions about occupations that are probably associated with RF exposure. If any of these screening questions were marked additional questions were asked about the job. Based on the literature and the evaluation by two industrial hygienists a classification into the following categories was performed: no RF exposure/not probably RF exposed/probably ER exposed/highly RF exposed. In total about 13% (299 cases and controls) were classified with at least possible RF exposure at the workplace. Analyses were adjusted for region, sex, age, SES, urban/rural residence, ionizing radiation exposure in the head/neck region. Mobile phone use was not considered as a confounder.

While overall RF exposure at workplaces showed no increased odds-ratios, high exposure and especially for durations of 10 years or more resulted in elevated risk estimates that were, however, not significant. This result was similar for meningioma (OR=1.55 for high exposure for 10 years or more) and glioma (OR=1.39).

The study tried to assess potential workplace exposure as precisely as possible in a personal interview, but still misclassification may have occurred especially in the probable and not probable categories while the high exposure group is likely to have had at least occasionally above average RF exposure. Odds ratios are in the range expected if exposure results in a substantial increase of growth rate. The small number of highly and long-term exposed cases (13 glioma and 6 meningioma) prohibit, however, far reaching conclusions.

IV. Evaluation of Evidence

Due to the varying endpoints, methods used and populations included and the small number of studies a formal meta-analysis is not possible. The following figure shows the results detailed in Table 2 in an easily comprehendible way.

Only few studies found clear indications of an association between RF exposure and brain tumors: one cohort study (Szmigielski 1996) and two case-control studies (Thomas et al. 1987, Grayson 1996). None of the ecological studies demonstrated a tendency for an increased risk in the vicinity of RF transmitters.

The discussion of the 15 published investigations revealed shortcomings in all studies. The greatest problem was encountered in the difficulties to reliably assess actual exposure. Even if we don't know the relevant aspect of the exposure, if any, that is responsible for an increased risk, the type, duration and amount of exposure must be determined in order to use the studies in derivations of exposure standards. None of the studies included a useful quantitative indicator of intensity of exposure and even duration of exposure was rarely addressed. Concerning type of exposure only quite crude and broad categories were used.
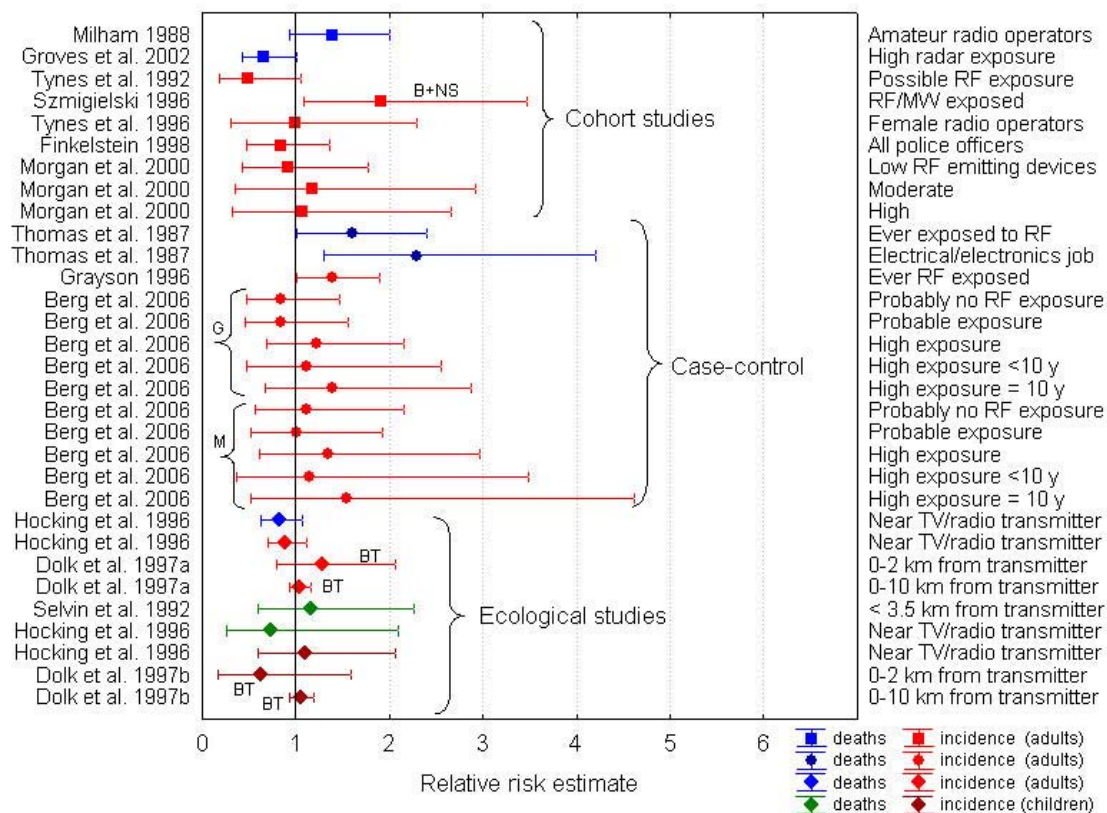
Fig. 1: Estimates of relative risk (and 95% confidence intervals) of various RF exposures with respect to brain tumors (B+NS…brain and nervous system tumors, BT…brain tumors, M…menigioma, G…glioma; all others primary malignant brain tumors)

In ecological studies, although for the studied population the exposure - despite considerable variations in time - is similar with respect to carrier frequency, modulation etc. it is quite different between various types of transmitters and hence results are not easily generalized. Considering the discussion of the different investigations and the fact that most biases encountered tend to dilute a potential risk, the compiled evidence from occupational cohorts is compatible with a moderately increased risk of RF exposure. Because of the lack of actual measurements but observing that exposure above guideline levels must have been a rare event a precautionary approach must result in a reduction of occupational exposure levels and organizational measures to avoid over-exposure. Although brain tumors are rare and the population attributable risk is low (assuming 13% of adults being occupationally exposed to RF fields as inferred from Berg et al. 2006, and assuming a relative risk of 1.3, about 4% of brain tumors can be attributed to RF exposure, i.e. 1,350 cases per years in the US).

V.   EVALUATION OF CANCER-RELATED ENDPOINTS (RF EXPOSURE)

A. Assessment of Epidemiological Evidence by IEEE (C95.1 Revision)

In their 2006 revision of the standard C95.1 IEEE has assessed the evidence from epidemiology for cancer related endpoints in chapter B.7.3. The assessment relies mainly on the reviews of Bergqvist (1997), Moulder et al. (1999) and Elwood (2003). These reviews and the IEEE overview share the same deficiencies. The main lines of argumentation would be impossible in any other field of environmental health and closely resemble the strategy used to dismiss a power frequency exposure/childhood leukemia association. In the following paragraphs the assessment by IEEE will be briefly discussed.

> *Cluster studies, such as the one performed in Sutton Coldfield in the U.K. in response to a cluster of leukemia and lymphoma in adults living close to an RF broadcasting transmitter (Dolk et al. [R624]), are inherently difficult to interpret because of the impossibility of assessing all of the effects that chance variation might have contributed to the cluster. In the initial Sutton Coldfield study, the authors correctly concluded that no causal association could be drawn between the presence of the cluster and RF exposure from broadcasting towers (Dolk et al. [R625]) (Cooper et al. [R760]). (IEEE C 95.1 – 2005, p.75)*

First of all the Sutton Coldfield study was no cluster study but an ecological investigation. It is true that it was initiated by an unconfirmed report of a cluster of leukemia and lymphoma in the vicinity of a broadcasting transmitter but it proceeded independently of this initial report and used registry data on the population living within a radius of 10 km around the transmitter. The statement that such studies are "inherently difficult to interpret because of the

impossibility of assessing all of the effects that chance variation might have contributed to the cluster" is ridiculous not only because the study is no cluster study but because it is impossible for any study to "assess all effects that chance variation might have contributed" to the endpoint under investigation. It is not mentioned that the study was supplemented by a larger investigation of another 20 high-power transmitters in Great Britain. The difficulties of interpreting ecological studies is related to the fact that potential confounders can only be related to a segment of the population but not to individuals and that in general duration and intensity of exposure are not known for individual members of the different strata. While evidence for an effect on brain tumor incidence from both studies (Dolk et al. 1997a, 1997b) is weak, there is consistent evidence for a relation to hematopoietic cancers. This evidence has been overlooked by the authors due their wrong assumption about the relation between proximity to the transmitter and exposure.

> *Inconsistent effects have been reported between residential proximity to other RF broadcast towers and adverse health endpoints (Bielski [R267]) (Maskarinec et al. [R579]) (Selvin and Merrill [R823]) (Michelozzi et al. [R858]) (Altpeter et al. [R977]) (Hallberg and Johansson [R995], [R996]) (Boscolo [R1012]), although many of these studies have significant flaws in their study design (making them difficult to interpret). (IEEE C 95.1 – 2005, p.75)*

Although it is not stated what these "inconsistent effects" might be, the statement is flawed in more than this respect. First of all the study by Bielski (1994) is an occupational investigation and not about residential proximity to RF broadcast towers, second three of these investigations (Selvin et al. 1992; Maskarinec et al. 1994; Michelozzi et al. 2002) included leukemia as an endpoint with indications of an increased incidence consistent with the studies from Great Britain (Dolk et al. 1997a, 1997b) and Australia (Hocking et al. 1996). Note that the study by Selvin et al. (1992), as stated previously, intended to compare different methods to assess the relationship between a point source and diseases and did erroneously assume a monotonous relationship between exposure and distance from a transmitter. Correcting this error there seems to be an increased probability of childhood leukemia in areas receiving the highest exposure from the Sutro tower. The other three investigations (Altpeter et al. 1995; Boscolo 2001; Hallberg & Johansson 2002) have nothing in common and hence cannot be inconsistent.

> *An increased incidence and mortality rate of childhood leukemia was reported in Australia with residential proximity to a specific RF broadcasting tower (Hocking et al. [R633]), although subsequent reanalysis of the data showed the results may have*

been influenced by other confounding variables within the study location (McKenzie et al. [R669]). (IEEE C 95.1 – 2005, p.75)

This is another example how carelessly and sloppy the evidence is dealt with by the IEEE committee. The study of Hocking et al. (1996) was not about "proximity to a specific RF broadcasting tower" but about an area where three broadcasting towers are located. While there is always the possibility of confounders influencing results of an epidemiologic investigation, the 'reanalysis' of McKenzie et al. (1998) is seriously flawed and cannot support the cited statement. Hocking et al. (1996) combined the districts near the broadcasting area and those further away based on homogeneity analyses, while McKenzie et al. (1998) omitted one area with high incidence (and highest exposure) based on inspection of data. Any statistical analysis subsequent to such data picking is useless.

> *While scattered reports of adverse health effects associated with occupational exposure to RF do exist (Demers et al. [R36]) (Kurt and Milham [R68]) (Pearce [R110]) (Speers et al. [R125]) (Thomas et al. [R128]) (Pearce et al. [R199], [R211]) (Hayes et al. [R207]) (Cantor et al. [R268]) (Davis and Mostofi [R563]) (Tynes et al. [R570], [R605]) (Grayson [R592]) (Richter et al. [R747]) (Holly et al. [R838]) these studies are largely inconsistent with each other in terms of the adverse health endpoints affected, and often show no clear dose response with RF exposure. Many have serious flaws in their study design, contain limited or insufficient RF exposure assessment, and are generally inconsistent with the absence of findings of an association from other occupational studies (Tornqvist et al. [R131]) (Coleman [R142]) (Lilienfeld et al. [R146]) (Robinette and Silverman [R147], [R148]) (Siekierzynski et al. [R151], [R152]) (Wright et al. [R213]) (Coleman et al. [R214]) (Muhm [R506]) (Czerski et al. [R542]) (Hill [R568]) (Lagorio et al. [R616]) (Kaplan et al. [R647]) (Morgan et al. [R701]) (Gallagher et al. [R822]) (Groves et al. [R853]) (Wiklund [R1013]) (Armstrong et al. [R1014]). (IEEE C 95.1 – 2005, p.75)*

Even allowing for restrictions of space for a discussion of the evidence, greater nonsense has not been produced so far in this field as condensed in these two sentences. Putting higgledy-piggledy all sorts of studies together and then wondering about endpoints being inconsistent is an intellectual masterpiece. Of the occupational studies mentioned, three (Thomas et al. 1987; Speers et al. 1988; Grayson 1996) were about brain cancer, three about hematopoietic cancers (Pearce et al. 1985; Kurt & Milham 1988; Pearce 1988), two about testicular cancer (Hayes et al. 1990; Davis & Mostofi 1993), one about male (Demers et al. 1991) and two about female breast cancer (Cantor et al. 1995, Tynes et al. 1996) the latter including other cancers as well, and one about intraocular melanoma (Holly et al. 1996). Three further studies (Pearce et al. 1989; Tynes et al. 1992; Richter et al. 2000) investigated several or all malignancies. These studies differ not only in endpoints, study type (cohort, case-control, and cluster) but also in

the methods of exposure assessment. Ignorance of the IEEE reviewers is underlined by the compilation of studies characterized by an "absence of findings of an association". Not only did several of these studies indeed indicate an association of cancer risk with EMF exposure (Lilienfeld et al. 1978; Robinette et al. 1980; Tornqvist et al. 1991; Armstrong et al. 1994; Lagorio et al. 1997; Groves et al. 2002) but two were no epidemiologic studies at all (Siekierzynski et al. 1974; Czerski et al. 1974) and several were rather addressing ELF exposure (Tornqvist et al. 1991; Wright et al. 1982; Coleman et al. 1983; Gallagher et al. 1991) and one (Wiklund 1981) was a cluster study in the telecommunication administration with uncertain type of exposure. Simply confronting studies finding an effect with others that were 'negative' is scientifically flawed and permits neither the conclusion that there is nor that there is no association between exposure and cancer risk. Even if all studies would have applied the same method, assessed the same endpoint and used the same exposure metric, studies reporting a significantly increased cancer risk are not outweighed by others that did not.

> *While micronuclei formation in workers occupationally exposed from broadcast antennas has been reported (Garaj-Vrhovac [R757]) (Lalic et al. [R791]), these findings were not verified in a larger study of more than 40 Australian linemen exposed under similar conditions (Garson et al. [R186]). (IEEE C 95.1 – 2005, pp.75-76)*

It goes without saying that also this statement is wrong. Garson et al. (1991) did not investigate micronuclei formation, their workers were considerably shorter exposed and it were not more than 40 linemen but 38 radio-lineman.

> *No clear association could be established between occupational exposures of parents to a number of agents, including RF, and effects (neuroblastoma) in their offspring (Spitz and Johnson [R289]) (De Roos et al. [R798]). (IEEE C 95.1 – 2005, p.76)*

What is meant by 'no clear association' is obscure. Spitz and Johnson (1985) found a significantly increased risk for paternal occupational exposure to electromagnetic fields, and also De Roos et al. (2001) found several jobs with paternal as well as maternal exposure to EMFs associated with an elevated risk for neuroblastoma in their children. However, broad groupings of occupations with ELF, RF EMF, as well as ionizing radiation (!) exposure did not reveal an increased risk.

> *One study reported a slight excess in brain tumors associated with combined exposure to RF and other exposures associated with electrical or electronic jobs, but not with RF alone (Thomas et al. [R128]). A study of a Polish military cohort reported a substantial excess of total cancer and several cancer sub-types with jobs associated*

with RF exposure (Szmigielski [R578]), (Szmigielski and Kubacki [R982]), although questions have been raised about severe bias in the exposure assessment of this study (Elwood [R665]) (Bergqvist [R1015]) (Stewart [R1133]). Studies by Milham of U.S. amateur radio operators reported an excess in one of nine types of leukemia assessed (see [R101], [R102], [R209], [R215], and [R569]), but not for total tumors, total leukemia, or brain tumors, and potential confounding factors might have included exposure to soldering fumes, degreasing agents and over-representation of a particular social class. (IEEE C 95.1 – 2005, p.76)

Again the evidence is incorrectly summarized for all cited investigations. Thomas et al. (1987) found a significantly elevated risk for brain tumors among all men exposed to RF fields and in particular in those exposed for 20 or more years. There were indications that this elevated risk is due to a subgroup with electrical or electronics jobs. The group of those exposed in other jobs is heterogeneous and may contain subjects with low or no exposure (e.g. some groups of welders) and therefore lack of an association could be due to a dilution effect from exposure misclassification.

As mentioned previously criticism of the Polish military cohort study about exposure assessment is unfounded. Bergqvist (1997), Elwood (1999) and Stewart (2000) criticized that the military health board assessed a number of potential risk factors only for cancer cases. However, they overlooked that the study was a cohort and not a case-control study and that at no stage information about these factors entered the analysis and therefore couldn't affect the results in any way.

The study by Milham (1988a, 1988b) of radio amateur operators revealed a significantly increased standardized mortality ratio (SMR) for acute myeloid leukemia while the overall mortality and cancer mortality was significantly reduced relative to the country mortality rates. As mentioned previously this points to a 'healthy worker' effect as well as to an influence of life-style factors (mortality related to smoking and overweight were reduced). From the mentioned nine types of leukemia three with expectancies below one and no case observed couldn't be assessed, from the six remaining types five had elevated SMRs with AML, the most frequent type in adults, being significantly elevated.

The last portion of the IEEE review of epidemiology studies is dedicated to mobile phone investigations that are discussed in another contribution.

The following citation presents the IEEE summary in its full length:

The epidemiological evidence to date does not show clear or consistent evidence to indicate a causal role of RF exposures in connection with human cancer or other disease endpoints. Many of the relevant studies, however, are weak in terms of their design, their lack of detailed exposure assessment, and have potential biases in the data. While the available results do not indicate a strong causal association, they cannot establish the absence of a hazard. They do indicate that for commonly encountered RF exposures, any health effects, if they exist, must be small. Even though epidemiological evidence cannot rule out a causal relationship, the overall weight-of-evidence is consistent with the results of the long term animal studies showing no evidence of physiological, pathological or disease-specific effects. (IEEE C95.1 - 2005; pp.76-77)

As already pointed out earlier (Kundi 2006) there is an intolerable tendency in the past years that confronted with an undeniable epidemiologic evidence of an association between an agent and adverse health effects such as cancer, interested parties take their resort to the concept of causality based on the wrong assumption evidence to "indicate a causal role" is a lot more difficult to provide. Unprecedented, however, is the notion of "a strong causal association". Whatever the meaning of this exceptional statement, the conclusion that, if health effects of commonly encountered RF exposures exist, they must be small, is wrong. To the contrary: considering the "lack of detailed exposure assessment" and other potential biases that predominantly lead to an underestimation of the risk, the evidence points to a quite substantial hazard. While the animal studies reviewed in another section of the IEEE standard document cannot be discussed here it should be underlined that they are generally insufficient to support either an increased risk or the lack of health relevant effects. Therefore they cannot be used in a weight-of-evidence statement as has been made by IEEE, that there is no evidence for adverse health effects of RF exposure.

VI.   CONCLUSIONS

•   Only few studies of long-term exposure to low levels of RF fields and brain tumors exist, all of which have methodological shortcomings including lack of quantitative exposure assessment. Given the crude exposure categories and the likelihood of a bias towards the null hypothesis of no association the body of evidence is consistent with a moderately elevated risk.

•   Occupational studies indicate that long term exposure at workplaces may be associated with an elevated brain tumor risk.

•   Although in some occupations and especially in military jobs current exposure guidelines may have sometimes been reached or exceeded, overall the evidence suggest that long-term exposure to levels generally lying below current guideline levels still carry the risk of increasing the incidence of brain tumors.

•   Although the population attributable risk is low (likely below 4%), still more than 1,000 cases per year in the US can be attributed to RF exposure at workplaces alone. Due to the lack of conclusive studies of environmental RF exposure and brain tumors the potential of these exposures to increase the risk cannot be estimated.

•   Epidemiological studies as reviewed in the IEEE C95.1 revision (2006) are deficient to the extent that the entire analysis is professionally unsupportable. IEEEs dismissal of epidemiological studies that link RF exposure to cancer endpoints should be disregarded, as well as any IEEE conclusions drawn from this flawed analysis of epidemiological studies.

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Table 1: Synopsis of epidemiologic studies of or including brain tumors (1987 – 2006)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Thomas et al. 1987	Northern New Jersey, Philadelphia, gulf coast of Lousiana/1979-1981/Case-control	Interviews with next-of-kin about occupational history – response rates: cases 74%, controls 63%; JEM (2 methods)	Death certificates verified through review of hospital records	age(m), (only males), year of death(m), area of residence(m), educational level, (lead, soldering fumes)	435/386	Cases: deaths of brain tumor or CNS tumors of white males (age>30) from death certificates Controls: deaths from other causes than brain tumors, epilepsy, etc.
Milham 1988	Washington, California/1979-1984/Cohort	Amateur radio operator license within 1/1979 to 6/1984	Mortality records	age, (only males), race, year of death	29	67829 operators, search of deaths in state registry through 1984
Selvin et al. 1992	San Francisco/1973-1988/Spatial cluster	Distance of center of census tract to microwave tower (Sutro tower)	SEER records	-	35	Search of cancer deaths of white individuals (age<21)
Tynes et al. 1992	Norway/1961-1985 /Occupational cohort	Job title in 1960 and 1970 censuses and expert categorization	Cancer registry	age, (only males)	119 overall, 6 in subgroup with possible RF exposure	Cohort of 37945 male workers identified that had jobs in 1960 with possible EMF exposure. among these 3017 with possible RF exposure
Grayson 1996	US Air Force/1970-1989/Nested case-control	Detailed job history and classification based on JEM (RF/MW exposure	Screening of hospital discharge records	age(m), race(m), military rank, (ELF and ionizing radiation	230/920	Cohort of ~880000 US Air Force members with at least one completed year of service within

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
		from frequent measurements)		exposure)		the study period, no follow up after subjects left service
Szmigielski 1996	Poland (military)/1971 - 1985/Occupational cohort	Allocation to RF/MW exposure group based on service records, documented measurements of military safety groups	Incident cases from central and regional military hospitals and military health departments	age, (only males)	~46	Annual number of ~127800 military career personnel, ~3720 RF/MW exposed per year
Hocking et al. 1996	Sydney (Australia)/ 1972-1990/Ecological	Municipalities within ~4 km of 3 TV broadcasting towers considered higher exposed as compared to 6 further away	Incident and death cases from cancer registry	age, sex, calendar period	740 (incident) 606 (mortality) 64 age<15 (incident) 30 age<15 (mortality)	Study population: inner area ~135000, outer area ~450000
Tynes et al. 1996	Norway/1961-1991/ Occupational cohort	Certified radio and telegraph operators 1920-1980 (98% worked on merchant ships); spot measurements on ships with old-	Cancer registry	age, (only females)	5	2619 women certified as radio or telegraph operators by Norwegian Telecom

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
		fashioned equipment				
Dolk et al. 1997a	Birmingham (GB)/ 1974-1986/Ecological	Living near a TV/FM radio transmitter (Sutton Coldfield)	Cancer registry	age, sex, calendar year, SES	332	Population (age≥15) ~408000 within 10 km of the transmitter
Dolk et al. 1997b	GB/1974-1986/ Ecological	Living near a high power (≥500 kW erp) transmitter (overall 21)	Cancer registry	age, sex, calendar year, SES	244	Population (age<15) within 10 km of one of 20 high power transmitters
Lagorio et al. 1997	Italy/1962-1992/ Occupational cohort	Working as RF heat-sealer operator	Cancer deaths from registry	age, (only females), calendar period, region	1	302 women employed 1962-1992 in a plastic-ware manufacturing plant as RF sealers
Finkelstein 1998	Ontario (Canada)/ 1964-1995/ Occupational cohort	Working as a police officer (possible handheld radar exposure)	Cancer registry	age, (only males), calendar year	16	20601 male officers of Ontario Police
Morgan et al. 2000	USA/1976-1996/ Occupational cohort	Jobs classified according to work with RF emitting devices with different output power	Death certificates from states' statistics offices	age, sex, period of hire	51	All U.S. Motorola employees with at least 1 day employment 1976-1996 (195775 workers, 2,7 million person-years)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Groves et al. 2002	USA/1950-1997/ Occupational cohort	6 occupational groups 3 with assumed low radar exposure (radar-, radio operator, aviation electrician's mate) and 3 with assumed high exposure (aviation electronics -, electronics -, fire control technician)	Death certificate from a state vital statistics office or National Death Index Plus	age at entry, (only males), attained age	88	40581 Navy Korean War veterans graduated 1950-54 from Navy technical schools; follow-up from graduation through 1997
Berg et al. 2006	Germany/2000-2003/ Case-control	JEM from occupational history collected in interview	Histological verified cases of glioma and meningioma	age(m), sex(m), region(m), SES, urban/rural, smoking, ionizing rad. exposure	Glioma 366/732 Meningioma 381/762	All histological confirmed cases of glioma and meningioma from 4 neurosurgical clinics (age: 30-69) (part.rate 84%); frequency matched controls from population registry (part.rate 63%)

SES…socio-economic status, JEM…job exposure matrix, erp…equivalent radiation power, RF/MW…radio frequency/microwaves, CNS…central nervous system, ELF…extremely low frequency

Table 2: Synopsis of main results of brain tumor studies (1987 – 2006)

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
Thomas et al. 1987	Brain tumor deaths (ICD not specified)	Ever exposed to RF	OR	1.6 [1.0 – 2.4]
		Electrical/electronics job	OR	2.3 [1.3 – 4.2]
		Unexposed*		
		Ever exposed < 5 y	OR	1.0
		5-19 y	OR	2.3
		20+ y	OR	2.0
Milham 1988	Brain cancer deaths (ICD-8: 191)	All	SMR	1.39 [0.93 – 2.00]
		Novice[a]	SMR	0.34
		Technician	SMR	1.12
		General	SMR	1.75
		Advanced	SMR	1.74
		Extra	SMR	1.14
Selvin et al. 1992	Brain cancer deaths (ICD-O: 191.2)	> 3.5 km distance from tower*		
		≤ 3.5 km[b]	RR	1.16 [0.60 – 2.26]
Tynes et al. 1992	Incident brain cancer (ICD-7: 193)	All with possible EMF exposure	SIR	1.09 [0.90 – 1.41]
		Subgroup possible RF exposure[c]	SIR	0.49 [0.18 – 1.06]
Grayson 1996	Incident brain cancer (ICD-9: 191)	Never RF/MW exposed*		
		Ever exposed	OR	1.39 [1.01 – 1.90]
Szmigielski 1996	Incident nervous system & brain tumors	RF/MW exposed	OER	1.91 [1.08 – 3.47]
Hocking et al. 1996	Brain cancer (ICD-9: 191)	Outer area*		
		Inner area (incident, overall)	RR	0.89 [0.71 – 1.11]
		Inner area (mortality, overall)	RR	0.82 [0.63 – 1.07]
		Inner area (incident, age<15)	RR	1.10 [0.59 – 2.06]
		Inner area (mortality, age<15)	RR	0.73 [0.26 – 2.10]
Tynes et al. 1996	Incident brain cancer (ICD-7: 193)	All	SIR	1.0 [0.3 – 2.3]
Dolk et al. 1997a	Incident brain tumors (ICD-8/9: 191, 192)	0-2 km from transmitter	OER	1.29 [0.80 – 2.06]
		0-10 km from transmitter	OER	1.04 [0.94 – 1.16]
Dolk et al. 1997b	Incident brain tumors (ICD-8/9: 191, 192)	0-2 km from transmitter	OER	0.62 [0.17 – 1.59]
		0-10 km from transmitter	OER	1.06 [0.93 – 1.20]
Lagorio et al. 1997	Brain cancer deaths (ICD-9: 191)	RF sealer operator	OER	1 : 0.1
Finkelstein 1998	Incident brain cancer (ICD-9: 191)	All police officers	SIR	0.84 [0.48 – 1.36]
Morgan et al. 2000	Incident brain cancer (ICD-9: 191)	No RF exposure*		
		Low[d]	RR	0.92 [0.43 – 1.77]

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
		Moderate	RR	1.18 [0.36 – 2.92]
		High	RR	1.07 [0.32 – 2.66]
Groves et al. 2002	Brain cancer deaths (ICD-9: 191)	Low radar exposure*		
		High radar exposure	RR	0.65 [0.43 – 1.01]
Berg et al. 2006	Incident glioma (ICD-O3: C71)	No occup. RF/MW exposure*		
		Probably no exposure	OR	0.84 [0.48 – 1.46]
		Probable exposure	OR	0.84 [0.46 – 1.56]
		High exposure	OR	1.22 [0.69 – 2.15]
		No high exposure*		
		High exposure <10 y	OR	1.11 [0.48 – 2.56]
		High exposure ≥ 10 y	OR	1.39 [0.67 – 2.88]
	Incident meningioma (ICD-O3: C70.0)	No occup. RF/MW exposure*		
		Probably no exposure	OR	1.11 [0.57 – 2.15]
		Probable exposure	OR	1.01 [0.52 – 1.93]
		High exposure	OR	1.34 [0.61 – 2.96]
		No high exposure*		
		High exposure <10 y	OR	1.15 [0.37 – 3.48]
		High exposure ≥ 10 y	OR	1.55 [0.52 – 4.62]

[a] From Milham 1988b, license classes as proxy for exposure duration

[b] Based on the assumption that exposure is higher near the microwave tower

[c] Computed based on Table 5 in Tynes et al. 1992

[d] Classification according to power output of equipment used for longest period of employment

OR…odds-ratio, SIR…standardized incidence ratio, SMR…standardized mortality ratio, RR…relative risk (rate ratio), OER…observed/expected ratio

SECTION 11

EVIDENCE FOR CHILDHOOD CANCERS (LEUKEMIA)

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Table of Contents

Tables

I. Introduction

Since the seminal work of Wertheimer and Leeper (1979) more than two dozen epidemiological studies of childhood cancer and residential exposure to power-frequency EMFs were published, not counting some studies about electrical appliances and cluster observations. Although these studies make up an impressive body of evidence, there is an ongoing controversy whether the observed relationships between exposure to power-frequency EMFs and childhood cancer (in particular leukemia) can be causally interpreted. Based on these comparatively few empirical studies virtually hundreds of commentaries, reviews and meta-analyses have been produced, more often than not increasing confusion instead of clarifying the issue. In 2000 two pooled analyses of childhood leukemia, the endpoint most often studied, have been published, one (Ahlbom et al., 2000) that was restricted to 9 studies that fulfilled a number of inclusion criteria (a defined population base for case ascertainment and control selection and using measurements or historical magnetic field calculations for exposure assessment), and another (Greenland et al., 2000; Greenland 2003) including also wire-code studies. Both pooled analyses got essentially the same result: a monotonously increasing risk with increasing power-frequency (50Hz/60Hz) magnetic field levels. As a consequence, the International Agency for Research on Cancer (IARC) concluded in 2001 that power-frequency EMFs are a possible human carcinogen (Group 2B). This classification was based on the evidence from epidemiological studies of childhood leukemia because the panel rated the evidence from all other types of cancer, from long-term animal experiments and mechanistic studies as inadequate.

Typically, if an agent is classified as a Group 2B carcinogen, precautionary measures are taken at workplaces and special care is recommended if it is present in consumer products (e.g. glass wool, lead, styrene, Lindane, welding fumes). Concerning power-frequency EMFs the WHO International EMF Program made the following exceptional statement: "In spite of the large number data base, some uncertainty remains as to whether magnetic field exposure or some other factor(s) might have accounted for the increased leukaemia incidence." (WHO Fact Sheet 263, 2001). This is the line of arguments that has been unswervingly followed by the electrical power industry since the early 1980's. An endless chain of factors allegedly responsible for the 'spurious' positive association between power-frequency EMF exposure and cancer has been put forward, leading to nothing except waste of energy and money. In the last years, due to the fact

that no confounding factor has been found that explains the increased leukemia risk, a slight change of arguments can be discerned that consists of pointing out the very low proportion of children (less than 1%) exposed to power frequency fields associated with a significantly increased risk. In fact, both pooled analyses concluded that there is little indication of an increased risk below 3 to 4 mG magnetic flux density.

In the following chapters we will present the epidemiological evidence, discuss potential biases and demonstrate that from a worst-case scenario the evidence compiled so far is consistent with the assumption of a much greater proportion of leukemia cases attributable to power frequency field exposure than previously assumed. The key problem identified is the lack of a bio-physical model of interaction between very weak ELF EMFs and the organism, tissues, cells, and biomolecules.

A. Epidemiological Studies of Power-Frequency EMF and Childhood Cancer

Table 11-2 gives a synopsis of studies on childhood cancer and exposure to power-frequency EMF, Table 11-3 presents the main findings of these investigations. Most often assessment of exposure was by measurements with 12 studies measuring for at least 24 hours up to 7 days, and 8 studies with spot measurements. Ten studies used distance from power lines as a proxy (some in combination with spot measurements) and 11 studies used wire codes classified according to the Wertheimer-Leeper or Kaune-Savitz methods. Several investigations covered more than one endpoint with hematopoietic cancers the most frequently included malignancies (overall 23 studies), followed by nervous system tumors (11 studies) and other cancers (8 studies). All childhood cancer cases were assessed by 8 investigations.

The most restrictive criteria for combining the evidence for an association between ELF magnetic fields (MF) exposure and childhood leukemia were applied by Ahlbom et al., (2000) that included 9 investigations. Table 11-1 shows the results of these investigations for the exposure category ≥ 4 mG (against < 1 mG as reference category). The studies included 3,203 children with leukemia, 44 of which were exposed to average flux densities of 4 mG or above. Thus only 1.4% of children with leukemia and less than 1% of all children in the studies were exposed that high in accordance with measurement samples from the general population in

Europe, Asia and America (Brix et al., 2001; Decat et al., 2005; Yang et al., 2004; Zaffanella, 1993; Zaffanella & Kalton, 1998).

Meta-analyses of wire-code studies (Greenland et al., 2000; Wartenberg, 2001) revealed similar results for childhood leukemia with estimates of risks around 2 for very high current codes but with considerable heterogeneity across studies.

Table 11-1: Results from nine studies included in Ahlbom et al. (2000) updated according to Schüz (2007) of residential MF exposure and risk of childhood leukemia

Country	Odds-Ratio[*] (95%-CI)	Observed Cases
Canada	1.55 (0.65−3.68)	13
USA	3.44 (1.24−9.54)	17
UK	1.00 (0.30−3.37)	4
Norway	0 cases / 10 controls	0
Germany	3.53 (1.01−12.3)	7
Sweden	3.74 (1.23−11.4)	5
Finland	6.21 (0.68−56.9)	1
Denmark	2 cases / 0 controls	2
New Zealand	0 cases / 0 controls	0
Overall	2.08 (1.30 – 3.33)	49

[*] 24-h geometric mean MF flux density of $\geq$ 4 mG against <1 mG

The only other endpoint except leukemia that has been investigated in several studies is nervous system tumors. The number of cases studied is too low to allow a differentiation according to diagnostic subgroups. Several papers have investigated childhood CNS tumors amongst other endpoints, including leukemia (Wertheimer & Leeper, 1979; Tomenius, 1986; Savitz et al., 1988; Feychting & Ahlbom, 1993; Olsen et al., 1993; Verkasalo et al., 1993; Tynes & Haldorsen, 1997; UKCCS, 1999; 2000), whereas others have solely investigated CNS tumors (Gurney et al., 1996; Preston-Martin et al., 1996; Schüz et al., 2001a). In most cases the time window was restricted to the postnatal period. Exposure was assessed based on residential proximity to overhead power lines, measurements and wiring configurations of houses. In a meta-analysis of childhood brain tumor studies (Wartenberg et al., 1998) estimates of risk were similar whether based on calculated fields (OR 1.4, 95% CI: 0.8 – 2.3), measured fields (OR 1.4,

95% CI: 0.8 – 2.4), wire codes (OR 1.2, 95% CI: 0.7 – 2.2), or proximity to electrical installations (OR 1.1, 95% CI: 0.7 – 1.7). The few studies published after this review do not change these figures substantially.

II. Discussion

Power frequency EMFs are among the most comprehensively studied risk factors for childhood leukemia. Except ionizing radiation no other environmental factor has been as firmly established to increase the risk of childhood leukemia, but for both there are ongoing controversies. Although data from atomic bomb survivors and radiotherapy of benign diseases (ringworm, ankylosing spondylitis, and thymus enlargement) clearly indicate a causal relationship between exposure and leukemia, for other conditions like living in the vicinity of nuclear power plants, diagnostic x-rays, exposure secondary to the Chernobyl incident evidence is less clear and therefore no agreement has been reached about these factors. Concerning power frequency EMFs few deny that the relationship is real and not due to chance, but still there is a controversy about the possibility that confounding, exposure misclassification, and selection bias is responsible for the observed relationship. Furthermore, it is often claimed that even if the exposure is causally related, due to the low attributable fraction no expensive measures to reduce exposure are warranted.

A.   Confounding

A confounder is a factor that is associated with the agent in question as well as with the disease. Hence a confounder must be a risk factor for the disease. Concerning childhood leukemia it was clear from the very beginning that any suggested confounder must be purely speculative since there is no established environmental risk factor except ionizing radiation. Even if a condition can be found that is strongly associated with exposure to power frequency fields, if it is not associated with childhood leukemia it cannot confound the relationship. In the homogenous case, i.e. the association between EMF exposure and the confounder does not depend on disease status and the confounder - leukemia association is independent of exposure to power frequency EMFs, even a stronger assertion can be proven: power frequency EMF remains a risk factor if the risk associated with the confounder is smaller than that associated with power frequency EMFs. Equation (1) gives the bias-factor for the homogenous case and dichotomous exposure variables (that can, however, easily be extended to categorical or continuous exposure variables):

$$B_F = \frac{1 + \pi_F(\Psi_{AF}\Psi_{DF} - 1)}{[1 + \pi_F(\Psi_{AF} - 1)][1 + \pi_F(\Psi_{DF} - 1)]} \tag{1}$$

(π_F is the prevalence of the confounder, Ψ_{DF} is the odds ratio for the confounder, and Ψ_{AF} is the odds ratio of the agent in question with respect to the confounder). From this equation it is

immediately clear that if either Ψ_{DF} or Ψ_{AF} or both are 1 there is no bias. This equation can be used to obtain limiting conditions for the odds ratio of the confounder given specific associations with power frequency fields. This has been done by Langholz (2001).

Langholz (2001) investigated factors that have been proposed as possible confounders based on data from Bracken et al. (1998). None of these factors on their own explain the power frequency EMF - leukemia relationship. It has been criticized (Greenland, 2003) that too far reaching conclusions have been drawn based on the failure to discover a single factor that may explain the relationship, because combinations of such factors have not been addressed. However, even considering combinations of confounders it is unlikely that confounding alone explains the relationship between power frequency EMFs and childhood leukemia. Because of the rather small relative risks of around two for average exposure to ≥ 3 to 4 mG magnetic flux density or very high current codes there is, however, a possibility that bias due to a combination of confounding and other errors account for the increased risk. It will be shown in the last section that the most important aspect is the exposure metric. A much higher risk may be associated with exposure to power frequency fields. If this is actually the case the problem of bias of other provenience disappears.

Because the increased risk from high levels of exposure to power frequency EMFs is found in America, Europe, and Japan a confounder explaining this increased risk must not be quite strong and associated with magnetic fields of various sources but must also be present around the world. It is virtually impossible that such a risk factor has not yet been detected. Therefore, confounding alone as an explanation for the relationship with leukemia can practically be ruled out.

B.   Exposure misclassification

Disregarding chance variations, non-differential exposure misclassification (i.e. misclassification that does not depend on disease status) always leads to an underestimation of the risk. The methods applied to calculate or measure MF in the residences of children are unlikely producing a bias that depends on the disease status. Hence, if exposure misclassification was present this will rather have reduced the overall risk estimate. Different effects must be considered whether sensitivity (the probability that a child that was exposed is correctly classified as exposed) or specificity (the probability that a child that was not exposed is correctly classified as not exposed) is affected by the assessment method. It can easily be shown that in the case of rare exposures the greater effect on the risk estimate is

introduced by reduced specificity (hence by the presence of false positives). This may explain why longer measurement periods show a tendency to higher risk estimates. However, if the true exposure condition is actually not rare, sensitivity is more important and misclassification will result in a substantial underestimation of the true risk.

C.   Selection bias

In studies that were relying on individual measurements selection bias may have played an important role. Participation rates were sometimes lower in controls and especially for families with lower SES. Schüz et al. (2001b) calculated in a simulation study that about two thirds of the increased risk could be due to selection bias. Although Wartenberg (2001) applying a meta-regression could not establish any aspect of study methodology that could account for the variation across studies, it is possible that the proportion of children exposed to high levels of MF has been underestimated in some studies.

D.   Exposure metric

After measurements of MF over 24 hours or even longer periods were introduced lower risk estimates for measured fields as compared to estimates from wire codes were noted. This observation was termed the "wire code paradox". Although much of the discrepancies disappeared after the pooled analyses (Ahlbom et al., 2000; Greenland et al., 2000) were published, and also the comprehensive meta-analysis of Wartenberg (2001) could find no support for a systematic effect, still in some investigations there was indeed a stronger relationship to estimates from wire codes as compared to measurement. Bowman et al. (1999) and Thomas et al. (1999) published a comprehensive analysis of this aspect based on data of the Californian childhood leukemia study (London et al., 1991). They correctly noted the different error structure associated with measured fields and calculated fields from the wire codes that are more stable over time. They further pointed to the fact that the bias introduced by basing the risk estimate on exposure variables that are unbiased but prone to statistical variation will be towards the null. It can be shown that this bias is inversely related to the conditional variance of the exposure metric. Hence the higher the variance of the used exposure metric, conditional on the true one, the greater the bias of the risk estimate.

Up to now most considerations put forward were directed towards identification of factors and methodological issues that would explain a spurious relationship between power frequency EMFs and childhood leukemia. Hardly anyone asked the question: "Why is the risk estimated up to now so low?" This question should, however, been asked because there

are a number of intriguing facts: First of all, in developing countries with low levels of electrification childhood leukemia incidence is manifold lower as compared to industrialized regions (Parkin et al., 1998). Although registry data in developing countries are less reliable and sparse the difference is too pronounced to be due to underreporting. The time trend of childhood leukemia in industrialized countries suggests that childhood leukemia in the age group below 4 to 5 years of age is essentially a new phenomenon that emerged in the 1920s. Milham and Ossiander (2001) suggest that the acute lymphoblastic leukemia peak is due to electrification. Given the evidence of the pooled analyses, risk increases as a function of average MF flux density reaching significance at the far end of the exposure distribution for children exposed to an average of 3 to 4 mG. This result is clearly not in line with the hypothesis that much if not all of childhood leukemia (at least for the most prevalent ALL type in the age group of 2 to 4 years) is due to power frequency EMFs. Obviously there are two conclusions possible: either the hypothesis is wrong or the data must be reinterpreted.

Another difficulty arises due to the fact that animal studies and in vitro tissue culture investigations provided equivocal evidence for a causal relationship between power frequency EMFs and cancer. There is a fundamental problem in clarifying the etiological role of the exposure in the development of leukemia. According to present theory (Greaves 1999; 2002; 2003; 2006; Wiemels et al., 1999) childhood leukemia is a consequence of several (at least two) genetic events one of which already occurred before birth. Factors affecting childhood leukemia may therefore be related to different critical exposure windows: the preconceptional, the prenatal, and the postnatal period. Preconceptional factors may affect the mother and the grandmother during pregnancy with the mother, as well as the father during spermatogenesis. During the prenatal period exposure of the mother during pregnancy and exposure of the fetus may differentially affect the first stage of the disease. In fact, there is convincing evidence that at birth around 1% of children show genetic deviations in cord blood cells (Wiemels et al., 1999; Eguchi-Ishimae et al., 2001; Mori et al., 2002) that could lead to leukemia conditional on them surviving and on additional events that lead to autonomous growth. Given this 100-fold higher incidence of early genetic events, a causal factor for childhood leukemia need not be directly genotoxic and not even mutagenic. A slight but continuous shift of the balance towards survival and proliferation of deviating clones will be sufficient to dramatically increase the incidence. Experimental investigations were generally insufficient to cover such effects.

Assuming that there is an exposure metric, intimately connected to average magnetic flux densities, and actually related to that condition responsible for the increased incidence of childhood leukemia, how does such a metric look like? Actually it is easy to derive the necessary conditions for such an exposure metric from bias considerations. There are only two such conditions that must be met:

      a. The conditional expectancy $E(x|z) = z$ (or equal to a linear function of z); where x is the unknown exposure metric and z is the logarithm of the true average magnetic flux density the child is exposed to.

      b. The conditional variance $V_{x|z}$ must be inversely related to z.

Based on the pooled analysis of Ahlbom et al. (2000) and assuming average magnetic flux density follows a log-normal distribution with mean 0.55 mG and a geometric standard deviation of 1, using the complete data set of cases and controls, the results of the pooled analysis can be reconstructed. However, *by varying the magnitude of the variance and the slope of the logistic function relating the purported exposure metric to the probability of developing childhood leukemia up to 80% of all cases can be attributed to the exposure.*

Fig.1 shows one of such Monte Carlo analyses. It can be seen that the bias of the risk estimate related to average MF flux density decreases as the level increases, however, the bias with respect to the assumed exposure metric reaches a factor of about 25 at levels above the third quartile.

While of course this analysis does not prove the assumption that most of childhood leukemia is due to electrification, it demonstrates that the data obtained so far do not contradict this assumption. It is of crucial importance to analyze existing measurement data for aspects of the exposure that are in line with conditions a. and b. stated above. These exposure conditions may be analyzed by in vitro studies to asses their potential the facilitate transformation of already genetically damaged cells.
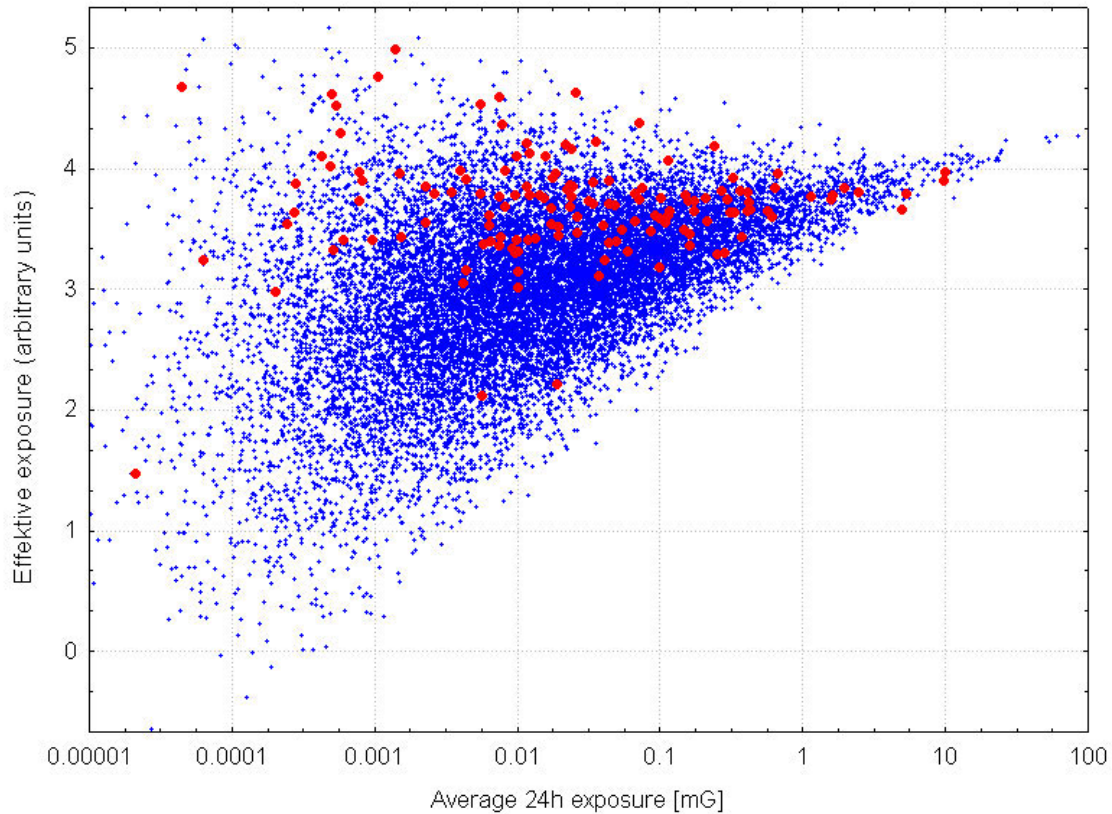
Fig. 1: Results of Monte Carlo simulation under the assumption of a log-normal distribution of average magnetic flux densities in the homes of children that are related to an assumed ‚effective' exposure metric that follows the conditions a. and b. mentioned in the text. Blue are controls and red children with leukemia. The purported ‚effective' exposure metric is associated with an attributable fraction of 80% and the odds-ratio for the highest quartile is around 50.

III. Conclusions

The only endpoint studied so far in sufficient detail is childhood leukemia. Brain and nervous system tumors were also studied in some detail but due to the diversity of these tumors no conclusions can be drawn.

Childhood leukemia is the most frequent childhood malignancy that peaks in the age group of 2 to about 5 years. This peak seems to have been newly evolved in the early quarter of the 20th century and may be due to electrification. This assumption is supported by the absence of this peak or it being much less pronounced in developing countries.

An overview of existing evidence from epidemiological studies indicates that there is a continuous increase of risk with increasing levels of average magnetic field exposure. Risk

estimates reach statistical significance at levels of 3 to 4 mG. A low number of children are exposed at these or higher levels.

Considering the possibility that aspects of exposure to power frequency EMFs that have not yet been detected may account for a great proportion of cases there are two necessary steps to be taken: Concerted efforts must be undertaken to scrutinize existing data and collect new ones that should reveal whether or not exposure metrics exist that show the necessary conditions for an effective exposure metric; and, second, precautionary measures must be delineated that result in a reduction of all aspects of exposure to power frequency EMFs.

Exposure guidelines of IEEE and ICNIRP are solely derived from immediate effects such as nerve and muscle excitations. These guidelines are indeed sufficient to protect from such acute effects (although indirect effects from contact currents cannot be ruled out). Evidence for long-term chronic effects has been collected in the past decades and has reached a state that it cannot longer be denied that these effects are real. Only under very exceptional and remote conditions of a combination of several unknown confounders, selection bias and differential exposure misclassification the established relationship could be spurious. There is no other risk factor identified so far for which such unlikely conditions have been put forward to postpone or deny the necessity to take steps towards exposure reduction. As one step in the direction of precaution, measures should be implemented to guarantee that exposure due to transmission and distribution lines is below an average of about 1 mG.

- The balance of evidence suggests that childhood leukemia is associated with exposure to power frequency EMFs either during early life or pregnancy.

- Considering only average MF flux densities the population attributable risk is low to moderate, however, there is a possibility that other exposure metrics are much stronger related to childhood leukemia and may account for a substantial proportion of cases. The population attributable fraction ranges between 1-4% (Kheifets et al., 2007) 2-4% (Greenland & Kheifets 2006), and 3.3% (Greenland 2001) assuming only exposures above 3 to 4 mG are relevant. However, if not average MF flux density is the metric causally related to childhood leukemia the attributable fraction can be much higher. Up to 80% of childhood leukemia may be caused by exposure to power frequency EMF.

- Other childhood cancers except leukemia have not been studied in sufficient detail to allow conclusions about the existence and magnitude of the risk.

- IEEE guideline levels are designed to protect from short-term immediate effects, long-term effects such as cancer are evoked by levels several orders of magnitudes below current guideline levels.

- Precautionary measures are warranted that should reduce all aspects of exposure, because at present we have no clear understanding of the etiologically relevant aspect of the exposure.

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Childhood Cancer and EMF

Table 11-2: Synopsis of childhood cancer epidemiologic studies (1979 – 2007)

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Wertheimer & Leeper 1979	Greater Denver area, Colorado/ 1950-1973/ Case-Control	wire-codes by inspection (not blinded) of surroundings of residences occupied at birth and time of death	retrospective (1976-1977) assessment	all assessments within 22 days	age (m), sex, urbanization, SES, family pattern, traffic	344 cancer deaths (age<19) from files, matched controls from next entry in birth register or from alphabetical list
Fulton et al. 1980	Rhode Island/1964-1978/Case-Control	power lines (<45.72m from residences) assessed and MF calculated as combined weighted average (based on Wertheimer-Leeper measurements)	retrospective (1979) assessment	all assessments within same period	age(m), SES	119 leukemia patients (age<20) from Rhode Island hospital files; 240 control addresses from birth register
Tomenius 1986	Stockholm county/ 1958-1973/ Case-Control	inspection of visible electrical constructions within 150m of dwellings occupied at birth and diagnosis date; spot measurements at the door of the dwellings (blinded to case status)	retrospective (~1981) assessment	all assessments within same period	age(m), sex(m), district(m)	716 tumor cases (660 malignant, 56 benign) from cancer registry (age<19), matched controls from entry into birth register just before or after index case from same church district

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Savitz et al. 1988	Five-county Denver area, Colorado/1976-1983/Case-Control	wire-code of homes occupied prior to diagnosis (blinded to case status); spot measurements at the front door, in child's and parent's bedrooms and other rooms of frequent occupancy; interviews of mothers (in some cases fathers or adopted mothers)	retrospective (~1985) assessment	all assessments within same period	age±3y (m), sex(m), area(m), SES, traffic density, maternal age, maternal smoking	356 cancer cases (age<15) from cancer registry (71% interviewed, 36% measurements, 90% wire codes); 278 controls (79% resp.rate) from RDD (80% interviewed, 75% measurements, 93% wire codes)
Coleman et al. 1989	Four boroughs near London/1965-1980/ Case-Control	historical exposure by type and distance of electricity supply within 100 m of residences; distance to center of building assessed blinded to case status; calculations according to peak winter load of the power lines	retrospective assessment	all assessments within same period	age(m), sex(m), year of diagnosis(m)	84 leukemia cases (age<18) and 141 cancer controls from cancer registry

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Myers et al. 1990	Yorkshire/1970-1979/ Case-Control	assessment of overhead power lines within a distance depending on type of power line (100-500m) of home at birth; flux densities calculated from line load data and distance to center of dwelling	retrospective (1981-1989) assessment	all assessments within same period	age(m), sex(m), district(m), house type	374 cancer cases (age<15) from registries; 588 controls from nearest entry in birth register of the same district
London et al. 1991	Los Angeles County, CA/1980-1987/Case-Control	24-h MF measurements (IREQ/ EMDEX) at location of child's bed; EF, MF and static magnetic field spot measurements; Wertheimer-Leeper wire code (all facilities within 46m; blinded to case status); interviews with parents about use of appliances etc.	measurements 1987-1989	all assessments within same period	age±1 or 2 or 3y(m), sex(m), ethnicity(m), indoor pesticides, hair dryers, black&white TV, fathers occupational exposure to chemicals	232 leukemia cases (70% part.rate) from LA County Cancer Surveillance Program (age<11); 232 matched controls (90% part.rate) – 65 as friends of cases, others by RDD (5 digits cases, last 2 random)

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Verkasalo et al. 1993	Finland/ 1970-1989/ Retrospective Cohort	estimated magnetic flux density from high-voltage power lines in the center of the building	cumulative and max. flux density any time between birth and diagnosis	n.a.	age, sex, calendar period	68300 boys and 66500 girls (age<20) identified having lived any time after birth in a house with a distance < 500m from a 110, 220, or 400 kV power line and an estimated flux density exceeding 0.1mG; 140 cancer cases from follow-up in cancer registry through 1990.
Feychting & Ahlbom 1993	Sweden/1960-1985/Nested Case-Control	calculations (blinded) based on historical load data, wire configuration and distance from 220 and 400kV power lines and spot measurements (several rooms, 5-min measurements, main current turned on and off)	the year closest to date of diagnosis	all assessments within same period	age(m), sex(m), parish(m), year of diagnosis, apartment/single house, traffic (NO_2)	142 cancer cases within the study base of children (age<16) living on a property <300m from any 220 or 400kV power line; 558 matched controls from the study base.

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Olsen et al. 1993	Denmark/1968-1986/ Case-Control	calculations based on estimated historical load of overhead transmission lines, transmission cables, and substations (50-400 kV)	retrospective up to 9 mo before birth	all assessments within same period	age(m), sex(m)	1707 cancer cases from registry (age<15) and 4788 matched controls from population register
Fajardo-Gutierrez et al. 1993	Mexico City/not specified/Case-Control	interview with parents including assessment of distance and type of transmission and distribution lines, power substations etc.	n.a.	n.a.	age±2y(m), SES	81 leukemia cases from two hospitals; 77 controls from orthopedics or traumatology department
Coghill et al. 1996	England/1986-1995/ Case-Control	E- and H-field probes designed for the study measured 24 h in the bedroom; data used only for the period 20:00 to 08:00	retrospective	parallel measurements in case and control homes	age(m), sex(m)	56 leukemia cases (age<15) from various sources (media advertising, self-help groups, Wessex Health Authority) and 56 controls (

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Gurney et al. 1996	Seattle area, Washington/1984-1990/Case-Control	wire-code by inspection of homes (blinded for case status) occupied within 3 y before diagnosis, electrical appliances by interview with mothers and mailed questionnaire	retrospective (1989-1994) assessment	all assessments within same period	age±2y(m), sex(m), area of residence(m), race, mothers education, family history of brain tumors, ETS, living on a farm, head/neck x-ray, head injury, epilepsy, fits	133 brain-tumor cases (age<20) (74% part.rate) by Cancer Surveillance System; 270 controls by RDD (79% part.rate)
Preston-Martin et al. 1996	Los Angeles County, California/1984-1991/ Case-Control	wire-code and outside spot measurements of homes occupied from conception to diagnosis (blinded for case status); 24h measurements in child's bedroom and another room for a subset; electrical appliances, occupation etc. by interviews with mothers	retrospective (1990-1992) assessment	all assessments within same period	age±1y(m), sex(m), year of diagnosis, SES, parents occupation, building type	298 brain tumor cases (age<20) (68% part.rate); 298 controls by RDD (70% part.rate)

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Tynes & Haldorsen 1997	Norway/1965-1989/Nested Case-Control	Cohort (age <15) living in a ward crossed by a high-voltage power line (≥45kV in urban, ≥100kV in rural areas) in at least one of the years 1960, 1970, 1980, 1985, 1987, 1989.	Calculated historical fields	n.a.	age(m), sex(m), munici-pality(m), SES, type of building, number of dwellings	500 cancer cases (94%) from cancer registry; 2004 controls (95%) randomly selected from cohort
Michaelis et al. 1997a	Lower Saxony, Germany/1988-1993/ Case-Control	24h measurements (EMDEX II) in the child's bedroom and living room in dwellings where the child lived longest (not blinded to case status); perimeter measurements (measurement wheel) with recordings every foot (~30cm) when walking through the rooms and outside the house where the child lived for at least 1y.	measurements 1992-1995	all measurements within same period	age±1y(m), sex(m), SES, urbanization	129 leukemia cases (age<15) (59% part.rate) from register; 328 controls (167 from same district, 161 from random district) (53% part.rate) from government registration files

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Michaelis et al. 1997b	Berlin/1991-1994/ Case-Control (pooled with data from Michaelis et al. 1997a)	as above	not specified	not specified	age±1y(m), sex(m), SES, urbanization, age at diagnosis, West/East Germany	47 leukemia cases (age<15) (59% part.rate) from register; 86 controls (28% part.rate) from government registration files
Linet et al. 1997	Illinois, Indiana, Iowa, Michigan, Minnesota, New Jersey, Ohio, Pennsylvania, and Wisconsin/1989-1994/Case-Control	24h measurements (EMDEX C) in child's bedroom (blinded to case status); spot measurements in the residences and at the front door; wire coding of residences of residentially stable case-control pairs	~2 years	all measurements within same period	age(m), ethnicity(m), 8-digits phone number(m), sex, SES, time of measurem., urbanization, type of residence, birth order, birth weight, mother's age, medical x-ray	638 ALL cases (age<15) from register of Children's Cancer Group (78% part.rate); 620 controls from RDD (63% part.rate).

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Li et al. 1998	Taipei Metropol.Area (3 districts), Taiwan/ 1987-1992/ Ecological	high voltage transmission lines (69 -345kV) were mapped to 124 administrative regions; households with ≥50% intersecting a buffer zone of 100m around transmission lines	n.a.	n.a.	age (5y groups), calendar year	28 leukemia cases from registry in a study base of ~121.000 children (age<15); 7 cases within 21 cases outside a 100m corridor each side of a transmission line
Dockerty et al. 1998	New Zealand/1990-1993/Case-Control	24h measurements (Positron) in child's bedroom and another room (only for leukemia cases); interview with mothers	1-2 years	all measurements within same period	age(m), sex(m), SES, maternal smoking, living on a farm	303 cancer cases (age<15) from 3 registries (88% part.rate) – 121 leukemia cases; 303 controls from birth register (68% part.rate)

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
UKCCS 1999	England, Scotland & Wales/1991(92)-1994(96)/Case-Control	spot measurements (EMDEX II) in child's bedroom, 90 min measurements in main family room, 48h measurements (20% of case-control pairs) at child's bedside; school measurements; weighted averages from info obtained by questionnaire; adjustments from historical load data	~2 years	<4 months in 98% of case-control pairs (spot), within 4 weeks (48h measurem.)	age (m), sex(m), district(m), deprivation index	2226 cancer cases (age<15) from registry (59% part.rate); 2226 matched controls from registry

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
McBride et al. 1999	Canada (5 provinces)/ 1990-1994(95)/Case-Control	48h personal measurements (Positron), 24h measurements in child's bedroom (75% cases, 86% controls); wire codes (78% cases, 85% controls) and residence perimeter and front door measurements (64% cases, 74% controls) (blinded to case status) (EMDEX C); interviews with parents	9 months average	2 months average	age±3-6mo (m), sex(m), area(m), maternal age, maternal education, income, ethnicity, number of residences	399 leukemia cases (age<15) (90% part.rate) from treatment centers and registry; 399 matched controls (76% part.rate) from health insurance/family allowance rolls
Green et al. 1999a	Greater Toronto Area, Canada/1985-1993/ Case-Control	48h personal measurements (Positron); spot measurements in child's bedroom and two other rooms; wire codes; interviews with parents	2-3 y average	~5 mo average	age±1y (m), sex(m), family income, siblingship, residential mobility, insecticides, mother's medication and exp. prior or during pregn.	201 leukemia cases (age<15) from hospital record (64% part.rate); 406 controls from telephone marketing list (10,000 residences) (63% part.rate)

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Green et al. 1999b	Greater Toronto Area, Canada/1985-1993/ Case-Control	as above	2-3 y average	~5 mo average	as above	88 leukemia cases (age<15) from hospital record; 133 controls from telephone marketing list (10,000 residences)
Schüz et al. 2001a	West Germany/1993(90)-1997(94)/Case-Control	24h measurements (FW2a) under mattress of child's bed; 24h measurements (EMDEX II) in living room; perimeter measurements with recordings every foot (~30cm) when walking through the rooms			age(m), sex(m), community(m), SES, year of birth, urbanization, residential mobility, season, type of residence	514 leukemia cases (age<15) from cancer registry (61% of eligible) and 1301 controls from population registry (61% of eligible)
Schüz et al. 2001b	Lower Saxony/1988 – 1993 & Western Germany/1992-1994/ Case-Control	as above			age(m), sex(m), community(m), SES, urbanization	64 cases of CNS tumors (age<15) from registry and 414 controls from population registry

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Mizoue et al. 2004	Japan/1992-2001/Ecological	classification of 294 districts according to their proximity to high voltage power lines (66 and 220V); proportion of area of district (0%, <50%, >50%) within ±300m of a power line	n.a.	n.a.	age (5y groups)	14 cases (age<15) of hematopoietic malignancies identified from two hospitals (all that treated these malignancies)
Draper et al. 2005	England & Wales/ 1962-1995/Case-Control	computed distance from nearest overhead power line (132kV, 275kV, 400kV) of residence at birth	n.a.	n.a.	age±6mo(m), sex(m), district(m), SES	29081 cancer cases (age<15) identified from several registries (88% of total); 29081 controls from birth registers
Kabuto et al. 2006	Tokyo, Nagoya, Kyoto, Osaka and Kitakyushu metropolitan areas (Japan)/1999-2001/Case-control	7 days continuous MF measurement (EMDEX Lite) in child's bedroom; spot measurements in- and outside the house (EMDEX II)	~13 mo	~3 days	age±(≤)1y(m), sex(m), region(m), population size(m), father's and mother's education	321 ALL/AML cases (age<15) from several registries of childhood cancer study groups (49% part.rate); 634 controls from residential registry (29% part.rate)

Childhood Cancer and EMF

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Mejia-Arangure et al. 2007	Mexico-City/1995-2003/Case-Control	spot measurements (EMDEX II) at the front door; wire coding (blinded to case status)	not specified	not specified	age, sex, SES, birth weight, maternal age, traffic, district, family history of cancer	42 ALL/AML cases (age<16) with Down syndrome from 4 (all) treating hospitals; 124 healthy controls with Down syndrome from 2 centers

RDD…Random Digit Dialing, n.a…not applicable, MF…magnetic field, SES…socio-economic status, ALL…acute lymphoblastic leukemia, AML…acute myeloid leukemia

Childhood Cancer and EMF

Table 11-3: Synopsis of main results of childhood cancer studies (1979 – 2007)

Study	Endpoint	Exposure category	Outcome [95% CI]
Wertheimer & Leeper 1979[a]	Leukemia	LCC* (birth address)	
		HCC	OR 2.28 [1.34 – 3.91]
	Lymphoma	LCC*	
		HCC	OR 2.48 [0.73 – 8.37]
	Nervous system tumors	LCC*	
		HCC	OR 2.36 [1.03 – 5.41]
	Others	LCC*	
		HCC	OR 2.38 [0.93 – 6.06]
	All hematopoietic	LCC*	
		HCC	OR 2.31 [1.41 – 3.77]
	All cancers	LCC*	
		HCC	OR 2.33 [1.59 – 3.42]
Fulton et al. 1980	Leukemia	Very low*[c]	
		Low	OR 1.1 [0.5 – 2.4]
		High	OR 1.2 [0.6 – 2.6]
		Very high	OR 1.0 [0.5 – 2.3]
Tomenius 1986	Leukemia	no 200 kV-line*	
		200 kV-line<150m	OR 1.09 [0.29 – 4.12]
	Lymphoma	no 200 kV-line*	
		200 kV-line<150m	OR 1.48 [0.35 – 6.35]
	Nervous system tumors	no 200 kV-line*	
		200 kV-line<150m	OR 3.96 [0.85 – 18.52]
	Others	no 200 kV-line*	
		200 kV-line<150m	OR 2.59 [0.70 – 9.66]
	All hematopoietic	no 200 kV-line*	
		200 kV-line<150m	OR 1.26 [0.47 – 3.34]
	All cancers	no 200 kV-line*	
		200 kV-line<150m	OR 2.15 [1.12 – 4.11]
	All cancers	<3mG birth dwelling*	
		≥3mG	OR 2.67 [1.18 – 6.08]

Childhood Cancer and EMF

Study	Endpoint	Exposure category	Outcome [95% CI]
	All cancers	<3mG diagn. dwelling*	
		≥3mG	OR 2.60 [1.20 – 5.67]
Savitz et al.1988	Leukemia	<2mG low power use*	
		2+ mG	OR 1.93 [0.67 – 5.56]
	Lymphoma	<2mG low power use*	
		2+ mG	OR 2.17 [0.46 – 10.31]
	Brain tumors	<2mG low power use*	
		2+ mG	OR 1.04 [0.22 – 4.82]
	Others	<2mG low power use*	
		2+ mG	OR 0.96 [0.31 – 2.98]
	All hematopoietic	<2mG low power use*	
		2+ mG	OR 1.99 [0.57 – 5.14]
	All cancers	<2mG low power use*	
		2+ mG	OR 1.35 [0.63 – 2.90]
	Leukemia	<2mG high power use*	
		2+ mG	OR 1.41 [0.57 – 3.50]
	Lymphoma	<2mG high power use*	
		2+ mG	OR 1.81 [0.48 – 6.88]
	Brain tumors	<2mG high power use*	
		2+ mG	OR 0.82 [0.23 – 2.93]
	Others	<2mG high power use*	
		2+ mG	OR 0.75 [0.30 – 1.92]
	All hematopoietic	<2mG high power use*	
		2+ mG	OR 1.51 [0.68 – 3.35]
	All cancers	<2mG high power use*	
		2+ mG	OR 1.04 [0.56 – 1.95]
	All cancers	0-0.64 mG low power use*	
		0.65-0.99 mG	OR 1.28 [0.67 – 2.42]
		1.0-2.49 mG	OR 1.25 [0.68 – 2.28]
		2.5+ mG	OR 1.49 [0.62 – 3.60]

Childhood Cancer and EMF

Study	Endpoint	Exposure category	Outcome [95% CI]
	All cancers	0-0.64 mG high power use*	
		0.65-0.99 mG	OR 1.13 [0.61 – 2.11]
		1.0-2.49 mG	OR 0.96 [0.56 – 1.65]
		2.5+ mG	OR 1.17 [0.54 – 2.57]
	Leukemia	LCC*	
		HCC	OR 1.41 [0.57 – 3.50]
	Lymphoma	LCC*	
		HCC	OR 1.81 [0.48 – 6.88]
	Brain tumors	LCC*	
		HCC	OR 0.82 [0.23 – 2.93]
	Others	LCC*	
		HCC	OR 0.75 [0.30 – 1.92]
	All hematopoietic	LCC*	
		HCC	OR 1.51 [0.68 – 3.35]
	All cancers	LCC*	
		HCC	OR 1.04 [0.56 – 1.95]
	All cancers	UG 2y before diagnosis*	
		VLCC	OR 0.96 [0.39 – 2.34]
		OLCC	OR 1.17 [0.65 – 2.08]
		OHCC	OR 1.40 [0.71 – 2.75]
		VHCC	OR 5.22 [1.18 – 23-09]
	All cancers	VLCC/OLCC*[b]	
		UG	OR 0.89 [0.51 – 1.55]
		OHCC	OR 1.25 [0.67 – 2.31]
		VHCC	OR 4.66 [0.95 – 22.76]
Coleman et al. 1989	Leukemia	≥100 m nearest substation*	
		50-99 m	OR 0.75 [0.40 – 1.38]
		25-49 m	OR 1.49 [0.61 – 3.64]
		0-24 m	OR 1.63 [0.32 – 8.38]

Childhood Cancer and EMF

Study	Endpoint	Exposure category	Outcome [95% CI]
Myers et al. 1990	All cancers	<0.1mG*	
		0.1-0.3mG	OR 0.96 [0.37 – 2.51]
		≥0.3mG	OR 1.73 [0.59 – 5.07]
London et al. 1991	Leukemia	<0.68mG* (24h.measurem.)	
		0.68-1.18mG	OR 0.68 [0.39 – 1.17]
		1.19-2.67mG	OR 0.89 [0.46 – 1.71]
		≥2.68mG	OR 1.48 [0.66 – 3.29]
		<0.32mG (spot bedroom)*	
		0.32-0.67mG	OR 1.01 [0.61 – 1.69]
		0.68-1.24mG	OR 1.37 [0.65 – 2.91]
		≥1.25mG	OR 1.22 [0.52 – 2.82]
		UG/VLCC*	
		OLCC	OR 0.95 [0.53 – 1.69]
		OHCC	OR 1.44 [0.81 – 2.56]
		VHCC	OR 2.15 [1.08 – 4.26]
Verkasalo et al. 1993	Leukemia	≥4mG any time	SIR 1.55 [0.32 - 4.54]
	Lymphoma	≥4mG any time	SIR [0.00 - 4.19]
	Nervous system tumors	≥4mG any time	SIR 2.31 [0.75 - 5.40]
	Others	≥4mG any time	SIR 1.24 [0.26 - 3.62]
	All hematopoietic	≥4mG any time	SIR 1.49 [0.74 - 2.66]
	All cancers	≥4mG any time	SIR 1.66 [0.34 - 4.84]
Feychting & Ahlbom 1993	Leukemia	<1mG* (calculated)	
		1-2mG	OR 2.1 [0.6 – 6.1]
		≥2mG	OR 2.7 [1.0 – 6.3]
	Lymphoma	<1mG* (calculated)	
		1-2mG	OR 0.9 [0.0 – 5.2]
		≥2mG	OR 1.3 [0.2 – 5.1]

Childhood Cancer and EMF

Study	Endpoint	Exposure category	Outcome [95% CI]
	Nervous system tumors	<1mG* (calculated)	
		1-2mG	OR 1.0 [0.2 – 3.8]
		≥2mG	OR 0.7 [0.1 – 2.7]
	Others	<1mG* (calculated)	
		1-2mG	OR 1.6 [0.6 – 4.3]
		≥2mG	OR 0.2 [0.0 – 1.7]
	All hematopoietic	<1mG* (calculated)	
		1-2mG	OR 1.7 [0.6 – 4.5]
		≥2mG	OR 2.2 [1.0 – 4.7]
	All cancers	<1mG* (calculated)	
		1-2mG	OR 1.5 [0.7 – 2.9]
		≥2mG	OR 1.1 [0.5 – 2.1]
Olsen et al. 1993	Leukemia	<1mG* (calculated)	
		1-4mG	OR 0.3 [0 – 2.0]
		≥4mG	OR 6.0 [0.8 – 44]
	Lymphoma	<1mG* (calculated)	
		1-4mG	OR 5.0 [0.7 – 36]
		≥4mG	OR 5.0 [0.3 – 82]
	CNS tumors	<1mG* (calculated)	
		1-4mG	OR 0.4 [0.1 – 2.8]
		≥4mG	OR 6.0 [0.7 – 44]
	All three combined	<1mG* (calculated)	
		1-4mG	OR 0.7 [0.2 – 2.0]
		≥4mG	OR 5.6 [1.6 – 19]
Fajardo-Gutierrez et al. 1993	Leukemia	Transformer station[d]	OR 1.56 [0.73 – 3.30]
		High voltage power line	OR 2.63 [1.26 – 5.36]
		Electric substation	OR 1.67 [0.65 – 4.35]
		Transmission line	OR 2.50 [0.97 – 6.67]

Childhood Cancer and EMF

Study	Endpoint	Exposure category	Outcome [95% CI]
Preston-Martin et al. 1996	Brain tumors	0.09-0.51 mG Md 24h *	
		0.52-1.02 mG	OR 1.5 [0.7 – 3.2]
		1.03-2.03 mG	OR 1.8 [0.7 – 4.5]
		2.04-10.4 mG	OR 1.2 [0.4 – 3.2]
		VLCC/OLCC*	
		UG	OR 1.9 [1.0 – 3.6]
		OHCC	OR 0.8 [0.6 – 1.2]
		VHCC	OR 1.2 [0.6 – 2.1]
Coghill et al. 1996	Leukemia	< 5 V/m E-field *	
		5-9 V/m	OR 1.49 [0.47 – 5.10]
		10-19 V/m	OR 2.40 [0.79 – 8.09]
		≥20 V/m	OR 4.69 [1.17 – 27.78]
Tynes & Haldorsen 1997	Leukemia	<0.5mG (TWA birth-diagn)*	OR 1.8 [0.7 – 4.2]
		0.5-1.4mG	OR 0.3 [0.0 – 2.1]
		≥1.4mG	
	Lymphoma	<0.5mG (TWA birth-diagn)*	OR 1.0 [0.1 – 8.7]
		0.5-1.4mG	OR 2.5 [0.4 – 15.5]
		≥1.4mG	
	Nervous system tumors	<0.5mG (TWA birth-diagn)*	OR 1.9 [0.8 – 4.6]
		0.5-1.4mG	OR 0.7 [0.2 – 2.1]
		≥1.4mG	
	Others	<0.5mG (TWA birth-diagn)*	OR 2.9 [1.0 – 8.4]
		0.5-1.4mG	OR 1.9 [0.6 – 6.0]
		≥1.4mG	

Childhood Cancer and EMF

Study	Endpoint	Exposure category	Outcome [95% CI]
	All hematopoietic	<0.5mG (TWA birth-diagn)* 0.5-1.4mG ≥1.4mG	 OR 1.4 [0.7 – 3.1] OR 0.7 [0.2 – 2.4]
	All cancers	<0.5mG (TWA birth-diagn)* 0.5-1.4mG ≥1.4mG	 OR 1.9 [1.2 – 3.3] OR 1.0 [0.5 – 1.8]
Michaelis et al. 1997a	Leukemia	<2mG (Median 24h)* ≥2mG	 OR 3.2 [0.7 – 14.9]
		<2mG (Median night)* ≥2mG	 OR 3.9 [0.9 – 16.9]
Michaelis et al. 1997b (pooled with previous)	Leukemia	<2mG (Median 24h)* ≥2mG	 OR 2.3 [0.8 – 6.7]
		<2mG (Median night)* ≥2mG	 OR 3.8 [1.2 – 11.9]
Linet et al. 1997	ALL	<0.65mG (TWA)* 0.65-1mG 1-2mG 2-3mG 3-4mG 4-5mG ≥5mG	 OR 0.96 [0.65 – 1.40] OR 1.15 [0.79 – 1.65] OR 1.31 [0.68 – 2.51] OR 1.46 [0.61 – 3.50] OR 6.41 [1.30 – 31.7] OR 1.01 [0.26 – 3.99]

Childhood Cancer and EMF

Study	Endpoint	Exposure category	Outcome [95% CI]
Dockerty et al. 1998	Leukemia	<1mG (24h bedroom AM)*	
		1-2mG	OR 1.4 [0.3 – 7.6]
		≥2mG	OR 15.5 [1.1 – 224]
		<1mG (24h daytime room)*	
		1-2mG	OR 3.7 [0.7 – 18.8]
		≥2mG	OR 5.2 [0.9 – 30.8]
Li et al.1998	Leukemia	≥100m from transm.line	
		<100m	SIR 2.43 [0.98 – 5.01]
		Total population<15y	
		≥100m from transm.line	SIR 1.05 [0.64 – 1.58]
		<100m	SIR 2.69 [1.08 – 5.55]
UKCCS 1999	Leukemia	<1mG (estim.AM exp.)*	
		1-2mG	OR 0.78 [0.55 – 1.12]
		2-4mG	OR 0.78 [0.40 – 1.52]
		≥4mG	OR 1.68 [0.40 – 7.10]
	Central nervous system cancers	<1mG (estim.AM exp.)*	
		1-2mG	OR 2.44 [1.17 – 5.11]
		2-4mG	OR 0.70 [0.16 – 3.17]
		≥4mG	OR --
	Others	<1mG (estim.AM exp.)*	
		1-2mG	OR 0.81 [0.52 – 1.28]
		2-4mG	OR 1.08 [0.45 – 2.56]
		≥4mG	OR 0.71 [0.16 – 3.19]
	All cancers	<1mG (estim.AM exp.)*	
		1-2mG	OR 0.93 [0.72 – 1.19]
		2-4mG	OR 0.87 [0.53 – 1.42]
		≥4mG	OR 0.89 [0.34 – 2.32]

Childhood Cancer and EMF

Study	Endpoint	Exposure category	Outcome [95% CI]
McBride et al. 1999	Leukemia	<0.8mG (lifetime predicted)*	OR 0.74 [0.48 – 1.13]
		0.8-1.5mG	OR 1.15 [0.70 – 1.88]
		1.5-2.7mG	OR 1.02 [0.56 – 1.86]
		≥2.7mG	
		Low (Kaune-Savitz)*	
		Medium	OR 1.12 [0.77 – 1.64]
		High	OR 1.17 [0.74 – 1.86]
Green et al. 1999a	Leukemia	<0.4mG (spot measurem.)*	
		0.4-0.9mG	OR 0.47 [0.12 – 1.89]
		0.9-1.5mG	OR 0.75 [0.19 – 3.02]
		≥1.5mG	OR 1.47 [0.44 – 4.85]
Green et al. 1999b	Leukemia	<0.3mG (48h measurem.)*	
		0.3-0.7mG	OR 2.0 [0.6 – 6.8]
		0.7-1.4mG	OR 4.0 [1.1 – 14.4]
		≥1.4mG	OR 4.5 [1.3 – 15.9]
		<0.4mG (spot measurem.)*	
		0.4-0.8mG	OR 1.8 [0.5 – 6.1]
		0.8-1.6mG	OR 2.8 [0.8 – 10.4]
		≥1.6mG	OR 4.0 [1.2 – 13.6]
Schüz et al. 2001a	Leukemia	<1mG (Md 24h)*	
		1-2mG	OR 1.15 [0.73 – 1.81]
		2-4mG	OR 1.16 [0.43 – 3.11]
		≥4mG	OR 5.81 [0.78 – 43.2]

Childhood Cancer and EMF

Study	Endpoint	Exposure category	Outcome [95% CI]
		<1mG (Md night-time)*	
		1-2mG	OR 1.42 [0.90 – 2.23]
		2-4mG	OR 2.53 [0.86 – 7.46]
		≥4mG	OR 5.53 [1.15 – 26.6]
Schüz et al. 2001b	CNS tumors	<2mG (Md 24h)*	
		≥2mG	OR 1.67 [0.32 – 8.84]
		<2mG (Md night-time)*	
		≥2 mG	OR 2.60 [0.45 – 14.9]
Mizoue et al. 2004	All hematopoietic	0% area intersection*	
		<50%	IRR 1.6 [0.5 – 5.1]
		>50%	IRR 2.2 [0.5 – 9.0]
Draper et al.2005	Leukemia	≥600m (from power line)*	
		200-600m	RR 1.22 [1.01 – 1.47]
		<200m	RR 1.68 [1.12 – 2.52]
	Brain tumors	≥600m (from power line)*	
		200-600m	RR 1.18 [0.95 – 1.48]
		<200m	RR 0.74 [0.47 – 1.15]
	Others	≥600m (from power line)*	
		200-600m	RR 0.96 [0.82 – 1.12]
		<200m	RR 0.88 [0.62 – 1.25]
Kabuto et al. 2006	ALL+AML	<1mG (1wk TWA)*	
		1-2mG	OR 0.93 [0.51 – 1.71]
		2-4mG	OR 1.08 [0.51 – 2.31]
		≥4mG	OR 2.77 [0.80 – 9.57]
	ALL+AML	<1mG (1wk night-time)*	
		1-2mG	OR 0.97 [0.52 – 1.79]
		2-4mG	OR 1.08 [0.47 – 2.47]
		≥4mG	OR 2.87 [0.84 – 9.88]

Childhood Cancer and EMF

Study	Endpoint	Exposure category	Outcome [95% CI]
	ALL	<1mG (1wk TWA)*	
		1-2mG	OR 0.87 [0.45 – 1.69]
		2-4mG	OR 1.03 [0.43 – 2.50]
		≥4mG	OR 4.67 [1.15 – 19.0]
Gurney et al.2006	Brain tumors	UG*	
		VLCC	OR 1.25 [0.74 – 2.13]
		OLCC	OR 0.74 [0.34 – 1.61]
		OHCC	OR 1.07 [0.55 – 2.06]
		VHCC	OR 0.51 [0.16 – 1.60]
		LCC*	
		HCC	OR 0.86 [0.50 – 1.48]
Mejia-Arangure et al. 2007	ALL+AML	<1mG (spot)*	
		1-4mG	OR 0.94 [0.37 – 2.4]
		4-6mG	OR 0.88 [0.15 – 5.1]
		≥6mG	OR 3.7 [1.05 – 13]
		Low (Kaune-Savitz)*	
		Medium	OR 5.8 [0.92 – 37]
		High	OR 4.1 [0.66 – 25]

* Reference category

[a] Computed from table 5 of the original publication (could be biased due to not considering individual matching)

[b] Computed from table 5 of the original publication

[c] Quartiles of exposure distribution of controls (exposure calculated)

[d] Reference categories: Without the respective appliance near the residence

OR…odds-ratio, SIR…standardized incidence ratio, RR…relative risk, IRR…incidence rate ratio, LCC…low-current code, HCC…high-current code, UG…underground cable, VLCC…very low current code, OLCC…ordinary low current code, OHCC…ordinary high current code, VHCC…very high current code, Md…median, TWA…time weighted average, AM…arithmetic mean, ALL…acute lymphoblastic leukemia, AML…acute myeloid leukemia

SECTION 12

Magnetic Field Exposure:
Melatonin Production; Alzheimer's Disease; Breast Cancer

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TABLE OF CONTENTS

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EXECUTIVE SUMMARY

Melatonin Production

Melatonin is a hormone produced primarily by the pineal gland, located in the center of the brain. Melatonin is evolutionarily conserved and is found in nearly all organisms. It has numerous properties which indicate that it helps prevent both Alzheimer's disease and breast cancer. There is strong evidence from epidemiologic studies that high ($\geq$ 10 milligauss or mG)*, longterm exposure to extremely low frequency (ELF, $\leq$ 60 Hz) magnetic fields (MF) is associated with a decrease in melatonin production(Section II.)

Alzheimer's Disease

Amyloid beta (Aβ) protein is generally considered the primary neurotoxic agent causally associated with Alzheimer's disease (AD). Aβ is produced by both brain and peripheral cells and can pass through the blood brain barrier.

1. There is longitudinal epidemiologic evidence that high peripheral blood levels of Aβ is a risk factor for Alzheimer's disease (AD). (Section III.A.)
2. There is epidemiologic evidence that extremely low frequency (ELF, $\leq$ 60 Hz) magnetic fields (MF) exposure up-regulates peripheral blood levels of Aβ. (Section III.A.)
3. There is evidence that melatonin can inhibit the development of AD and, thus, low melatonin may increase the risk of AD (Section III.B.)
4. There is strong epidemiologic evidence that significant (i.e., high), occupational ELF MF exposure can lead to the down-regulation of melatonin production. The precise components of the magnetic fields causing this down-regulation are unknown. Other factors which may influence the relationship between MF exposure and melatonin production are unknown, but certain medications may play a role. (Section II.)
5. There is strong epidemiologic evidence that high occupational MF exposure is a risk factor for AD, based on case-control studies which used expert diagnoses and a restrictive classification of MF exposure. (Section III.C.)
6. There are no epidemiologic studies of AD and radiofrequency MF exposure and only one of non-acute radiofrequency MF exposure and melatonin, so conclusions are not yet appropriate. (Sections III.D and II.)

Breast Cancer

The only biological hypothesis which has been epidemiologically investigated to explain the relationship between MF exposure and breast cancer is that high* MF exposure can lower melatonin production, which in turn can lead to changes in the various biological systems which melatonin influences, including increased estrogen production and subsequent deleterious interactions with DNA, and decreased antiproliferative, antioxidant, DNA repair, and immune response capabilities. Thus lowered melatonin production can be expected to lead to increased risk of breast cancer.

1. *In vitro* and animal studies have demonstrated that (i) melatonin is a potent scavenger of oxygen and nitrogen radicals that cause DNA damage, (ii) melatonin interferes with estrogen's deleterious interactions with DNA, and (iii) melatonin inhibits the development of mammary tumors. (Section IV.A.)
2. Human studies indicate that MF exposure can decrease melatonin production. (Section II)
3. Human studies have found that low melatonin production is a likely risk factor for breast cancer. (Section IV.B.)
4. Human studies have shown that light-at-night and night shift work reduce melatonin production and are both risk factors for breast cancer. (Section IV.D.)
5. Occupational studies indicate that high MF exposure increases the risk of breast cancer. This is particularly true for a recent, large, and well-designed study from Poland (funded by the NCI, administered for the NCI by Westat, and conducted by Polish scientists).

   A recent, large, and well-designed, Swedish case-control study used a new ELF MF job exposure matrix, developed by the same group, which is nearly completely at odds with earlier exposure classifications. The female occupation generally thought to be the one with the highest ELF MF exposure (seamstress) was considered to have medium-low exposure, while several lower MF exposed occupations were considered high. The case-control study consequently found no risk associated with high MF occupations as rated by the new matrix, but did find that seamstresses had a statistically elevated risk of breast cancer. This job exposure matrix is likely inappropriate in many important instances and needs to be thoroughly reviewed. (Section IV.E.)
6. Studies of residential MF exposure and breast cancer have been generally negative. Measured residential MF exposure may not be related to actual individual exposure. Residential exposure is most often low, is usually not measured in residences that may be related to the latency period of breast cancer, does not take into consideration point sources of strong magnetic fields which may be related to real exposure, and thus often does not relate to actual exposure. Residential exposure studies are therefore not considered to be of importance for the purposes of this report. (Section IV.F.)
7. Quality radiofrequency studies are lacking. (Section IV.G.)

Seamstresses

As a group, seamstresses have proven to constitute an important occupation for the demonstration of a relationship between ELF MF exposure and both Alzheimer's disease and breast cancer. Seamstresses who use industrial sewing machines have very high and relatively constant MF exposure. This is because the motors of older AC machines are large and produce high levels of MFs, and are on and producing such fields even when no sewing is being done. The AC/DC transformers of DC industrial machines always produce a high field even when the machine is turned off (but not unplugged). In addition, rooms, in which a large number of such machines are used, even have relatively high ambient MF levels. Home sewing machines generally produce smaller MFs, but even these weaker MFs are substantial.

RECOMMENDATION Using the Precautionary Principal, mitigating exposure is a proper goal. Mean <u>occupational</u> exposures over 10 mG or intermittent exposures above 100 mG should be lowered to the extent possible. In situations where this is not feasible, the daily length of exposure should curtailed. Lowering MF exposure can be done by improved placement of the source(s) of magnetic fields (e.g., electric motors in sewing machines, AC/DC converters), shielding, and redesign. It is clear that re-engineering products can greatly lessen MF exposure, and possibly result in important innovations. It is noted that certain automotive models produce medium to high MFs, as do steel-belted radial tires (Milham *et al.*, 1999).

I.   INTRODUCTION

All of the studies discussed have based exposure classifications using magnetic field (MF) measurements, not electric field (EF) measurements. We separately discuss extremely low frequency (ELF, ≤ 60 Hz) MFs and radiofrequency (RF) MFs. Furthermore, the discussion is primarily limited to investigations related to ELF MF exposure as a possible risk factor for Alzheimer's disease (AD), <u>female</u> breast cancer (BC), and the possible biological pathways linking ELF MF exposure to AD and BC incidence.

<u>Exposure Concerns</u>

Epidemiologic investigations are sensitive to errors in exposure assessment and errors in case-control designation. This is particularly true for MF exposure and for AD classification. With respect to occupational exposures, all job exposure matrices (JEM) are based on the measurement of a relatively small number of subjects in each job type. However, extensive measurements have been performed for workers in the electric utility industry and for seamstresses. Note, however, that the Swedish breast cancer study by Forssén *et al.* (2005) used only 5 essentially part-time seamstresses to determine exposure classification (Forssén *et al.* (2004).

The geometric mean MF exposure over the time period of observation is generally used for classification. For ordinal classifications, individual subjects in jobs with mean MF exposure measured close to a boundary value, e.g., between low and medium or between medium and high MF exposure, will frequently be incorrectly classified. This misclassification will generally lead to bias in the estimated risk towards 1, i.e., no risk.

For residential exposures, which do not include living near high power lines, measurements of necessity need to be taken at the current residence. Measurements are usually taken in several rooms at various locations, sometimes with and without electrical equipment turned on, but rarely (if ever) with water lines turned on. Thus, individualized exposures, e.g., sitting near a fuse box, being near one or more AC/DC transformers, use of specific brands and models of home sewing machines, being near a microwave oven in operation, and a myriad of other point sources are missed. Previous residences are usually not measured. Consequently, exposure classification is problematic for studies interested in risk associated with residential MF exposure.

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*   Unless otherwise specified, 'MF' or 'magnetic fields' refer to ELF MF fields. Also, unless otherwise specified, "high" MF exposure as used in this report means an exposure of at least 10 mG or (relatively frequenct) intermittent exposure above 100 mG, while "medium" exposure is an average exposure of between 2 and 10 mG or (relatively frequent) intermittent exposure above 10 mG. "Long-term exposure" means exposure over a period of years. Often, other researchers used a cut-point of around 2-3 mG, or sometimes even less, as a "high" average. The reviews of each study presented here detail the specific cut-point(s) used.

**   Unless otherwise specified, 'MF' or 'magnetic fields' refer to ELF MF fields. Also, unless otherwise specified, "high" MF exposure as used in this report means an exposure of at least 10 mG, while exposure means exposure over a period of years. **

Diagnostic Concerns

AD is difficult to correctly diagnose. Non-specialists frequently incorrectly diagnose a patient as having AD. Exposure assessment and case-control classification errors bias the odds ratio (OR) estimator, when based on dichotomous exposure classification, towards the null hypothesis. When based on three (3) or more classification groups, exposure assessment and case-control classification errors in the types of analyses used most likely also lead to bias towards the null hypothesis.

> With respect to AD, unless the diagnosis is made by experts, there is a very large false positive rate. That is, community-based physicians often incorrectly diagnose dementia (versus depression, for example) and are particularly poor at determining the correct differential diagnosis of dementia. Most subjects with a diagnosis of dementia are simply assumed to have AD. This means that around 40% of all AD diagnoses by physicians who are not experts are incorrect. Diagnostic information on death certificates is even worse. Such a large error in caseness clearly biases the OR estimator towards the null hypothesis. (Many cases of AD go undiagnosed, especially early stage AD. However, this likely does not lead to a significant error rate in classification of controls.)

> With respect to breast cancer, the sub-type of breast cancer is generally recorded, e.g., estrogen receptor positive (ER+) or negative (ER-), which may very well be important with respect to MF exposure. However, sub-group analyses have not usually been performed.

Therefore, in reviewing published studies, particular emphasis is placed on these errors or caveats. Studies which assessed occupational exposures and those which assessed residential exposures are both discussed. Various algorithms for "MF exposure" have been used, and these will also be discussed. Not all studies, exposure data, and exposure algorithms are of equal value.

For both AD and BC, a possible biological pathway of particular importance is down- regulation of melatonin production as a result of longterm MF exposure. This is discussed in detail in this review.

A second possible biological pathway relates specifically to Alzheimer's disease. Longterm MF exposure may increase the production of amyloid beta (Aβ), both in the brain and peripherally. Aβ, particularly the form with 42 amino acids (Aβ_{1-42}), is considered the primary neurotoxic compound causing AD. This pathway was proposed by Sobel and Davanipour (1996a). Two recent epidemiologic studies have provided some degree of confirmation. Thus, MF exposure may be a risk factor for AD through two complementary biological pathways. (See Sections III.A. and III.B.)

There may certainly be other potential biological pathways that will be identified. For example, melatonin interacts with certain cytokines which appear to affect immune responses. This may

be relevant to the early elimination of cells which are either pre-malignant or malignant, thus preventing the development of overt breast or other cancers. However, the two pathways outlined above can most easily be evaluated in human studies, both population-based studies and clinical trials.

There are also several epidemiologic studies of melatonin production among workers with longterm occupational exposure to magnetic fields and a single study of women with high (vs low) residential MF exposure. These studies generally indicate that longterm MF exposure can lead to lowered melatonin production.

II.   ELF Magnetic Field EXPOSURE and MELATONIN PRODUCTION

> *Conclusion: Eleven (11) of the 13 published epidemiologic residential and occupational studies are considered to provide (positive) evidence that high MF exposure can result in decreased melatonin production. The two negative studies had important deficiencies that may certainly have biased the results. There is sufficient evidence to conclude that longterm relatively high ELF MF exposure can result in a decrease in melatonin production. It has not been determined to what extent personal characteristics, e.g., medications, interact with ELF MF exposure in decreasing melatonin production.*

Eightly-five percent (85%) to 90% of pineal melatonin production is at night. Laboratory-based studies, using pure sinusoidal magnetic fields under experimental conditions have not found an effect on melatonin production (Graham *et al.*, 1996, 1997; *Brainard et al.*, 1999). However, several studies among subjects chronically exposed in occupational and residential environments have found an effect, while a few have not. The lack of an effect in laboratory settings may be because the MF exposure was too "clean" or because the duration of exposure was not sufficiently long, e.g., days, weeks, months.

The evidence indicates that high and MF exposures may lead to a decrease in melatonin production. Whether this decrease is reversible with a cessation of exposure is unknown. The extent of the decrease is hard to evaluate. It is also not yet possible to identify individual susceptibility to such a decrease in melatonin production.

Melatonin production is generally measured using its primary urinary metabolite, 6-sulphatoxymelatonin (aMT6s). Total overnight melatonin production is best estimated using complete overnight urine samples. Creatinine-adjusted aMT6s is slightly more correlated with cumulative melatonin estimates obtained from sequential overnight blood samples than is unadjusted aMT6s (Cook *et al.*, 2000; Graham *et al.*, 1998).

The human studies in occupational or residential environments which identified an effect are summarized below.

Positive Studies

- Assessment in the Finnish Garment Industry As a follow-up component to a Finnish study of MF exposures among garment factory workers, a small study of nighttime melatonin production was carried out (Juutilainen *et al.*, 1999). aMT6s excretion and creatinine were measured using complete overnight urine samples. Seamstresses (n=31), other garment workers (n=8), and non-exposed outside workers (n=21) participated. Observations were taken using complete overnight urine collections beginning on a Thursday night through the first morning void on Friday and on the subsequent Sunday night through the first morning void on Monday. There was very little variation between the two time period observations within each group, indicating that if there is an effect of MF exposure, it does not disappear over the weekend, at least among seamstresses using older industrial alternating current machines. The average Thursday-Friday non-adjusted aMT6s excretion level and the average aMT6s excretion level adjusted for creatinine were both statistically significantly lower (p< 0.05) among the workers in the garment factory compared to the controls, even after controlling for other factors associated with a lowering of melatonin levels: creatinine-adjusted aMT6s - 16.4 vs 27.4 ng/mg; unadjusted aMT6s - 5.1 vs 10.0 ng. There was no indication of a dose-response relationship among the garment factory workers.

  In a follow-up study, Juutilainen and Kumlin analyzed the same data in conjunction with a dichotomization of a measure of light-at-night (LAN), obtained from items in the original study questionnaire concerning use of a bedroom light at night, street lights outside the bedroom windows, and use of curtains which do or do not let light filter through. There was a significant interaction between the dichotomized MF exposure (high/low, i.e., cases vs controls) and LAN (yes/no). aMT6s was significantly lower for subjects with high MF with or without LAN. In addition, aMT6s was significantly lower among subjects with high MF and LAN exposure versus subjects with high MF and no LAN exposure. Alternatively, aMT6s was essentially identical for subjects with low MF exposure, regardless of the LAN status.

- Washington State Residential MF Exposure and Melatonin Study Women, aged 20 to 74, were selected for a study of the relationship of bedroom 60 Hz magnetic field levels and melatonin production (Kaune *et al.*, 1997a,b; Davis *et al.*, 2001a). Approximately 200 women were recruited based on magnetic field exposure information from a case-control study of breast cancer (PI: S Davis). About 100 women were sought whose bedrooms were at the high end of magnetic field level in the original study and about 100 were sought who were at the low end. Concurrent measurements of light at night in the bedrooms of these women were also obtained using a specially modified EMDEX II system. Mean magnetic field levels in the two groups differed by less than 1 mG. Thus, compared to MF exposures in many occupations, the women had quite low MF exposures. However, there was an inverse association between bedroom magnetic field levels and urinary aMT6s adjusted for creatinine levels on the same night, after adjusting for time of year, age, alcohol consumption, and use of medications. The association was strongest at those times of the year with the longest length of daylight and in women who

were using medications that themselves were expected to attenuate melatonin production, e.g., beta and calcium channel blockers and psychotropic drugs.

- <u>Crossover Trial of MF Exposure at Night and Melatonin Production</u> Davis *et al.* (2006) conducted a randomized crossover trial among 115 pre-menopausal women with regular periods between 25 and 35 days apart, a body mass index between 18 and 30 kg/m^2, not using hormonal contraceptives or other hormones for at least 30 days before the study period, no history of breast cancer, no history of chemotherapy or tamoxifen therapy, not having been pregnant or breast-feeding within the previous year, not working any night shifts, not taking supplemental melatonin, phytoestrogens or isoflavones, and not eating more than 5 servings of soy-based foods within any one week. MF exposure or sham exposure was for 5 consecutive days. A random half of these women received MF exposure and then sham exposure one month later. The other random half had the exposures reversed. Ovulation was determined in the first, second and third months. The initial exposure (MF or sham) was in the second month during days 3-7 post-ovulation. The second exposure (sham or MF) was during the same days in the third month. The charging base of an electric toothbrush which produced a steady magnetic field was used. It was placed under the subject's bed at the head level so that the subject's head received 5-10 mG exposure above baseline. Complete overnight urine samples were collected on the night of the last exposure (MF or sham) in each of the two exposure periods. There were 2 subjects who did not ovulate during either exposure month and 13 who did not ovulate in one of the two months. Statistical adjustment was made for age, hours of darkness, body mass index, medication use, any alcohol consumption, and number of alcoholic beverages consumed. Because each subject was her own control, these adjustments probably did not affect the point estimates much. A regression analysis was undertaken. The 95% confidence interval (CI) of the regression slope was [-3.0 – +0.7] for all subjects and [-4.1 – -0.2] when the 15 subjects with "minor" protocol violations were eliminated from the analysis. These violations were (a) more than 40 days between the two assessments, (b) urine collections not on the same post-ovulation day, and (c) menstrual period started early. Only (b) appears to be really relevant because these subjects could have had less MF exposure. However, this information is not provided. Separate analyses were conducted for "medication users" (n=14) and non-users (n=101). The slope point estimate for the users was numerically smaller (-3.1) than for the non-users (-1.0). The authors state that the study "found that nocturnal exposure to 60-Hz magnetic fields 5 to 10 mG greater than ambient levels in the bedroom is associated with decreased urinary concentrations of (aMT6s)". It should be noted that the p-value of the slope estimate in the primary analysis (all participants) was greater than 0.05. However, the 95% CI, [-3.0 – +0.7], was quite unbalanced, with 0 being much closer to the upper end of the CI than the lower end. Also, the 95% CI, when the 15 subjects with minor protocol violations are eliminated is entirely below 0, and thus the point estimate is statistically significant at the 0.05 level. The authors also state the following: "(t)he more pronounced effect of magnetic field exposure on melatonin levels seen in medication users and in those with an anovulatory cycle suggest {sic} that individuals who have decreased melatonin levels already may be more susceptible to the effects of magnetic field exposure in further decreasing melatonin levels." The justification for this statement is not based on statistical testing.

- Residential High Power Lines, MF Exposure and aMT6s in the Quebec City Study
  Levallois *et al.* (2001) evaluated aMT6s among 221 women living near 735-kV power
  lines compared to 195 age matched women who live far away from such lines. The
  subjects wore magnetic field dosimeters for 36 consecutive hours to measure their actual
  MF exposure. The geometric mean 24-hour MF exposure was 3.3 mG among women
  living near a high power line and 1.3 mG among those who did not live near a high power
  line. Similarly, geometric mean exposure during sleep was 2.9 mG versus 0.8 mG for the
  two groups. No direct effect of MF exposure on creatinine-adjusted aMT6s was
  identified. However, living near a high power line and MF exposure interacted with age
  and body mass index (BMI; kg/m^2). Living near a high power line was associated with a
  significant decline in creatinine-adjusted aMT6s among older subjects and subjects with
  higher BMI. There were similar significant decreases related to age and BMI for women
  in the lowest quartile versus highest quartile. All analyses were adjusted for age, BMI,
  alcohol consumption in the previous 24 hours, medication use in the previous 24 hours,
  light at night, and education.

- Assessment in the Electric Utility Industry Burch *et al.* (1996, 1998, 1999, 2000, 2002)
  have reported on the association between levels of occupational daytime magnetic field
  exposure, non-work MF exposure, and the excretion of total overnight and daytime
  aMT6s among electric utility workers in several studies. These studies are among the
  largest to evaluate the relationships between MF exposure and melatonin production in
  humans, and are the only studies to use personal exposure monitoring of both MF and
  ambient light with a repeated measures design.

  - ✓ In their 1996 abstract, analyses were conducted for 35 of 142 electric utility
    workers enrolled in a larger study. MF exposure was assessed continuously at 15
    second intervals for three 24-hour periods, with logs kept to identify work, sleep
    and other non-work time periods. Ambient light intensity was also individually
    measured. Complete overnight urine samples and post-work spot urine samples
    were collected at the same times over the 3 days. There were statistically
    significant inverse relationships between nocturnal aMT6s levels and log-
    transformed worktime mean MF exposure (p=0.013), geometric worktime mean
    MF exposure (p=0.024), and cumulative worktime MF exposure (p=0.008). There
    was no association, however, between sleep time and other time MF exposure
    levels and aMT6s levels during the daytime or nighttime, even though average
    cumulative MF levels were only somewhat higher during work: 18.3 mG-hours
    (work); 13.1 mG-hours (non-work); 12.6 mG-hours (sleep).

  - ✓ In their 1998 study, further results related to nocturnal aMT6s urinary excretion in
    relation to MF exposure were presented, using all 142 electric utility workers. The
    MF exposure metrics were geometric mean intensity, a rate-of-change metric
    (RCM), and the standardized rate-of-change metric (RCMS). RC was used as a
    measure of intermittence, while RCMS was used as a measure of the temporal
    stability of the serially recorded personal MF exposures. Statistical adjustments
    were made for age, month, and personal ambient light exposure. 24-hour mean MF

exposure intensity, RCM, and RCMS were not associated with either nocturnal aMT6s or creatinine-adjusted aMT6s. However, there was an inverse relationship between residential RCMS and nocturnal aMT6s. The interaction between residential intensity and RCMS was inversely associated with total overnight urinary aMT6s excretion and with creatinine-adjusted nocturnal aMT6s excretion. There was a "modest" reduction in nocturnal aMT6s with more temporally stable MF exposures at work. The effect on nocturnal aMT6s was greatest when residential and workplace RCMS exposures were combined. The authors concluded that their study provides evidence that temporally stable MF exposure (i.e., lower RCMS) are associated with decreased nocturnal urinary aMT6s levels. Given the strong correlation between cumulative overnight serum melatonin levels and both total overnight urinary aMT6s and creatinine-adjusted aMT6s levels, these results indicate a reduction in overnight melatonin production.

✓ In their 1999 study, data from the same 142 electric utility workers were further analyzed. Personal exposure to workplace geometric mean and RCMS were evaluated for their effect on post-work urinary aMT6s measurements. No association between creatinine-adjusted aMT6s and the geometric mean MF exposure, before or after adjustment for age, calendar month and light exposure was found. However, MF temporal stability was associated with a statistically significant reduction in adjusted mean post-work aMT6s concentrations on the second (p=0.02) and third (p=0.03) days of observation. Light exposure modified the MF exposure effect. Overall, there was a significant (p=0.02) interaction between RCMS and ambient light exposure. Reductions in post-work aMT6s levels were associated with temporally stable MF exposures among workers in the lowest quartile of ambient light exposure (mostly office workers), whereas there was no RCMS effect among workers with intermediate or elevated ambient light exposure.

✓ In their 2000 study, Burch *et al.* examined aMT6s levels among a completely different population of 149 electrical workers, 60 in substations, 50 in 3-phase environments, and 39 in other jobs, using the same data collection strategy as was used in the previous study, but with the added characterization of specific work environments. The rationale for this study was based on previous observations in experimental animals suggesting that non-linear field polarization was critical in the reduction of melatonin production. These types of fields were expected to be present within substations and in the vicinity of 3-phase electrical conductors. Other conductors (1-phase, linear polarization) were selected as a control condition because they had not previously been associated with an alteration of melatonin production in laboratory animal studies. Thus, participating workers recorded the times they spent in these environments over the 3-day data collection period. Comparisons were made separately for subjects working in substation or 3-phase environments, or among those working in 1-phase environments. Adjusted mean aMT6s levels were compared statistically among workers in the lowest and highest tertiles of MF exposure, using either the geometric mean or the RCMS measurements. Among workers in either a substation or 3-phase environment for

more than 2 hours, nocturnal aMT6s decreased 43% (p=0.03) when tertiles were based on geometric mean exposure and decreased 42% (p=0.01) when tertiles were based on RCMS. With RCMS tertiles, total overnight aMT6s excretion also decreased 42% (p=0.03) and post-work creatinine-adjusted aMT6s decreased 49% (p=0.02). With geometric mean tertiles, total overnight aMT6s excretion decreased 39% and post-work creatinine-adjusted aMT6s decrease 34%. However, neither of these decreases was statistically significant. No MF-related effects were observed among workers with less than 2 hours time spent in substation/3-phase environments. Similarly, no reduction in aMT6s levels were observed among workers in 1-phase environments.

✓ In 2002, Burch *et al.* studied two consecutive cohorts of electric utility workers using the same data collection strategy to evaluate the effects of cellular telephone use and personal 60 Hz MF exposure on aMT6s excretion. The sample sizes were 149 for Cohort 1 (from the 2000 study) and 77 for Cohort 2. Total overnight and post-work urine samples and self-reported workplace cell phone use were obtained over three (3) consecutive workdays. ELF MF and ambient light exposure were also measured with specially adapted personal dosimeters. The outcome of interest was melatonin production as measured by aMT6s. The cut-point for high versus low cell phone use was 25 minutes per day. Only 5 worker-days of cell phone use more then 25 minutes were reported in Cohort 1 versus 13 worker-days in Cohort 2. No differences in aMT6s production were found in Cohort 1. However, for Cohort 2 there were significant linear trends of decreasing overnight aMT6s and creatinine-adjusted aMT6s levels with increasing cell phone use. There was also a marginally significant increasing trend in post-work creatinine-adjusted aMT6s with increasing cell phone use. Finally, there was a combined effect of cell phone use and ELF MF exposure on aMT6s excretion: among workers in the highest tertile of ELF MF exposure, those who used a cell phone for more than 10 minutes had the lowest overnight aMT6s and creatinine-adjusted aMT6s levels compared to those with lower ELF MF exposure or cell phone use. All analyses used a repeated measures method and were adjusted for age, month of participation, and light exposure.

• Swiss Railway Worker Study Pfluger and Minder (1996) studied 66 railway engineers operating 16.7 Hz electric powered locomotives and 42 "controls". Mean MF exposure at the thorax for the engineers was above 150 mG and approximately 10 mG for the controls. Thus most controls also had high MF exposure, certainly compared to residential and most occupational MF exposures. Morning and early evening (post-work) urine samples were used to measure aMT6s. Evening aMT6s values were significantly lower following work periods (early, normal or late shifts) compared to leisure periods for the engineers, but not for the controls. Also, morning samples did not differ between leisure and work mornings. This indicates that there was at least somewhat of a recovery from the worktime MF exposures. Evening aMT6s values did not differ between work time and leisure time for either engineers or controls. However, there was a rebound in morning aMT6s between a work period and leisure period. Pfluger and Minder did not

report the results of a comparison of nighttime aMT6s levels between engineers and controls.

- Video Display Unit Studies Non-panel video display screens, e.g., computer monitors, produce significant MF exposure despite improvements over the last decade or so. Arnetz and Berg (1996) studied 47 Swedish office workers who used video display units (VDU) in their work in the 1980s. Circulating melatonin levels significantly decreased during work, but not during a day of "leisure" in the same environment. Nighttime melatonin production was not observed. In 2003, Santini *et al.* conducted a similar, but quite small, study of 13 young female office workers, 6 of whom worked for at least 4 hours per day in front of a video screen. Overnight urine samples were used to measure aMT6s. The aMT6s values of the exposed workers was 54% lower (p<0.01) compared to the non-exposed workers.

Negative Studies

- Italian Study of Workers Gobba *et al.* (2006) recruited 59 workers, 55.9% of whom were women, for a study of melatonin production and MF exposure. Actually more workers were recruited, but urine samples for only those subjects who did not get up to urinate during sleep time were assayed. Creatinine-adjusted aMT6s was measured using a Friday morning urine sample and the following Monday morning urine sample. Mean age was 44.4 years (standard deviation, 9.2). Exposure during worktime was measured over a three-day period. The logarithm of the time weighted average (TWA) and the percent of time above 2 mG were used as the measures of exposure. 2 mG was the cut-point between low and high exposure. 52.5% were in the low exposed group; a larger percentage of men than women were in the low exposed group. Occupations included clothing production (n=26), utility companies (14), teachers (6), engineering industry (5), and miscellaneous (8). There were no significant differences in creatinine-adjusted aMT6s values based on the logarithm of the TWA or percent of observations above 2 mG.

- Occupational MF Exposures among 30 Males Subjects in France Touitou *et al.* (2003) studied 15 men exposed to occupational magnetic fields for between 1 and 20 years and age-matched15 controls. All subjects were free of acute or chronic diseases, had regular sleep habits, did not do night work, took no transmeridian airplane flights during the preceding 2 months, took no drugs, were nonsmokers, and used alcohol and coffee in moderate amounts. Furthermore, they did not use electric razors or hair dryers during the study or in the 24 hours prior to blood sampling. All of the 15 MF exposed men worked in high voltage electrical substations. They also lived near substations. None of the controls had an occupation associated with MF exposure. Exposed subjects had a mean exposure of 6.4 mG during work and 8.2 mG during other times. For the control subjects, the mean exposure was 0.04 mG, both during the day and at other times. Blood samples were taken hourly from 8:00 pm until 8:00 am in a standard manner. All urine between these times was collected. Melatonin concentration (pg/ml) was measured in each blood sample. The study was done in the autumn. The 12 hour melatonin blood concentration curves for the exposed and non-exposed subjects are almost identical. The creatinine-

adjusted aMT6s levels are also nearly identical. No analyses were conducted based on length of time in the occupation.

III. ALZHEIMER'S DISEASE

A.   **Alzheimer's Disease Specific Pathway: Over-Production of Peripheral Amyloid Beta Caused by MF Exposure**

Conclusion: There is now evidence that (i) high levels of peripheral amyloid beta are a risk factor for AD and (ii) medium to high MF exposure can increase peripheral amyloid beta. High brain levels of amyloid beta are also a risk factor for AD and medium to high MF exposure to brain cells likely also increases these cells' production of amyloid beta.

Sobel and Davanipour (1996a) have published a biologically plausible hypothesis relating MF exposure to AD, based on the unrelated work of many researchers in several different fields. The hypothesized process involves increased peripheral or brain production of amyloid beta ($A\beta$) as a result of MF exposure, and subsequent transportation of peripheral $A\beta$ across the blood brain barrier. Figure 1 provides a schematic outline of the hypothesis. Each step in the proposed pathway is supported by *in vitro* studies.

Two versions of the amyloid beta protein have been identified. They are identical, except one is longer, 42 versus 40 amino acids. These are specified, respectively, by $A\beta_{1-42}$ and $A\beta_{1-40}$. $A\beta_{1-42}$ is considered the more neurotoxic of the two.

This hypothesis has not yet been fully tested. However, two recent studies of elderly subjects and electrical workers, respectively, have provided important initial support. The Mayeux *et al*. (1999, 2003) papers demonstrate that higher levels peripheral $A\beta_{1-42}$ are a risk factor for AD. The Noonal et al. (2002a) paper demonstrates that MF exposure can increase the peripheral levels of $A\beta_{1-42}$ and that contemporaneous blood levels of melatonin are inversely associated with peripheral levels of $A\beta_{1-42}$.

- Mayeux *et al*. (1999, 2003) conducted a population-based, longitudinal study of elderly subjects who were cognitively normal at baseline and found that higher peripheral blood levels of $A\beta_{1-42}$ were prognostic of subsequent development of AD. The 2003 paper had a longer follow-up period and 282 additional subjects (169 vs 451).

  In the first paper, 105 subjects, cognitively normal at baseline, were followed for an average of 3.6 years. The mean age at baseline was 74.3 +/- 5.3 years. Sixty-four (64) subjects developed AD. Table 1 provides the baseline and follow-up means for age, education, $A\beta_{1-42}$, $A\beta_{1-40}$, and the ratio $A\beta_{1-42}/ A\beta_{1-40}$. The subjects who developed AD were older at baseline, had nearly two years less education, and higher $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$. All mean differences were significant at the p=0.001 level, except for the ratio, which was significant at the p=0.05 level.

For $A\beta_{1-42}$, the OR for AD, based on the actual $A\beta_{1-42}$ values, was 1.0114, p = 0.006. Thus, for example, the OR for an individual with an $A\beta_{1-42}$ value 10 pg/ml above the cutpoint for the 1st quartile (24.6 pg/ml) is estimated to be $(1.0114)^{10} = 1.12$, an increase of 12%; for an individual with an $A\beta_{1-42}$ value 40 points above this cutpoint, the estimated increase in risk is 57%. A similar analysis for $A\beta_{1-40}$ did not yield a significant result.

Subjects were then divided into quartiles based on their $A\beta_{1-42}$ values. For $A\beta_{1-42}$ there was a highly significant (p=0.004) trend across quartiles. The adjusted odds ratios (OR) for the 2nd – 4th quartiles were 2.9, 3.6, and 4.0, using logistic regression. The latter two were statistically significant at the 0.05 level. The ranges for the 3rd and 4th quartiles were 45.9 – 85.0 pg/ml and > 85.0 pg/ml, respectively. For the 2nd quartile, the significance level of the OR was not provided; however, the 95% confidence interval (CI) was [0.9 – 6.8]. Perhaps because the per unit analysis was not significant for $A\beta_{1-40}$, an analysis using quartiles was not reported.

In the second paper (Mayeux *et al*., 2003), follow-up of patients was up to 10 years and there were 451 patients who were cognitively normal at baseline, versus 169 in the initial paper. Table 2 contains the same information for this study as is provided in Table 1 for the initial study. Eighty-six (86) of the 451 subjects developed AD. Presumably, the additional subjects had had their peripheral amyloid beta assayed after the submission of the original paper. Again, the $A\beta_{1-42}$ values were divided into quartiles, based on the 451 subjects who were cognitively normal at their last follow-up. The adjusted relative risk (RR) estimates for the 2nd – 4th quartiles were 1.3, 1.9, and 2.4, using Cox survival analysis. The latter two were statistically significant at the 0.05 and 0.006 levels, respectively. The ranges for the 3rd and 4th quartiles were 60.2 – 84.15 pg/ml and $\geq$ 84.15 pg/ml, respectively. For the 2nd quartile, the significance level of the OR was again not provided; however, the 95% confidence interval (CI) was [0.6 – 2.1].

> The mean levels of $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-42}$/ $A\beta_{1-40}$ at baseline in the second paper were 133.9 pg/ml, 62.2 pg/ml, and 0.50. In the initial paper, the comparable figures were 120.5 pg/ml, 63.2 pg/ml, and 0.57. The means for $A\beta_{1-42}$ and $A\beta_{1-42}$/ $A\beta_{1-40}$ are quite similar in the two studies. However, the means for $A\beta_{1-40}$ are quite different, so there were most likely several subjects who were not in the initial report, and who had $A\beta_{1-40}$ assays which were very high. These subjects were evidently almost all in the cognitively normal group. This is because in the AD groups, the $A\beta_{1-40}$ means were 134.7 and 136.2 pg/ml. However, in the cognitively normal group, the means were 111.8 and 133.3 pg/ml. Thus, the additional 260 subjects with did not develop AD (365-105=260) had an average $A\beta_{1-40}$ of 142.0 pg/ml. Such a large difference is left unexplained in the Mayeux *et al*. (2003) paper.

Mayeux *et al*. (1999) comment that "cerebral deposition of $A\beta_{1-42}$ is unlikely to result directly from increased plasma $A\beta_{1-42}$". However, studies by Zlokovic and colleagues provide a basis for concluding that, in fact, peripheral $A\beta_{1-42}$ is likely to cross the blood brain barrier, perhaps chaperoned by apolipoprotein E (ApoE), particularly the ε4 isoform

(see Sobel & Davanipour, 1996a). Currently, the relative amounts of peripheral and cerebral $A\beta_{1-42}$ or $A\beta_{1-40}$ which aggregate are unknown.

Two newly developed PET scan techniques, however, provide the ability to investigate the relative amounts in humans (Klunk *et al.*, 2004; Ziolko *et al.*, 2006; Small *et al.*, 2006) . It is also straightforward to use labeled amyloid beta to determine the rate at which peripheral amyloid beta is transported to the brain, at least in animal models and perhaps also in humans.

- Noonan *et al.* (2002a) examined 60 electric utility workers in studying the relationship between measured MF exposure during the work day and serum $A\beta_{1-42}$ and $A\beta_{1-40}$ (square root transformed) levels. MF exposure was individually determined by wearing a dosimeter at the waist during work time. Blood samples were obtained between 2:50 pm and 4:50 pm. The primary findings were as follows:
    - **i.** there was an inverse association between physical work and $A\beta$ levels;
    - **ii.** there was an apparent trend for the $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$ levels to be higher for higher magnetic field exposure (significance not provided); and
    - **iii.** the differences (Table 3) in $A\beta$ levels between the highest (≥ 2 milliGauss (mG), n=7) and lowest (< 0.5 mG, n=20) exposure categories were 156 vs 125 pg/ml (p=0.10) for $A\beta_{1-40}$, 262 vs 136 pg/m (p=0.14) for $A\beta_{1-42}$, and 1.46 vs 1.03 for $A\beta_{1-42}/A\beta_{1-40}$ (significance not provided).

There was a 93% increase in $A\beta_{1-42}$, a 25% increase in $A\beta_{1-40}$, and a 42% increase in the ratio $A\beta_{1-42}/A\beta_{1-40}$ between the lowest and highest MF exposure categories. The 2 mG cutpoint for the highest category is the cutpoint generally used for medium (or at times high) MF exposure in epidemiologic studies. Thus, while the sample size was small, this study provides some evidence that MF exposure may result in higher peripheral production of $A\beta$ for exposures above 2mG.

Melatonin production was estimated using urinary 6-sulphatoxymelatonin (aMT6s) adjusted for creatinine (Graham *et al.*, 1998). aMT6s is the primary urinary metabolite of melatonin. A complete overnight urine sample was used to estimate overnight melatonin production, normally about 85-90% of total 24-hour production. A post-work urine sample, taken on the same day as the post-work blood sample, was used to estimate work time melatonin blood levels. The overnight creatinine-adjusted aMT6s levels were, on average, about 5 times higher than the post-work creatinine-adjusted aMT6s levels. Noonan *et al.* state that the correlations between overnight creatinine-adjusted aMT6s and amyloid beta levels were not significant. No data were provided. However, post-work creatinine-adjusted aMT6s levels were negatively correlated with both the $A\beta_{1-42}$ and the $A\beta_{1-42}/A\beta_{1-40}$ post-work levels. The Spearman correlation coefficients were -0.22 (p=0.08) and -0.21 (p=0.10), respectively. With adjustment for age and physical work, the correlation with $A\beta_{1-42}$ was marginally stronger (-0.25, p=0.057). The timing of the urine sample with respect to the blood sample appears to be important. Table 4 provides the Spearman correlations, adjusted for age and physical work, based on the time difference between blood and urine samples, which were all obtained after the blood draw. Some of the workers had their urine sample in the early evening. It is clear that the correlation is strongest when the samples are taken close to one another in time.

In an unadjusted analysis, the post-work creatinine-adjusted aMT6s levels were split into tertiles. Subjects in the highest tertile had the lowest levels of $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$ (Table 5). However, subjects in the middle tertile had higher levels than subjects in the lowest tertile.

- In an *in vitro* study, Del Giudice *et al.* (2007) used human neuroglioma cells (H4/APPswe), which stably overexpress a specific human mutant amyloid precursor protein (APP, to examine the effect of ELF MF exposure. ELF MF or sham exposure was 3.1 mT (31,000 mG) for 18 hours. Total Aβ and total Aβ$_{1-42}$ production was statistically significantly elevated among the ELF MF exposed cells compared to the cells with sham exposure. No gross morphological changes or changes in viability were observed in the ELF MF exposed cells. The 3.1 mT exposure level is 2-3 orders of magnitude higher than the highest occupational mean exposures. The authors state that such high levels were administered because occupational exposures are "much more prolonged than the one described in our experimental setting". There was no indication that any longer duration exposure at lower levels was studied.

B. **Alzheimer's Disease Alternative/Complementary Pathway: Lowered Melatonin Production**

Conclusion: There is considerable in vitro and animal evidence that melatonin protects against AD. Therefore it is certainly possible that low levels of melatonin production are associated with an increase in the risk of AD.

Several *in vitro* and animal studies indicate that melatonin may be protective against AD and thus low or lowered melatonin production may be a risk factor for AD. These studies have generally found that supplemental melatonin has the following effects:
- the neurotoxicity and cytotoxicity of Aβ is inhibited, including mitochondria (Pappolla *et al.*, 1997, 1999, 2002; Shen YX *et al.*, 2002a; Zatta *et al.*, 2003; Jang *et al.*, 2005);
- the formation of β-pleated sheet structures and Aβ fibrils is inhibited (Pappolla *et al.*, 1998; Poeggeler *et al.*, 2001; Skribanek *et al.*, 2001; Matsubara *et al.*, 2003; Feng *et al.*, 2004; Cheng and van Breemen, 2005);
- the profibrillogenic activity of apolipoprotein E ε4, an isoform conferring increased risk of AD, is reversed (Poeggeler *et al.*, 2001);
- oxidative stress *in vitro* and in transgenic mouse models of AD is inhibited if given early (Clapp-Lilly *et al.*, 2001a; Matsubara *et al.*, 2003; Feng *et al.*, 2006), but not necessarily if given to old mice (Quinn *et al.*, 2005);
- survival time is increased in mouse models of AD (Matsubara *et al.*, 2003);
- oxidative stress and proinflammatory cytokines induced by Aβ$_{1-40}$ in rat brain are reduced *in vitro* and *in vivo* (Clapp-Lilly *et al*, 2001b; Shen YX *et al.*, 2002b; Rosales-Corral *et al.*, 2003);
- the prevalence of Aβ$_{1-40}$ and Aβ$_{1-42}$ in the brain is decreased in young and middle aged mice (Lahiri *et al.*, 2004);
- memory and learning is improved in rat models of AD pathology (Shen YX *et al.*, 2001; Weinstock and Shoham, 2004), but not necessarily in Aβ-infused rat models (Tang *et al.*, 2002).

Note that transgenic mouse models of AD mimic senile plaque accumulation, neuronal loss, and memory impairment. See Pappolla *et al.* (2000), Cardinali *et al.* (2005), Srinivasan *et al.* (2006), Cheng *et al.* (2006), and Wang and Wang (2006) for reviews. Thus, chronic low levels of melatonin production may be etiologically related to AD incidence.

C.   **Epidemiologic Studies of Alzheimer's Disease and ELF MF Exposure**

Conclusion: There is strong epidemiologic evidence that exposure to ELF MF is a risk factor for AD. There are seven studies of ELF MF exposure and AD. Six of these studies are more of less positive and only one is negative. The negative study has a serious deficiency in ELF MF exposure classification that results in subjects with rather low exposure being considered as having significant exposure. There are insufficient studies to formulate an opinion as to whether radiofrequency MF exposure is a risk factor for AD.

C.1.   **Introduction**

First, it is necessary to point out that there are no case-control studies of melatonin as a risk factor for AD. This is primarily because AD results in a precipitous decline in melatonin production due to the destruction of specific neuronal structures and therefore it is inappropriate to use "current" melatonin production of cases as a surrogate estimate of the pre-AD melatonin production. Also there have yet to be any longitudinal studies of melatonin production. This is probably because neither urine nor blood have been collected appropriately to measure nocturnal melatonin production.

If MF exposure is a true risk factor, there are several problematic areas in evaluation and comparison of epidemiologic studies related to occupational MF exposure and Alzheimer's disease, particularly the following.

1. Diagnosis – false positive diagnoses will bias the odds ratio estimator towards 1.0
2. Occupational exposure assessment – inclusion of subjects with low exposure in the "exposed" categories likely biases the odds ratio estimator towards 1.0
   - Definition of MF exposure – published studies have differing definitions of MF exposure, potentially resulting in "exposure" categories with significant proportions of subjects with low exposure
   - Cut-points for non-exposure/exposure categories – some studies use numerical estimates of exposure developed from earlier exposure studies (job exposure matrices) in certain occupations and use average estimates and/or low cut-points to determine "medium" exposure
   - Ever versus never exposed – at least one study used ever exposed, with a low threshold for exposure
   - Categorized occupational data – categorized data from governmental databases leads to relatively large variation in "exposure" within occupational categories, which results in subjects with low exposure being classified as having been exposed.

Table 6 provides the data on the percentages of MF exposed subjects in the published studies to date. There is a wide range of percentages, due primarily to variation in exposure definition, use of average or mean job-specific estimates, and secondarily to the use of varying job exposure matrices. Table 7 provides the odds ratio estimates of studies discussed in some detail below. The studies

which used death certificates or other non-expert databases for the identification of AD cases are not included in Table 7.

The role of seamstresses among workers with high occupational MF exposure in the two *et al.* studies (1995, 1996b) and the Davanipour *et al.* study (2007) is discussed.

C.2.   **Death Certificates-Governmental Databases: Alzheimer's Disease Diagnosis**

The use of death certificates or governmental databases to identify AD cases is certainly problematic. False positive diagnoses tend to bias the OR estimator towards 1.0. Most diagnoses of AD have been and still are made by physicians who are not experts in AD, and who seldom have sufficient clinical time to make a proper diagnosis. The determination of dementia and subsequent differential diagnosis of AD by someone other than an expert has a high false positive rate. In addition, many physicians do not think that AD is a "cause of death", which results in an increase in the false negative rate.

Therefore the recent "positive" Feychting *et al.* (2003), Håkansson *et al.* (2003), and Park *et al.* (2005) studies and the "negative" Savitz *et al.* (1998a,b) and Noonan *et al.* (2002b) studies have been excluded from the discussion below of individual studies. The Johansen *et al.* study (2000) has also been excluded because it depended upon the clinical hospital discharge diagnoses of an historical cohort to determine a "diagnosis" of "presenile" AD or "dementia". Evidently, in that study, late-onset (age at least 65) AD was included under "dementia". (It should be noted that Johansen *et al*. found an increased risk of "dementia", but not "presenile" AD, associated with higher MF exposure.)

C.3.   **MF Exposure Assessment Rates and Analytic Results**

The Sobel *et al.* (1995, 1996b), the Davanipour *et al.* (2007), and the Harmanci *et al.* (2003) studies have followed nearly the same protocol for MF exposure assessment and classification into low, medium and high MF occupations. In these studies, medium exposure was defined as mean MF occupational exposure above 2 mG, but less than 10 mG, or intermittent exposures above 10 mG, while high exposure was defined as mean MF exposure above 10 mG or intermittent exposures above 100 mG. The rates of medium or high (M/H) exposure in these studies are considerably lower than the rates in the Feychting *et al.* (1998a), Graves *et al.* ((1999), Qiu *et al.* (2004), and Savitz *et al.* (1998b) studies and somewhat lower than the Feychting *et al.* (2003) study. The remaining three studies (Häkansson *et al.*, 2003; Savitz *et al.*, 1998a; Johansen, 2000) utilized subjects from electrical industries and therefore understandably have high rates of MF exposure. (See Table 6 for these rates.)

Thus, it is likely that a substantial percentage of MF "exposed" subjects in 4 of the 6 comparable studies (Feychting *et al.*, 1998a; Graves *et al.*, 1999; Qiu *et al.*, 2004) (Table 7) had a high rate of somewhat minimal exposure in the "exposed" category, due to classification methodologies, compared to the "exposed" categories in the Davanipour *et al.* (2007), Harmanci *et al.* (2003), and the Sobel *et al.* (1995, 1996b) studies. This would tend to lead to an OR estimate closer to 1.0 in the 4 former studies.

C.3.1.   **Sobel *et al.* (1995) Study – <u>Positive Study</u>**

The initial publication of an apparent association between AD and having worked in occupations with likely MF exposure consisted of three case-control studies, two from Helsinki, Finland, and one from Los Angeles, USA (Sobel *et al.*, 1995). Control groups varied: the first case-control study analyzed used VaD patients; the second (and largest study) used non-neurologic hospital patients; and the third (and second largest study) used non-demented well subjects. The study-specific ORs were 2.9, 3.1, and 3.0, while the combined OR was 3.0 (95% CI = [1.6 – 5.4], $p < 0.001$), with no confounder adjustments necessary. The occupational information was apparently primarily related to the last occupation, e.g., judge, high ranking military officer. A total of 386 cases and 575 controls was analyzed in these studies. 9.3% of the cases and 3.4% of the controls were judged to have had an occupation with likely medium or high MF exposure. Among women, 31 (5.3%) were exposed to M/H occupational MF, of whom 29 (95%) were seamstresses, who were classified as having high exposure based on measurements taken during the study. Seamstresses have subsequently been shown to have very high MF exposures (e.g., Hansen *et al.*, 2000; Kelsh *et al.*, 2003; Szabó *et al.*, 2006).

C.3.2.   **Sobel *et al.* (1996b) and Davanipour *et al.* (2007) Studies – <u>Positive Studies</u>**

These two studies utilized the databases of the nine (9) State of California funded Alzheimer's Disease Diagnosis and Treatment Centers (ADDTC). Sobel *et al.* (1996b), the second published study of occupational MF and AD, used the Rancho Los Amigos (RLA) ADDTC database. There were 316 cases and 135 controls. Twelve percent (12%) of the cases and 5.3% of the controls had had a medium or high "primary" exposed (MF) occupation. The Davanipour *et al.*, 2007) study used the databases of the other 8 ADDTCs. Seven and one-half percent (7.5%) of the cases and 3.8% of the controls had had a medium or high MF "primary" occupation. Among the women in the RLA ADDTC study, 26 (8.4%) had M/H exposure, of whom 17 (65.4%) were seamstresses. In the Davanipour *et al.* study, among women, 50 (3.8%) had M/H MF exposure, of whom 34 (68%) were seamstresses. This difference is statistically significant ($p < 0.001$). Among the men in the RLA ADDTC study, 14.8% had a medium or high MF exposed occupation, while in the Davanipour *et al.* ADDTC study, 13.5% had a medium or high MF exposed occupation. This difference is not significant. It thus appears that the women in the combined populations from which the ADDTCs in the Davanipour *et al.* study have drawn their patients have a lower rate of MF exposed occupations than the population from which the RLA ADDTC draws its patients. This is not too surprising because Los Angeles has a large apparel manufacturing industry.

The OR (adjusted for age-at-onset, gender, and education) for medium or high MF exposure in the RLA ADDTC study was 3.9 (95% CI = [1.5 – 10.6], $p = 0.006$). The ORs for medium or high MF exposure in the Davanipour *et al.* ADDTC study were lower: 2.2 ($p < 0.02$; 95% CI = [1.2 – 3.9]) and 1.9 ($p < 0.04$; 95% CI = [1.04 – 3.6]), using age-at-exam and age-at-onset, respectively, plus gender and history of stroke in the model. These ORs are all statistically significant. In the two studies, the 95% CIs greatly overlap and, under the assumption of normality of the natural logarithms of the odds ratios estimators and a straightforward hypothesis

test that the means of two independent normally distributed variables are equal, the null hypothesis that the corresponding ORs are equal cannot be rejected at the 0.05 level.

C.3.3. **Other AD and Occupational ELF MF Exposure Studies**

Studies with Positive Results

Qiu et al. (2004) *Study* Qiu *et al.* (2004) studied a Swedish cohort of 931 subjects, aged 75+ at baseline, followed for up to 7 years. Job history was usually obtained from the next-of-kin, but only after 4 years of follow-up. MF exposure assessment was estimated using previous occupational exposure studies, specific measurements (e.g., seamstresses and tailors), and expert opinion. During the follow-up period, 265 subjects developed dementia, with 202 receiving an AD diagnosis. Numerical exposure estimates were obtained using both the longest held occupation, last occupation, and any occupation. The estimated average daily MF exposure was used to classify individual exposure.

Exposure for a sample of seamstresses and tailors was measured at the head. They were classified as having low exposure. Exposures of seamstresses who used industrial sewing machines and workers who used home sewing machines likely were under estimated by Qiu *et al.* (2004): 5.5 mG for "industrial seamstresses" and 1.9 for tailors. Qui *et al.* only considered home sewing machines, which at the head had a mean exposure of 10 mG. For "industrial seamstresses, they assumed that 50% of the workday was at a 10 mG exposure and 50% was at background, 1 mG. This gives an average exposure of 5.5 mG. For tailors, they assumed that only 10% of the workday was spent sewing, so the mean exposure was 1.9 mG. There are several problems with this determination of exposure for seamstresses and tailors:

1. exposures to the head are among the lowest body exposures and are not necessarily the sole important exposure;
2. even in Sweden, it is unlikely that home sewing machines were exclusively used. It is more likely that most of the machines were industrial machines, which produce much higher fields constantly, even when sewing is not occurring;
3. seamstresses have exposure most of the workday;
4. ambient exposure levels in industrial settings have been measured at up to 6 mG (Sobel and Davanipour, unpublished Finnish data);
5. tailors would not make a living sewing only 0.8 hours per day.

Hansen *et al.* (2000) found that, at the side of the waist, mean full-shift exposure for industrial machines was approximately 30 mG, while Qiu used a figure of 10 mG. Based on unpublished measurements on AC home sewing machines, Sobel and Davanipour (1996c) found that exposures to the head were usually the lowest measurements, while the chest, pelvic area, thigh, knee, right arm and hand had much higher exposures (Table 8). In addition, foot pedals can produce high magnetic fields (Table 8). Also, AC/DC converters in the handles (right side) of computerized home sewing machines constantly produce high magnetic fields – about 75 mG at 2 inches away from the handle. The right hand, lower right arm, and knee regularly receive high exposures (Table 8). Thus, the 10% sewing time assumed by Qiu *et al.* (2004) does not mean that significant exposure is not over a longer time period. The biological plausibility of hypotheses discussed above

provides an argument that exposure to other body parts may also be deleterious. The numbers or percentages of industrial seamstresses and/or home sewing machine workers were not provided by Qui *et al.*

Nevertheless, for the principal occupation, but not for the last occupation or cumulative lifetime exposure, Qiu *et al.* (2004) found statistically significant ORs: OR=2.3 (95% CI = [1.0 – 5.1]) for AD and OR=2.0 (95% CI = [1.1 – 3.7]) for any dementia for men with average exposures greater than 2 mG. For women, no increase in risk was found for the principal occupation, last occupation, and all occupations combined. The average lengths of time in the last and principal occupations were not provided. Thus, comparison with the Feychting *et al.* study (1998a) could not be made.

The proportions of subjects with at least 2 mG exposure were 28.2% for AD cases and 28.8% for controls for the principal occupation (Table 6). For all occupations combined, the proportions were higher. For men, with cases and controls combined, the proportions were 43.1% and 33.0%, respectively, for principal occupation and all occupations combined. For women, the proportions were 24.3% and 32.1%. In the Sobel *et al.* (1995, 1996b) and Davanipour *et al.* (2007) studies, the proportion of female cases and controls with medium or high exposure (considered above 2 mG) was only 5.5%, 80% of whom were seamstresses or had allied professions with significant MF exposure, e.g., cutter. Thus, in these three publications, the exposure category for women contained a higher percentage of subjects with very high exposure. This may explain the lack of findings among women. The occupations which were in the exposure categories 'at least 2 mG' (dichotomized exposure) or 'at least 1.8 mG' (trichotomized) were not provided by Qiu *et al.* (2004).

Harmanci et al. (2003) Study Harmanci *et al.* (2003) conducted a cross-sectional, population-based study of Alzheimer's disease by selecting a random sample of 1067 subjects at least age 70, among whom 1019 (96%) agreed to participate in the study. AD was determined in a two-step process: a screening exam using the Turkish version of the Mini-Mental State Exam (MMSE), followed by an expert clinical exam among those whose MMSE scored indicated cognitive impairment. Two hundred twenty three (223) were asked to have a clinical exam, and 155 (69.5%) agreed. Among the subjects with a "normal" score on the MMSE, 126 were randomly selected for a clinical examination. Among these 281 subjects, 57 were clinically diagnosed as having possible AD, and 127 were determined to be cognitively normal. These subjects were included in the case-control study. M/H MF exposed occupations were stenographers and typists, carpenters and joiners, metal molders and core makers, tailors, dressmakers, and hatters. Except for stenographers, these occupations were considered to result in medium or high MF exposure in the Sobel *et al.* (1995, 1996b) and current study. A stepwise backwards logistic regression analysis was used. Medium/high MF exposure occupations had an adjusted OR of 4.0, with a 95% CI of [1.02 – 15.78]. It is interesting to note that use of electrical residential heating was also a risk factor (OR = 2.8, 95% CI = [1.1 – 6.9]).

Feychting et al. (1998a) Study In the case-control study by Feychting *et al.* (1998a), MF exposure during the last occupation, but not during the longest held occupation, was a risk factor for dementia not caused by a single stroke. The last occupation was held an average of 24.8 years among cases and 25.9 and 25.1 years among subjects within the two control groups. Consequently exposure during the last occupation was over a significant period of time. Using

the two control groups, the ORs for dementia were 3.3 and 3.8 with 95% CIs of [1.3 – 8.6] and [1.4 – 10.2] for occupations with geometric mean MF exposures estimated to be at least 2 mG. Housewives were excluded from the analyses. The ORs for Alzheimer's disease were somewhat lower (2.4 and 2.7). When the analysis was restricted to subjects aged 75 and below at onset or examination, the ORs (5.0 and 4.8) for AD were statistically significant. Also, for subjects of all ages with occupations likely to have resulted in an average MF exposure above 5 mG, the ORs for AD were both high, but significant for one referent group (OR = 8.3), and not for the other (OR = 4.1). The Feychting *et al.* study was small: 44 dementia cases had occupational data, 29 of whom were diagnosed with AD. 43% of the cases were in the MF exposed group, while 23% and 19% of the controls were in this exposure group. Given these high percentages, it is clear that some lower MF exposed occupations were classified in the exposed category than were classified in this study and the earlier Sobel *et al.* studies (1995, 1996b).

Study with Only Negative Results

Graves et al. (1999) *Study* Graves *et al.* (1999) studied 89 matched case-control pairs. Complete occupational histories were obtained. MF exposure in a given occupation was defined as having at least "probable intermittent exposures (a few minutes)" above 3 mG. A high exposure category was defined as exposure of "1 to several hours" above 3 mG. Two industrial hygienists rated the occupations. Thus, many exposed subjects likely had a low average exposure. 19.1% and 21.4% of the cases were considered to have been 'ever' exposed, while 21.4% and 22.5% of the controls were considered 'ever' exposed. An unknown number of subjects, classified as having experienced MF exposure, would not have been so classified in most or all of the other studies of neurodegenerative diseases or cancer. The estimated adjusted ORs for 'ever' having been exposed were 0.74 and 0.95, depending upon which industrial hygienist's classification was used (Graves *et al.*, 1999).

As noted above, the Feychting *et al.* (1998a) study found elevated odds ratios associated with the last occupation, and in the Sobel *et al.* studies (1995, 1996b) and the Davanipour *et al.* (2007) study, occupational information most likely related to the last occupation. Also, Feychting *et al.* (1998a) did not find an increased risk associated with measures which included earlier occupations, e.g., highest exposed occupation and longest held occupation. Qui *et al.* (2004) found elevated risk associated with the principal occupation for males. Consequently, 'ever' vs 'never' exposed, as used by Graves *et al.* (1999), may not be an appropriate comparison.

Graves *et al.* (1999) also used a cumulative exposure index, the weighted sum of the numbers of years in each occupation with the weights being 0, 1 and 2 for no exposure, only "intermittent exposures" above 3 mG, and exposure for "1 to several hours" above 3 mG, respectively. Using the non-zero cumulative index values, exposure was dichotomized at the median as 'low' or 'high'. Adjusted ORs for 'low' or 'high' cumulative exposure versus no exposure were also close to 1.0. The last or the primary occupation was not separately analyzed.

In summary, the non-significance of the ORs in the Graves *et al.* (1999) study may be due to three reasons: (1) less restrictive definitions of magnetic field exposure resulting in minimally exposed subjects being classified as having been 'ever exposed' or even highly exposed; (2) equal weight given to exposure during any age period, e.g., age 25-45 and age 45-65; (3) a cumulative exposure metric which equates what can be negligible exposure with significant exposure, e.g., negligible

exposure for 20 years equals significant exposure for 10 years. In addition, there were no seamstresses among their subjects, who were from an HMO established primarily for union families. Seamstresses are seldom in a union.

D.   Epidemiologic Studies of Alzheimer's Disease and RF Exposure

There are no studies of AD and RF to discuss. The single published epidemiologic study of RF and melatonin is discussed in Section II (Burch *et al.*, 2002).

IV.   BREAST CANCER

Figure 2 provides a schematic outline of the areas of study providing evidence that ELF MF exposure can lead to breast cancer through an effect on melatonin production levels, and, of course, possible but unknown other pathways. Section references are provided in Figure 2.

There is now accumulating evidence that low melatonin production may increase the risk of breast cancer (BC). This evidence comes from *in vitro*, animal, and two longitudinal human studies. The *in vitro* and animal study literature is quite extensive, so only a highlight review is provided. There are numerous published case-control studies of residential and occupational MF exposure as a risk factor for breast cancer. No epidemiologic studies of radiofrequency MF exposures and breast cancer have been published, which do not include ELF MF exposure, and which have reasonable data on RF exposure.

For a review of melatonin from basic research to cancer treatment, see Vjayalaxmi *et al.*, 2002.

- *Conclusion: There is sufficient evidence from in vitro and animal studies, from human biomarker studies, from occupational and light at night studies, and a single longitudinal study with appropriate collection of urine samples to conclude that high MF exposure may certainly be a risk factor for breast cancer. Most of the residential MF exposure studies have been negative. This may be because "high" residential exposures are actually not very high. Individual exposures may be of importance, e.g., home sewing machines, hair dryers, AC/DC converters near the head of the bed, water pipes causing intermittent high exposures near living room or TV room sofas and easy chairs.*

A.   *In Vitro* and Animal Studies Relating to Melatonin as a Protective Factor against Breast Cancer

A.1.   *In Vitro* Studies Related to Prevention of Oxidative Damage; Comparative *in vivo* Studies with Vitamin C and Vitamin E

Melatonin has been found to neutralize hydroxyl radicals and to reduce oxidative damage in over 800 publications (Reiter et al., 1995; Tan *et al.*, 2002). Melatonin has also been shown to act synergistically with vitamin C, vitamin E and glutathione (Tan *et al.*, 2000) and stimulates the antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione reductase (Reiter *et al.*, 2002).

- Using a cell-free system, Tan et al and others have demonstrated that melatonin neutralizes hydroxyl radicals more efficiently than does reduced glutathione Tan *et al.*, 1993a; Bromme *et al.*, 2000).
- Melatonin reduces oxidative damage to macromolecules in the presence of free radicals (Reiter *et al.*, 1997, 2001a). One mode of action is as a free radical scavenger (Reiter *et al.*, 2001b).
- Melatonin increases the effectiveness of other antioxidants, e.g., superoxide dismutase, glutathione peroxidase, and catalase (Antolin *et al.*, 1996; Kotler *et al.*, 1998; Pablos *et al.*, 1995; Barlow-Walden *et al.*, 1995; Montilla *et al.*, 1997).
- Melatonin has protective effects against ultraviolet and ionizing radiation (e.g., Vijayalaxmi *et al.*, 1995). Vijayalaxmi et al studied the effects of melatonin on radiation induced chromosomal damage in human peripheral blood lymphocytes (Vijayalaxmi *et al.*, 1996). Blood from human volunteers was collected before and after administration of a single 300 mg oral dose of melatonin. The post-administration samples of both serum and leukocytes had increased concentration of melatonin compared to the samples prior to melatonin administration. After gamma radiation and mitogen exposure, a sample of cells was cultured for 48-72 hours. Lymphocytes from the sample after melatonin was administered had significantly fewer chromosomal aberrations and micronuclei. Primary DNA damage was reduced. Vijayalaxmi et al hypothesized that melatonin, in addition to its hydroxyl radical scavenging, may also stimulate or activate DNA repair processes (Vijayalaxmi *et al.*, 1998).

Melatonin has been found to be a more potent protector from oxidative injury than vitamin C or vitamin E (micromoles/kg) in several *in vivo* studies (for a review, see: Tan *et al.*, 2002). Melatonin was also found *in vitro* to scavenge peroxyl radicals more effectively than vitamin E, vitamin C or reduced glutathione (Pieri *et al.*, 1994; Reiter *et al.* 1995), although melatonin is not a very strong scavenger of peroxyl radicals (Reiter *et al.*, 2001b).

A.2.   **Animal Studies of Mammary Tumor Prevention with Melatonin**

Several studies have found that melatonin inhibits the incidence of mammary tumors in laboratory animals either prone to such tumors or exposed to a carcinogen (e.g., Tamarkin *et al.*, 1981; Shah *et al.*, 1984; Kothari *et al.*, 1984; Subramanian and Kothari, 1991a,b; Blask *et al.*, 1991). In 1981, Tamarkin et al found that supplemental melatonin, given on the same day as 7,12-dimethylbenz(alpha)-anthracene (DMBA) and continued for 90 days, lowered the incidence of mammary tumors from 79% in controls to 20% (p<0.002) in the melatonin treated Sprague-Dawley rats (Tamarkin *et al.*, 1981). When they treated pinealectomized rats with DMBA, the incidence of mammary tumors increased to 88%, indicating a possible effect on endogenous melatonin on tumor incidence. Similar results, but with somewhat different study designs, using female Holtzman rats given the carcinogen 9,10-dimethylbenzanthracene have been found (Shah *et al.*, 1984; Kothari *et al.*, 1984). Subramanian and Kothari studied the suppressive effect by melatonin in rats treated similarly with DMBA under varying light:dark schedules and time of melatonin administration in both intact and pinealectomized female Holtzman rats (Subramanian and Kothari, 1991a). They found that when administered during the initiation phase, melatonin only suppressed tumor development in intact animals. However, when administered during the

promotion phase, melatonin had suppressive effects regardless of the presence or absence of the pineal gland. Subramanian and Kothari (1991b) also studied C3H/Jax mice and spontaneous mammary tumor development. Mammary tumors developed in 23.1% of mice provided with melatonin from 21 to 44 days of age, but in 62.5% of control mice (p<0.02). Furthermore, there was a decrease in serum 17-beta-estradiol levels in the melatonin treated mice (p<0.05). In a N-methyl-N-nitrosourea (NMU) model of hormone-responsive Sprague-Dawley rat mammary carcinogenesis, Blask *et al.* (1991) found that melatonin, given during the promotion phase, reduced the incidence of tumors and antagonized estradiol's stimulation of NMU-induced tumor incidence and growth. They, however, did not find a decrease in estradiol in the melatonin treated rats.

In two studies, Tan *et al.* (1993b, 1994) found that melatonin protected Sprague-Dawley rats from safrole induced liver DNA adduct formation. The protection was found at both physiological and pharmacological levels of supplementation. The level of protection was dose dependent. Intraperitoneal injection of paraquat causes lipid peroxidation, a decrease in total glutathione, and an increase in oxidized glutathione in Sprague-Dawley rats. Melchiorri et al found that melatonin inhibits these effects (Melchiorri *et al.*, 1995). In addition, melatonin and retinoic acid appear to act synergistically in the chemoprevention of animal model tumors (Teplitzky *et al.*, 2001) and *in vitro* systems (e.g., Eck-Enriquez *et al.*, 2000).

A.3.   Animal Studies Related to Prevention of Oxidative DNA Damage by Estradiol and Radiation

Karbownik *et al.* (2001) found that melatonin protects against DNA damage in the liver and kidney of male hamsters caused by estradiol treatment. They also found that in the testes, estradiol did not increase DNA damage, but that melatonin was protective against the natural level of oxidative DNA damage, as indicated by 8-hydrodeoxyguanosine (8-oxodG) levels. Several studies have found that laboratory animals are protected by melatonin from lethal doses of ionizing radiation (e.g., Blickenstaff *et al.*, 1994; Vijayalaxmi *et al.*, 1999; Karbownik *et al.*, 2000). Vijayalaxmi *et al.* (1999) and Karbownik *et al.* (2000) investigated markers of oxidative DNA damage and found that significant decreases in these markers in the melatonin treated animals.

A.4.   Melatonin: Scavenger of $^{\bullet}$OH and Other ROS

Melatonin is a powerful, endogenously produced scavenger of reactive oxygen species (ROS), particularly the hydroxyl radical ($^{\bullet}$OH). Other ROS which melatonin scavenges include hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\bullet}$), peroxynitrite anion ($ONOO^-$), hypochlorous acid (HOCl), and singlet oxygen ($^1O_2$) (Reiter, 1991; Tan *et al.*, 2000, Hardeland *et al.*, 1995; Antolin *et al.*, 1997; Stasica *et al.*, 1998). $^{\bullet}$OH is produced at high levels by natural aerobic activity. ROS are also produced by various biological activities or result from certain environmental and lifestyle (e.g., smoking) exposures.

Hydrogen peroxide does not appear to react directly with DNA (Halliwell, 1998), but does undergo chemical reactions within the cell nucleus which produce $^{\bullet}$OH, e.g., with Fe^{+2}. On the

other hand, $^1O_2$ readily oxidizes the guanine base. HOCl, ONOO$^-$, NO$^\bullet$ damage in various patterns (Halliwell, 1998).

However, $^\bullet$OH is the most reactive and cytotoxic of the ROS (Halliwell *et al.*, 1986). $^\bullet$OH appears not to be removed by antioxidative enzymes, but is only detoxified by certain direct radical scavengers (Tan *et al.*, 1999) such as melatonin.

Melatonin is found in every cell of the body and readily crosses the blood-brain barrier. It scavenges ROS at both physiologic and pharmacologic concentrations. In the literature, "physiologic" refers to blood level concentrations of melatonin, while "pharmacologic" indicates 2-3 orders of magnitude higher concentration. Recently, intracellular levels of melatonin, especially within the nucleus, have been shown to be naturally at "pharmacologic" levels for all cellular organelles studied to date (Maestroni, 1999; Reiter *et al.*, 2000).

Tan *et al.* (2002) review the underlying basis for melatonin's scavenging of ROS, which is briefly discussed here. From the known structure-activity relationships, the reactive center of the interaction between oxidants and the melatonin molecule is its indole moiety. This is due to its high resonance stability and quite low activation energy barrier towards free radical reactions. In addition, the methoxy and amide side chains contribute significantly to melatonin's antioxidant activity. The methoxy group in the C5 component of the molecule appears to prevent prooxidative activity. If this methoxy group is replaced by a hydroxyl group, under some *in vitro* conditions, melatonin may exhibit prooxidant capability. The mechanisms of melatonin's scavenging ROS appear to involve the donation of an electron to form a melatoninyl cation radical or a radical addition at site C3 of the melatonin molecule. (There are other possibilities also.) All known intermediates generated by the scavenging of a ROS by melatonin are also free radical scavengers. This is known (by some) as the 'free radical scavenging cascade reaction', which allows one melatonin molecule to scavenge 4 or more ROS. (See Tan *et al.*, 2007, for details).

B.   **Longitudinal Human Studies of Low Overnight Melatonin Production as a Risk Factor for Breast Cancer**

Conclusion: Two longitudinal studies have been conducted of low melatonin production as a risk factor for breast cancer. Neither study collected urine samples in an optimal manner to estimate the important component of melatonin production – overnight production. No published longitudinal study has collected complete overnight urine. However, one used first morning void, which is close to optimal, but the other had to use 24-hour collection, which hides possible non-circadian rhythm. The study with the first morning void was positive, the other was negative. Thus, there is some longitudinal evidence that low melatonin production is a risk factor for breast cancer.

There have been only two longitudinal studies of low melatonin production as a risk factor for breast cancer. Note that many breast cancers are associated with a decrease in melatonin production (Bartsch *et al.*, 1997). There is often a "rebound" after excision of the tumor, but it is not known if post-excision melatonin production is near the pre-tumor production level (Bartsch

et al., 1997). Thus, as with AD, it is not appropriate to use post-tumor melatonin levels in a case-control study of low melatonin as a risk factor for breast cancer.

DNA damage is the pathway through which normal cells become malignant. Thus, the greater the amount of DNA, the greater the probability of a malignant transformation and the development of a cancer. Davanipour *et al.* (2007) have conducted a study on the association between endogenous melatonin levels and oxidative guanine DNA damage among mothers and their oldest sampled daughters. The mothers' age range was 43-80, while the oldest daughter's age range was 18-51. Nearly all of the mothers, but few of the daughters were postmenopausal. Complete overnight urine samples were obtained. Creatinine-adjusted aMT6s and 6-hydrodeoxyguanosine (8-oxodG) were assayed. 8-oxodG is a measure of the level of oxidative DNA damage. Creatinine-adjustment is not necessary because the 8-oxodG level using complete overnight urine is a measure of the total repair of oxidized DNA guanine during the night. There was a statistically significant (p=0.02) inverse association between the level of nocturnal melatonin production (aMT6s/creatinine) and 8-oxodG for the mothers, but not for the daughters. Statistical adjustment was made for age and weight; however, there was little difference in the results with or without adjustment. The correlation between creatinine-adjusted aMT6s and 8-oxodG was 0.35 (p=0.01).

Positive Study

Schernhammer and Hankinson (2005) reported on the association between urinary melatonin levels and breast cancer risk in the Nurses' Health Study II. The study had collected first morning void urine samples prior to the diagnosis of any cancer in a sub-sample of the women in the study. Assays of aMT6s and creatinine for 147 women who developed invasive breast cancer, and 291 age-matched controls, plus 43 women who developed in situ breast cancer and 85 matched controls were analyzed. Analyses were based on quartiles of creatinine-adjusted aMT6s developed from the control data, with subjects in the lowest quartile as the referent group. (Thus, the analyses were conducted with a view that higher levels of melatonin production might be protective.) Unadjusted analyses, estradiol level adjusted analyses, and analyses adjusted for age-at-menarche, parity, age-at-first birth, family history of BC and benign breast disease, alcohol use, antidepressant use, and body mass index were conducted. It should be noted that low levels of melatonin are causally associated with earlier age-at-menarche (e.g., Cohen *et al.*, 1978; Sizonenko, 1987). Thus, inclusion of age-at-menarche in the adjustment is perhaps not appropriate. Analyses of cases and controls from the lowest and the highest quartile were statistically significant for each level of adjustment. The odds ratios (OR) were all 0.59. (In terms of risk associated with low melatonin production, the OR was 1/0.59 = 1.69.) Inclusion of the the cases with in situ breast cancer led to OR between 0.68 and 0.70. Significance levels were not provided. However, the 95% CI's for invasive breast cancer did not contain 1.0, while the 95% CIs when in situ breast cancer cases were included just barely contained 1.0.

** It should be noted that the first morning void, especially when the subject has had urine voids during sleep time, is not as good as complete overnight urine collection in estimating nocturnal melatonin production. **

Negative Study

Travis *et al.* (2004) conducted a study of melatonin and breast cancer using the Island of Guernsey or Guernsey III longitudinal study. This study recruited women for an eight and one-half year period, ending in 1985. During the follow-up period, 127 women developed breast cancer. Three hundred fifty three (353) controls were selected with matching based on age, recruitment date, menopausal status, day of menstrual cycle (if applicable) when the urine sample was obtained, and number of years post-menopausal (if applicable). Twenty-four (24) hour urine samples were collected. These samples were evidently not divided between overnight and other time-of-day sub-samples. None of the analyses (all cases-controls, only pre-menopausal cases-controls, or only post-menopausal cases-controls) showed any hint of an increase risk associated with low 24-hour melatonin production.

** It is unfortunate that the 24-hour urine samples were not subdivided by time of day. It is the nocturnal blood level of melatonin that is important. About 85%-90% of pineal melatonin is produced nocturnally. The circadian rhythm appears to be vital for the effects of melatonin in regulation of important biologic functions, including immune response. This particular problem with the study makes the results suspect. (See Hrushesky and Blask, 2004, for further details.) **

C.   **No Case-Control Studies of Low Melatonin Production as a Risk Factor for Breast Cancer**

As mentioned previously, breast cancer itself often causes a decrease in melatonin production, e.g., Bartsch *et al.* (1997). It is therefore inappropriate to use current levels of melatonin production of breast cancer cases in a case-control study of whether low levels of melatonin are a risk factor for breast cancer, and none have been published.

D.   **Light-at-Night and Night Shift Work Studies as a Risk Factor for Breast Cancer – Surrogates for Low Melatonin Production**

Conclusion: There is moderately strong evidence that both longterm light-at-night and night shift work increase the risk of breast cancer. Five (5) studies are reviewed, 4 of which are positive. The negative study did find an increased risk for light-at-night, but not shift work. This study classified subjects as having had rather short shift work as exposed. Only very few subjects had at least 8 years of shift work: 8 (1.6%) of cases and 19 (3.7%) of controls.

Several studies have found an increase in risk of breast cancer among women who have rotating night shift work or who otherwise experience light at night. Light at night (LAN) is well-known to cause a decrease in nocturnal melatonin production (e.g., Lewy *et al.*, 1980; Lowden *et al.*, 2004; Schernhammer *et al.*, 2004). Note that occupational studies of MF exposure (Section E, below) have included jobs with night shift work, e.g., flight attendant and radio/telegraph operators.

Positive Studies

- Lie *et al.* (2006) studied the occurrence of breast cancer among Norwegian nurses. All data were obtained from government registers. Among a cohort 44,835 nurses, who graduated from a 3-year nursing program between 1914 and 1980 and who were alive on January 1, 1953, or born after this date, 537 breast cancer cases which occurred between 1960 and 1982 were identified. (1960 was chosen because that was the first year for which fertility data were available.) Four (4) controls, alive and cancer free, for each case were selected from the nurse cohort, matched by year of birth ($\pm$ 1 year). Controls were required to have graduated or started their initial job no later than the year the corresponding case was diagnosed with BC. Number of years of night shift work was estimated from work history and work locations. Statistical adjustments in OR estimates included total employment time and parity. The OR for 30+ years of night shift employment versus 0 years, was 2.21 (p<0.05), 95% CI = [1.10 – 4.45]. The p-value for trend was 0.01. When the analysis was limited to nurses aged 50+, the OR was 2.01 (p>0.05), 95% CI = [0.95 – 4.26]. The number of cases without night shift work was only 50 for all ages, and was 29 for nurses over age 50. The number of cases with at least 30 years of night shift work was 24. (No case below age 50 had 30+ years of night shift work.)

- Schernhammer *et al.* (2001) examined rotating night shift work as a possible risk factor for breast cancer in the Nurses' Health Study. The total number of years in which a subject had worked rotating night shifts of at least 3 nights per month was obtained in 1988. The sample was quite large: 31,761 nurses had not had any years meeting the night shift criterion; 40,993 had had 1-14 years; 4,426 had had 15-29 years; and 1,382 had had 30+ years. During the following 10 year period, 2,441 incident cases of breast cancer were identified. Compared to nurses who had had no qualifying years, the adjusted relative risk (RR) for nurses with 30+ years of rotating night shift work was 1.36, with a 95% CI of [1.04 – 1.78]. All subjects with 30+ of rotating night shift work were post-menopausal Analyses were also conducted within pre- and post-menopausal groups. The RR and 95% CI were the same for 30+ years of exposure, because the number of nurses with no exposure decreased slightly (from 925 down to 801). While not statistically significant, perhaps due to sample size, pre-menopausal nurses who had at least 15 years of shift work had an adjusted RR of 1.34, 95% CI = [0.77 – 2.33], essentially the same RR as post-menopausal women (RR=1.36, 95% CI = [1.04 – 1.78]) who worked night shift for at least 30 years. There were only 14 pre-menopausal nurses with 15+ years of exposure. The trend in RR for increasing years of exposure was statistically significant for post-menopausal nurses and all nurses. Adjustments were made for age, weight change between age 18 and menopause, and many other variables associated with breast cancer. The increase in risk was almost totally due to hormone-receptor positive breast cancers. This was the first prospective night shift and breast cancer study.

- Davis *et al.* (2001b) studied 813 breast cancer patients, aged 20-74, and 793 controls. The controls were obtained through random digit dialing and were frequency matched by 5-year age intervals. Lifetime occupational history, bedroom lighting, and sleep

habits were obtained by interview for the 10 years prior to diagnosis. Not sleeping during nocturnal periods (when melatonin production is usually at its peak) had an OR of 1.14 for each night per week. The 95% CI was [1.01 – 1.28]. Night shift work had an OR of 1.6, 95% CI = [1.0 – 2.5]. There was a significant upward trend (p = 0.02) in the OR with increasing years and more hours per week in night shifts. Statistical adjustments were made for parity, family history of BC, oral contraceptive use (ever), and recent (but discontinued) use of hormone replacement therapy.

- Hansen (2001) studied BC risk among younger Danish women whose work was mostly at night. All women born between 1935 and 1959, and 30-54 years of age, were identified though the Danish Cancer Registry. The number of such women was 7,565. One control per case was randomly selected from the Danish Central Population Registry. Controls were (i) living, (ii) apparently cancer free, and (iii) working before the date of diagnosis of the corresponding case. Work history was obtained from the Danish pension fund database. No work history was found for 530 cases, so the number of case-control pairs for the study was 7,035. Using a national survey (1976) of women and working conditions, 4 occupational categories were identified in which at least 60% of the female employees so some work at night. These were manufacturing of beverages, land transport services, catering, and air transport services. For hospitals, furniture manufacturing, water transport services, and cleaning services, between 40% and 59% of the women work some night shifts. Comparisons were made between occupations in which 60%+ of the women work night shifts and occupations in which less than 40% work night shifts. Only occupations within 5 years of diagnosis were considered. This limit was based on suspected induction time for breast cancer. To be placed in the "exposed" category a women had to have worked at least 6 months in a night shift occupation. Statistical adjustments were made for age, social class, ages at birth of first and last child, and parity. The OR for all "exposed" occupations was statistically significant (p<0.05): OR=1.5, 95% CI = [1.3 – 1.7]. For women who worked at least 6 years in "exposed" occupations, the OR was 1.7 (p<0.05). The results were essentially driven by the catering and air transport service occupations. (It should be noted that these two occupations may also result in higher MF exposure, compared to manufacture of beverages and land transport services.) The authors state that "(w)hen the 5-year induction time was ignored, the ORT decreased marginally".

Negative Study

- O'Leary *et al.* (2006) studied night shift work, light-at-night and BC in Long Island, NY, as part of the Electromagnetic Fields and Breast Cancer on Long Island Study (EFBCLIS) Group. There were 487 cases and 509 population-based controls, frequency matched to the expected age distribution of the cases in the study. These subjects had to have participated in the earlier Long Island Breast Cancer Study Project (LIBCSP). Each case had to have lived in the same home for at least 15 years prior to the diagnosis of breast cancer, while each control had to have lived in the same residence for at least 15 years prior to recruitment. Cases had to have had their BC diagnosis within the 12 month period beginning August 1, 1996. Controls were concurrently recruited. The LIBCSP had collected, via direct interview, complete job history information, including

shift work – all jobs held for at least 6 months beginning at age 16, full time or part-time. The EFBCLIS repeated the job history interview, without the shift work information, for the period 15 years prior to the date of BC diagnosis (cases) or recruitment (controls). Military assignments were included. Light-at-night information was obtained by interview, and included information about sleep hours, frequency and length of having lights on during sleep time for the 5 year period prior to the reference date.

Exposure to shift work was defined as ever having had a job (≥ 6 months, either part or full time) with at least 1 day per week of shift work, during the 15 years prior to the reference date. Sub-groups were defined as follows: ever had an evening shift job; ever had an overnight shift job; ever had an evening shift, but never an overnight job; ever had an overnight shift; but never an even shift job. Statistical analyses were adjusted for reference date, parity, family history of BC, education, history of benign breast disease.

For any of the various categories of shift work during the 15 years prior to the reference date, there was no elevated risk of BC. However, 'any overnight shift work' had a statistically significant OR below one. The referent group included subjects with a jobs having less than 1 shift work day per week. Such a job could have been held for many years. The OR for at least 8 years of overnight shift work was statistically significantly below 1. For light-at-night within 5 years prior to the reference date, the only statistically significant finding was an OR = 1.65 for waking up and turning on lights at least 2 times per night versus doing so no more than 3 times per month.

The authors conclude that their study "provides mixed evidence for the light-at-night hypothesis". Analyses of shift work within 5 years of the reference date, the "induction" period used by Hansen (2001), were not presented. Overnight shift work was in the work history of only 26 cases and 50 controls; a duration of at least 8 years of overnight shift work was experienced by only 6 cases and 19 controls. Thus, the effective, "exposed" sample size was quite small. Information as to when this shift work occurred relative to the reference date was not provided.

E.   **Occupational Case-Control Studies of MF Exposure as a Risk Factor for Breast Cancer**

Conclusion: There is rather strong evidence from case-control studies that longterm, high occupational exposure to ELF magnetic fields is a risk factor for breast cancer. Six (6) independent studies are reviewed. Four (4) have positive conclusions, while two (2) are negative. The latest study is particularly strong. The two negative studies have serious shortcomings in exposure classification and come from the same research group.

There have been several case-control studies of occupations with more or less high MF exposure and the risk of breast cancer. These studies have been generally positive, in the sense that there appears to be an increased risk. Earlier studies generally lack appropriate exposure information (e.g., Wertheimer and Leeper, 1994).

Positive Studies

- Peplonska *et al.* (2007) have conducted a large, population-based, case-control study of breast cancer and 73 occupational categories. All incident cases of cytologically or histologically confirmed breast cancer among women aged 20-74 in Warsaw and Lódź, Poland, in 2000-2002 were identified. 2,502 controls were randomly selected using the Polish Electronic System of Population Evidence, which maintains records on all citizens of Poland. Controls were matched to cases by city of residence and age ± 5years. A structured questionnaire was completed by 79% of the cases and 69% of the controls. The questionnaire included items related to demographics, reproductive and menstrual history, hormone use history, physical activity, occupational history for all jobs held at least 6 months, smoking, alcohol use, diet, cancer history in female relatives, medical and screening history, prenatal exposures, and history of weight and height development. Occupational information included job title, start and stop dates, employer, company products and/or services, work activities and duties, physical activity related to work, passive smoking, and exposures to a list of chemicals. The study was funded by the U.S. National Cancer Institute (NCI) and managed by Westat (Rockville, MD).

  Statistical adjustment was made for age, age-at-menarche (≤ 12; 13-14; ≥ 15), menopausal status; age-at-menopause, parity ≤ 1; 2; ≥ 3), body mass index (< 25; 25-30; ≥ 30 kg/m^2), first degree female family history of BC, education ($<$ high school; high school; some college or professional training; college degree), previous mammographic screening, and city of residence. Oral contraceptive use, marital status, tobacco and alcohol use, age-at-first full term birth, breastfeeding, recreational and occupational history were not used for adjustment in the final analyses because they had "little impact" on the results.

  In the primary analyses, for each specific job category/industry, the referent group consisted of all subjects who did not work in that job/industry for at least 6 months. For each specific "white-collar" occupation, additional analyses using all other white-collar jobs as the referent group were conducted. This was thought to provide at least a partial account for socio-economic factors not accounted for by education. Similar blue-collar job analyses were not conducted. Several job categories containing occupations with elevated MF exposure had statistically significantly elevated ORs.

  ** These ORs were significantly elevated despite the fact that all other occupations with elevated MF exposure were placed in the referent group. **

  ELF MF exposure was determined using a job exposure matrix developed within NCI for a brain cancer study. No, low, medium and high categories were developed by "experienced industrial hygienists". (No reference was provided.) The highest MF exposure category of all jobs for an individual was used in analyses. 99% of the high exposed subjects were so ranked due to employment as machine operators and tenders

in the textile apparel and furnishing industry. Information on which occupations were classified as low or medium MF exposure were not provided.

** It should be noted that (1) 'tenders' generally provide maintenance to machinery and (2) operators of machines other than sewing machines, e.g., cutters, both have lower MF exposure than seamstresses. **

The OR for high MF exposure versus no exposure was significant: OR = 1.5, 95% CI = [1.1 – 2.0]. For low exposure, the OR was also significant: OR = 1.2, 95% CI = [1.0 – 1.5]. For medium exposure the OR was also 1.2, but the 95% CI was [0.9 – 1.5]. Additional data analyses were not provided. The OR for high exposure among textile apparel machine operators and tenders is in line with the statistically significantly increased OR for seamstresses in the Forssén *et al.* (2005) study (see below under "negative studies") discussed below. In the Forssén *et al.* study (2004), seamstresses were classified as having medium-low MF exposure.

Specific ORs for occupations classified (surprisingly and for some likely incorrectly) as having high (as opposed to low or at most medium) MF exposure by Forssén *et al.* (2004) (see below) were calculated: cooks (OR=1.0); computer scientists (OR=1.3); computer and peripheral equipment operators (OR=0.7); data entry keyers (OR=0.3); dentists (OR=0.6); dental nurses (OR=1.0); counter clerks and cashiers (OR=1.1); and telephone operators (OR=0.9).

- Labréche *et al.* (2003) studied occupational ELF MF exposure and post-menopausal breast cancer. Cases and controls were identified through pathology department records at 18 hospitals in Montreal, Canada. These hospitals treat most of the breast cancer cases in the area. Age was restricted to 50-75 at the time of initial diagnosis of primary BC. Cases had to be residents of the region and the diagnosis had to have been in 1996 or 1997. Controls had one of 32 other cancer diagnoses and were frequency matched by age and hospital. The following cancers were excluded: liver, intrahepatic bile duct, pancreas, lung, bronchus, trachea, brain, central nervous system, leukemia, lymphoma, and non-melanoma skin cancer, but not gastrointestinal (Schernhammer *et al.*, 2003) or colorectal cancer (Bubenik, 2001).

  Complete occupational history, including task descriptions, and other personal information was obtained by personal interview, either of the subject or a surrogate if the subject was deceased or otherwise unavailable. Specialized occupational questionnaires were used for specific occupations, including sewing machine operators, cooks and nurses. The development of these questionnaires was lead by Jack Siemiatycki. See, for example, Siemiatycki *et al.* (1991, 1997). ELF MF exposures were estimated from detailed descriptions of tasks, equipment used, and the work environment by industrial hygienists intimately familiar with Montreal workplaces. The MF exposure categories and primary occupations were as follows: no exposure (< 2 mG; low exposure (2-5 mG, "typical jobs", including VDT operators, electric typewriter operators); medium exposure (5-10 mG; denturists, machinists); and high exposure (≥ 10 mG; sewing machine operators, textile workers). The industrial hygienists

"confidence" in each subject's exposure assessment was obtained as definitely no exposure, or low, medium, and high confidence of exposure.

Exposures to benzene, perchloroethylene, and alphatic aldehyes, chemicals found in the textile industry, were also considered.

Statistical adjustments were made for age at diagnosis, family history of breast cancer, education, ethnicity, age-at-bilateral oophorectomy, age-at-menarche, age-at-first full-term pregnancy, oral contraception use, duration of HRT, total duration of breast feeding, alcohol use, smoking, and body mass index, as appropriate. Adjustment was also made for proxy versus personal responses because proxies tend to report fewer jobs. In addition, duration of employment in the textile industry was an adjustment variable. As mentioned previously, adjustment for age-at-menarche is probably not appropriate due to melatonin's causal relationship with age-at-menarche.

In addition to the categorical analyses, the number of hours of medium or high exposure was used as a risk factor. The number of hours from the lower limit of the second quartile to the upper limit of the third quartile of medium/high exposure was 6000 hours. ORs were presented for a difference of 6000 hours.

All analyses, e.g., no exposure vs ever exposed, prior to 10 years before diagnosis, or before age 35, were non-signficant and non-elevated except for the following ones, adjusted for textile industry employment and other factors:

- ✓ No exposure vs medium-to-high exposure – OR = 1.90, 95% CI = [0.99 – 3.85];
- ✓ 6000 hour increase in medium-to-high exposure – OR = 1.21, 95% CI = [0.97 – 1.49];
- ✓ 6000 hour increase in medium-to-high exposure prior to 10 years before diagnosis – OR = 1.31 (p<0.05);
- ✓ 6000 hour increase in medium-to-high exposure prior to age 35 – OR = 1.54 (p<0.05).

The significant results appear to be primarily due to MF association with progesterone positive and/or estrogen positive breast cancers.

The use of a 10 year lag eliminates exposure periods which may be too near the diagnosis time to be etiologically relevant. The analysis of exposures prior to age 35 identifies the time period when the development of female breast cells appears to cease.

The use of textile industry employment (yes/no) or length of time in the textile industry, as appropriate, as a covariate provides some adjustment for chemical exposures. Thus, the increase in the ORs when adjustment was also made for textile industry employment relates to MF exposure.

Finally, controls also had cancer. While many of the excluded cancers may conceivably have ELF MF as a risk factor, some of the non-excluded ones may also. This is

especially true if the melatonin hypothesis is correct. Thus, the OR estimates may be biased towards 1.

- Kliukiene *et al.* (1999, 2003, 2004) and Tynes *et al.* (1996) studied occupational MF exposure and breast cancer among Norwegian women in general and radio and telegraph operators in particular. These were follow-up studies. A population-based cohort of 1.1 million women was developed using the 1960, 1970, and 1980 censuses. All women were working at the time of enrollment and had a potential for occupational MF exposure. The follow-up period was from 1961-1992. Date of birth, and census information about occupation and socioeconomic status was obtained. Incidence of breast cancer was obtained from the Cancer Register of Norway. Out-migration information was obtained.

  For the countrywide, all occupations study (1999), MF occupational exposure assessment was not optimal, but was as follows. The first method used expert opinion. An expert panel, using written guidelines, decided whether a given occupation had MF exposure above 1 mG for than 4 hours per week, between 4 and 24 hours per week, or more than 24 hours per week. Occupations were identified by a 3-5 digit industry code and a 3-digit occupation code. For cumulative exposure, the mean of each of the three (3) levels of exposure were used: 2 hours; 14 hours, 32 hours (based on a 40 hour week). It was assumed that each subject was in the same occupation from census to census, unless she died, emigrated or turned age 65.

  The second method used the Swedish job exposure matrix used in the Forssén *et al.* (2000) study (below), which was constructed from observations of male workers. Cumulative exposure was categorized as below 9 mG-years, between 9 and 14 mG-years, between 14 and 30 mG-years, and above 30 mG-years. Exposure was also classified by number of work hours of exposure above background (1 mG): below 900 hours; 900-999 hours; 1000-1999 hours; 2000 or more hours.

  Poisson regression, with adjustment for age, time period, and socioeconomic status, was used to estimate the relative risk (RR) of breast cancer. 22,543 breast cancer cases were diagnosed during the follow-up period. In the total cohort and the two sub-cohorts for those below or at least 50 years of age at inclusion in the cohort (Kliukiene *et al.*, 2004), the RRs were statistically significantly above 1.0 for each category of number of exposed hours, with below 900 hours as the reference category. For each cumulative exposure category above the reference category (below 9 mG-years, the RR for the total was statistically elevated. For the two sub-cohorts, the RRs were significantly elevated for the 9–14 and 14–30 mG-years categories. For the 30+ mG-years category the RRs were elevated, but lower bounds of the 95% CIs were 0.98 and 0.99.

  These studies did not have very good occupational data.

  For the radio and telegraph operators studies, the same cohort and occupational determination method was used. The Kliukiene *et al.* (2003) study was identical to the Tynes *et al.* (1996) study, except for a longer follow-up. By the end of May 2002, there

were 99 breast cancer cases among the 2619 radio and/or telegraph operators in the cohort. The standardized incidence ratio was 1.30, 95% CI = [1.05 – 1.58].

A nested case-control study was also conducted, using the 99 BC cases and 4 controls per case matched on year of birth ± 5 years for cases born prior to 1920 and ± 1 year for cases born in 1920 or later. It was an update of an earlier study by Tynes *et al.* (1996). The reference category consisted of subjects (all radio and/or telegraph operators) who were not registered in the Norwegian Seamen Registry, i.e., had no history of working on merchant ships. MF exposure was not particularly explicit. It seems to have been assumed that that women who had no history of working on merchant ships had lower MF exposure (ELF and radiofrequency) than those with a history of such work. Spot ELF MF and radiofrequency MF measurements in the radio/telegraph rooms of 2 and 3 ships, respectively, were performed. RF magnetic and electric fields were below the detection level of the instruments at the operator's desks. ELF magnetic fields varied from 0.2 mG to 60 mG at the operator's desks. However, the highest exposures were only to the stretched out leg. "Normal" exposure to the body varied from 1 mG to 2 mG. Thus, exposure was certainly not high.

Tertiles of cumulative exposure at sea were used in the statistical analyses, with adjustment for age-at-first birth and parity. Detailed job histories on each ship were available for each 'exposed' subject. For each ship, the amount of time spent in the radio/telegraph room was estimated by an experienced researcher. A rank of 1-3 was assigned: 1 – 'long voyage' for tankers or dry-cargo ships with longer stays as sea; 2 – 'many calls' for trade ships with several loading and discharge ports; 3 – larger passenger ships. Increasing rank implies increasing percentage of time spent in the radio/telegraph room. Exposure was then calculated by summing the product of the number years of service on ships of each rank by the rank of the ships.

Analyses were conducted for total exposure, and for total exposure with lag times of 10 and 20 years prior to BC diagnosis. Analyses were conducted for (1) all cases and controls, for cases and controls below age 50 in the reference year, and for cases and controls at least age 50 in the reference year, and (2) ER+ and ER- cases.

No OR was statistically significant for any analysis without consideration of ER status. However, there was a statistically significant increasing trend in the ORs over cumulative exposure categories in the analyses for all cases, cases younger than 50, and cases at least age 50. There was also a significant upward trend for a 10 year lag time using all cases. The ORs for the highest exposure category were all elevated, but not significant perhaps because of the sample size.

For analyses by ER status, the only significant finding was for ER- cases, age 50+ in the highest exposure category. There were elevated ORs for all exposure categories for all ER- cases, and for the highest exposure category for ER+ cases and for ER+ cases below age 50.

The authors concluded that "occupational exposure to electromagnetic fields increases the risk of (female) breast cancer" (Kliukiene *et al.*, 2003).

- Loomis *et al.* (1994) investigated BC mortality among female electrical utility workers. This study used U.S. national death certificate information, 1985-1989, to identify cases and controls (without leukemia or brain cancer as a cause or contributing cause of death) and occupations. There were 27,814 women with breast cancer and sufficient occupational information, of whom 68 had an "electrical" occupation. There were 110,750 controls, of whom 199 had an "electrical" occupation. The primary factor limiting the sample size was the availability of occupational information. It should be noted that use of occupational data from death certificates is far from optimal. Statistical adjustments were made for age, ethnicity, and social class. Loomis *et al.* found an elevated risk associated with having an electrical occupation recorded on the death certificate: OR=1.38 (p<0.05). The only specific occupation with a statistically significant elevated risk was telephone installers, repairers and line workers: OR=2.17. Electrical engineers and electrical technicians had 'elevated', but not significant risk estimates (OR=1.73 and 1.28). On the other hand, air traffic controllers, telephone operators, data keyers, computer operators, computer programmers did not have 'elevated' risk estimates.

  In a letter commenting on the Loomis *et al.* paper, Kantor *et al.* (1995) analyzed essentially the same data set, with the inclusion of data from 1984. They used an industrial hygienist to estimate the probability of occupational ELF MF exposure or video display terminals (0, low, medium or high) among white and black women. The ORs were statistically significant (but not particularly high) for medium or high probability of exposure for both white and black women. When the hygienist actually categorized the level of ELF MF exposure, only medium exposure was associated with a statistically significant OR. High exposure had somewhat lower ORs.

Negative Studies

- Forssén *et al.* (2005) published a case-control study of occupational MF exposure and breast cancer. This study may be considered influential, unless reviewed in detail. So considerable detail is provided.

  The Forssén *et al.* (2005) study found no association between occupational MF exposure, as determined by Forssén *et al.* (2005) , and breast cancer. The study is singled out because (1) it is essentially well designed, and (2) has a completely inappropriate ELF MF occupational classification scheme based on either non-representative workers in specific occupations or what should be considered quite suspect individual measurements (Forssén *et al.*, 2004). Many occupational groups which are generally considered to contain higher MF exposed occupations have been classified as low or medium-low exposure.

** Forssén *et al.* (2005) did find that seamstresses had statistically
significantly elevated risk of breast cancer. However, they classified
seamstresses as having medium-low MF exposure. **

Forssén *et al.* (2005) used newly collected exposure data for occupations in which
women commonly work (Forssén *et al.*, 2004). The exposure study assessed
occupations identified within the Swedish 1980 census. Forty-nine (49) specific
occupational titles were identified. Volunteers working in each of these occupations
were then ascertained by methods which are not specified. Personal 24-hour ELF MF
measurements were obtained on what was presumably supposed to be a typical 24-hour
day, using a dosimeter worn at the waist. The volunteers kept a diary so that time
periods at work, at home, and elsewhere could be identified. The number of subjects
with measurements by occupation ranged from 5 to 24. The total number of subjects
measured was 471. There were only 5 observations for Seamstresses, and 5 Radio and
Television Assemblers and Repairwomen. The workday measurements were used for
classification purposes. In the epidemiologic study of breast cancer, 4 categories of
exposure were used: Low (< 1 mG); Medium-Low (1-1.9 mG); Medium-High (2-2.9
mG); and High (≥ 3 mG). The occupations in the categories above 'low' are provided
in Table 9. The arithmetic rate of change measure was also calculated. Seamstresses
and Radio and Television Assemblers and Repairwomen were both classified as
medium-low exposed occupations. The 5 seamstresses measured for exposure had their
own small businesses and did not work in apparel manufacturing. They evidently also
did not do much sewing. They spent 55% of their workday in fields below 1 mG and
only 15% in fields above 3mG. This is only an average of 1 hour and 12 minutes of
'high' exposure during a working day. In the two counties in Sweden in which both the
measurement study and the breast cancer case-control study were performed, there was
almost no apparel manufacturing (Forssén *et al.*, 2004; personal communication, M.
Feychting, 2007). Still, it is difficult to imagine such low exposures among women who
actually work as seamstresses.

The cases and controls were obtained from all women who were employed at any time
between 1976 and 1999, based on any of the censuses between 1960 and 1990, in either
Stockholm or Gotland counties, Sweden. Subjects entered the study in either 1976 or
their 15th birthday, which ever came first, and were followed through 1999 or to the date
of their initial breast cancer diagnosis. Cases were identified through the Regional
Cancer Registry in Stockholm. The referent year was the year of the case's diagnosis.
Controls were selected randomly by age and calendar year, apparently matched to cases.
Cases could not also be controls. Both cases and controls had to be living in Stockholm
or Gotland counties during the referent year. All information, including occupational
history, was obtained from registries. 20,400 cases and 116,227 controls were enrolled
in the study. Varying numbers of cases and controls were used in the analyses,
depending on the availability of occupational and other data. Statistical adjustment was
made for age, referent year, parity, and socioeconomic status.

For statistical analyses, exposure was assessed in various ways: (1) MF exposure for the
occupation closest to the time prior to the referent year; (2) MF exposure at the most

recent census which was at least10 years prior to the referent date; (3) MF exposure at the most recent census when the subject was at least age 35. Analyses were also carried out by (4) splitting the study period at 1985, by (5) only using subjects who either always had low exposure or ever having had high exposure, and by (6) defining low exposure as a median less than 1 mG and a third quartile of less than 1.7 mG and high exposure as a median greater than 2.5 mG and a first quartile including 1.7 mG. With these definitions, high exposed occupations were cashiers, working proprietors in retail trade, air stewardesses, dental nurses, cooks, post office clerks, and kitchen maids. No time latency period was used in the analyses related to (3).

There were no significant or elevated adjusted ORs for analysis (1) using the 4 categories of exposure, either for all BC cases, ER positive cases, or ER negative cases, for age below or at least 50. The referent group had MF exposure below 1 mG. There were no significant or elevated adjusted ORs for analysis (1) using low versus high (separated) exposure categories defined by (6), above.

Finally, in a series of analyses based on exposure 10+ years before the referent year, before age 35 for post-menopausal women, referent year before or after 1985, maximum point exposure, rate of change, and proportion of time exposure was above 3 mG, only a single adjusted OR was significant. The significant OR=0.87 and was for medium-high MF exposure among post-menopausal women before age 35.

It is thus fair to say that Forssén *et al.* (2005) found no relationship between their assessment of MF exposure and breast cancer. The authors do recognize that "(t)he major concern in the study is exposure misclassification".

Their job exposure classification is at odds with other classifications. Forssén *et al.* (2004, 2005) have classified Dental Nurses, Cashiers in Retail Stores and Restaurants, Working Proprietors in Retail Trade, Cooks, and Air Stewardesses as high MF exposure occupations. None of these occupations would be classified as having high MF exposure in any other classification scheme. The common cut-point for high exposure is 10 mG. Cashiers, cooks, and air stewardesses may at times have medium or high exposure, depending on (1) the exposure from scanners, (2) the exposure from microwave ovens, mixers, other motorized kitchen equipment, and (3) the exposure time from sitting near electrical panels on takeoff and landing and in the airplane's kitchen areas.

> ** Forssén *et al.* should conduct a sub-study to determine the actual environment in which the seamstresses in their study worked, the type of machines used (industrial, home; AC or DC operation), and the percent of time spent actually sewing. They also should conduct a study of seamstresses in general in Stockholm and Gotland counties and the in-migration rates. Also, the authors note an occupational category labeled 'textile occupations', which certainly includes seamstresses, but is otherwise undefined in the paper. Textile occupations need to be specified and studied individually, as was done by Hansen *et al.*, 2000. It is important to determine

whether the "seamstresses" in the Forssén *et al.* (2005) study have fundamentally different levels of exposure than seamstresses in other studies.**

The only significant occupational finding in this study related to seamstresses. Two analyses were conducted related to seamstresses (Table 10), probably because their exposure assessment was so at odds with every other series of exposure measurements of seamstresses. First, the OR for 'textile occupations', undefined in the paper, versus low MF exposed occupations was 1.37, 95% CI = [1.11 – 1.68]. Second, the OR for 'textile occupations' versus all other occupations, regardless of MF exposure assessment, was 1.33, 95% CI = [1.10 – 1.62]. The authors state that their results "suggest that the increased risk for breast cancer in these occupations might be related to some exposure other than magnetic fields".

'Textile occupations' were not defined, but could certainly have included a multitude of occupations with quite varying chemical exposures, and generally medium or high MF exposures. However, none of the 49 occupational categories, other than seamstress, used in the study appear to relate to textile occupations, if sales and administration are excluded.

The numbers of seamstresses as cases or controls in the study are not provided. However, in the AD studies by Sobel and Davanipour (1995, 1996, 2007), approximately 2% of the controls were seamstresses. Thus, there may have been at least 2000 seamstresses among the controls. Assuming that most, if not all women in "textile occupations" were seamstresses, and based on the OR of "textile occupations" vs MF exposure below 1 mG, the number of seamstresses with BC in the study can be estimated as approximately 475. Rough calculations indicate that if seamstresses are reclassified as having high MF exposure (> 3 mG), the adjusted OR for high occupational MF versus low occupational MF exposure would be about 1.10 and statistically significant. It is worth repeating that the Forssén *et al.* (2004) occupational classification for high MF exposure is (1) not as high as usual and (2) measured workday exposures are unusual for such occupations.

- Forssén *et al.* (2000) conducted an earlier case-control study of occupational and residential MF exposure and breast cancer. The cohort from which the study population was obtained consisted of all Swedish residents who lived within 300 meters of a (high power, 220 or 400 kilovolt) transmission line for at least one year between 1960 and 1985 and were at least age 16 sometime in the period. Subjects in this group living further away from transmission lines essentially had no exposure from such lines. Cases were identified through cancer registries. Controls were randomly selected and matched by age group, residence in the same parish at the time of diagnosis of the case and in the same type of house (single-family/apartment further than 300 meters from the same power line. (The parish/power line criteria were relaxed for 95 cases; a control could not be found for 7 cases.) Residential exposure was calculated from the MF generated by power lines. Occupation information was obtained from census data. An older job-exposure matrix was used to assess occupational MF exposure. Low (< 1.2 mG),

medium (1.2 – 1.9 mG), and high (≥ 2.0 mG) exposure categories were selected, based on quartiles. Exposure greater or equal to 2.5 mG was also considered.

Statistical adjustments were made for the matching variables. Only occupational exposure immediately prior to the diagnosis of BC and only residential exposure at the time of diagnosis was used in the analyses. No information concerning occupations of the subjects was provided. It is unlikely that seamstresses were included in the analyses.

No significant findings were identified.

Of 1767 cases and 1766 controls, only 711 and 709, respectively, had residential exposure information, only 744 and 764 had occupational exposure information, and only 197 and 200 had both types of exposure information. For the actual analyses of occupational exposures, with matching variable adjustment, there was complete information for only 440 cases and 439 controls. For analyses using both occupation and residential exposures, and matching variables, there was complete information for only 87 cases and 83 controls.

F.   **Residential Case-Control Studies of MF Exposure as a Risk Factor for Breast Cancer**

Residential MF exposure studies and BC have either used wire configuration coding, proximity to high voltage lines, various protocols of room measurements, or a combination of these methods. These studies have generally not found any increased risk of breast cancer (e.g., Feychting *et al.*, 1998; Davis *et al.*, 2002; London *et al.*, 2003; Schoenfeld *et al.*, 2003). Residential studies have measured actual magnetic fields only in current homes of cases and controls, thus homes which might be etiologically relevant are often or usually without actual measurements. Wire configurations and proximity to high voltage lines were at times used for surrogate measures of exposure related to previous homes. Each of these three methods of assessment of the level of exposure leads to significant classification errors. In addition, residential exposures are, almost always, surely relatively low. Individualized exposure, due for example to home sewing, sitting or sleeping near a panel of circuit breakers, sitting near a water pipe (e.g., in the floor or ceiling), is not identified. For homes near high voltage lines, rooms can have dramatically different ambient levels of MF. For these reasons, these studies are not relevant to the purposes of this review.

G.   **Radiofrequency Exposure and Breast Cancer**

There are no epidemiologic studies of radiofrequency MF exposure and breast cancer which do not include ELF MF exposure and which have reasonable data on RF   exposure, e.g., Kliukiene *et al.* (2003), above.

V. SEAMSTRESSES

> *Conclusion: Seamstresses are, in fact, one of the most highly MF exposed*
> *occupations, with exposure levels generally above 10 mG over a significant*
> *proportion of the workday. They have also been consistently found to be at higher*
> *risk of Alzheimer's disease and (female) breast cancer. This occupation deserves*
> *specific attention in future studies.*

Seamstress was the primary occupation among women with high MF exposure in the Sobel *et al.* (1995, 1996b) and Davanipour *et al.* (2007) studies related to AD. No other published AD study has evidently involved populations in which sewing was a somewhat common occupation. In the 5 independent case-control studies presented in the 3 Sobel & Davanipour papers, most of the high MF exposed women (cases and controls) were seamstresses. (Among women in these case-control studies, the Mantel-Haenszel AD odds ratio for seamstresses is 3.13, p < 0.01). Information about sewing as a hobby, which at least used to be common, was unavailable. Seamstresses have been shown to have very high ELF MF exposures (e.g., Szabó *et al.*, 2006; Kelsey *et al.*, 2003; Deadman and Infante-Rivard, 2002; Hansen *et al.*, 2000). Forssén *et al.* (2004) measured 5 "seamstresses" who owned independent small businesses and found what they classified as medium-low exposure – a mean of 1.7 mG. These 5 individuals used home sewing machines and evidently did not sew much. Peplonska *et al.* (2007), using a NCI occupational MF classification scheme found that, at least among women, nearly all high exposures occurred among textile machine operators and tenders. Both Forssén *et al.* (2005) and Peplonska *et al.* (2007) found statistically significantly elevated ORs for breast cancer among seamstresses/textile machine operators and tenders.

Sobel and Davanipour (1996c) measured ELF MF exposure from several home sewing machine models, both AC and DC models, to several parts of the body. The results are provided in Table 8. These results show that (1) high ELF MF exposure occurs to many parts of the body, (2) exposures vary by manufacturer, model, and even by machines of the same model, and (3) exposures depend on whether the machine operates by AC or DC current. For Alzheimer's disease and for breast cancer, it is not known where exposures may be most important. The peripheral Abeta hypothesis, if correct, would indicate that exposure to any location is important for AD. To affect pineal production of melatonin, it is not known whether exposure to the pineal gland is what is most important. For example, a majority of breast cancers causally lower pineal melatonin production. Because the melatonin production rebounds after excision of the tumor, the tumor itself must be secreting something that leads to the decline in melatonin production. Thus, it is conceivable that MF exposure may, at least in some individuals, also lead to the peripheral production of something that also causes a lowering of melatonin production. It is also not known whether MF exposure directly to the breast is etiologically important. Note that the right breast receives higher MF exposure from home sewing machines. No studies of right versus left breast cancer and use of home sewing machines have been published.

ACKNOWLEDGEMENT

Figure 1: Hypothesized Biological Pathway from MF Exposure to AD Development (from Sobel & Davanipour, 1996a)
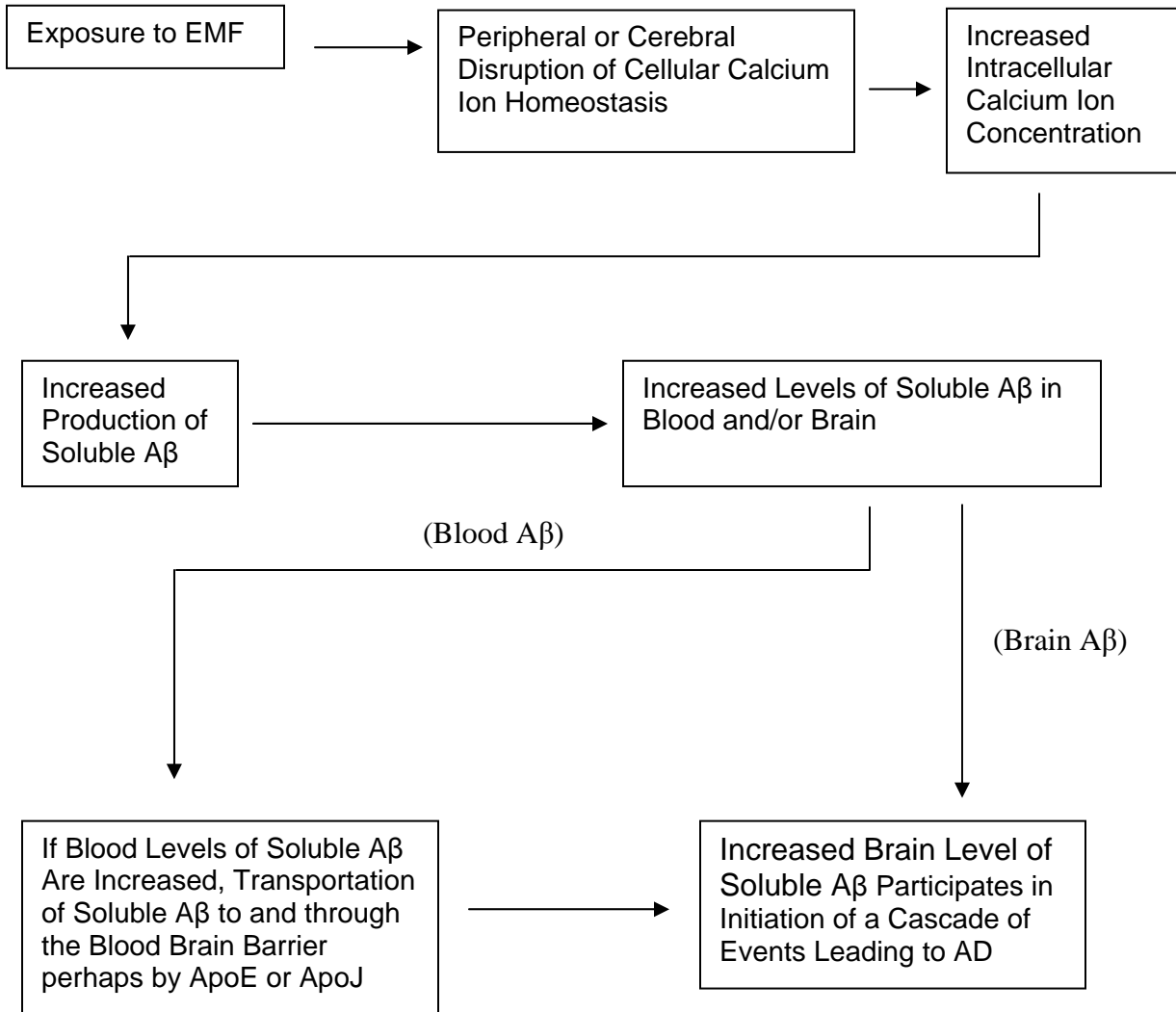
Figure 2: Outline of the Evidence that ELF MF Exposure Causes Breast Cancer through Decreases in Melatonin Production – with Section References


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┌──────────────┐                    ┌──────────────┐   (Section IV.D.)
│   ELF MF     │  ─────────────▶    │ Decrease in  │ ◀──────────────┐
│  Exposure    │                    │  Melatonin   │                │
└──────────────┘   (Section II)     │  Production  │                │
     ┊                              └──────────────┘      ┌──────────────┐
     ┊                                     ┊              │ Light-at-Night│
     ┊                        (Sections  IV.A., IV.B)     │     or        │
     ┊                                     ┊              │  Shift Work   │
     ┊                                     ▼              └──────────────┘
     ┊                              ┌──────────────┐             ┊
     └ ─ ─ ─ ─ ─ ─ ─ ─ ▶          │ Breast Cancer│             ┊
        (Section IV.E.)            │  Occurrence  │ ◀ ─ ─ ─ ─ ─ ┘
                                   └──────────────┘
                                        (Section IV.D.)
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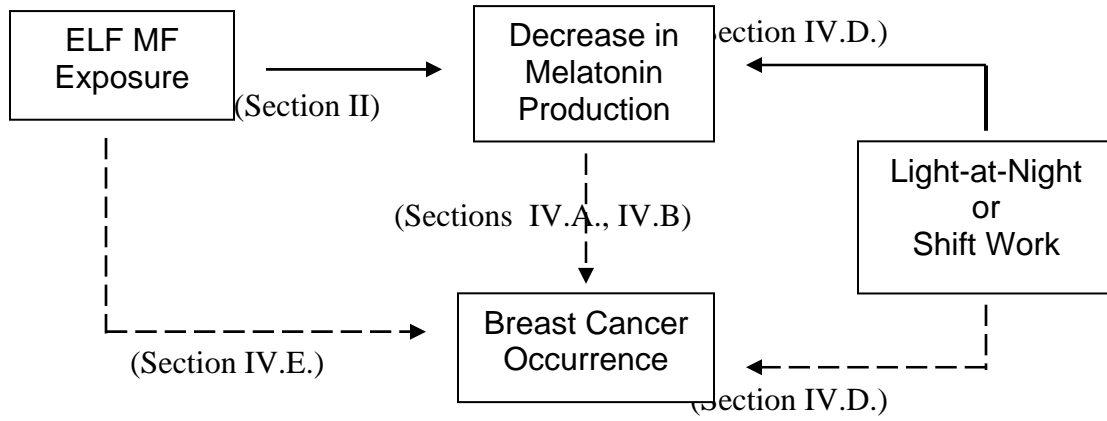
Note: Dashed lines indicate studies directly relating ELF MF exposure, light-at-night, or shift work to breast cancer occurrence.

Table 1: Baseline Data Results from the 1999 Mayeux *et al*. Paper: Means (Standard
Deviation)

Variable	Cognitively Normal at Follow-Up	Developed AD (3.6 Year Average Follow-Up)
Sample Size (n)	105	64
Age	73.4 (5.3)	77.4 (5.9)[a]
Education	9.3 (4.6)	7.5 (3.8)[a]
$A\beta_{1-40}$ (pg/ml)	111.8 (44.1)	134.7 (46.4)[a]
$A\beta_{1-42}$ (pg/ml0	51.5 (42.0)	82.4 (68.8)[a]
$A\beta_{1-42}/ A\beta_{1-42}$	0.51 (0.41)	0.67 (0.56)[b]

Notes: Cognitively normal was determined at baseline by the global Cognitive Dementia
Rating (CDR) scale with CDR=0 being normal. AD was diagnosed based on a CDR of 0.5
or 1.0, and clinical, functional and neuropsychological assessment as specified by the
NINCDS-ADRDA criteria. [a] $p \leq 0.0001$; [b] $p < 0.05$.

Table 2: Baseline Data Results from the 2003 Mayeux *et al*. Paper: Means (Standard
Deviation)

Variable	Cognitively Normal At Follow-Up	Developed AD (Up to 10 Year Follow-Up)
Sample Size (n)	365	86
Age	75.5 (5.9)	79.3 (6.6)[a]
Education	9.0 (4.6)	6.8 (4.5)[a]
$A\beta_{1-40}$ (pg/ml)	133.3 (61.9)	136.2 (46.7)[c]
$A\beta_{1-42}$ (pg/ml0	58.8 (32.9)	76.5 (59.8)[b]
$A\beta_{1-42}/ A\beta_{1-42}$	0.48 (0.3)	0.61 (0.53)[b]

Notes: Cognitively normal was determined at baseline by the global Cognitive Dementia
Rating (CDR) scale with CDR=0 being normal. AD was diagnosed based on a CDR of 0.5
or 1.0, and clinical, functional and neuropsychological assessment as specified by the
NINCDS-ADRDA criteria. [a] $p \leq 0.001$; [b] $p < 0.05$; [c] Not Significant.

Table 3:   Post-Work Levels of $A\beta_{1-40}$, $A\beta_{1-42}$, $A\beta_{1-42}/A\beta_{1-42}$ by MF exposure among Electrical Workers in the Noonan *et al*. (2002a) Study

MF Exposure	$A\beta_{1-40}$ (pg/ml)	$A\beta_{1-42}$ (pg/ml)	$A\beta_{1-42}/A\beta_{1-42}$	Sample Size
< 0.5 mG	125	136	1.03	20
0.5 – 0.99 mG	137	163	1.11	25
1.0 – 1.99 mG	128	166	1.19	8
$\geq$ 2.0 mG	156	262	1.46	7

Table 4:   Correlation (Corr) between Post-Work Creatinine-Adjusted aMT6s and Amyloid Beta by Number of Minutes between Samples in the Noonan *et al*. (2002a) Study

Number of Minutes	Sample Size	$A\beta_{1-42}$		$A\beta_{1-40}$		$A\beta_{1-42}/A\beta_{1-40}$	
		Corr	p-Value	Corr	p-Value	Corr	p-Value
All Subjects	60	-0.25	0.057	-0.19	0.144	-0.23	0.080
$\leq$ 90	46	-0.30	0.047	-0.22	0.154	-0.27	0.080
$\leq$ 60	37	-0.37	0.027	-0.25	0.150	-0.37	0.029
$\leq$ 30	23	-0.43	0.054	-0.28	0.224	-0.42	0.059

Table 5: Amyloid Beta Levels by Tertile of Post-Shift Creatinine-Adjusted aMT6s Levels in the Noonan *et al*. (2002a) Study

aMT6s/Cr Tertiles* (ng/mg)	$A\beta_{1-42}$		$A\beta_{1-40}$		$A\beta_{1-42}/A\beta_{1-40}$	
	Mean**	95% CI	Mean**	95% CI	Mean**	95% CI
≤ 1.38	177	[112–258]	133	[111–156]	1.30	[0.86–1.74]
1.39–3.3	214	[120–334]	147	[125–170]	1.33	[0.85–1.90]
> 3.3	123	[58–180]	123	[108–139]	0.82	[0.49–1.26]

*   n=60 subjects in each tertile
** geometric mean averaged over the work shift

Table 6:   Percentages of Subjects with Medium to High MF Occupations Exposure

STUDY	CASES	CONTROLS
Sobel *et al.* (1995a)	9.3 %	3.4 %
Sobel *et al.* (1996b)	12.0 %	5.3 %
Davanipour *et al.* (2007)	7.4 %	3.8 %
Harmanci *et al.* (2003)	10.5 %	3.1 %
Feychting *et al.* (1998a)	43.0 %	23.0 % & 19.0 %[#]
Graves *et al.* (1999)	19.1 % & 21.4 %	21.4 % & 22.5 %[^]
Qiu *et al.* (2004)	28.2 %[*]	28.8 %[*]
	34.2 %[**]	42.7 %[**]

Cases & Controls Combined

Feychting *et al.* (1998)	11.1 %
Håkansson *et al.* (2003)	80.5 % - likely exposed engineering industry workers
Johansen *et al.* (2000)	56 % - electrical company workers
Savitz *et al.* (1998a)	electric utility cohort – percentage not supplied
Savitz *et al.* (1998b)	23.9 %

\# Two control groups;
^ Two industrial hygienists
* Based on estimated daily exposure in principal occupation;
** Based on estimated daily exposure in all occupations

Table 7:   Odds Ratios for the MF and AD Studies*

Study	Risk Estimate (OR)	95% CI	p-value
Sobel *et al.* (1995) (late-onset; L vs M/H)			
	3.0	1.6 – 5.4	< 0.001
Sobel *et al.* (1996b) (late-onset; L vs M/H)			
	3.9	1.5 – 10.6	0.006
Feychting *et al.* (1998)(mostly late-onset; last occupation; by control group)			
(exposure ≥ 2 mG)	2.4	0.8 – 6.9	--**
	2.7	0.9 – 7.8	--**
(exposure ≥ 5 mG)	4.1	0.7 – 23.5	--**
	8.3	1.1 – 62.7	--**
Graves *et al.* (1999) (late-onset; ever exposed)			
	0.95	0.4 – 2.4	--**
	0.74	0.3 – 2.4	--**
Harmanci *et al.* (2003) (late-onset) 4.0		1.0 – 15.8	--**
(exposure as defined in Sobel *et al.* (1995, 1996b))			
Qiu *et al.* (2004) (age ≥ 75; exposure: ≥ 2 mG)			
Men	2.3	1.0 – 5.1	--**
Women	0.8	0.5 – 1.1	--**
Davanipour *et al.*, (2007) (exposure as defined in Sobel *et al.* (1995, 1996b))			
M/H vs L 2.2		1.2 - 3.9	< 0.02
H vs L 2.7		0.8 - 9.1	< 0.11

  * Studies use various types of controls and definitions of MF exposure. See text.
  ** p-values were not provided.

Table 8: Mean MF Exposures (mG) for Home Sewing Machines by Body Location: Continuous 2-Minute Measurements (Sobel & Davanipour, 1996c)

Sewing Machine	Background	Head	Breast Left	Breast Right	Pelvic Area	Thigh Left	Thigh Right	Knee Left	Knee Right	Lower Right Arm	Right Hand	Foot Pedal
Alternating Current Machines (older machines)												
Bernina 811	0.6	18.6	5.6	12.9	26.9	11.7	90.1	8.9	13.5	251.1	57.0	86.1
Bernina 811	0.9	1.7	2.6	5.4	8.2	4.5	11.6	6.8	36.5	77.1	31.7	102.0
Bernina 817	0.6	8.4	9.6	23.5	41.9	19.1	30.6	9.2	35.4	724.6	135.6	NA
Bernina 817	1.2	12.1	14.2	33.9	51.0	10.3	588.5	8.8	125.7	753.0	132.4	NA
Brother 920D	0.7	2.4	2.1	2.3	1.1	1.3	1.5	1.9	2.3	8.5	16.0	6.2
Necchi Type 525	0.3	5.1	2.0	1.1	2.5	1.1	2.4	2.0	5.1	25.9	22.6	5.9
Sears Kenmore	0.2	1.2	1.9	4.9	5.5	2.2	5.3	2.5	15.8	26.0	17.9	13.8
Singer 625	0.3	4.6	3.6	5.6	5.5	3.9	6.6	6.4	17.2	...	...	...
Singer 5932	0.5	1.2	0.9	2.0	2.7	1.1	2.5	1.0	4.1	8.6	23.0	2.9
Singer 6212C	0.3	7.0	2.8	6.4	2.0	1.4	2.2	1.4	1.9	31.0	26.2	4.4
Viking Husqvarna 6020	0.8	1.5	1.3	1.5	2.7	1.4	2.0	3.1	9.1	5.9	24.9	62.3
White 1410	0.2	2.2	1.6	1.1	1.1	3.2	10.8	4.2	67.5	20.8	18.3	2.8
Direct Current Machines (newer machines)												
Bernina 1000	1.0	1.3	1.6	2.3	2.9	1.9	2.5	2.8	11.2	8.1	41.2	798.0
Bernina 1090S	1.0	1.2	1.6	1.6	1.7	1.2	1.3	1.5	7.7	3.3	22.9	1.0
Elna Diva 900	1.6	5.1	3.9	4.1	4.1	3.0	3.1	3.2	8.4	40.4	57.1	1.8
Singer 3317C	0.7	3.4	1.6	2.9	2.2	2.1	2.2	1.5	11.3	22.1	25.8	5.8
Singer 9015	0.7	2.5	1.9	3.3	4.9	1.7	4.3	2.1	26.2	7.0	28.9	2.3
Viking Husqvarna 500	1.0	3.7	2.7	5.0	3.9	1.8	2.8	2.7	13.8	24.9	39.4	1.1
Percent > 2.0 mG	0%	67%	50%	78%	83%	50%	89%	72%	94%	100%	100%	80%

<u>Note</u>: The Bernina 1000, Bernina 1090S, Elna Diva 900, Singer 3317C, Singer 9015 and Viking Husqvarna 500 were brand new. The Singer 5932, Singer 6212C, and Brother 920D were 3-10 years old. The Bernina 811 and 817 machines, the Sears Kenmore, the Singer 625 the Viking Husqvarna 6020 are probably at least 15 years old. Both the White and the Necchi are fairly old. NA = not applicable, i.e., there was no foot pedal. "..." = no measurements were taken, e.g., because of machine malfunction.

Percent > 2.0 mG	0%	67%	50%	78%	83%	50%	89%	72%	94%	100%	100%	80%

Note: The Bernina 1000, Bernina 1090S, Elna Diva 900, Singer 3317C, Singer 9015 and Viking Husqvarna 500 were brand new. The Singer 5932, Singer 6212C, and Brother 920D were 3-10 years old. The Bernina 811 and 817 machines, the Sears Kenmore, the Singer 625 the Viking Husqvarna 6020 are probably at least 15 years old. Both the White and the Necchi are fairly old. NA = not applicable, i.e., there was no foot pedal. "..." = no measurements were taken, e.g., because of machine malfunction.

Table 9:   Classification of Occupations in Forssén *et al.* (2005)

Classification	Occupation	24-Hour Geometric Mean Average (mG)
High (≥ 3 mG)	Dental Nurse	3.0
	Air Stewardesses	3.0
	Cooks	3.1
	Working Proprietors in Retail Trade	3.4
	Cashiers in Retail Stores and Restaurants	4.5
Medium-High (2 – 2.9 mG)	Computer Operators	2.0
	Motor Vehicle Drivers	2.0
	Shop Managers	2.1
	Shop Assistants	2.1
	Hairdressers and Beauticians	2.1
	Bank Clerks	2.2
	Kitchen Supervisors	2.4
	Post Office Clerks	2.5
	Waitresses in Restaurants and School Kitchens	2.5
	Kitchen Maids	2.8
Medium-Low (1 – 1.9 mG)	Registered Nurses	1.0
	System Analysts and Programmers	1.2
	Telephone Operators	1.5
	Radio & Television Assemblers and Repairwomen	
	Seamstresses	1.6

Table 10:   Odds Ratio Estimates for Textile Occupations in the Forssén *et al.* (2005) Paper

_____

Comparison	OR	95% Confidence Interval
Textile Occupations vs Occupations with 24-Hour Exposure Below 1 mG	1.37	[1.11 , 1.68]
Textile Occupations vs All Other Occupations (Regardless of MF Exposure)	1.33	[1.10 , 1.62]

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SECTION 13

EVIDENCE FOR BREAST CANCER PROMOTION

(Melatonin Studies in Cells and Animals)

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TABLE OF CONTENTS

Introduction

The subject of breast cancer and studies of melatonin has a long and rich history replete with destroyed scientific reputations and career-ending charges of misconduct of scientists who have contributed stellar scientific work that has proved extremely inconvenient for governmental agencies and military and industrial interests (Liburdy). References are given in each section below to facilitate locating the pertinent references for each section.

II. Melatonin and ELF-EMF

Evidence which supports a possible mechanism for ELF-EMF and breast cancer is the consistent finding (in five separate labs) that environmental levels of ELF-EMF can act at the cellular level to enhance breast cancer proliferation by blocking melatonin's natural oncostatic action in MCF-7 cells (Liburdy, 1993; Luben et al, 1996; Morris et al, 1998; Blackman et al, 2001; Ishido, et al, 2001). ELF-EMF levels between 0.6 and 1.2 µT have been shown to consistently block the protective effects of melatonin.

The series of papers reporting increased breast cancer cell proliferation when ELF-EMF at environmental levels negatively affects the oncostatic actions of melatonin in MCF-7 cells should warrant new public exposure guidelines or planning target limits for the public, and for various susceptible segments of the population.

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Girgert et al (2005) reported that *"the anti-estrogenic activity of tamoxifen is reduced in two subclones of MCF-7 cells under the influence of ELF/EMF to different extent. Dose-response curves of the growth-inhibitory effect of tamoxifen are shifted towards higher concentrations leading to a reduced growth inhibition at a given concentration.   Our observations confirm results from a previous report describing a reduced inhibitory effect of tamoxifen at 1^{-7} M from 40% to only 17% by exposure to an EMF of 1.2 µT"* (Harland t al, 1997).   Further, Girgert et al conclude that "*From a medical point of view, it is disturbing that maximal induction of cell proliferation by tamoxifen at a field strength of 1.2 µT is observed at concentration of 10^{-6} M. This is exactly the serum concentration achieved in BC patients under standard oral therapy."* (De Cupis et al, 1997).

The Girgert et al paper confirms prior findings that environmental level ELF-EMF inhibits the antiproliferative action of tamoxifen in MCF-7 human breast cancer cells. Four other papers reporting this effect include Liburdy et al, 1997; Harland et al, 1997; Harland et al, 1999; and Blackman et al, 2001).

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VII. Conclusions

Conclusion: The constellation of relevant scientific papers providing mutually-reinforcing evidence for an association between power-frequency electromagnetic fields (ELF-EMF) and breast cancer is strongly supported in the scientific literature.

Conclusion: ELF at environmental levels negatively affects the oncostatic effects of both melatonin and tamoxifen on human breast cancer cells. Numerous epidemiological studies over the last two decades have reported increased risk of male and female breast cancer with exposures to residential and occupational levels of ELF. Animal studies have reported increased mammary tumor size and incidence in association with ELF exposure.

Conclusion: ELF limits for public exposure should be revised to reflect increased risk of breast cancer at environmental levels possibly as low as 2 mG or 3 mG; certainly as low as 4 mG.

SECTION 14

**EVIDENCE FOR DISRUPTION BY THE
MODULATING SIGNAL**

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Table of Contents

I.   Introduction

II.   The Old Standards (Based on Heating and Electric Current Flow in Tissues)

III. Laboratory Studies

Fundamental exposure parameters that must be considered when establishing a mode (or mechanism) of action for non-thermal EMF-induced biological effects.

    A. **Intensity**
    B. **Frequency**
    C. **Static Magnetic Field**
    D. **Electric & Magnetic Components**
    E. **Sine and Pulsed Waves**
    F. **Mechanisms**

IV. Problems with Segregation of Effects by Artificial Frequency Bands that Ignore Modulation

    A.   **Suggested Research**

V.   Conclusions

VI.   References

I.   Introduction

Modulation signals are one important component in the delivery of EMF signals to which cells, tissues, organs and individuals can respond biologically. At the most basic level, modulation can be considered a pattern of pulses or repeating signals which have specific meaning in defining that signal apart from all others. Modulated signals have a specific 'beat' defined by how the signal varies periodically over time. Pulsed signals occur in an on-off pattern, which can either be smooth and rhythmic, or sharply pulsed in quick bursts. Amplitude and frequency modulation involves two very different processes where the high-frequency signal, called the carrier wave, has a low-frequency signal that is superimposed on or 'rides' on the carrier frequency. In amplitude modulation, the lower-frequency signal is embedded on the carrier wave as changes in its amplitude as a function of time, whereas in frequency modulation, the lower-frequency signal is embedded as slight changes in the frequency of the carrier wave. Each type of low-frequency modulation conveys specific 'information', and some modulation patterns are more effective (more bioactive) than others depending on the biological reactivity of the exposed material. This enhanced interaction can be a good thing for therapeutic purposes in medicine, but can be deleterious to health where such signals could stimulate disease-related processes, such as increased cell proliferation in precancerous lesions. Modulation signals may interfere with normal, non-linear biological functions. More recent studies of modulated RF signals report changes in human cognition, reaction time, brainwave activity, sleep disruption and immune function. These studies have tested the RF and ELF-modulated RF signals from emerging wireless technologies (cell phones) that rely on pulse modulated RF to transmit signals. Thus modulation can be considered as information content embedded in the higher frequency carrier wave that may have health consequences beyond any effect from the carrier wave directly.

In mobile telephony, for example, modulation is one of the underlying ways to categorize the radiofrequency signal of one telecom carrier from another (TDMA from CDMA from GSM). Modulation is likely a key factor in determining whether and when biological reactivity might be occurring, for example in the new technologies which make use of modulated signals, some modulation (the packaging for delivery for an EMF 'message')

may be bioactive, for example, frequencies are similar to those found in brain wave patterns. If a new technology happens to use brain wave frequencies, the chances are higher that it will have effects, in comparison, for example, to choosing some lower or higher modulation frequency to carry the same EMF information to its target. This chapter will show that other EMF factors may also be involved in determining if a given low-frequency signal directly or as a modulation of a radiofrequency wave can be bioactive. Such is the evolving nature of information about modulation. It argues for great care in defining standards that are intended to be protective of public health and well-being. This section describes some features of exposure and physiological conditions that are required in general for non-thermal effects to be produced, and specifically *to illustrate how modulation is a fundamental factor which should be taken into account in public safety standards*.

II.   The Old Standards (Based on Heating and Electric Current Flow in Tissues)

It is universally accepted that radiofrequency radiation (RFR) can cause tissue heating and that extremely low frequency (ELF) fields, e.g., 50 and 60 Hz, can cause electrical current flows that shock and even damage or destroy tissues. These factors alone are the underlying bases for present exposure standards. EMF exposures that cause biological effects at intensities that do not cause obvious thermal changes, that is, effects via non-thermal mechanisms, have been widely reported in the scientific literature over the last several decades. The current public safety limits do not take modulation into account and thus are no longer sufficiently protective of public health where chronic exposure to pulsed or pulse-modulated signal is involved, and where sub-populations of more susceptible individuals may be at risk from such exposures.

III.   Laboratory Studies

Published laboratory studies have provided evidence for more than 40 years on bioeffects at much lower intensities than cited in the various widely publicized guidelines for limits

to prevent harmful effects. Many of these reports show EMF-caused changes in processes associated with cell growth control, differentiation and proliferation which are biological processes of considerable interest to scientists who study the molecular and cellular basis of cancer. EMF effects have been reported in gene induction, transmembrane signaling cascades, gap junction communication, immune system action, rates of cell transformation, and breast cancer cell growth. These reports have cell growth control as a common theme. Other more recent studies on brainwave activity, cognition and human reaction time lend credence to modulation (pulsed RF and ELF-modulated RF) as a concern for wireless technologies, most prominently from cell phone use.

Experimental results are described below to illustrate the influence of each EMF parameter, while also demonstrating that it is highly unlikely the effects are due to EMF-caused current flow or heating.

Several papers in the 1960s and early 1970s reported that ELF fields could alter circadian rhythms in laboratory animals and humans. In the latter 1960s, a paper reported that the EMF environment in planned space capsules could cause human response time changes, i.e., the interval between a signal and the human response (Hamer, 1968). Subsequent experiments by that research group were conducted with monkeys, and showed similar response time changes and also EEG pattern changes (Gavalas, 1970; Gavalas-Medici, 1976). The investigators shifted the research subject to cats and observed EEG pattern changes, ability to sense and behaviorally respond to the ELF component of RFR, and the ability of minor electric current to stimulate the release of an inhibitory neurotransmitter, GABA, and simultaneous release of a surrogate measure, calcium ions, from the cortex (Kaczmarek, 1973, 1974). At this time the investigators adopted newly hatch chickens as sources of brain tissue and observed changes in the release of calcium ions from in vitro specimens as a function of ELF frequency directly or as amplitude modulation ('am') of RFR (RFRam) (Bawin, 1975, 1976, 1978a, 1978b; Sheppard, 1979). Tests of both EMF frequency and intensity dependences demonstrated a single sensitive region (termed 'window') over the range of frequency and intensity examined. This series of papers showed that EMF-induced changes could occur in several species (human, monkey, cat

and chicken), that calcium ions could be used as surrogate measures for a neurotransmitter, that ELF fields could produce effects similar to RFRam (note: without the 'am', there was no effect although the RFR intensity was the same), and that the dose and frequency response consisted of a single sensitivity window.

An independent research group published a series of papers replicating and extending this earlier work (Blackman et al., 1979, 1980a, 1980b, 1981, 1982, 1985, 1988a, 1988b, 1989, 1990; Joines and Blackman et al., 1981a, 1981b, 1986). These papers reported multiple windows in intensity and in frequency within which calcium changes were observed in the chick brain experimental systems under EMF exposure. Three other independent groups reported intensity and frequency windows for calcium, neurotransmitter or enolase release under EMF exposure of human and animal nervous system-derived cells in vitro (Dutta et al., 1984, 1989, 1992, 1994), of rat pancreatic tissue slices (Albert et al., 1980), and of frog heart (Schwartz et al., 1990) but not atrial strips in vitro (Schwartz et al., 1993). This series of papers showed that multiple frequency and intensity windows were a common phenomenon that required the development of new theoretical concepts to provide a mechanism of action paradigm.

Additional aspects of the EMF experiments with the chick brain described by Blackman and colleagues, above, also revealed critical co-factors that influenced the action of EMF to cause changes in calcium, including the influence of the local static magnetic field, and the influence of physico-chemical parameters, pH, temperature and ionic strength of the bathing solution surrounding the brain tissue during exposure. This information provides clues for and constraints on any theoretical mechanism that is to be developed to explain the phenomenon. These factors demonstrate that the current risk assessment paradigms, which ignore them, are incomplete and thus may not provide the level of protection currently assumed.

The detailed set of frequency and intensity combinations under which effects were observed, were all obtained from chickens incubated for 21 days in an electrically heated chamber containing 60-Hz fields. Tests were performed to determine if the 60-Hz

frequency of ELF fields (10 volts per meter in air) during incubation, i.e., during embryogenesis and organogenesis, would alter the subsequent calcium change responses of the brain tissue to EMF exposure. The published papers (Blackman et al., 1988b; Joines et al., 1986) showed that the brain tissue response was changed when the field during the incubation period was 50 Hz rather than 60 Hz. This result is consistent with an anecdotal report of adult humans, who were institutionalized because of chemical sensitivities, were also responsive to EMF fields that were present in the countries where they were born and raised (Blackman, 2006). This information indicates there may be animal and human exposure situations where EMF imprinting could be an important factor in laboratory and epidemiological situations. EMF imprinting, which may only become manifest when a human is subjected to chemical or biological stresses, could reduce ability to fight disease and toxic insult from environmental pollution, resulting in a population in need of more medical services, with resulting lost days at work.

Fundamental exposure parameters that must be considered when establishing a mode (or mechanism) of action for non-thermal EMF-induced biological effects.

A. Intensity

There are numerous reports of biological effects that show intensity "windows", that is, regions of intensity that cause changes surrounded by higher and lower intensities that show no effects from exposure. One very clear effect is 16-Hz, sine wave-induced changes in calcium efflux from brain tissue in a test tube because it shows two very distinct and clearly separated intensity windows of effects surrounded by regions of intensities that caused no effects (Blackman et al., 1982).   There are other reports for similar multiple windows of intensity in the radiofrequency range (Blackman et al., 1989; Dutta et al., 1989, 1992; Schwartz et al., 1990). Note that calcium ions are a secondary signal transduction agent active in many cellular pathways. These results show that intensity windows exist, they display an unusual and unanticipated "non linear" (non-linear and non-monotonic) phenomenon that has been mostly ignored in all risk assessment and standard setting exercises, save the National Council for Radiation Protection and Measurements. (NCRP) 1986 publication. Protection from multiple

intensity windows has never been incorporated into any risk assessment; to do so would call for a major change in thinking. These results mean that lower intensity is not necessarily less bioactive, or less harmful.

Multiple intensity windows appeared as an unexpected phenomenon in the late 1970s and 1980s. There has been one limited attempt to model the phenomenon (Thompson et al., 2000). However, there are publications from two independent research groups showing multiple intensity windows for 50 MHz, 147 MHz, and 450 MHz fields when amplitude-modulated at 16 Hz using the calcium ion release endpoint in chicken brains, in vitro. The incident intensities (measured in air) for the windows at the different carrier frequencies do not align at the same values. However, Joines et al., (1981a, 1981b) and Blackman et al. (1981) noted the windows of intensity align across different carrier frequencies if one converts the incident intensity to the intensity expected within the sample at the brain surface, but correcting for the different dielectric constants in the samples at the different carrier frequencies. The uniqueness of this response provides a substantial clue to theoreticians but it is interesting that no publications have appeared attempting to address this relationship. It is obvious that this phenomenon is one that needs further study.

B. Frequency

Frequency-dependent phenomena are common occurrences in nature. For example, the human ear only hears a portion of the sound that is in the environment, typically from 20 to 20000 Hz, which is a frequency "window." Another biological frequency window can be observed for plants grown indoors. Given normal indoor lighting the plants may grow to produce lush vegetation but not produce flowers unless illuminated with a lamp that emits a different spectrum of light. Similarly, there are examples of EMF-caused biological effects that occur as a result of EMF of concern to us in a frequency-dependent manner that cannot be explained by current flow or heating. The examples include reports of calcium ion efflux from brain tissue in vitro at low frequency (Blackman et al., 1988a, 1988b) and at high frequency (Blackman et al., 1981; Joines and Blackman, 1981). The bioactive frequency regions observed in these studies have never been

explicitly considered for use in any EMF risk assessments, thus demonstrating the incomplete nature of current exposure limits.

There are also EMF frequency-dependent alterations in the action of nerve growth factor (NGF) to stimulate neurite outgrowth (growth of primitive axons or dendrites) from a peripheral-nerve-derived cell (PC-12) in culture (Blackman et al., 1995, 1999; Trillo et al., 1996). The combined effect of frequency and intensity is also a common occurrence in both the sound and the light examples given above. Too much or too little of either frequency or intensity show either no or undesirable effects. Similarly, in low intensity EMF work, "islands" of effective combinations of intensity and frequency are surrounded by a "sea" of null effects (Blackman et al., 1988a). Although the mechanisms responsible for these effects have not been establish, the effects represent a heretofore unknown phenomenon that may have ramifications for risk assessment and standard setting. Nerve growth and neurotransmitter release that can be altered by different combinations of EMF frequencies and intensities, especially in developing organisms like children, could conceivably produce over time a subsequent altered ability to successfully or fully respond behaviorally to natural stressors in the adult environment; research is urgently need to test this possibility in animal systems.

Nevertheless, this phenomenon is ignored in the development of present exposure standards that rely primarily on biological responses to intensities within a relatively narrow band of frequencies, based on an energy deposition endpoint.

C. Static Magnetic Field

The magnetic field of the earth at any given location has a relatively constant intensity as a function of time. However, the intensity value, and the inclination of the field with respect to the gravity vector, varies considerable over the face of the earth. More locally, these features of the earth's magnetic field can also vary by more than 20% inside man-made structures, particularly those with steel support structures. There are many reports of EMF-caused effects being dependent on the static magnetic field intensity (cf. Blackman et al., 1985) and of its orientation, with respect to an oscillating magnetic field

(Blackman et al., 1990; Blackman et al., 1996). One aspect common to many of these reports is that the location in the active frequency band is determined by the intensity of the static magnetic field. There have been many attempts to explain this phenomenon but none has been universally accepted. However, it is clear that if a biological response depends on the static magnetic field intensity, and even its orientation with respect to an oscillating field, then the conditions necessary to reproduce the phenomenon are very specific and might easily escape detection (cf. Blackman and Most, 1993). The consequences of these results are that there may be exposure situations that are truly detrimental (or beneficial) to organisms but that are insufficiently common on a large scale that they would not be observed in epidemiological studies; they need to be studied under controlled laboratory conditions to determine impact on health and wellbeing.

D. Electric & Magnetic Components

Both the electric and the magnetic components have been shown to directly and independently cause biological changes. There is one report that clearly distinguishes the distinct biological responses caused by the electric field and by the magnetic field. Marron et al. (1988) show that electric field exposure can increase the negative surface charge density of an amoeba, Physarum polycephalum, and that magnetic field exposure of the same organism causes changes in the surface of the organism to reduce its hydrophobic character. Other scientists have used concentric growth surfaces of different radii and vertical magnetic fields to determine if the magnetic or the induced electric component is the agent causing biological change.   Liburdy (1992), examining calcium influx in lymphocytes, and Greene et al. (1991), monitoring ornithine decarboxylase (ODC) activity in cell culture, showed that the induced electric component was responsible for their results. In contrast, Blackman et al. (1993a, 1993b) monitoring neurite outgrowth from two different clones of PC-12 cells and using the same exposure technique used by Liburdy and by Greene showed the magnetic component was the critical agent in their experiments. EMF-induced changes on the cell surface, where it interacts with its environment, can dramatically alter the homeostatic mechanisms in tissues, whereas changes in ODC activity are associated with the induction of cell proliferation, a desirable outcome if one is concerned about wound healing, but

undesirable if the concern is tumor cell growth. This information demonstrates the multiple, different ways that EMF can affect biological systems. Current analyses for risk assessment and standard setting have ignored this information, thus making their conclusions of limited value.

E. Sine and Pulsed Waves

Important characteristics of pulsed waves that influenced the number and characteristics of the sine wave representations include the following: 1) frequency, 2) pulse width, 3) intensity, 4) rise and fall time, and 5) the frequency, if any, within the pulse ON time. Chiabrera et al. (1979) showed that pulsed fields caused de-differentiation of amphibian red blood cells. Scarfi et al. (1997) showed enhanced micronuclei formation in lymphocytes of patients with Turner's syndrome (only one X chromosome) but no change in micronuclei formation when the lymphocytes were exposed to sine waves (Scarfi et al., 1996). Takahashi et al. (1986) monitored thymidine incorporation in Chinese hamster cells and explored the influence of pulse frequency (two windows of enhancement seen), pulse width (one window of enhancement seen) and intensity (two windows of enhancement seen followed by a reduction in incorporation). Ubeda et al. (1983) showed the influence of difference rise and fall times of pulsed waves on chick embryo development.

It is important to note that the frequency spectrum of pulsed waves can be represented by a sum of sine waves which, to borrow a chemical analogy, would represent a mixture or a soup of chemicals, anyone of which could be biologically active. Risk assessment and exposure limits have been established for specific chemicals or chemical classes of compounds that have been shown to cause undesirable biological effects. Risk assessors and the general public are sophisticated enough to recognize that it is impossible to declare all chemicals safe or hazardous; consider the difference between food and poisons, both of which are chemicals. A similar situation occurs for EMF; it is critical to determine which combinations of EMF conditions have the potential to cause biological harm and which do not.

Obviously, pulse wave exposures represent an entire genre of exposure conditions, with additional difficulty for exact independent replication of exposures, and thus of results, but with increased opportunities for the production of biological effects. Current standards were not developed with explicit knowledge of these additional consequences for biological responses.

F. Mechanisms

Two recent papers have the possibility of advancing understanding in this research area. Chiabrera et al. (2000) created a theoretical model for EMF effects on an ion's interaction with protein that includes the influence of thermal energy and of metabolism. Before this publication, theoreticians assumed that biological effects in living systems could not occur if the electric signal is below the signal caused by thermal noise, in spite of experimental evidence to the contrary. In this paper, the authors show that this limitation is not absolute, and that different amounts of metabolic energy can influence the amount and parametric response of biological systems to EMF. The second paper, by Marino et al. (2000), presents a new analytical approach to examine endpoints in systems exposed to EMF. The authors, focusing on exposure-induced lyphoid phenotypes, report that EMF may not cause changes in mean values of endpoints, but rather in variances in those same endpoints. They provide further evidence using immunological endpoints from exposed and sham treated mice (Marino et al., 2001a, 2001b, 2001c). Additional research has emerged from this laboratory on EMF-induced animal and human brain activity changes that provides more evidence for the value of their research approach (Marino et al., 2002, 2003, 2004; Carrubba et al., 2006, 2007a, 2007b). *It is apparent that much remains to be examined and explained in EMF biological effects research through more creative methods of analysis than have been used before. The models described above need to be incorporated into risk assessment determinations.*

IV. Problems with Segregation of Effects by Artificial Frequency Bands that Ignore Modulation

One fundamental limitation of most reviews of EMF biological effects is that exposures are segregated by the physical (engineering/technical) concept of frequency bands favored by the engineering community. This is a default approach that follows the historical context established in the past by the incremental addition of newer technologies that generate increasingly higher frequencies.   However, this approach fails to consider unique responses from biological systems that are widely reported at various combinations of frequencies, modulations and intensities.

When common biological responses are observed without regard for the particular, engineering-defined EMF frequency band in which the effects occur, this reorganization of the results can highlight the commonalities in biological responses caused by exposures to EMF across the different frequently bands. An attempt to introduce this concept to escape the limitations of the engineering-defined structure occurred with the development of the 1986 NCRP radiofrequency exposure guidelines because published papers from the early 1970s to the mid 1980s (to be discussed below) demonstrated the need to include amplitude modulation as a factor in setting of maximum exposure limits. The 1986 NCRP guideline was the one and only risk evaluation that included an exception for modulated fields.

The current situation argues strongly for a change in the way risk assessment is conducted, especially for the last 15 to 20 years.   Unfortunately, subsequent risk evaluations did not follow the NCRP example, but returned to the former engineering-defined analysis conditions, in part because scientists who reported non-thermal effects were not placed on the review committees, and in the terms of Slovic (1999) "Risk assessment is inherently subjective and represent a blend of science and judgment with important psychological, social, cultural, and political factors. … Whoever controls the definition of risk controls the rational solution to the problem at hand. … Defining risk is thus an exercise in power." It appears that by excluding scientists experienced with

producing non-thermal biological effects, the usually sound judgment by the selected committees was severely limited in its breadth-of-experience, thereby causing the members to retreat to their own limited areas of expertise when forced to make judgments, as described by Slovic (1999), "Public views are also influenced by worldviews, ideologies, and values; so are scientists' views, particularly when they are working at limits of their expertise." The current practice of segregating scientific investigations (and resulting public health limits) by artificial divisions of frequency dramatically dilutes the impact of the basic science results, thereby reducing and distorting the weight of evidence in any evaluation process (see evaluations of bias by Havas 2000, referring to NRC 1997 compared to NIEHS 1998 and NIEHS 1999).

A. Suggested Research

Are there substitute approaches that would improve on the health-effects evaluation situation? As mentioned above, it may be useful in certain cases to develop a biologically based clustering of the data to focus on and enrich understanding of certain aspects of biological responses. Some examples to consider for biological clustering include: 1) EMF features, such as frequency and intensity inter-dependencies, 2) common cofactors, such as the earth's magnetic field or co-incident application of chemical agents to perturb and perhaps sensitize the biological system to EMF, or 3) physiological state of the biological specimen, such as age or, sensitive sub-populations, including genetic predisposition (Fedrowitz et al., 2004, 2005).

To determine if this approach has merit, one could combine reports of biological effects found in the ELF (including sub-ELF) band with effects found in the RF band when the RF exposures are amplitude modulated (AM) using frequencies in the ELF band. The following data should be used: 1) human response time changes under ELF exposure (Hamer, 1968), 2) monkey response time and EEG changes under ELF exposure (Gavalas et al., 1970; Gavales-Medici & Day-Magdaleno, 1976), 3) cat brain EEG, GABA and calcium ion changes induced by ELF and AM-RF (Kaczmarek and Adey, 1973, 1974; Bawin et al. 1973), 4) calcium ion changes in chick brain tissue under ELF and AM-RF (Bawin et al., 1975, 1976, 1978a, 1978b; Sheppard et al., 1979; Joines and

Blackman et al., , 1981a, 1981b, 1986; Blackman et al., 1979, 1980a, 1980b, 1981, 1982, 1985, 1988a, 1988b, 1989, 1990), and 5) calcium changes under AM-RF in brain cells in culture (Dutta et al., 1984, 1989, 1992) and in frog heart under AM-RF (Schwartz et al., 1990). The potential usefulness of applying biological clustering in the example given above even though AM is used, is that the results may have relevance to assist in the examination of some of the effects reportedly caused by cellular phone exposures which include more complex types of modulation of RF. This suggestion is reasonable because three groups have recently reported human responses to cell phone emissions that include changes in reaction times (Preece et al., 1998, 1999; Koivisto et al. 2000a, 2000b; Krause et al., 2000a, 2000b) or to brain wave potentials that may be associated with reaction time changes (Freude et al., 1998, 2000).

The papers described above, published in the 1960s through 1991, foreshadowed the more recent publications in 1999 and 2000 showing response time changes, or associated measures, in human subjects during exposure to cell phone-generated radiation (although none of the earlier studies was acknowledged in these recent reports on cognition and reaction time). Without guidance from this extensive earlier work, the development of the mechanistic bases for non-thermal effects from EMF exposures will be substantially delayed.

V. Conclusions

• There is substantial scientific evidence that some modulated fields (pulsed or repeated signals) are bioactive, which increases the likelihood that they could have health impacts with chronic exposure even at very low exposure levels. Modulation signals may interfere with normal, non-linear biological processes.

• Modulation is a fundamental factor that should be taken into account in new public safety standards; at present it is not even a contributing factor.

• To properly evaluate the biological and health impacts of exposure to modulated RFR (carrier waves), it is also essential to study the impact of the modulating signal (lower frequency fields or ELF-modulated RF).

• Current standards have ignored modulation as a factor in human health impacts, and thus are inadequate in the protection of the public in terms of chronic exposure to some forms of ELF-modulated RF signals.

• The current IEEE and ICNIRP standards are not sufficiently protective of public health with respect to chronic exposure to modulated fields (particularly new technologies that are pulse-modulated and heavily used in cellular telephony).

• The collective papers on modulation appear to be omitted from consideration in the recent WHO and IEEE science reviews. This body of research has been ignored by current standard setting bodies that rely only on traditional energy-based (thermal) concepts.

• More research is needed to determine which modulation factors, and combinations are bioactive and deleterious at low intensities, and are likely to result in disease-related processes and/or health risks; however this should not delay preventative actions supporting public health and wellness.

• If signals need to be modulated in the development of new wireless technologies, for example, it makes sense to use what existing scientific information is available to avoid the most obviously deleterious exposure parameters and select others that may be less likely to interfere with normal biological processes in life.

• The current membership on Risk Assessment committees needs to be made more inclusive, by adding scientists experienced with producing non-thermal biological effects.

• The current practice of segregating scientific investigations (and resulting public health limits) by artificial divisions of frequency needs to be changed because this approach dramatically dilutes the impact of the basic science results and eliminates consideration of modulation signals, thereby reducing and distorting the weight of evidence in any evaluation process.

Disclaimer: the opinions expressed in this text are those of its author, and are not necessarily those of his employer.

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SECTION 15

EVIDENCE BASED ON EMF MEDICAL THERAPEUTICS

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Table of Contents

I. Introduction

Electromagnetic fields are widely used in therapeutic medical applications. Proof of effectiveness has been demonstrated in numerous clinical applications of low-intensity ELF-EMF and RF-EMF, each treatment employing specific characteristics of frequency, modulation and intensity to achieve its efficacy.   On the other hand, higher levels of EMFs encountered in the environment which are indiscriminately generated by technologies of the 20th and 21st centuries may result in harm.   EMF levels which are allowable today under thermally-based public exposure standards do not take into account these clear indications of the sensitivities of the human body to EMFs. If we are to promulgate public exposure standards that are protective of public health, then this body of evidence on healing with EMFs is of primary importance in developing biologically-based public exposure standards.

 "Although incompletely understood, tissue free radical interactions may extend to zero field levels. Emergent concepts of tissue thresholds to imposed and intrinsic magnetic fields address ensemble or domain functions of populations of cells, cooperatively whispering together in intercellular communication and organized hierarchically at atomic and molecular levels." 10

II. Therapeutic Uses for Electromagnetic Fields

Since EMFs have been shown to be effective in treating conditions of disease at energy levels far below current public exposure standards, this body of evidence forms a strong warning that indiscriminate EMF exposure is ill advised. Health concerns from indiscriminate exposure to EMF, as opposed to EMF exposures done with clinical oversight, could lead to harm as can the unsupervised use of pharmaceutical drugs. The consequence of multiple sources of EMF exposure in daily life, with no regard to cumulative exposures or to potentially harmful combinations of EMF exposures will pose

future difficulties in identifying sources of disease (because of multiple and overlapping exposures) and time-varying and geography-varying differences from person to person. Just as ionizing radiation can be used to effectively diagnose disease and treat cancer, it is also a cause of cancer under different exposure conditions. Since EMFs are both a cause of disease, and also used for treatment of disease, it is vitally important that public exposure standards reflect our current understanding of the biological potency of EMF exposures.

"there is an abundance of experimental and clinical data demonstrating that exogenous EMFs of surprisingly low levels can have a profound effect on a large variety of biological systems. Both electrical and electromagnetic devices have been demonstrated to positively affect the healing process in fresh fractures, delayed and nonunions, osteotomies, and spine fusion in orthopedics and for chronic and acute wound repair. These clinical results have been validated by well-designed and statistically powered double-blind clinical trials and have survived meta-analyses. The FDA has approved labeling for these biophysical devices, limited at present to these indications." "The potential clinical applications of EMF therapeutics extend far beyond those considered here and the clinical rewards are certain to be huge." "Cancer, cardiac muscle regeneration, diabetes, arthritis, and neurological disorders are just some of the pathologies that have already been shown to be responsive to EMF therapy. Successful applications of low-frequency EMFs have been reported for treatment of bronchial asthma, myocardial infarction, and venous and varicose ulcers. There is emerging research on EMF effects on angiogenesis and the manner in which this may increase stem cell survival in the treatment of Altzeimer's (sic) and Parkinson's diseases. There are also many studies that point to the possibility of the use of EMF for peripheral nerve regeneration" and *" the treatment of cancer." "EMF therapy modalities are simple, safe and significantly less costly to the health care system. They offer the ability to treat the underlying pathology rather than simply the symptoms. The time is particularly opportune given the increased incidence of side effects from the use of pharmacological agents. EMF therapeutics will have a profound impact upon health and wellness and their costs worldwide."* [1]

A. Bone Repair

Clinical use of pulsed EMF has been demonstrated to achieve bone repair, particularly in fractures that do not heal on their own. Bone healing is stimulated by very weak electromagnetic fields that are far lower in strength than would produce tissue heating. The FDA approved pulsed EMF for use in bone healing in 1979. Since that time, many millions of patients have benefited from this therapy. Since PEMF treatments are non-invasive and clinically effective, it has advantages to the patient in terms of reduced pain and suffering, reduction in health care costs, and effectiveness where other methods have failed to produce adequate clinical results.

"It is now commonplace to learn the successful use of weak, nonthermal electromagnetic fields (EMF) in the quest to heal, or relieve the symptoms of a variety of debilitating ailments. This chapter attempts to give the reader an introduction and assessment of EMF modalities that have demonstrated therapeutic benefit for bone and wound repair and chronic and acute pain." [2]

Pilla provides extensive discussion of the "clinical evidence that time-varying magnetic fields (EMF) can modulate molecular, cellular and tissue functions in a physiologically significant manner." [2]   A description of the various waveforms and EMF modalities which are effective in bone and wound repair are beyond the scope of this paper, but are well documented. [2]   In addition to documenting that bone repair in fractures is achieved with pulsed EMF at low intensities, Pilla also reports that pulsed EMF has been successful in promoting bone repair and healing of spine fusions for the treatment of chronic back pain from worn and/or damaged spinal discs. [3] The FDA has approved pulsed EMFs for bone healing and this is a widely recognized treatment, particularly for fractures that are slow to heal, or do not repair with conventional medical treatment. It represents one of the best documented cases in science where the body clearly responds to low-intensity EMF signals for healing purposes; these EMF signals are far below current public exposure standards and are proof of the bioactivity (in a beneficial form as applied).

Liboff describes signal shapes in electromagnetic therapies that contribute greatly to our understanding of the various forms of EMF signal delivery that are fundamental to eliciting specific bioeffects. He simply and elegantly describes electric and magnetic signal characteristics, their signature shapes and methods of delivery (time-varying, oscillatory, or modulated) which create special interactions with human tissues and organs for healing. [4]

"It is likely that the future will see combinations of such signals in therapeutic applications, especially as more information filters back from the laboratory elaborating on the nature of electromagnetic interactions with living tissue." [4]

B. Wound Repair

The clinical application of pulsed EMF has been shown to enhance wound repair and healing. [2,5] Devices that use pulsed EMF have been approved for use in the United States by the FDA. Pilla reports "*the clear clinical effectiveness of PEMF signals has resulted in significantly increased use*" in treating wounds that do not heal. [5] In Pilla's extensive summary presented on beneficial effects of EMF on wound healing, he reports pulsed EMF has been reported to reduce edema, increase blood flow, modulate upregulated growth factor receptors, enhance neutrophil and macrophage attraction and epidermal cell migration, and increase fibroblast and granulation tissue proliferation. Most wound studies were conducted on arterial or venous skin ulcers, diabetic ulcers, pressure ulcers, and surgical and burn wounds. [5] Wound repair under the influence of very low level pulsed EMFs is a second solid documentation in science that very low level EMFs are bioactive (in this case, beneficial) when applied in very specific clinical applications where the exposure variables are carefully selected.

Oschman provides an overview of the evolution of energy medicine and electromagnetic energy treatments related to bone repair, wound healing, pain relief, depression, insomnia, inflammation of tissues and other medical conditions. [6] He also underscores the counter-intuitive thesis that low-intensity EMFs can be more effective in eliciting

healing responses than larger intensity exposures; and that understanding of the subtle

energies and their specific interactions with human functioning is imperative.

(l)iving tissues are far more sensitive to external fields than previously realized. After a period when physicists were certain that observed sensitivities to nonionizing and nonthermal radiations wer physically impossible, we now know that biological systems defy the simple logic that larger stimuli should produce larger responses.For many living systems, extremely weak fields can be more effective than strong fields." [6]

C. Pain Management

Pulsed magnetic field (PMF) devices are also used with FDA approval for *"relief of acute and chronic pain and the reduction of edema* (swelling*), all symptoms of wounds from post-surgical procedures, musculoskeletal injuries, muscle and joint overuse, as well as for chronic wounds."* [5]

Pulsed EMF has been shown to be effective in relief of chronic pain associated with connective tissue injury (cartilage, tendon, ligaments and bone) and soft-tissue injuries associated with the joints.   Both acute and chronic pain may be successfully treated with EMFs as an alternative to non-steroidal anti-inflammatory drugs (NSAIDs). Relief from chronic pain due to osteoarthritis has been reported with treatment by EMFs. [2]

Markov reports that EMF is used in treatment of pain associated with tendonitis, multiple sclerosis, carpal tunnel syndrome and some forms of arthritis. He discusses the use of pulsed EMF for headache and migraine pain relief; neck and whiplash injuries, postoperative pain, sprains, chronic pelvic pain, and nerve regeneration. Pain reduction by clinical application of pulsed EMF is achieved with non-thermal levels of exposure, and produces a nonthermal biological effect. [8]

D. Depression, Anxiety Disorders, Insomnia

"Today (2002) we are at a threshold for the acceptance of electromagnetic therapy as a clinically accepted form of therapy for such diverse diseases as unipolar depression, Parkinson's disease, and sleep disorders and the treatment of debilitating chronic and acute pain." [8]

Shealy et al (2007) detail clinical findings for treatment of depression and mood management, reduction in anxiety, and treatment of insomnia. 10 Electrical energy stimulators that deliver very low-level EMF have been reported to be clinically effective in the alteration of neurobioechemicals including serotonin and cortisol.   Depression, mood disorders and insomnia have been related to disregulation of serotonin levels. Use of EMFs to reduce symptoms of depression, anxiety and insomnia are authorized by the FDA, and have been in use since the 1970's.   Shealy reports that transcranial stimulation by EMFs led to a significant relief of depression in 85% of patients who had failed pharmacological agents, and was at least twice as effective as any known antidepressant drugs and without complications. [10]

E. Protection from Anoxia (Protection for the Heart)

The work of Albertini, Litovitz and di Carlo, Goodman and Blank, Han, Pipkin, Rasmark and Kwee, [11-17] has shown that very weak ELF-EMF and RF-EMF exposures can actually help to protect cells against tissue damage. They can induce an adaptive stress response in cells, which in turn helps the cell fight damage. The response is production of stress proteins (heat shock proteins or HSP). These stress proteins help to protect the cells against injury and death. A 20-minute exposure to electromagnetic fields at only 80 mG will start stress protein production, which helps to fight cellular damage from lack of oxygen, for example. Protection from anoxia (or lack of oxygen) is important in heart attacks. Pre-treatment with ELF-EMF (and also RF-ELF) before blocking oxygen to cells has been shown to be protective against the lack of oxygen to heart tissues. The exposure level is on the order of 80 mG ELF-EMF or far below any

possible thermal heating.

This means that there are clinical applications for protection against heart attack damage that can be provided by very low-dose EMF exposures. Such protection could be vitally important in reducing damage from oxygen loss during heart attacks. It is another line of proof that low-intensity electromagnetic fields are bioactive, and when applied in specific therapeutic ways, are beneficial. It also underscores that the body can detect and decode these very weak signals, providing further evidence that thermally-based standards are incomplete because they do not take into account the sensitivity of the human body to non-thermal levels of EMF exposure.

IV. Conclusions

Since EMFs have been shown to be effective in treating conditions of disease at energy levels far below current public exposure standards, this body of evidence forms a strong warning that indiscriminate EMF exposure is ill advised.

Based on extensive clinical applications of low-intensity EMFs since at least the 1970s, it has been demonstrated beyond argument that some forms of EMFs can be medically effective in treating a wide variety of human health disorders and injuries. Since all of these treatments are conducted at energy levels that do not involve tissue heating per se, it is convincing proof that the human body both reacts to and can be affected by exposures to EMFs.   Exposures can be beneficial when EMFs are applied with conscious knowledge of the exposure factors that are proven to lead to specific biological (healing) consequences. The intensity of such therapeutic exposures nearly always falls below current public exposure standards as discussed in Section 3.

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SECTION 16

"Late Lessons From Early Warnings:

Towards realism and precaution with EMF?"

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Disclaimer.

The views expressed are those of the author and do not represent the views of the EEA or its Management Board. The author has no competing financial interest in the matters dealt with.

TABLE OF CONTENTS

I.   INTRODUCTION

The histories of selected public and environmental hazards, from the first scientifically based early warnings about potential harm, to the subsequent precautionary and preventive measures, have been reviewed by the European Environment Agency.("Late Lessons from Early Warnings: the Precautionary Principle 1896-2000", EEA,2001). This paper summarises some of the definitional and interpretative issues that arise from the report and subsequent debates, such as the contingent nature of knowledge; the definitions of precaution, prevention, risk, uncertainty, and ignorance; the use of differential levels of proof; and the nature and main direction of the methodological and cultural biases within the environmental health sciences.   These issues are relevant to EMF.

II.   THE TWELVE "LATE LESSONS FROM EARLY WARNINGS

The paper does not address the specifics of EMF hazards, leaving it to the reader to apply, or not, the "Twelve late Lessons" that conclude the report. These lessons attempt to synthesise the fourteen historical experiences from the very different case study chapters into generic knowledge that can help inform policy-making on current issues such as GMO, nanotechnologies, mobile phones, and endocrine disrupting substances where the luxuries of hindsight are not yet available but where exposures are already widespread and rising.

The idea of the twelve late lessons is to make the most of past experience to help anticipate future surprises whilst recognising that history never exactly repeats itself. When adopted alongside the best available science the lessons aim to help minimize hazards without compromising innovation. The "lessons" are reproduced below.

A. "Identify/Clarify the Framing and Assumptions"
1. Manage "risk", "uncertainty" and "ignorance"
2. Identify/reduce "blind spots" in the science
3. Assess/account for all pros and cons of action/inaction
4. Analyse/evaluate alternative options

5. Take account of stakeholder values

6. Avoid "paralysis by analysis" by acting to reduce hazards via the precautionary principle.

B. "Broaden Assessment Information"

7. Identify/reduce interdisciplinary obstacles to learning

8. Identify/reduce institutional obstacles to learning

9. Use "lay", local as well as specialist knowledge

10. Identify/anticipate "real world" conditions

11. Ensure regulatory and informational independence

12. Use more long-term (ie. decades) monitoring and research

III. EARLY USE OF PRECAUTION

The Vorsorgeprinzip, or "foresight" principle, only emerged as a specific policy tool during the German debates on the possible role of air pollution as a cause of "forest death" in the 1970-80s. However, John Graham, one of Bush's science policy advisors, and trenchant critic of the precautionary principle, has noted that:

> *"Precaution, whether or not described as a formal principle, has served mankind well in the past and the history of public health instructs us to keep the spirit of precaution alive and well"*. (Graham 2002).

Graham might have been thinking of the cholera episode of 1854 when precaution did indeed serve the people of London well. Dr. John Snow, a London physician, used the spirit of precaution to advise banning access to the polluted water of the Broad St. pump which he suspected was the cause of the cholera outbreak. He based his recommendation on the evidence he had been accumulating for some years including his study of S. London populations served by both piped and well water. Snow's views on cholera causation were not shared by The Royal College of Physicians who considered Snow's thesis and rejected it as 'untenable' as they and other "authorities" of the day believed that cholera was caused by airborne contamination. This particular scientific "certainty" soon turned out to be certainly mistaken, with the last remaining doubt being removed when Koch in Germany isolated the cholera vibrio in 1883.

From the *association* between exposure to water polluted with human faeces, and cholera, observed by Snow in 1854, to Koch's discovery of the *"mechanism of action"*, took 30 years of further scientific inquiry. Such a long time lag between acknowledging compelling associations and understanding their mechanisms of action is a common feature of scientific inquiry, as the histories of TBT, PCBs, DES, the Great Lakes pollution, beef hormones and the other cases in the EEA report illustrate.

IV. KNOWLEDGE AND IGNORANCE REQUIRES BOTH PREVENTION AND PRECAUTION

The Broad St. pump, TBT, DES, PCBs and Great Lakes Pollution examples described here also serve to illustrate the contingent nature of knowledge. Today's scientific certainties can be tomorrow's mistakes, and today's research can both reduce and increase scientific uncertainties, as the boundaries of the "known" and the unknown expand. Waiting for the results of more research before taking action to reduce threatening exposures may not only take decades but the new knowledge may identify previously unknown sources of both uncertainty and ignorance, as awareness of what we do not know expands, thereby supplying further reasons for inaction. "Paralysis by Analysis " can then follow.

"The more we know, the more we realise what we don't know" is not an uncommon scientific experience. Socrates observed some time ago:
"I am the wisest man alive, for I know one thing, and that is that I know nothing".
(Plato's Apology 1.21).

This was an early lesson in humility that has been lately forgotten by many scientists and politicians, who often put what turns out to be "misplaced certainty" in today's scientific knowledge: or assume that uncertainty can only be reduced, and not increased, by further research.

The distinction between uncertainty and ignorance is important. (Stirling, 1999) Ignorance is knowing that today's knowledge is very limited: it is the source of scientific surprises, such as the hole in the ozone layer, the mesothelioma cancer from asbestos, imposex in sea snails etc.   It is distinct from the uncertainties that arise from

gaps in knowledge and from variances in sampling and monitoring; parameter variability; model assumptions; and from the other attempts to approximate, model and predict unfolding realities.

Foreseeing and preventing hazards in the context of ignorance presents particular challenges to decision-makers. At first sight it looks impossible to do anything to avoid or mitigate "surprises". And ignorance ensures that there will always be surprises. However, some measures that could help limit the consequences of ignorance and the impacts of surprises are:

- using intrinsic properties as generic predictors for unknown but possible impacts e.g. the persistence, bioaccumulation and spatial range potential of chemical substances. (Stroebe et al., 2004)
- reducing specific exposures to potentially harmful agents on the basis of credible 'early warnings' of *initial* harmful impacts, thus limiting the size of any other 'surprise' impacts from the same agent, such as the asbestos cancers that followed asbestosis; and the PCB neurotoxicological effects that followed its wildlife impacts.
- promoting a diversity of robust and adaptable technological and social options to meet needs, which limits technological 'monopolies' (such as those like asbestos, CFCs, PCBs etc.), and therefore reduces the scale of any 'surprise' from any one technological option.
- using more long-term research and monitoring of what appear to be "surprise sensitive sentinels", such as frogs and foetuses.

A. Prevention and Precaution

The distinction between *prevention* and *precaution* is also important. Preventing hazards from "known" risks is relatively easy and does not require precaution. Banning smoking, or asbestos, today requires only acts of prevention to avoid the well-known risks. However, it would have needed precaution, (or foresight, based on a sufficiency of evidence), to have justified acts to avoid exposure to the then uncertain hazards of asbestos in the 1930s –50s, or of tobacco smoke in the 1960's). Such precautionary acts then, if implemented successfully, would have saved many more lives in Europe than today's bans on asbestos and smoking are doing. As

Cogliano has recently pointed out, the difference between prevention and precaution can be further illustrated by showing that *prevention* is used to justify the restriction of exposure to an IARC Category 1 carcinogen whereas *precaution* is necessary to justify restricting exposure to a Category 2A or B carcinogen, where the evidence is less strong. The section below. on different levels of proof , further elaborates this point.

For EMF, the question is, does the existing strength of evidence justify *precautionary* actions now? Or will exposure reduction be delayed until the evidence is clear enough to justify the more belated and overall less protective *prevention* of "known" causes, so that EMF replicates the fate of asbestos ,smoking and most of the other cases in the EEA report?

Some commentators, who have a long and distinguished history in preventing occupational and environmental risks, have queried the added value of the precautionary principle in the field of public health, with its long traditions of prevention. (Goldstein, 2007).

The key to understanding the added value of the PP requires a) acknowledging the distinction between prevention and precaution described above; b) an appreciation of the further distinctions between the primary, secondary and tertiary preventative *measures* that have long between adopted in public health, and the prior *justification* for any such measure, which the PP brings; and c) a recognition of the increased legitimacy and transparency that arises from the articulation and adoption of the PP in legal texts, international agreements and conventions, as opposed to being merely part of general practice.

More empirically, the evidence that many scientific disciples, legal scholars (de Sadeleer, 2007),and international policymakers, have, since the 1970s, recognised the need for legitimising the PP as a new policy tool that is better able to deal with systems complexities, ignorance and uncertainties, suggests that the PP brings added value to the protection of the environment and the public.

There is much discussion generated by the different meanings often attached to the common terms "prevention", "precaution", "risk", "uncertainty" and "ignorance". **Table 1** attempts to clarify these so as to help reduce unnecessary argumentation.

Table 1: Clarification of Key Terms

Situation	*State and dates of knowledge*	*"Nature of the justification for Action"*
Risk	**'Known' impacts; 'known' probabilities e.g. asbestos**	**Prevention: action taken to reduce known hazards e.g. eliminate exposure to asbestos dust**
Uncertainty	**'Known' impacts; 'unknown' probabilities e.g. antibiotics in animal feed and associated human resistance to those antibiotics**	**Precautionary prevention: action taken to reduce exposure to potential hazards**
Ignorance	**'Unknown' impacts and therefore 'unknown' probabilities eg the 'surprises' of chlorofluorocarbons (CFCs) pre 1974**	**Precaution: action taken to anticipate, identify and reduce the impact of 'surprises'**

Source: Reproduced, with amendment, from the Late Lessons Report, EEA 2001.

V. THE PRECAUTIONARY PRINCIPLE: DEFINITIONS AND INTERRPRETATIONS

There are some relatively rare but successful acts of "precautionary prevention" in the EEA report such as on cholera in1854, on TBT in France in the 1980s, and on CFCs in the 1970s. Together with the many other examples of the failure to use the precautionary principle in the other case studies (EEA, 2001), these illustrate the wisdom of taking appropriate precautionary actions to avoid plausible and serious threats to health or environments, especially when the impacts are irreversible and likely to be much more costly to society than the precautionary measures.

Some commentators have stressed the need for policymakers to take account of the foreseeable, or plausible, countervailing (secondary) costs of otherwise genuine precautionary attempts to protect the environment and health. (Rushton, 2007). This

consideration of countervailing costs has long been recognised by the better policymakers, even if it is difficult in practice to anticipate and account for all consequences of actions. And of course there are the secondary benefits of precautionary actions as well, which tend to be less stressed, such as the benefit of reduced respiratory and cardiovascular disease from the reduced combustion of fossil fuels: a large and early secondary benefit of that climate change measure.

The outcomes of some controversial actions based on the PP, such as the EU ban on antibiotics as growth promoters, which is a Late Lessons case study, have since been scrutinised, and have been considered sound ,or unsound, depending on the science used and its interpretation by different interests. (Cox, 2007, Angulo et al., 2004).

Any policy effectiveness analysis of measures taken to deal with such multi-causal and long term hazards as antibiotics as growth promoters is fraught with methodological difficulties and is hampered by long latencies and complex biological systems: untangling the causal impact of one stressor amongst many inter-dependent ones is virtually impossible. The value of applying more probabilistic and value of information data to such conundrums is recognised by many risk managers. However, this cannot remove the need for scientific and political judgment about how to take appropriate and proportionate action in the face of irreducible uncertainties, ignorance and plausible hazards which could have serious, widespread and irreversible impacts and for which probabilities are not possible at the time when they are most needed. This is the current case with many EMF exposures.

A. Some Definitions and Interpretations of the Precautionary Principle

The increasing awareness of complexity and uncertainty during the 1980/90's led to the German debates on the Vorsorgeprinzip shifting to the international level, initially in the field of conservation (World Charter for Nature UN 1982), but then particularly in marine pollution, where an overload of data accompanied an insufficiency of knowledge. (Marine Pollution Bulletin, 1997). This generated the need to act with precaution to reduce the large amounts of chemical pollution entering the North Sea. Since then many international treaties have included the PP (including the often cited version from the Third North Sea Ministerial Conference, 1990, have included

reference to the precautionary principle, or, as they refer to it in the USA, the precautionary approach.

The N.Sea declaration called for *"action to avoid potentially damaging impacts of substances, even where there is no scientific evidence to prove a causal link between emissions and effects"*.

This definition has often, and sometimes mischievously, been used to deride the precautionary principle by claims that it appears to justify action even when there is "no scientific evidence" that associates exposures with effects. However, the N. Sea Conference definition clearly links the words "no scientific evidence" with the words. "to prove a causal link". We have already seen with the Broad St. pump and TBT examples that there is a significant difference between evidence about an "association" and evidence that is robust enough to establish a "causal" link. (Hill, 1965).

The Treaty of the European Union also cites the precautionary principle, as well as the other key principles of sound public policy on health:

> *"Community policy on the environment … shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should, as a priority, be rectified at the source and the polluter should pay"* (Treaty on European Union, 1992).

Other parts of the EU Treaty ,and cases taken at the European Court of Justice, make it clear that these principles also apply to environmental and consumer protection issues.

These principles, as well as the important and legally required *proportionality principle,* which limits disproportion between the costs and benefits of prevention, are not defined in the Treaty but are illuminated by their practical application in case law. However, all serious applications of the precautionary principle require some scientific evidence of a plausible association between exposures and current, or potential, impacts.

There is still much disagreement and discussion about the interpretation and practical application of the precautionary principle, due, in part, to this lack of clarity and consistency over its definition. For example, many definitions in the Treaties and Conventions use a double negative to define the precautionary principle: that is, they

identify reasons that cannot be used to justify not acting, but without specifying that a sufficiency of evidence is needed to justify taking action.

B. Reasonable Grounds for Concern?

The Communication from the EU on the precautionary principle (European Commission 2000) does specify that "reasonable grounds for concern" are needed to justify action under the precautionary principle, but it does not make explicit that these grounds will be case specific: nor does it explicitly distinguish between risk, uncertainty and ignorance. Since the EC Communication, the EU Council of Misisters, EU case law, and the regulation establishing the new European Food Safety Authority, EFSA, (General Food Law Regulation, EC No 178/2002), have further clarified the circumstances of use and application of the precautionary principle.
For example, the judgement of the European Court of Justice in the BSE case contained a general definition which authoritative commentators think contain many of the necessary elements of the precautionary principle that are applicable in all areas of the EC law:

> *"Where there is uncertainty as to the existence or extent of risks to human health, the institutions may take protective measures without having to wait until the reality and seriousness of those risks become fully apparent"* (Christoforou, 2002).

The WHO Declaration from the Fourth Ministerial Conference on Environment and Health (WHO, 2004a) refers explicitly to the precautionary principle with the recommendation:

> *"that it should be applied where the possibility of serious or irreversible damage to health or the environment has been identified and where scientific evaluation, based on available data, proves inconclusive for assessing the existence of risk and its level but is deemed to be sufficient to warrant passing from inactivity to policy alternatives"* (WHO, 2004b).

The American Public Health Association (APHA) affirmed endorsement of the precautionary principle as a cornerstone of public health for the protection of children's health. In a 2000 policy statement, the APHA encouraged governments, the private sector and health professionals to promote and use the precautionary principle to protect the health of developing children (APHA, 2001).

C. The EEA working definition of the Precautionary Principle.

The working definition used in the European Environment Agency that has been developed during debates since 2001 is explicit about specifying both uncertainty and ignorance, as contexts for applying the principle, and in acknowledging that a case-specific sufficiency of scientific evidence is needed to justify public policy actions:

> '*The Precautionary Principle provides justification for public policy actions in situations of scientific complexity, uncertainty and ignorance, where there may be a need to act in order to avoid, or reduce, potentially serious or irreversible threats to health or the environment, using an appropriate level of scientific evidence, and taking into account the likely pros and cons of action and inaction*' (Gee, 2006).

The definition is also explicit about the trade off between action and inaction, and widens the conventionally narrow, and usually quantifiable, interpretation of costs and benefits to embrace the wider and sometimes unquantifiable, "pros and cons".   Some of these wider issues, such as loss of the ozone layer, or of public trust in science, are unquantifiable, but they can sometimes be more damaging to society than the quantifiable impacts: and they need to be included in any comprehensive risk assessment. The EEA definition is proving to be useful in clarifying the use and interpretation of the PP, especially in emerging issues such as EMF.

VI. DIFFERENT LEVELS OF PROOF FOR DIFFERENT PURPOSES

The level of proof (or strength of scientific evidence) that would be appropriate to justify public action in each case varies with the pros and cons of action or inaction. These include the nature and distribution of potential harm; the justification for, and the benefits of the agent or activity under suspicion; the availability of feasible alternatives; and the overall goals of public policy. Such policy goals can include the achievement of the "high levels of protection" of public health, of consumer safety, and of the environment, required by the EU Treaty.

The use of different levels of proof is not a new idea: societies often use different levels of proof like for different purposes.

For example, a high level of proof (or strength of evidence) such as "beyond all reasonable doubt" is used to achieve good science where A is seen to cause B only when the evidence is very strong. Such a high level of proof is also used to minimise the costs of being wrong in the criminal trial of a suspected murderer, where it is usually regarded as better to let several guilty men go free than it is to wrongly convict an innocent man. However, in a different, civil trial setting, where, say, a citizen seeks compensation for neglectful treatment at work, which has resulted in an accident or ill health, the court often uses a lower level of proof commensurate with the costs of being wrong in this different situation. I n compensation cases an already injured party is usually given the benefit of the doubt by the use of a medium level of proof, such as "balance of evidence or probability". It is seen as being less damaging (or less costly in the wider sense) to give compensation to someone who was *not* treated negligently than it is to *not* provide compensation to someone who was treated negligently. The "broad shoulders" of insurance companies are seen as able to bear the costs of mistaken judgements rather better than the much narrower shoulders of an injured citizen. In each of these two illustrations it is the nature and distribution of the costs of being wrong that determines the level of proof (or strength of evidence) that is "appropriate" to the particular case.

Bradford Hill, cited above, was very concerned about the social responsibility of scientists and he concluded his classic 1965 paper on association and causation in environmental health , which was prepared at the height of the smoking controversy, with a "call for action" in which, *inter alia*, he also proposed the concept of case specific and differential levels of proof. His three examples ranged from "relatively slight" to "very strong" evidence, depending on the nature of the potential impacts and of the pros and cons in each specific case, i.e., possibly teratogenic medicine for pregnant women; a probable carcinogen in the workplace; and government restrictions on public smoking or diets. (Bradford Hill 1965).

Identifying an appropriate level of proof has also been an important issue in the climate change debates. The International Panel on Climate Change (IPCC) discussed

at length this issue before formulating their 1995 conclusion that "on the balance of evidence" mankind is disturbing the global climate. They further elaborated on this issue in their 2001 report where they identified 7 levels of proof (or strengths of evidence) that can be used to characterise the scientific evidence for a particular climate change hypothesis.

Table 2 provides the middle 5 of these levels of proof from the IPPC and illustrates their practical application to a variety of different societal purposes. In the cancer field the International Agency for Research on Cancer also uses several strengths of evidence to characterise the scientific evidence on carcinogens. (Cogliano, 2007)

Different Levels of Proof for Different Purposes: Some Examples and Illustrations

Probability	Quantitative descriptor (Probability bands based on IPCC 2001)	Qualitative Descriptor	Illustrations
100 % probability	Very likely (90-99 %)	• "Statistical significance"	• Part of strong scientific evidence for "causation"
		• "Beyond all reasonable doubt"	• Most criminal law. And the Swedish Chemical law, 1973, for evidence of "safety" of substances under suspicion- burden of proof on manufacturers
90 %	Likely (66-90 %)	• "Reasonable certainty"	• Food Quality Protection Act, 1996 (US)
		• "Sufficient scientific evidence"	• To justify a trade restriction designed to protect human, animal or plant health under World Trade Organisation Sanitary and Phytosanitary (SPS) Agreement, Art. 2.2, 1995
50 %	Medium Likelihood (33-66 %)	• "Balance of evidence"	• Intergovernmental Panel on Climate Change 1995 & 2001
		• "Balance of probabilities"	• Much Civil and some administrative law

		• "Reasonable grounds for concern"	• European Commission Communication on the Precautionary Principle 2000
		• "Strong possibility"	• British Nuclear Fuels occupational radiation compensation scheme, 1984 (20-50% probabilities triggering different awards up to 50% +, which then triggers full compensation)
	Low Likelihood (10-33 %)	• "Scientific suspicion of risk"	• Swedish Chemical law, 1973, for sufficient evidence to take precautionary action on potential harm from substances-burden of proof on regulators
0 %		• "Available pertinent information"	• To justify a provisional trade restriction under WTO SPS Agreement, Art. 5.7 where "scientific information is insuffiicient"
	Very Unlikely (1-10 %)	• Low risk	• Household fire insurance
0 % probability		• "Negligible and insignificant"	• Food Quality Protection Act, 1996 (US)

Source: EEA, 2001

VII.   FALSE NEGATIVES AND FALSE POSITIVES.

All of the 14 case studies (tributylin or TBT, benzene, PCBs, CFCs, MTBE, SO_2. Great Lakes pollution. DES, and beef hormones. asbestos. medical x-rays, BSE and Fisheries are all examples of "false negatives" in the sense that the agents or activities were regarded as not harmful for some time before evidence showed that they were indeed hazardous.

We tried to include a "false positive" case study in the report (i.e., where actions to reduce potential hazards turned out to be unnecessary), but failed to find either authors or sufficiently robust examples to use.   Providing evidence of "false positives" is more difficult than with "false negatives" (Mazur, 2004). How robust, and over what periods of time, does the evidence on the absence of harm have to be before concluding that a restricted substance or activity is without significant risk?

Volume 2 of "Late Lessons", which the EEA intends to publish in 2008, will explore the issues raised by false positives. including lessons to be learned from such apparent examples as the EU ban on food irradiation and hazardous labelling on saccharin in the US. The Y2K computer bug story may also carry some interesting lessons.

Why are there so many "false negatives" to write about, and how might this be relevant to EMF?   Conclusions based on the first Late lessons volume of case studies point to two main answers: the bias within the health and environmental sciences towards avoiding "false positives", thereby generating more "false negatives", and the dominance within decision-making of short-term, specific, economic and political interests over the longer term, diffuse, and overall welfare interests of society.

The latter point needs to be further explored, particularly within the political sciences. Researchers could examine the ways in which society's long-term interests can be more effectively located within political and institutional arrangements that have, or could have, an explicit mandate to look after the longer term welfare of society, and thereby to better resist the short-term pressures of particular economic or political interests. The judiciary in democracies can play part of this role, as can long running

and independent advisory bodies, such as the Royal Commission on Environmental Pollution (UK), or the German Advisory Council on Global Change.

The current and increasing dominance of the short-term in markets and in parliamentary democracies makes this an important issue. The experiments we are conducting with planet earth, its eco-systems and the health of its species, including humans, require, *inter alia*, more long-term monitoring of "surprise-sensitive" parameters which could, hopefully, give us early warnings of impending harm. Such long-term monitoring requires long-term funding, via appropriately designed institutions: such funding and institutions are in short supply. The case studies in Vol. 1 of "Late Lessons" illustrate both the great value, (e.g. in the TBT, DES, Great Lakes and CFCs stories), yet relative paucity, of long-term monitoring of both health and environments. Such monitoring can contribute to the "patient science" that slowly evolving natural systems require for their better understanding.

Since the publication of "Late Lessons" we have further explored the second cause of "false negatives" i.e. the issue of bias within the health and environmental sciences. Table 3 lists sixteen common features of methods and culture in the environmental and health sciences and shows their main directions of error. Of these, only three features tend towards generating "false positives" whereas twelve tend towards generating "false negatives". (Clearly, the weighting of these different biases would be the next step but has not yet been tried).

Table 3

ON BEING WRONG:
Environmental and Health Sciences and Their Directions of Error

SCIENTIFIC STUDIES	SOME METHODOLOGICAL FEATURES	MAIN[1] DIRECTIONS OF ERROR-INCREASES CHANCES OF DETECTING A:
Experimental Studies	• High doses	• False positive
	• Short (in biological terms) range of doses	• False negative
(Animal	• Low genetic variability	• False negative
Laboratory)	• Few exposures to mixtures	• False negative
	• Few Foetal-lifetime exposures	• False negative
	• High fertility strains	• False negative (Developmental/reproductive endpoints)
Observational	• Confounders	• False positive
Studies	• Inappropriate controls	• False positive/negative
(Wildlife &	• Non-differential exposure misclassification	• False negative
Humans)	• Inadequate follow-up	• False negative
	• Lost cases	• False negative
	• Simple models that do not reflect complexity	• False negative
Both	• Publication bias towards positives	• False positive
Experimental And	• Scientific cultural pressure to avoid false positives	• False negative
Observational Studies	• Low statistical power (e.g. From small studies)	• False negative
	• Use of 5 % probability level to minimise chances of false positives	• False negative

Source: Gee, 2006

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[1] Some features can go either way (e.g. inappropriate controls) but most of the features mainly err in the direction shown in the table

The general bias towards the null helps to produce robust science, basing it on strong foundations of knowledge, but this bias can encourage poor public health or environmental policy. The goals of science and public policy-making on health and environmental hazards are different: science puts a greater priority on avoiding "false positives" by accepting only very high levels of proof of "causality", whereas public policy tries to prioritize the avoidance of "false negatives" on the basis of a sufficiency of evidence of potential harm.

Table 3 is derived from papers presented to a conference on the precautionary principle organised by the Collegium Ramazzini, the EEA, the WHO and NIEHS in 2002. (Grandjean et al., 2003). It provides a first and tentative step in trying to capture and communicate the main directions of this bias within the environmental and health sciences, a bias which decision makers and the public should be aware of. As they debate the evidence on emerging hazards such as EMF.

The appropriate balance between false negatives and positives was addressed at a JRC/EEA workshop on the precautionary principle and scientific uncertainty which was held during the "Bridging the Gap" Conference, 2001, organised by the Swedish Presidency of the EU, in partnership with the EEA and DG Research. It drew the following conclusion:

> *"Improved scientific methods to achieve a more ethically acceptable and economically efficient balance between the generation of "false negatives" and "false positives" are needed"*. (Swedish EPA 2001).

VIII.   SOME CRITERIA FOR ESTABLISHING CAUSATION

Bradford Hill established nine criteria for helping to move from association to causation in environmental health which have been, and still are, widely used in debates on issues such as EMF
Two of the apparently more robust of the "criteria" may not be so robust in the context of multi-causality, complexity and gene/host variability.
For example, *"consistency"* of study findings is not always to be expected. As Prof. Needleman, who provided the first of what could be called the second generation of early warnings on lead in petrol in 1979 has observed:

> *"Consistency in nature does not require that all or even a majority of studies find the same effect. If all studies of lead showed the same relationship between variables, one would be startled, perhaps justifiably suspicious"* (Needlemann , 1995).

It follows that the *presence* of consistency of results between studies on the same hazard can provide robust evidence for a causal link, but the *absence* of such consistency may not provide very robust evidence for the absence of a real association. In other words, the "criterion" of consistency is asymmetrical, like most of the other Bradford Hill "criteria".

Similarly, the criterion of ***"temporality",*** which says that the putative cause X of harm Y must come before Y appears, is robust in a simple, uni-causal world. In a multi-causal, complex world of common biological end points that have several chains of causation this may not necessarily be so. For example, falling sperm counts can have multiple, co-causal factors, some of which may have been effective at increasing the incidence of the biological end point in question in advance of the stressors in focus, thereby confusing the analysis of temporality. The resulting overall sperm count trends could then be rising, falling or static, depending on the combined direction and strengths of the co-causal factors and the time lags of their impacts. It follows that say, chlorine chemicals, may or may not be co-causal factors in falling sperm counts: but the use of the "temporality" argument by the WHO, who observed that sperm counts began to fall before chlorine chemistry production took off, does not provide robust evidence that they are not causally involved.

The presence of "temporality", like "consistency" may be robust evidence *for* an association being causal, but its *absence* may not provide robust evidence *against* an association. Bradford Hill was explicitly aware of the asymmetrical nature of his "criteria": his followers have not always been so aware.
During 2005, the 40th anniversary year of the Bradford Hill "criteria", the EEA convened a panel of experts to review the "criteria" and their use in light of advances in knowledge, particularly multi-causality, since 1965. A report will be published in 2007.

How this goal can be achieved without compromising science remains to be explored, (Grandjean 2004; Grandjean et al., 2004). It is clearly necessary, particularly when dealing with EMF, for scientific methods to not only take account of thisfalse negative/positive bias in methodologies but also to more clearly reflect other realities such as multi-causality; thresholds; timing of dose; sensitive sub-populations, such as children, (Jarosinska and Gee, 2007); sex, age, and immune conditions of the host; information physics; effects below the thresholds of "acute" impacts, such as tissue heating; non-linear dose/response relationships; "low dose" effects; and the effects arising from disturbing the balance between opposing elements in complex biological systems. The evidence on EMF needs to take full account of these realities, as well as of the methodological biases of Table 3.

1X.   PUBLIC PARTICIPATION IN RISK ANALYSIS

Choosing an appropriate level of proof for a particular case is clearly based, *inter alia,* on value judgements about the acceptability of the costs, and of their distribution, of being wrong in both directions, i.e. of acting or not acting to reduce threatening exposures. This is why it is necessary to involve the public in decisions about serious hazards and their avoidance: and to do so for all stages of the risk analysis process.

Three of the "twelve late lessons" (number 5, number 9 and number 10) explicitly invite early involvement of the public and other stakeholders at all stages of risk analysis, a development which has been actively encouraged in many other influential reports during the last decade. (NRC 1994; US Presidential Commission on Risk Assessment and Risk Management 1997; Royal Commission on Environmental Pollution 1998; CEC Communication on the Precautionary Principle 2000; German Advisory Council on Global Change 2001).

The best available science is therefore only a necessary but not a sufficient condition for sound public policy making on potential threats to health and the environment. Where there is scientific uncertainty and ignorance "it is primarily the task of the risk managers to provide risk assessors with guidance on the science policy to apply in their risk assessments." (Christoforou, 2003). The content of this science policy advice, as well as the nature and scope of the questions to be addressed by the risk

assessors, need to be formulated by the risk managers and relevant stakeholders at the initial stages of the risk analysis.

Involving the public in not only all stages of risk analysis, but also in helping to set research agendas and technological trajectories, (Wilsdon and Willis, 2004) is not easy. Many experiments, in both Europe and the USA, with focus groups, deliberative polling, citizens' juries, and extended peer review, (Funtovicz and Ravetz, 1990/92) are exploring appropriate ways forward.

The issue of time is also a critical issue for risk analysis and application of the precautionary principle. For example, the time from the first scientifically based early warnings (1896 for medical X rays, 1897 for benzene, 1898 for asbestos) to the time of policy action that effectively reduced damage was often 30-100 years. Some consequences of the failures to act in good time (e.g. on CFCs or asbestos) continue to cause damage over even longer time periods. For example, the ozone hole will cause many thousands of extra skin cancers in today's children but the cancers will only peak around the middle of this century because of the long latent period between exposure and effect. Such long-term but foreseeable impacts raise liability and compensation issues, including appropriate discount rates (if any) on future costs and benefits, which being value-laden choices. need also to be discussed by stakeholder groups. Again, experience in the climate change field with these long-term issues may be helpful in managing them with respect to electromagnetic fields (ELF and RF).

The wider involvement of stakeholders has also been recognised more recently by the International Risk Governance Council (IRGC, 2005) and the EU report on Science and Governance, (Wynne et al., 2007). Whether wider involvement of stakeholders results in better and more acceptable decisions needs to studied: early indications (Beierle, 2002), and lessons from history, suggests that is. In many cases several decades will be necessary to confidently judge outcomes, given latencies and complexities.

X.   SOME EXAMPLES OF EARLY WARNINGS

The 14 case studies in the Late Lessons Report (EEA 2001) include examples some chemicals (tributylin or TBT, benzene, PCBs, CFCs, MTBE, SO_2 and Great Lakes pollution); two other pharmaceuticals (DES, and beef hormones); two physical agents (asbestos and medical x-rays); one pathogen (BSE); and Fisheries (overfishing).

The main issues discussed so far, such as the contingent nature of knowledge; ignorance and "surprises"; appropriate levels of evidence for policy actions; and public participation in risk analysis are critical to the successful application of both scientific knowledge and the precautionary principle to public policy-making. They are therefore relevant to discussions about the potentially new hazards that are now emerging e.g. from nanotechnology, (Royal Society 2003); from the non-ionising radiations arising from the use of mobile phones, (Stewart Reports 2000, 2004), and from endocrine disrupting substances or EDSs. (WHO, 2002).

With such newly emerging hazards it can be helpful to use historical examples to illustrate what a scientifically based early warning looks like as it is often difficult to properly recognise such warnings at the time they occur.   A good example is that provided by the UK Medical Research Council's Swann Committee in 1969. They were asked to assess the evidence for risks of resistance to antibiotics in humans following the prolonged ingestion of trace amounts of antibiotics arising from their use as growth promoters in animal feed. (Edqvist and Pedersen 2001). They concluded that:

> *"Despite the gaps in our knowledge .. we believe … on the basis of evidence*
> *presented to us, that this assessment is a sufficiently sound basis for action .. The cry*
> *for more research should not be allowed to hold up our*
> *recommendations'.…'sales/use of AFA should be strictly controlled via tight criteria,*
> *despite not knowing mechanisms of action, nor foreseeing all effect"*. (Swann 1969).

A. Antibiotics in Animal Feed

The Swann Committee also concluded that it would be more rewarding and innovative to improve animal husbandry as a means of encouraging disease free animal growth rather than to the cruder approach of diets containing antimicrobials. Despite the gaps in knowledge, the need for much more research, and considerable ignorance about the mechanisms of action, a sufficiency of evidence was identified and described by the Swann Report that justified the need for public authorities to restrict the possibility of exposures to antibiotics from animal growth promoters. This early warning was initially heeded, but was then progressively ignored by the pharmaceutical companies and regulatory authorities, who wanted more scientific justification for restricting anti-microbial growth promoters. However, in 1985 in Sweden, and then in the EU in 1999, the use of antibiotics as growth promoters was finally banned. Pfizer, the main supplier of such antibiotics in Europe, appealed against the European Commission banning decision, pleading, *inter alia*, an insufficiency of scientific evidence. They lost this case at the European Court of Justice (Case T-13/99-Pfizer 2002), a case which further clarified the proper use and application of the precautionary principle in circumstances of scientific uncertainty and of widespread, if low, public exposures to a potentially serious threat.

B. Lead in Gasoline

A US example of an early warning comes from the lead in gasoline story: a warning that was largely ignored for over 50 years, resulting in much damage to the intelligence and behaviour of children in America, Europe and the rest of the motorised world. Yandell Hendersson, Chair of the Medical Research Board, US Aviation Service, who had been asked to look at the scientific evidence on the possible hazards of tetraethyl lead during the temporary ban on lead in petrol, in 1925, concluded:

> *"It seems likely that the development of lead poisoning will come on so insidiously that leaded gasoline will be in nearly universal use … before the public and the government awakens to the situation"*. (Rosner and Markowitz, 2002).

Motorised societies would have gained much in dollars, brainpower and social cohesion had they heeded this foresight.

C. Tributylin (TBR) – A Marine Antifoulant for Ships

The case study on tributylin (TBT) and DES illustrate the surprises that arise from real life complexities and which may carry some lessons for the EMF debate. For example, the unfolding of the TBT story was accompanied by an increased appreciation of scientific complexity arising from the discoveries that adverse impacts were caused by very low doses (i.e. in parts/trillion); that high exposure concentrations were found in unexpected places e.g. in the marine micro-layer; and that bioaccumulation in higher marine animals, including sea-food for human consumption, was greater than expected. The early actions on exposure reduction in France and the UK in 1982-85 were based on a 'strength of evidence' for the 'association' only: knowledge about 'causality', 'mechanisms of action' and other the complexities above came much later.

We were lucky in some ways with the TBT story: a highly specific, initially uncommon impact (imposex) was quickly linked to one chemical, TBT. This relatively easily identified linkage is not likely to be repeated for the more common and multi-causal impacts where, for example, neurodevelopmental diseases and dysfuntions, or common cancers, are the impacts under suspicion.

D. Diethylstilbestrol (DES)

Key lessons from the DES story are also instructive, as it provides the clearest example of endocrine disruption in humans. Diethylstilbestrol, commonly referred to as DES, is a synthetic estrogen . It was originally prescribed to prevent miscarriage, but did not. Later, sons and daughters of mothers given DES to prevent miscarriage developed cancers, reproductive tract anomalies, and had more pre-term babies themselves as a result. The effects of DES include the absence of visible and immediate teratogenic effects **not** being robust evidence for the absence of reproductive toxicity; and the 'timing of the dose clearly determining the poison', in contrast to the 'dose determines the poison' dictum of Paracelsus. Timing is also relevant to other biological end points:

> *"the time of life when exposures take place may be critical in defining dose-response*
> *relationships of EDSs for breast cancer as well as for other health effects",*
> (WHO/IPCS, 2002).

Although the exposure levels were higher than the usual environmental levels of other EDSs, the DES story provides a clear warning about the potential dangers of perturbing the endocrine system with synthetic chemicals.

With over 20,000 publications, DES is now a well-studied compound, yet many doubts persist about its mechanisms of action. Since no dose-effect relationship has been found in humans, it cannot be excluded that DES could have been toxic at low doses, and that other less potent xenoestrogens could have similar effects.

If we still have few certainties about DES after so much time and research, what should our attitude be towards emerging hazards, such as other endocrine disrupting substances (EDSs) and EMF?

XI.   CONCLUSION

The lessons of history from the EEA report, and subsequent debates and events, indicate that they have much relevance to the EMF issue, as well as to other emerging issues such as nanotechnology, (Royal Society, 2003) and endocrine disrupting substances or EDSs (WHO, 2002).   The public health assessment of EMF could apply these lessons, approaches, terms of discussion and interpretations to the precautionary and preventative actions on the different parts of the EMF exposure problem.

There are of course large differences between smoking and EMF. The smoking hazard had at least 10 times the relative risk increase in the exposed population compared to the leukaemia risk from power line exposure; and the size of the smoking exposed population, and its exposure above that needed to generate a doubling of the risk, are both very much greater than with power lines. The larger relative risk for smoking and lung cancer seems to arise from comparing smokers with non, or never, smokers whilst the relative risk of 2 to 3 that arises between moderate and heavy smokers, or between second hand smokers and non smokers, is more relevant to the EMF issue,

where there is an absence of unexposed controls.   The lower relative risks of 2 or 3 for EMF are biased towards the null to unknown extent by the absence of such controls (Milham, 1998).   However, the parallel between the slow recognition of the smoking hazard and power line EMF hazard is interesting.

The parallel with the history of X rays is also pertinent. The initial discovery, by Alice Stewart in the early 50s, that a few x rays of a pregnant woman in the sensitive period of her pregnancy gave a 2 fold excess of leukaemia, was greeted with much strident disbelief, particularly from the male doctors whose latest toy was under threat. It took another 20 years or so before her result became generally accepted, and only after several negative studies that were conducted in the early response to her study. Many studies of X rays in pregnant women now exist, and, as with the power line studies, the relative risk remains at about 2. (EEA, 2001) What will the history of EMF look like in 2020?

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SECTION 17

KEY SCIENTIFIC EVIDENCE AND
PUBLIC HEALTH POLICY RECOMMENDATIONS

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Table of Contents

I.   KEY SCIENTIFIC EVIDENCE

Exposure to electromagnetic fields (EMF) has been linked to a variety of adverse health outcomes.   The health endpoints that have been reported to be associated with ELF and/or RF include childhood leukemia, adult brain tumors, childhood brain tumors, genotoxic effects (DNA damage and micronucleation), neurological effects and neurodegenerative disease, immune system disregulation, allergic and inflammatory responses, breast cancer in men and women, miscarriage and some cardiovascular effects. Effects are not specifically segregated for ELF or RF, since many overlapping exposures occur in daily life; and because this is an artificial division based on frequencies as defined in physics that has little bearing on the biological effects. Both ELF and RF, for example have been shown to cause cells to generate stress proteins, a universal sign of distress in plant, animal and human cells.

The number of people exposed to elevated levels of EMF has been estimated in various studies, and there is general agreement among them. In the United States, few people have chronic or prolonged exposures over 4 mG (0.4 µT) (Kheifets et al, 2005b). Section 20 has information on average residential and occupational ELF levels. The highest exposure category in most all studies is ≥ 4 mG (≥ 0.4 µT). Many people have daily exposures to ELF in various ways, some of them up to several hundred milligauss for short periods of time, but relatively few people with the exception of some occupational workers habitually experience ELF exposures greater than 1-2 mG (0.2 – 0.3 µT - App. 20-A).

The exposure of children to EMF has not been studied extensively; in fact, the FCC standards for exposure to radiofrequency radiation are based on the height, weight and stature of a 6-foot tall man, not scaled to children or adults of smaller stature. They do not take into account the unique susceptibility of growing children to exposures

(SCENIHR, 2007; Jarosinska and Gee, 2007), nor are there studies of particular relevance to children.

Differences in exposure patterns between infants, children and adults; 2) special susceptibilities of infants and children to the effects of EMF; and 3) interactions between chemical contaminants and EMF are lacking; as are studies on chronic exposure for both children and adults. There is reason to believe that children may be more susceptible to the effects of EMF exposure since they are growing, their rate of cellular activity and division is more rapid, and they may be more at risk for DNA damage and subsequent cancers. Growth and development of the central nervous system is still occurring well into the teenage years so that neurological changes may be of great importance to normal development, cognition, learning, and behavior. Prenatal exposure to EMF have been identified as possible risk factor for childhood leukemia. Children are largely unable to remove themselves from exposures to harmful substances in their environments.   Their exposure is involuntary.

Like second-hand smoke, EMF is a complex mixture, where different frequencies, intensities, durations of exposure(s), modulation, waveform and other factors is known to produce variable effects. Many years of scientific study has produced substantial evidence that EMF may be considered to be both carcinogenic and neurotoxic. The weight of evidence is discussed in this report, including epidemiological evidence and studies on laboratory animals.

Relative risk estimates associated with some of these endpoints are small and the disease is fairly rare (for childhood leukemia, for example), For other diseases, the risk estimates are small but the diseases are common and EMF exposures at levels associated with increased risks are widespread and chronic so the overall public health impacts may be very large.

A. Weight of Evidence Assessment and Criteria for Causality

A weight-of-evidence approach has been used to describe the body of evidence between health endpoints and exposure to electromagnetic fields (ELF and RF).

The number and quality of epidemiological studies, as well as other sources of data on biological plausibility are considered in making scientific and public health policy judgments. Methodological issues that were considered in the review of the epidemiological literature include 1) quality of exposure assessment. 2) sample size of the study, which detects the power to detect an effect, 3) extent to which the analysis or design takes into account potential confounders or other risk factors, 4) selection bias, 5) the potential for bias in determining exposure. Assessment of the epidemiological literature is consistent with guidelines from Hill (1971), Rothman and Greenland (1998) and the Surgeon General's Reports on Smoking (US DHHS, 2004), and California Air Resources Board (2005). Factors that were considered in reaching conclusions about the weight of evidence overall included strength of the association, consistency of association, temporality, biological plausibility, dose-response and issues with non-linear dose-response, specificity and experimental evidence.

There is a relatively large amount of human epidemiological information with real world exposures, including data from occupational studies. There is less animal data in most cases, except for the genotoxicity studies. Human epidemiological evidence has be given the greatest weight in making judgments about weight-of-evidence, where the results across high quality studies give relatively consistent positive results. Meta-analyses of childhood leukemia, adult leukemia, adult brain tumors, childhood brain tumors, male and female breast cancer and Alzheimer's disease were relied upon in assessing the overall strength of epidemiological study results. Sections 5 – 15 provide analysis of the relevant scientific studies that are key evidence in making public health policy recommendations with respect to exposure to electromagnetic fields (both ELF and RF).

B.   Summary of Evidence

1. Childhood Leukemia

Several meta-analyses have been conducted to assess risks of childhood leukemia from exposure to ELF. The results of these studies that combine or pool results of many individual studies (including studies that report both effects and no effects) consistently report increased risks.

Meta-Analysis: Studies of Childhood Leukemia and EMF

Greenland et al., (2000) reported a significantly elevated risk of 1.68 [95% CI 1.23-2.31] based on pooled results from 12 studies using a time-weighted average of exposure greater than 3 mG (0.3 µT). This is a 68% increased risk of childhood leukemia.

Ahlbom et al., (2000) reported a doubling of risk based on a meta-analysis of nine (9) studies.   The results reported an elevated risk of 2.0 [95% CI 1.27-3.13] for EMF exposures equal to or greater than 4 mG (0.4 µT) as compared to less than 1 mG (0.1 µT)

Other Relevant Evidence

In 2002, the International Agency for Cancer Research (IARC) designated EMF as a "possible human carcinogen" or Group 2B Carcinogen based on consistent epidemiological evidence. The exposure levels at which increased risks of childhood leukemia are reported in individual studies range from above 1.4 mG or 0.14 µT (Green et al., 1999).
for younger children to age six (6) to 4 mG (0.4 µT). Many individual studies with cutpoints of 2 mG or 3 mG (0.2-0.3 µT)) report increased risks. Plausible biological mechanisms exist that may reasonably account for a causal relationship between EMF exposure and childhood leukemia.

Recurrence of Childhood Leukemia and Poorer Survival Rates with Continued EMF Exposure

Foliart reported more than a four-fold (450% increased risk) of adverse outcome (poorer survival rate) for children with acute lymphoblastic leukemia (ALL) who were recovering in EMF environments of 3 mG (0.3 µT) and above (OR 4.5, CI 1.5-13.8). Svendsen reported a poorer survival rate of children with acute lymphoblastic leukemia (ALL) in children exposed to 2 mG (0.2 µT) and above. These children were three times more likely (300% increased risk) to die than children recovering in fields of less than 1 mG (OR 3.0, CI 0.9.8). Children recovering in EMF environments between 1- 2 mG (0.1-0.2 µT) also had poorer survival rates, where the increased risk was 280% (OR 2.8, CI 1.2-6.2).

Higher Lifetime Cancer Risks with Childhood EMF Exposure

Lowenthal (2007) reported that children raised for the first five years in home environments exposed to EMF within 300 meters of a high voltage power line have a five-fold (a 500 percent increased risk of developing some kinds of cancers sometime in later life. For children from newborn to 15 years of age; it is a three-fold risk of developing cancer later in life (Lowenthal et al., 2007). There is suggestive evidence for a link between adult leukemia and EMF exposure.

Attributable Risk

Wartenberg estimates that 8% to 11% of childhood leukemia cases may be related to ELF exposure. This translates into an additional 175 to 240 cases of childhood leukemia based on 2200 US cases per year. The worldwide total of annual childhood leukemias is estimated to be 49,000, giving an estimate of nearly 4000 to 5400 cases per year. Other researchers have estimated higher numbers that could reach to 80% of all cases (Milham, 2001).

2. Childhood Brain Tumors

Childhood Brain Tumors

There is suggestive evidence that other childhood cancers may be related to EMF exposure. The meta-analysis by Wartenberg et al., (1998) reported increased risks for childhood brain tumors.   Risks are quite similar whether based on calculated EMF fields (OR = 1.4, 95% CI = 0.8 – 2.3] or based on measured EMF fields (OR = 1.4, 95% CI = 0.8 – 2.4).

3. Adult Brain Tumors

Brain Tumors in Electrical Workers and in Electrical Occupations (Meta-analysis)

A significant excess risk for adult brain tumors in electrical workers and those adults with occupational EMF exposure was reported (Kheifets et al., 1995). This is about the same size risk for lung cancer and second hand smoke (US DHHS, 2006). A total of 29 studies with populations from 12 countries were included in this meta-analysis.   The relative risk was reported as 1.16 (CI = 1.08 – 1.24) or a 16% increased risk for all brain tumors. For gliomas, the risk estimate was reported to be 1.39 (1.07 – 1.82) or a 39% increased risk for those in electrical occupations.   A second meta-analysis published by Kheifets et al., ((2001) added results of 9 new studies published after 1995. It reported a new pooled estimate (OR = 1.16, 1.08 – 1.01) that showed little change in the risk estimate overall from 1995.

4. Brain Tumors and Acoustic Neuromas in Cell Phone and Cordless Phone Users (Meta-Analysis)

Glioma and Acoustic Neuroma

Hardell et al., (2007) reported in a meta-analysis statistically significant increased risk for glioma with exposure of 10 years or greater in persons using cell phones. Risks were estimated to be 1.2 (0.8 – 1.9) for all use; but when ipsilateral use was assessed (mainly on same side of head) it increased the risk of glioma to 2.0 (1.2 – 3.4) for 10 years and greater use.

For acoustic neuromas, Hardell et al., (2007) reported the increased risk with 10 years or more of exposure to a cell phone at 1.3 (0.6 – 2.8) but this risk increased to 2.4 (1.1 – 5.3) with ipsilateral use (mainly on the same side of the head).   There is a consistent pattern of increased risk for brain tumors (glioma) and acoustic neuromas at 10 years and greater exposure to cell phones.

The meta-analysis by Lakhola et al., (2006) reported that brain tumor risk was 1.3 (0.99 – 1.9) for ipsilateral use of a cell phone, but no data was given for exposures at 10 years or greater (all exposures were of shorter duration).

The meta-analysis by Kan et al., (2007) reported "no overall risk" but found elevated risk of brain tumors (RR = 1.25, CI 1.01 – 1.54) $\geq$ 10 years, reinforcing the findings of other pooled estimates of risk. No estimates of increased risk with ipsilateral use were provided, which would have likely increased reported risks.

5. Neurodegenerative Diseases

Alzheimer's Disease and ALS

Evidence for a relationship between exposure and the neurodegenerative diseases, Alzheimer's and amyotrophic lateral sclerosis (ALS), is strong and relatively consistent. While not every publication shows a statistically significant relationship between exposure and disease, ORs of 2.3 (95% CI = 1.0-5.1 in Qio et al., 2004), of 2.3 (95% CI = 1.6-3.3 in Feychting et al., 2003) and of 4.0 (95% CI = 1.4-11.7 in Hakansson et al., 2003) for Alzheimer's Disease.

Hakansson et al., report more than a doubling of risk for ALS 2.2 (95% CI = 1.0-4.7).

Savitz et al., (1998) reports more than a tripling of risk for ALS (3.1, CI = 1.0 – 9.8).

6. Breast Cancer (Men and Women)

A meta-analysis by Erren (2001) on EMF and breast cancer reported pooled relative risks based on studies of both men and women. A total of 38 publications were reviewed; there were 23 studies on men; 25 studies on women; and 10 studies on both men and women. The pooled relative risk for women exposed to EMF was 1.12 (CI 1.09 – 1.15) or a 12% increased risk, Erren observed that variations between the contributing results are not easily attributable to chance (P = 0.0365). For men and breast cancer, he reported a fairly homogeneous increased risk (a pooled relative risk of 1.37 [CI 1.11 – 1.71]*).

This analysis is well conducted. The results were stratified according to measured or assumed intensity of exposure to EMF; and the estimate of risk for the most heavily exposed group was extracted. Independent estimates of RRs were grouped according to gender, type of study (case-control and cohort), country where the study was conducted and method used to assess exposure. Pooled estimates of RRs and their 95% confidence intervals (CI) referring to various combinations of these factors were calculated according to appropriate statistical methods (Greenland, 1987).   Misclassification possibilities were thoroughly assessed, and whether the results were sole endpoints or there were multiple endpoints in each study did not affect the RRs.

Erren qualifies his findings by discussing that latencies for cancers can be 20 to 30 years, Further, he notes that studies of total EMF exposures from both home, travel and workplace are rarely available, and these EMF sources are ubiquitous. Both could result in underestimation of risks. Another way in which risks might be masked is by variations in age of study participants. Forssen and colleagues (2000) reported no increased RRs for breast cancer in women of all ages when they combined residential and occupational EMF exposures (RR = 0.9, CI 0.3 – 2.7). However, when risks for the women younger than 50 years of age were separated out and calculated, the RR increased to 7.3 (CI 0.7 – 78.3) although with wide confidence intervals based on only four cases. Erren notes

"When possibly relevant exposures to EMF in the whole environment are assessed only partially, errors in the categorization of exposure status are likely to occur. If such misclassification is random and thus similar in subrgroups being compared (nondifferential), then the error will tend to introduce bias towards the null. Substantial random misclassification of exposures would then tend to generate spurious reports of 'little or no effect'. Note for example that estimates of smoking-associated lung cancer risks in the early 1950's could have been seriously distorted if exposure assessment had not considered smoking either at work or at home."

"Collectively, the data are consistent with the idea that exposures to EMF, as defined, are associated with some increase in breast cancer risks, albeit the excess risk is small."
Erren (2001)

7. Combined Effects of Toxic Agents and ELF

ELF and Toxic Chemical Exposures

There is also the issue of what weight to give the evidence for synergistic effects of toxic chemical exposure and EMF exposure. Juuilainen et al., (2006) reported that the combined effects of toxic agents and ELF magnetic fields together enhances damage as compared to the toxic exposure alone. In a meta-analysis of 65 studies; overall results showed 91% of the *in vivo* studies and 68% of the *in vitro* studies had worse outcomes (were positive for changes indicating synergistic damage) with ELF exposure in combination with toxic agents. The percentage of the 65 studies with positive effects was highest when the EMF exposure preceded the other exposure. The radical pair mechanism (oxidative damage due to free radicals) is cited as a good candidate to explain these results. Reconsideration of exposure limits for ELF is warranted based on this evidence.

II. FALLACIES AND ANSWERS IN THE DEBATE OVER EMF EVIDENCE

There are several arguments (false, in our view) that have been presented by those who minimize the strength of the relationship between exposure to both 50-60Hz ELF and RF EMFs. These are as follows:

A. "Only a small number of children are affected."

This argument is not correct because we do not know precisely how many children are affected. In 1988 Carpenter and Ahlbom attempted to answer this question based on the results of the New York State Powerlines Project and the results of the study of Savitz et al. (1988), and concluded that if the magnetic fields homes in the US were similar to those in Denver, Colorado fully 10 to 15% of US childhood leukemia (about 1,000 cases) could be associated with residential magnetic field exposure. They then concluded that exposure to magnetic fields from non-residential sources (particularly appliances) must be at least equal in magnitude, and that if so these two sources of exposure would account for 20-35% of childhood leukemia.

There have been several meta-analyses of the childhood leukemia data (Wartenberg, 1998; Greenland et al., 2000; Ahlbom et al., 2000). All have concluded that there is a significant association between residential exposure to magnetic fields and elevated risk of leukemia in children. Greenland et al. (2000)

performed a meta-analysis of 15 studies of magnetic field or wire code investigations of childhood leukemia, and calculated the attributable fraction of cases of childhood leukemia from residential magnetic field exposure in the US was 3%. Ahlbom et al. (2000) conducted a different meta-analysis that concluded there was a significant 2-fold elevation of risk at exposure levels of 4 mG (0.4 µT) or greater. Kheifets et al. (2006) attempted to calculate the attributable fraction of worldwide childhood leukemia due to EMFs, based on the meta-analyses of Ahlbom et al. (2000) and Greenland et al., (2000). They concluded that the attributable fraction of leukemia was between <1% to 4%. The recent WHO Environmental Health Criteria ELF Monograph #238 (2007) states "(A)ssuming that the association is causal, the number of cases of childhood leukaemia worldwide that might be attributable to exposure can be estimated to range from 100 to 2,400 cases per year. However this represents 0.2 to 4.9% of the total annual incidence of leukaemia cases, estimated to be 49,000 worldwide in 2000. Thus, in a global context, the impact on public health, if any, would be limited and uncertain."

These reports are important, in that they show consistency in there being a clearly elevated risk of leukemia in children with EMF exposure from power line fields in homes. These meta-analyses lead to the conclusion, reflected in the WHO report, that there is an association between childhood cancer and exposure to elevated magnetic fields in homes. We strongly disagree, however, with the overall conclusion that these calculations indicate that the fraction of childhood leukemia attributable to EMFs is so small as to not have serious public health implications.

There are several reasons why the WHO ELF Environmental Health Criteria Monograph conclusion is not justified. These studies all considered either only measured magnetic fields in homes or wire codes from power lines, ignoring exposure from appliances, wireless devices and all exposures outside of the home. Thus these metrics do not come close to accounting for any individual's cumulative exposure to EMFs. If residential magnetic fields cause cancer, then those from other sources will add to the risk. The failure to measure total EMF exposure would tend to obscure the relationship and lead to gross underestimation of the true relationship between exposure and disease. While the evidence for a relationship between exposure and childhood leukemia may be considered to be definitive at exposure levels of 3 or 4 mG (0.3 or 0.4 µT) or higher; there is evidence from some (but not all) of the other studies for an elevated risk at levels not greater than 2 mG (0.2 µT) (Savitz et al., 1988; Green, 1999). There is absolutely no evidence that exposures at lower levels are "safe", since persons with these exposures are usually the "control" group. Therefore this WHO statement fails to acknowledge the true magnitude of the problem, even when considering only childhood leukemia. The global attributable risk of childhood leukemia as a result of exposure to EMFs must be significantly greater than that calculated from consideration of only residential 50/60 Hz magnetic fields in studies where there is no unexposed control.

 As detailed in other chapters in this report (Chapter 10), there is some evidence for a relationship between EMF exposure and brain cancers in children. We have almost no understanding of the mechanisms behind the development of brain cancers, and any cancer in a child is a tragedy. While evidence for a relationship between EMF exposure and childhood brain cancer is not as strong as for leukemia, it is of concern and deserves more study. Of even greater concern, given the clear evidence for elevated risk of childhood leukemia upon exposure to 50/60 Hz EMFs, is the relative lack of a comparable body of information on the effects of radiofrequency EMFs on the health of children. A recent study of South Korean children (1,928 with leukemia, 956 with brain cancer and 3,082 controls) living near to AM radio transmitters reports an OR of 2.15 (95% CI = 1.19-2.11) for risk of leukemia in children living within 2 km of the nearest AM transmitter as compared to those living more than 20 km from it (Ha et al., 2007). No relation was found for brain cancer. This study is consistent with the hypothesis that radiofrequency EMFs have similar effects to 50/60 Hz EMFs, but more study is needed. Since radiofrequency EMFs have higher energy than do power line frequencies, one might expect that they would be even more likely to cause disease. The enormous and very recent increase in use of cell phones by children is particularly worrisome. However there is little information at present on the long-term consequences of cell phone use, especially by children.

B.   "There is insufficient evidence that adult diseases are secondary to EMF exposure."

It is correct that the level of evidence definitively proving an association between exposure to EMFs and various adult diseases is less strong that the relationship with childhood leukemia. However there are multiple studies which show statistically significant relationships between occupational exposure and leukemia in adults (see Chapter 11), in spite of major limitations in the exposure assessment. A very recent study by Lowenthal et al. (2007) investigated leukemia in adults in relation to residence near to high-voltage power lines. While they found elevated risk in all adults living near to the high voltage power lines, they found an OR of 3.23 (95% CI = 1.26-8.29) for individuals who spent the first 15 years of life within 300 m of the power line. This study provides support for two important conclusions: adult leukemia is also associated with EMF exposure, and exposure during childhood increases risk of adult disease. Thus protecting children from exposure should be a priority.

The evidence for a relationship between exposure and breast cancer is relatively strong in men (Erren, 2001), and some (by no means all) studies show female breast cancer also to be elevated with increased exposure (see Chapter 12). Brain tumors and acoustic neuromas are more common in exposed persons (see Chapter 10). There is less published evidence on other cancers, but Charles et al. (2003) report that workers in the highest 10% category for EMF exposure were twice as

likely to die of prostate cancer as those exposed at lower levels (OR 2.02, 95% CI = 1.34-3.04). Villeneuve et al. (2000) report statistically significant elevations of non-Hodgkin's lymphoma in electric utility workers in relation to EMF exposure, while Tynes et al. (2003) report elevated rates of malignant melanoma in persons living near to high voltage power lines. While these observations need replication, they suggest a relationship between exposure and cancer in adults beyond leukemia.

Evidence for a relationship between exposure and the neurodegenerative diseases, Alzheimer's and amyotrophic lateral sclerosis (ALS), is strong and relatively consistent (see Chapter 12). While not every publication shows a statistically significant relationship between exposure and disease, ORs of 2.3 (95% CI = 1.0-5.1 in Qio et al., 2004), of 2.3 (95% CI = 1.6-3.3 in Feychting et al., 2003) and of 4.0 (95% CI = 1.4-11.7 in Hakansson et al., 2003) for Alzheimer's Disease, and of 3.1 (95% CI = 1.0-9.8 in Savitz et al., 1998) and 2.2 (95% CI = 1.0-4.7 in Hakansson et al., 2003) for ALS cannot be simply ignored.

In total the scientific evidence for adult disease associated with EMF exposure, given all of the difficulties in exposure assessment, is sufficiently strong that preventive steps are appropriate, even if not all reports have shown exactly the same positive relationship. While there are many possible sources of false positive results in epidemiological studies, there are even more possible reasons for false negative results, depending on sample size, exposure assessment and a variety of other confounders. It is inappropriate to discount the positive studies just because not every investigation shows a positive result. While further research is needed, with better exposure assessment and control of confounders; the evidence for a relationship between EMF exposure and adult cancers and neurodegenerative diseases is sufficiently strong at present to merit preventive actions to reduce EMF exposure.

C. "The risk is low."

This argument is incorrect because at present it is not possible to determine the magnitude of the risk. Clearly as far as EMFs are concerned there is no unexposed population. Therefore one can only compare groups with different levels of exposure. We can perhaps say with confidence that the elevated risk of leukemia from residential exposure of children to magnetic fields is "low" (meaning ORs in the range of 2-4), but this does not consider the child's exposure to appliances, exposure in automobiles and at daycare or school, exposures in playgrounds and at all of the other places that a child spends time. Even if the risk to one individual is low, the societal impact when everyone is exposed may be very significant.

In addition the exposure assessment is grossly inadequate, even in the best of studies. Most reports deal only with either characterization of the fields within

residences or with job titles in occupational settings. Some studies attempt to quantitate other sources of exposure, such as frequency of cell phone usage or use of other appliances, but these studies almost always do not consider residential exposure from power lines. In no investigation has it been possible to follow the exposures of a large number of people over a number of years with accurate monitoring of total exposure to EMFs. This would of course be almost impossible to do for the very good reason that as a person moves through his or her environment the exposures vary from place to place and from moment to moment. However to truly and objectively determine the risk of exposure to EMFs it is essential to consider residential, occupational (or school) and recreational exposures to the full range of the electromagnetic spectrum, including appliances and wireless devices. This has not been accomplished in any study, and without such information it is not possible to determine the overall magnitude of the risk. It is possible, indeed likely, that upon consideration of both childhood and adult diseases that the risk is not low.

D. "There is no animal evidence".

It is correct that there is no adequate animal model system that reproducibly demonstrates the development of cancer in response to exposure to EMFs at the various frequencies of concern. McCann et al. (1997) reviewed the animal studies, and while they found most to be negative there were several that showed suggestive positive results. They also clearly identified issues that need to be improved in further animal carcinogenesis investigations. However Kheifets et al. (2005a) in a policy review noted that "even consistent negative toxicological data cannot completely overcome consistent epidemiological studies. First, a good animal model for childhood leukemia has been lacking. Second, particularly for ELF, the complex exposures that humans encounter on a daily basis and a lack of understanding of the biologically relevant exposure calls into question the relevance of exposures applied in toxicology. Another limitation of toxicologic studies is that animals cannot be exposed to fields that are orders of magnitude more powerful than those encountered by humans, decreasing their power to detect small risks." Further, they conclude that "(A)lthough the body of evidence is always considered as a whole, based on the weight of evidence approach and incorporating different lines of scientific enquiry, epidemiologic evidence, as most relevant, is given the greatest weight."

One positive animal study is that by Rapacholi et al. (1997), who demonstrated that lymphoma-prone transgenic mice developed significantly more lymphoma after exposure to 900 MHz fields (lymphoma being the animal equivalent of human leukemia) than did unexposed animals. More striking is the report from Denver, Colorado using the wire-code characterization originally developed by Wertheimer and Leeper (1979) showing that pet dogs living in homes characterized as having high or very high wire codes, as compared to those with low or very low wire codes or buried power lines, showed a OR of 1.8 (95% CI =

0.9-3.4) for development of lymphoma after adjustment for potential confounders, whereas dogs that lived in homes with very high wire codes had an OR of 6.8 (95% CI = 1.6-28.5) (Reif et al., 1995). This study is impressive because the exposure of the dogs reflects the environment in which exposure has been associated with elevated risk of human cancer in two independent investigations (Wertheimer and Leeper, 1979: Savitz et al., 1988).

It is curious that in many legal situations the courts are reluctant to accept only evidence that substance X causes cancer in animals without corresponding evidence in humans. In the case of EMFs we have strong evidence that EMFs cause cancer in human, but much less evidence from animal models. The US Supreme Court, in the case of Daubert vs. Merrell Dow Pharmaceuticals, effectively ruled that animal studies were not relevant to human health, and that the only admissible evidence must be from human epidemiological studies! While this is certainly not a justifiable conclusion, the situation with regards to EMF health effects is that we have strong evidence for human cancer from epidemiological studies, but do not have good evidence for cancer in experimental animals. But it is humans that we should be concerned about, not the laboratory rats.

E. "We do not know a mechanism."

We do not know the mechanism of cancer in general, although we know a lot about cancer. It came as a major surprise to most scientists when Lichtenstein et al., (2000) reported that genetic factors play a minor role in causing most types of cancer, since it was commonly assumed that genetics was the major cause. However Lichtenstein et al. concluded from their study of identical twins that environmental factors were the initiating event in the great majority of cancers. This does not, of course, mean that genetic susceptibility to environmental contaminants is unimportant, but only that genetic factors alone do not result in cancer. We know mechanisms of action for some carcinogenic substances, but for most cancers we know neither the environmental trigger nor the mechanism of action. So there is no reason to negate the evidence that EMFs cause cancer just because we do not know a single mechanism to explain it's mode of action.

We do not know the mechanism or cause for development of Alzheimer's Disease or ALS. We do know that both are more common in individuals in certain occupations, and that exposure to certain metals appears to be associated with increased risk (Kamel et al., 2002; Shcherbatykh and Carpenter, 2007). In the case of Alzheimer's Disease there are abnormalities of amyloid β and tau protein (Goedert and Spillantini, 2006), but very limited understanding of why or how they form. Neither the association with metals nor the presence of abnormal proteins constitutes a mechanism for cause of disease. So rather than discounting the relationship between EMF exposure and neurodegenerative diseases we should be using this information as a tool to better understand the etiology of these diseases.

There is clear evidence from animal and cell culture studies that ELF and RFR have biological effects. Furthermore, these effects occur at intensities commonly experienced by humans. We know a number of ways in which EMFs alter cell physiology and function, as detailed in various chapters in this report. EMFs affect gene transcription (Chapter 5 and 6), cause the synthesis of stress proteins (Chapter 7) and cause breakage of DNA, probably through generation of reactive oxygen species (Chapter 6 and 9 - Lai and Singh, 2004).   Any one of these actions might be responsible for the carcinogenic and neurodegenerative actions of EMFs. However, as with many environmental agents, it would be a mistake to assume that there is only one target or mechanism of action. It is unlikely, for example, that the effects on the nervous system and behavior are secondary to exactly the same cellular targets and actions that lead to cancer. It is likely that there are multiple mechanisms of action leading to disease. But the lack of complete understanding of basic mechanisms does not alter the importance of the relationships.

F. Vested Interests: How They Shape the Public Health Debate

There is no question but that global implementation of the safety standards proposed in this report has the potential to not only be very expensive but also could be disruptive of life and economy as we know it if implemented abruptly and without careful planning. Action must be a balance of risk to cost to benefit. However, "deny and deploy" strategies by industry should not be rewarded in future risk assessment calculations.   For example, if significant economic investments in the roll-out of risky technologies persist beyond the time that there is reasonable suspicion of risk available to all who look, then such costs should not be borne by ratepayers (in the case of new powerlines) or by compensating industry for bad corporate choices. Such investments in the deployment of new sources of exposure for ELF and RF should not count toward the balance sheet when regulatory agencies perform risk assessments. Mistakes may be made, but industry should make mid-course corrections to inform and protect the public, rather than deny effects pending "proof". Whether the costs of remedial action are worth the societal benefits is a formula that should reward precautionary behavior. Prudent corporate policies should be expected to address and avoid future risks and liabilities. Otherwise, there is no market incentive to produce safe (and safer) products.

The deployment of new technologies is running ahead of any reasonable estimation of possible health impacts and estimates of probabilities, let alone a solid assessment of risk. However what has been missing with regard to EMF has been an acknowledgement of the risk that is demonstrated by the scientific studies. As discussed in earlier sections, in this case there is clear evidence of risk, although the magnitude of the risk is uncertain, and the magnitude of doing

nothing on the health effects cost to society is similarly uncertain. This situation is very similar to our history of dealing with the hazards of smoking decades ago, where the power of the industry to influence governments and even conflicts of interest within the public health community delayed action for more than a generation, with consequent loss of life and enormous extra health care costs to society.

Just because a problem is difficult to solve is not a reason to deny that a problem exists. In fact solutions to difficult issues usually can't be expected until the issues are known and creative thinking is brought to bear to find a solution.

The most contentious issue regarding public and occupational exposures to ELF and RF involves the resolute adherence to existing ICNIRP and IEEE standards by many countries, in the face of growing scientific evidence of health risks at far lower levels. Furthermore there is widespread belief that governments are ignoring this evidence. There are two obvious factors that work against governments taking action to set exposure guidelines based on current scientific evidence of risk. These are: 1) contemporary societies are very dependent upon electricity usage and RF communications, and anything that restricts current and future usage potentially has serious economic consequences and 2) the electric power and communications industries have enormous political clout and even provide support for a significant fraction of what research is done on EMF. This results in legislation that protects the status quo and scientific publications whose conclusions are not always based on only the observations of the research. It hinders wise public health policy actions and implementation of prevention strategies because of the huge financial investments already made in these technologies.

In 1989, in an editorial for Science Magazine, Philip H. Abelson called for more research into low-frequency electromagnetic fields. At that time, he confirmed that a US Office of Technology Assessment (OTA) study had determined that *"(o)verall, the evidence is too weak to allow firm conclusions either way"* but a policy of prudent avoidance strategy was suggested, Abelson defined this as "*to systematically look for strategies which can keep people out of 60 Hz fields*". Both policy actions were developed in the midst of scientific uncertainty, but rising concern for possible health impacts to the public. At that time, with high level of unknowns, the appropriate level of policy action was prudent avoidance or precautionary action. Nearly two decades later, the level of action warranted is higher – based on many new scientific publications confirming risks may exist – and justifying prevention or preventative action.

III. EMF EXPOSURE AND PRUDENT PUBLIC HEALTH PLANNING

• *The scientific evidence is sufficient to warrant regulatory action for ELF; and it is substantial enough to warrant preventative actions for RF.*

• *The standard of evidence for judging the emerging scientific evidence necessary to take action should be proportionate to the impacts on health and well-being*

• *The exposures are widespread.*

• *Widely accepted standards for judging the science are used in this assessment.*

Public exposure to electromagnetic radiation (power-line frequencies, radiofrequency and microwave) is growing exponentially worldwide. There is a rapid increase in electrification in developing countries, even in rural areas. Most members of society now have and use cordless phones, cellular phones, and pagers. In addition, most populations are also exposed to antennas in communities designed to transmit wireless RF signals. Some developing countries have even given up running land lines because of expense and the easy access to cell phones. Long-term and cumulative exposure to such massively increased RF has no precedent in human history. Furthermore, the most pronounced change is for children, who now routinely spend hours each day on the cell phone. Everyone is exposed to a greater or lesser extent. No one can avoid exposure, since even if they live on a mountain-top without electricity there will likely be exposure to communication-frequency RF exposure. Vulnerable populations (pregnant women, very young children, elderly persons, the poor) are exposed to the same degree as the general population. Therefore it is imperative to consider ways in which to evaluate risk and reduce exposure. Good public health policy requires preventative action proportionate to the potential risk of harm and the public health consequence of taking no action.

IV. RECOMMENDED ACTIONS

A. Defining new exposure standards for ELF

This chapter concludes that new ELF limits are warranted based on a public health analysis of the overall existing scientific evidence. The public health view is that new ELF limits are needed now. They should reflect environmental levels of ELF that have been demonstrated to increase risk for childhood leukemia, and possibly other cancers and neurological diseases. ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor. It is no longer acceptable to build new power lines and electrical facilities that place people in ELF environments that have been determined to be risky. These levels are in the 2 to 4 milligauss* (mG) range (0.2 – 0.4 µT), not in the 10s of mG or 100s of mG. The existing ICNIRP limit is 1000 mG (100 µT) and 904 mG (90.4 µT) in the US for ELF is outdated and based on faulty assumptions.   These limits are can no longer be said to be protective of public health and they should be replaced. A safety buffer or safety factor should also be applied to a new, biologically-based ELF limit, and the conventional approach is to add a safety factor lower than the risk level.

While new ELF limits are being developed and implemented, a reasonable approach would be a 1 mG (0.1 µT) planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG (0.2 µT) limit for all other new construction. It is also recommended for that a 1 mG (0.1 µT) limit be established for existing habitable space for children and/or women who are pregnant (because of the possible link between childhood leukemia and *in utero* exposure to ELF). This recommendation is based on the assumption that a higher burden of protection is required for children who cannot protect themselves, and who are at risk for childhood leukemia at rates that are traditionally high enough to trigger regulatory action. This situation in particular warrants extending the 1 mG (0.1 µT) limit to existing occupied space. "Establish" in this case probably means formal public advisories from relevant health agencies. While it is not realistic to reconstruct all existing electrical distribution systems, in the short term; steps to reduce exposure from these existing systems need to be initiated, especially in places where children spend time, and should be encouraged. These limits should reflect the exposures that are commonly

associated with increased risk of child hood leukemia (in the 2 to 5 mG (0.2 to 0.5 µT) range for all children, and over 1.4 mG (0.14 µT) for children age 6 and younger). Nearly all of the occupational studies for adult cancers and neurological diseases report their highest exposure category is 4 mG (0.4 µT) and above, so that new ELF limits should target the exposure ranges of interest, and not necessarily higher ranges.

Avoiding chronic ELF exposure in schools, homes and the workplace above levels associated with increased risk of disease will also avoid most of the possible bioactive parameters of ELF discussed in the relevant literature.

It is not prudent public health policy to wait any longer to adopt new public safety limits for ELF. These limits should reflect the exposures that are commonly associated with increased risk of childhood leukemia (in the 2 to 5 mG (0.2-0.5 µT) range for all children, and over 1.4 mG (0.14 µT) for children age 6 and younger). Avoiding chronic ELF exposure in schools, homes and the workplace above levels associated with increased risk of disease will also avoid most of the possible bioactive parameters of ELF discussed in the relevant literature.

B. Defining preventative actions for reduction in RF exposures

Given the scientific evidence at hand, the rapid deployment of new wireless technologies that chronically expose people to pulsed RF at levels reported to cause bioeffects, whicih in turn, could reasonably be presumed to lead to serious health impacts, is a public health concern. A public health action level that implements preventative action now is warranted, based on the collective evidence. There is suggestive to strongly suggestive evidence that RF exposures may cause changes in cell membrane function, cell communication, metabolism, activation of proto-oncogenes and can trigger the production of stress proteins at exposure levels below current regulatory limits. Resulting effects can include DNA breaks and chromosome aberrations, cell death including death of brain neurons, increased free radical production, activation of the endogenous opioid system, cell stress and premature aging, changes in brain function

including memory loss, retarded learning, performance impairment in children, headaches and fatigue, sleep disorders, neurodegenerative conditions, reduction in melatonin secretion and cancers (Chapters 5, 6, 7, 8, 9, 10, and 12).

As early as 2000, some experts in bioelectromagnetics promoted a 0.1 µW/cm2 limit (which is 0.614 Volts per meter) for ambient outdoor exposure to pulsed RF, so generally in cities, the public would have adequate protection against involuntary exposure to pulsed radiofrequency (e.g., from cell towers, and other wireless technologies). The Salzburg Resolution of 2000 set a target of 0.1 µW/cm2 (or 0.614 V/m) for public exposure to pulsed radiofrequency. Since then, there are many credible anecdotal reports of unwellness and illness in the vicinity of wireless transmitters (wireless voice and data communication antennas) at lower levels. Effects include sleep disruption, impairment of memory and concentration, fatigue, headache, skin disorders, visual symptoms (floaters), nausea, loss of appetite, tinnitus, and cardiac problems (racing heartbeat), There are some credible articles from researchers reporting that cell tower -level RF exposures (estimated to be between 0.01 and 0.5 µW/cm2) produce ill-effects in populations living up to several hundred meters from wireless antenna sites,

This information now argues for thresholds or guidelines that are substantially below current FCC and ICNIPR standards for whole body exposure. Uncertainty about how low such standards might have to go to be prudent from a public health standpoint should not prevent reasonable efforts to respond to the information at hand.   No lower limit for bioeffects and adverse health effects from RF has been established, so the possible health risks of wireless WLAN and WI-FI systems, for example, will require further research and no assertion of safety at any level of wireless exposure (chronic exposure) can be made at this time. The lower limit for reported human health effects has dropped 100-fold below the safety standard (for mobile phones and PDAs); 1000- to 10,000-fold for other wireless (cell towers at distance; WI-FI and WLAN devices). The entire basis for safety standards is called into question, and it is not unreasonable to question the safety of RF at any level.

A cautionary target level for pulsed RF exposures for ambient wireless that could be applied to RF sources from cell tower antennas, WI-FI, WI-MAX and other similar sources is proposed. The recommended cautionary target level is 0.1 microwatts per centimeter squared (µW/cm2)** (or 0.614 Volts per meter or V/m)** for pulsed RF where these exposures affect the general public; this advisory is proportionate to the evidence and in accord with prudent public health policy. A precautionary limit of 0.1 µW/cm2 should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers and PDAs and other sources of radiofrequency radiation. An outdoor precautionary limit of 0.1 µW/cm2 would mean an even lower exposure level inside buildings, perhaps as low as 0.01 µW/cm2. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however, for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of WI-FI technologies, we also recommend that wired alternatives to WI-FI be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts. This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future.

Broadcast facilities that chronically expose nearby residents to elevated RF levels from AM, FM and television antenna transmission are also of public health concern given the potential for very high RF exposures near these facilities (antenna farms). RF levels can be in the 10s to several 100's of µW/cm2 in residential areas within half a mile of some broadcast sites (for example, Lookout Mountain, Colorado and Awbrey Butte, Bend, Oregon). Like wireless communication facilities, RF emissions from broadcast facilities that are located in, or expose residential populations and schools to elevated levels of RF will very likely need to be re-evaluated for safety.

For emissions from wireless devices (cell phones, personal digital assistant or PDA devices, etc) there is enough evidence for increased risk of brain tumors and acoustic neuromas now to warrant intervention with respect to their use. Redesign of cell phones and PDAs could prevent direct

head and eye exposure, for example, by designing new units so that they work only with a wired headset or on speakerphone mode.

These effects can reasonably be presumed to result in adverse health effects and disease with chronic and uncontrolled exposures, and children may be particularly vulnerable. The young are also largely unable to remove themselves from such environments. Second-hand radiation, like second-hand smoke is an issue of public health concern based on the evidence at hand.

V.   CONCLUSIONS

• We cannot afford 'business as usual" any longer.   It is time that planning for new power lines and for new homes, schools and other habitable spaces around them is done with routine provision for low-ELF environments . The business-as-usual deployment of new wireless technologies is likely to be risky and harder to change if society does not make some educated decisions about limits soon. Research must continue to define what levels of RF related to new wireless technologies are acceptable; but more research should not prevent or delay substantive changes today that might save money, lives and societal disruption tomorrow.

• New regulatory limits for ELF based on biologically relevant levels of ELF are warranted. ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor. It is no longer acceptable to build new power lines and electrical facilities that place people in ELF environments that have been determined to be risky (at levels generally at 2 mG (0.2 µT) and above).

• While new ELF limits are being developed and implemented, a reasonable approach would be a 1 mG (0.1 µT) planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG (0.2 µT) limit for all other new construction,   It is also recommended for that a 1 mG (0.1 µT) limit be established for existing habitable space for children and/or women who are pregnant . This recommendation is

based on the assumption that a higher burden of protection is required for children who cannot protect themselves, and who are at risk for childhood leukemia at rates that are traditionally high enough to trigger regulatory action. This situation in particular warrants extending the 1 mG (0.1 µT) limit to existing occupied space. "Establish" in this case probably means formal public advisories from relevant health agencies.

• While it is not realistic to reconstruct all existing electrical distributions systems, in the short term; steps to reduce exposure from these existing systems need to be initiated, especially in places where children spend time, and should be encouraged.

• A precautionary limit of 0.1 (µW/cm2 (which is also 0.614 Volts per meter) should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers and PDAs and other sources of radiofrequency radiation. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however, for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of WI-FI technologies, we also recommend that wired alternatives to WI-FI be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts. This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future.

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SECTION 19

GLOSSARY OF TERMS AND ABBREVIATIONS

Absorption. In radio wave propagation, attenuation of a radio wave due to dissipation of its energy, i.e., conversion of its energy into another form, such as heat.

Athermal effect. Any effect of electromagnetic energy on a body that is not a heat-related effect.

Blood–brain barrier. A functional concept developed to explain why many substances that are transported by blood readily enter other tissues but do not enter the brain; the "barrier" functions as if it were a continuous membrane lining the vasculature of the brain. These brain capillary endothelial cells form a nearly continuous barrier to entry of substances into the brain from the vasculature.

Conductance. The reciprocal of resistance. Expressed in siemens (S).

Conductivity: A property of materials that determines the magnitude of the electric current density when an electric field is impressed on the material.

Continuous wave. A wave whose successive oscillations are identical under steady-state conditions.

Current density. A vector of which the integral over a given surface is equal to the current flowing through the surface; the mean density in a linear conductor is equal to the current divided by the cross-sectional area of the conductor. Expressed in ampere per square metre (A m^{-2}).

Depth of penetration. For a plane wave electromagnetic field (EMF), incident on the boundary of a good conductor, depth of penetration of the wave is the depth at which the field strength of the wave has been reduced to 1/e, or to approximately 37% of its original value.

Dielectric properties: In the context of this document the properties of materials conductivity and permeability.

Dosimetry. Measurement, or determination by calculation, of internal electric field strength or induced current density, of the specific energy absorption, or specific energy absorption rate distribution, in humans or animals exposed to electromagnetic fields.

Electric field strength. The force (**E**) on a stationary unit positive charge at a point in an electric field; measured in volt per metre (V m^{-1}).

Electrosensitivity (Electrohypersensitivity): A working definition of EHS from Bergqvist et al. (1997) is "a phenomenon where individuals experience adverse health effects while using or being in the vicinity of devices emanating electric, magnetic or electromagnetic fields (EMFs)".

Electromagnetic energy. The energy stored in an electromagnetic field. Expressed in joule (J).

Electric field strength (E): The magnitude of a field vector at a point that represents the force (F) on a charge (q). E is defined as $E = F/q$ and is expressed in units of Volt per meter (V/m).

Electromagnetic field: Electromagnetic phenomena expressed in vector functions of space and time.

Electromagnetic radiation: The propagation of energy in the form of electromagnetic waves through space.

EMF. Electric, magnetic, and electromagnetic fields.

Exposure: Exposure occurs wherever a person is subjected to electric, magnetic or electromagnetic fields or contact currents other than those originating from physiological processes in the body.

Extra low frequency (ELF): Extra low frequency fields include, in this document, electromagnetic fields from 1 to 300 Hz. Alternately, **ELF-** Extremely low frequency where the European convention is extremely low frequncy, US is extra-low frequency.

Frequency modulation (FM): Frequency Modulation is a type of modulation representing information as variations in the frequency of a carrier wave. FM is often used at VHF frequencies (30 to 300 MHz) for broadcasting music and speech.

Far field. The region where the distance from a radiating antenna exceeds the wavelength of the radiated EMF; in the far-field, field components (**E** and **H**) and the direction of propagation are mutually perpendicular, and the shape of the field pattern is independent of the distance from the source at which it is taken.

Frequency. The number of sinusoidal cycles completed by electromagnetic waves in 1 second; usually expressed in hertz (Hz).

Impedance, wave. The ratio of the complex number (vector) representing the transverse electric field at a point to that representing the transverse magnetic field at that point. Expressed in ohm (S).

Magnetic flux density (B): The magnitude of a field vector at a point that results in a

force (F) on a charge (q) moving with the velocity (v). The force F is defined by F = q*(v x B) and is expressed in units of Tesla (T).

Magnetic field strength (H): The magnitude of a field vector that is equal to the magnetic flux density (B) divided by the permeability (μ) of the medium. H is defined as H = B/μ and is expressed in units of Ampere per metre (A/m).

Magnetic permeability. The scalar or vector quantity which, when multiplied by the magnetic field strength, yields magnetic flux density; expressed in henry per metre (H m$^{!1}$). *Note:* For isotropic media, magnetic permeability is a scalar; for anisotropic media, it is a tensor quantity.

Microwaves: Microwaves are defined in the frame of this expertise as electromagnetic waves with wavelengths of approximately 30 cm (1 GHz) to 1 mm (300 GHz).

Milligauss (mG)

A milligauss is a measure of ELF intensity and is abbreviated mG. This is used to describe electromagnetic fields from appliances, power lines, interior electrical wiring.

Milliwatt (mW): A unit of power equal to 10^{-3}.

Microwatt (uW): A unit of power equal to 10^{-6}

Microwatts per centimeter squared (µW/cm2)

Radiofrequency radiation in terms of power density is measured in microwatts per centimeter squared and abbreviated (µW/cm2). It is used when talking about emissions from wireless facilities, and when describing ambient RF in the environment. The amount of allowable RF near a cell tower is 1000 µW/cm2 for some cell phone frequencies, for example.

Nanowatt (nW): A unit of power equal to 10^{-9} Watt.

Non – thermal effects (or athermal effects): An effect which can only be explained in terms of mechanisms other than increased molecular motion (i.e. heating), or occurs at absorbed power levels so low, that a thermal mechanism seems unlikely, or displays so unexpected a dependence upon some experimental variable that it is difficult to see how heating could be the cause.

Near field. The region where the distance from a radiating antenna is less than the wavelength of the radiated EMF. *Note:* The magnetic field strength (multiplied by the impedance of space) and the electric field strength are unequal and, at distances less than one-tenth of a wavelength from an antenna, vary inversely as the square or cube of the distance if the antenna is small compared with this distance. Near field exposures are unreliable for estimation of exposures by calculation. The can zero out or be additive and

nearly infinite, thus creating problems for exposure assessment.

Non-ionizing electromagnetic radiation (NIER). Includes all radiations and fields of the electromagnetic spectrum that do not normally have sufficient energy to produce ionization in matter; characterized by energy per photon less than about 12 eV, wavelengths greater than 100 nm, and frequencies lower than 3×10^{15} Hz.

Occupational exposure. All exposure to EMF experienced by individuals in the course of performing their work. Safety limits are five times higher for allowable occupational exposures than for general public exposures in the US.

Permeability (μ): A property of materials that indicates how much polarisation occurs when an electric field is applied.

Permittivity. A constant defining the influence of an isotropic medium on the forces of attraction or repulsion between electrified bodies, and expressed in farad per metre (F m^{-1}); *relative permittivity* is the permittivity of a material or medium divided by the permittivity of vacuum.

Public Exposure. All exposure to EMF experienced by the general public excluding exposure during medical procedures and occupational work environments. Public exposure limits in the US are five times lower than for occupational exposures, where informed consent by employees is required.

Power Density. The power as measured in free space (ambient) as opposed to measured by SAR or specific absorption rate (within tissues or the body). The unit of measurement can be watts per square meter, milliwatts per square meter or microwatts per centimeter squared. Radiofrequency (RF). Any frequency at which electromagnetic radiation is useful for telecommunications, or broadcasting for radio and television. Frequency range is usually defined as 300 Hz (300 hertz) to 300 GHz (300 gigahertz).

Radiofrequency (RF): The frequencies between 100 kHz and 300 GHz of the electromagnetic spectrum.

Reasonance. The change in amplitude occurring as the frequency of the wave approaches or coincides with a natural frequency of the medium; whole body absorption of electromagnetic waves presents its highest value, i.e., the reasonance. for frequencies (in MHz or megahertz) corresponding to approximately 114/L where L is the height of the individual in meters. Reasonance can also be applicable to organs, tissues, or other body parts.

Specific Absorption Rate (SAR is measured in watts per kilogram or W/Kg)

SAR stands for specific absorption rate. It is a calculation of how much RF energy is absorbed into the body, for example when a cell phone or cordless phone is pressed to the head. SAR is expressed in watts per kilogram of tissue (W/Kg). The amount of allowable energy into 1 gram of brain tissue from a cell phone is 1.6 W/Kg in the US. For whole body exposure, the exposure is 0.8 W/Kg averaged over 30 minutes for the general public. International standards in most countries are similar, but not exactly the same.

Static electric field: Static fields produced by fixed potential differences.

Static magnetic fields: Static fields established by permanent magnets and by steady currents.

VDU: Video display units for computers, videos, TV and some measurement devices using cathode ray tubes

WI-FI: Stands for wireless fidelity. WI-FI systems create zones of wireless RF that allow access to wireless internet for computers, internet phone access and other wireless services. Access points that provide WI-FI to access Land Area Networks (LANs) can be installed on streets (for city-wide coverage) or indoors in buildings, Restaurants, hotels, coffee shops, airports, malls and other commercial enterprises are widely installing WI-FI. The range of typical WI-FI systems is about 300 feet.

WI-MAX: Stands for "Wireless interoperability for Microwave Access" and is a telecommunications technology aimed at providing wireless data over long distances. Like WI-FI, WI-MAX systems are designed to provide wireless access but over much broader geographic areas, with some systems transmitting signal up to 10 miles. Higher levels of RF are produced at the wireless transmission facilities than for WI-FI.s

Section 20   LIST OF ABBREVIATIONS

µT	microtesla
µW	microwatt
AC	Alternating current
ALS	Amyotrophic Lateral Sclerosis
AM	Amplitude modulation
B	Magnetic flux density
BBB	Blood-Brain-Barrier
CENELEC	European Committee for Electrotechnical Standardization
CI	Confidence Interval
CNS	Central Nervous System
CW	Continuous wave
DC	Direct current

DECT	Digital Enhanced Cordless Telephone
DMBA	7,12-dimethylbenz[a]anthracene
DNA	Deoxyribonucleic acid
EEG	Electroencephalogram
EHS	Electromagnetic hypersensitivity
ELF	Extra low frequency (also ELF-EMF)
EMF	Electromagnetic field
FM	Frequency Modulation
GSM	Global System for Mobile Communication
H	Magnetic field strength
HSP	Heat-shock proteins (stress proteins)
Hz	Frequency in Hertz
IARC	International Agency for Research on Cancer
IL	Interleukin
kg	Kilogram
kHz	Kilohertz
kV	Kilovolt
MF	Magnetic Field (sometimes MF-ELF)
MHz	Megahertz
ms	Milliseconds
mT	Millitesla
mG	Milligauss
mW	Milliwatt
nT	Nanotesla
nW	Nanowatt
NRPB	National Radiation Protection Board (HPA)
OR	Odds Ratio (measure of increased risk of disease)
REFLEX	European Research Program for Radiofrequency Hazards
RF	Radiofrequency Radiation (also written as RFR or RF-EMF)
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks

TNO   Nederlandse Onderzoek (Netherlands Organisation Applied Scientific Research
UMTS   Universal Mobile Telephony System **UNEP** United Nations Environmental
VDT   Video display terminal (VDU – for computers, videos, TV, that use cathode
ray tubes).

Wi-Fi   Short for wireless fidelity – wireless internet access - works for short-
distances for cell phone and laptop computer access without wires.

WLAN   Wireless Local Area Network (wireless internet coverage usually up to 300’

provided by access points that create elevated radiofrequency radiation for that service zone.

WiMAX Worldwide Interoperability for Microwave Access (wireless service up to 10 miles in comparison to Wi-Fi that may serve 300' area)

WHO World Health Organisation

FCC The Federal Communications Commission (FCC) is an independent United States government agency, created, directed, and empowered by Congressional statute to oversee the regulation of radio and TV broadcasting and wireless technologies. It is not a health agency.

HPA Health Protection Agency (UK) that was formerly the National Radiation Protection Division Board). The Health Protection Agency (HPA) is an independent body that protects the health and well-being of the population. The Agency plays a critical role in protecting people from infectious diseases and in preventing harm when hazards involving chemicals, poisons or radiation occur.

DNA Deoxyribonucleic acid, or DNA is a nucleic acid molecule that contains the genetic instructions used in the development and functioning of all living things.

Melatonin Melatonin is a hormone produced in the brain by the pineal gland, It is a potent anti-oxidant that protects against oxidative damage from free radicals that can cause DNA damage.

Alzheimer's Alzheimer's disease is a progressive brain disorder that gradually destroys a person's memory and ability to learn, reason, make judgments, communicate and carry out daily activities. As Alzheimer's progresses, individuals may also experience changes in personality and behavior, such as anxiety, suspiciousness or agitation, as well as delusions or hallucinations.

RFIAWG Radiofrequency Interagency Working Group (US) composed of members from federal agencies with some interest in radiofrequency radiation issues. This Working Group was made up of representatives from the US government's National Institute for Occupational Safety and Health (NIOSH), the Federal Communications Commission (FCC), Occupational Health and Safety Administration (OSHA), the Environmental Protection Agency (US EPA), the National Telecommunication and Information Administration, and the US Food and Drug Administration (FDA).

ICNIRP International Commission on Non-Ionizing Radiation. It is a body of independent scientific experts consisting of a main Commission of 14 members, 4 Scientific Standing Committees covering Epidemiology, Biology, Dosimetry and Optical Radiation and a number of consulting

experts. This expertise is brought to bear on addressing the important issues of possible adverse effects on human health of exposure to non-ionising radiation.

SECTION 20   **What are Ambient ELF and RF Levels?**

Average Residential Exposures to ELF (Power Frequency Fields)

A nation-wide survey in the United States by Zaffanella et al (1993) collected
engineering data on sources and levels of 60 Hz electric power magnetic fields that exist
inside residences in the United States.

Approximately 1000 residences were randomly selected for the survey. The goals were
to 1) identify all significant sources of magnetic field, 2) estimate for each source the
percentage of residences where magnetic fields exceeded specified levels, 3) to determine
the relation between magnetic field and sources and 4) to characterize the field varations
in time.

The median field was identified as 0.5 mG and the average field was 0.9 mG. Thus, this
confirms that average residential magnetic fields based on the 1000-home study is less
than 1 mG.

Appliances produce magnetic fields but these diminish rapidly with distance (at $1/R^3$),

Power lines generally produce the largest average residential magnetic field when the
entire living space of a residence and a 24-hour period are considered. Power line
magnetic field exceeds 1 mG in 17%, exceed 2.5 mG in 9.5% and exceed 5 mG in 0.3%
of all the residences surveyed.

Zaffanella (1998) conducted measurements to characterize typical EMF exposure levels
in persons living in the United States - a study called the 1000-Person Study. Table A-
S.2 shows that about half of all people in the US have EMF exposures at home under
0.75 mG; in bed are 0.48 mg; at school 0.60 mG; at work 0.99 mG; and 0.87 mG is the
median EMF exposure for an average 24-hour day.

Table A-S.2

Table S.2 Descriptive Statistics for Different Activity Periods

Parameter	Home not in Bed	In Bed	Work	School	Travel	24-Hour
Number of Valid Data Sets	1011	996	525	139	765	1012
1st Percentile	0.10 mG	0.01 mG	0.14 mG	0.13 mG	0.13 mG	0.18 mG
5th Percentile	0.20 mG	0.08 mG	0.24 mG	0.18 mG	0.29 mG	0.27 mG
10th Percentile	0.27 mG	0.12 mG	0.30 mG	0.29 mG	0.41 mG	0.35 mG
25th Percentile	0.44 mG	0.24 mG	0.60 mG	0.35 mG	0.66 mG	0.51 mG
50th Percentile	0.75 mG	0.48 mG	0.99 mG	0.60 mG	0.98 mG	0.87 mG
75th Percentile	1.39 mG	1.24 mG	1.78 mG	1.01 mG	1.46 mG	1.41 mG
90th Percentile	2.49 mG	2.44 mG	3.32 mG	1.64 mG	2.18 mG	2.38 mG
95th Percentile	3.89 mG	3.63 mG	5.00 mG	1.77 mG	2.73 mG	3.38 mG
99th Percentile	9.50 mG	9.19 mG	13.5 mG	3.55 mG	5.43 mG	6.16 mG
Mean	1.29 mG	1.11 mG	1.73 mG	0.82 mG	1.22 mG	1.25 mG
Standard Deviation	2.54 mG	2.06 mG	3.09 mG	0.70 mG	0.99 mG	1.51 mG
Geometric Mean	0.80 mG	0.52 mG	1.03 mG	0.64 mG	0.96 mG	0.89 mG
Geometric Standard Deviation	2.50	3.52	2.57	2.06	2.03	2.18

In Sweden, Mild et al (1996) report that overall mean residential ELF exposures are 0.4 mG, and in Norway are 0.13 mG.

Average Occupational Exposures to ELF

Average occupational exposures in commercial office buildings are 1-2 mG or less and have been reported fairly consistently across numerous studies of exposure assessment (Table 1). Powerline and electrical workers have higher average occupational exposures from 10 mG to 16.6 mG.

Table A-2: Average Occupational Exposures to ELF

EMF RAPID Program – Questions and Answers, NIEHS,
June 2002

Office buildings (median)	0.6 mG
Support staff	0.5 mG
Professional staff	0.6 mG
Maintenance staff	0.6 mG
Visitors	0.6 mG

EMF RAPID Program Engineering Project #3 Executive
Summary, May 1996

Office building (average)	0.7 mG
Office building (median)	0.4 mG

Electric and Magnetic Field Fundamentals (EPRI Resource Paper, March 1994)

Typical magnetic fields in offices	1 – 2 mG
Power line workers	10 mG

Occupational EMF Exposure Assessment (EPRI Resource Paper, February 1994)

Office Worker Comparison Group	1.6 mG
All Occupationally Exposed Utility Workers	16.6 mG

Table 7 – Other Studies Cited

Bracken Study (1990)	1.0 mG
Deadman Study (1988)	1.6 mG
Bowman Study (1992)	0.9 – 1.8 mG

Limits on Operation of Sensitive Electronic Equipment

Companies that manufacture or use equipment in nanotechnology and biotechnology and found 1.0 mG is generally the limit for proper operation of electron beam devices (mass spectrometers, scanning electron microscopes, lithography, etc) used in these technologies. Ten (10) milligauss (mG) is the EMF limit for normal computers – above 10 mG can introduce "computer jitter" and other problems.

What are Ambient Radiofrequency Radiation/Microwave Levels?

Prior to the rapid development of wireless communications for personal and business usage, RF power density levels were primarily related to AM, FM and television broadcasting signal in both urban and rural areas of the United States. Microwave frequencies used for wireless communications were negligible.

Original extra-planetary sources of microwave radiation were infinitesimally small, on the order of a billionth of a microwatt per centimeter squared (10^{-12} uW/cm^2). Human evolution took place without any appreciable exposure to microwave radiation from background sources. The human body has no evolutionary protection against microwave radiation, as it does for ultraviolet radiation from the sun (Johannson, 2000). Wireless voice and communications have introduced unprecedented levels of public exposure in the last decade.

Mantiply (1997) measured and reported common sources and levels of RF in the environment. He identified areas near cellular base stations on the ground near towers to be from 0.003 to 0.3 µW/cm2. Background level ambient RF exposures in cities and suburbs in the 1990's were generally reported to be below 0.003 µW/cm2.

Hamnerius (2000) reported that ambient RF power density measurements in twelve (12) large cities in Sweden were roughly ten times higher than in the United States for equivalent measurement locations by Mantiply in 1978 (when no cellular phone service existed in the US). He reported a total mean value of 26 measured sites in the study was 0.05 µW/cm2 and the median value was 40 µW/cm2. An office location with a base station nearby at about 300 feet distance tested 150 µW/cm2. A train station with antennas mounted indoors tested at about 3 µW/cm2. Both indoor and outdoor ambient RF power density measurements showed high variability depending on proximity to transmitting antennas.

Sage Associates reported on microwave frequency RF power density levels at outdoor locations both near and far from wireless antenna sites in the United States (Sage, 2000). Within the first 100-300 feet, power density levels have been measured at 0.01 to 3.0 µW/cm2. Elevated RF power density levels from a major wireless antenna site can often be detected at 1000 feet or more. Power density levels away from wireless antenna sites measure between 0.001 µW/cm2 to 0.000001 µW/cm2.

Vegetation often reduces signal (and therefore the reach of elevated RF exposures) but dry building materials used to visually screen wireless sites do not appreciably diminish signal transmission. Therefore, many sites that are "out-of-sight" because of stealth design can still produce elevated RF levels in nearby areas where people live, work and go to school. For purposes of this evaluation, a 10 dB attenuation has been incorporated to take building material shielding effects into account.

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