CSC-Docket No. 461

Field Point Estate Townhouses

Interrogatories-Submitted for Eversource Response

1. What are the projected EMF readings at various distances from the proposed substation on 290 Railroad Ave?
2. Please explain why the Siting Council and Greenwich residents should treat the testimony of Dr. Gabor Mezei as unbiased and independent when his employment history and research funding has many connections to the energy industry, e.g., EPRI and Exponent? (See Exhibits 1 & 2 (the latter is provided to show how experts with conflicts of interest can corrupt the outcomes of careful deliberation))
3. Please explain why the Siting Council and Greenwich residents should treat the testimony of Exponent as unbiased and independent when serious concerns of its neutrality exist? This is the same company whose experts stated that *dioxin* is not carcinogenic! (See Exhibits 3 & 4)
4. Please explain why the Siting Council and Greenwich residents should accept that EMF is safe for the community given the following summary findings which say otherwise: 1. the table showing that the NIEHS Working Group, the IARC (WHO committee), the National Radiological Protection Board (UK), and two-thirds of the California DHS committee conclude that the research data shows the existence of a "possible link between EMF and childhood leukemia" (See Exhibit 5) and 2. California DHS's risk assessment of various health effects as summarized by Professor Denis Henshaw at University of Bristol (See Exhibit 6)
5. How should we regard the work of Drs. Henry Lai, Narendra Singh, and Martin Blank showing the link between ELF and DNA damage? (See Exhibits 7 & 8)
6. How should we regard Dr. Henry Lai's literature review of research conducted between 2007-2014 which shows a preponderance of studies showing genetic effects from EMF? (See Exhibit 9)
7. Why is the project in-service date so important to adhere to when the town's population growth rate and energy growth rate is so low and when the date causes more acceptable (from the town's perspective) siting options (like 330 Railroad Ave) to be dismissed?
8. Did ISO-New England consider the summers of 2012 and 2013 to be unusually warm? What quantitative haircut factors did ISO-NE apply to peak energy usage in MW to create a usage history normalized for weather for Greenwich (or comparable region)?

Exhibit 1

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Failure Analysis Associates®

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Gabor Mezei, M.D., Ph.D.
Senior Managing Scientist

Professional Profile

Dr. Gabor Mezei is a Senior Managing Scientist in Exponent's Health Sciences Center for Epidemiology and Computational Biology. Dr. Mezei has over 25 years of experience in health research including epidemiological studies of both clinical outcomes and environmental and occupational health issues. His current work primarily focuses on health research related to electromagnetic fields (EMF) and asbestos exposures, and wearable electronics. He has considerable experience in conducting complex health assessment and exposure characterization studies related to power frequency and radiofrequency EMF. Previously, at the Electric Power Research Institute, he was responsible for leading a multidisciplinary scientific research program aimed at addressing potential human health effects associated with residential and occupational exposure to power frequency and radiofrequency EMF. Dr. Mezei oversaw studies on potential EMF effects on animal health and welfare and directed occupational health and safety research focusing on injury surveillance, ergonomics evaluations of electric utility workers' tasks, and occupational exposure assessments within the electric power industry. Earlier, as a research associate at the Toronto Western Hospital, University of Toronto, he conducted research studies on adverse clinical outcomes and hospital readmissions following ambulatory surgery.

Dr. Mezei trained as a medical doctor (M.D.) at the Semmelweis University of Medicine in Budapest, Hungary, and as an epidemiologist (Ph.D.) at the School of Public Health of the University of California in Los Angeles (UCLA). He was the recipient of Fogarty and Fulbright Fellowships. He served as an affiliate associate professor in the Department of Environmental and Occupational Health Sciences of the University of Washington in Seattle, Washington, and as a visiting scientist at the Hungarian National Research Institute for Radiobiology and Radiohygiene in Budapest, Hungary. Dr. Mezei lectured at Stanford University, the UCLA School of Public Health, and the Electrotechnical Committee of the Hungarian Academy of Sciences. Dr. Mezei appeared as an EMF health expert in hearings at several state (US) and provincial (Canada) public utility commissions and a parliamentary committee in Ireland.

Dr. Mezei is an author or co-author of over 60 scientific publications and book chapters on topics related to epidemiology of environmental and occupational exposures and chronic diseases (such as cancer and neurodegenerative diseases), adverse clinical outcomes, and environmental exposure assessment.

Academic Credentials and Professional Honors

Ph.D., Epidemiology, University of California, Los Angeles, 1995
M.D., Medicine, Semmelweis University of Medicine, Hungary, 1990

Exhibit 2

GM reaches $900 million settlement with Justice Department over defective ignition switches

THE WALL STREET JOURNAL.

http://www.wsj.com/articles/SB116831654647871083

LEADER (U.S.) | COURT OF OPINION

Amid Suits Over Mold, Experts Wear Two Hats

Authors of Science Paper Often Cited by Defense Also Help in Litigation

By **DAVID ARMSTRONG**
Updated Jan. 9, 2007 11:59 p.m. ET

(See Corrections & Amplifications item below.)

Soon after moving into a New York City apartment, Colin and Pamela Fraser say, they began to suffer headaches, rashes, respiratory infections and fatigue. They attributed it to mold.

But their lawsuit against the cooperative that owns the building hit a roadblock when the court wouldn't let their medical expert testify that mold caused their problems. This is "unsupported by the scientific literature," the state trial judge said.

She relied in part on a position paper from the American College of Occupational and Environmental Medicine, or ACOEM. Citing a substance some molds produce called mycotoxins, the paper said "scientific evidence does not support the proposition that human health has been adversely affected by inhaled

mycotoxins in the home, school, or office environment."

Two Views of Mold

Passages from papers by two professional societies:

American College of Occupational and Environmental Medicine
"Scientific evidence does not support the proposition that human health has been adversely affected by inhaled mycotoxins [from mold] in the home, school, or office environment."

Institute of Medicine
"Studies have demonstrated adverse effects—including immunotoxic, neurologic, respiratory and dermal responses—after exposure to specific toxins, bacteria, molds or their products."

The paper has become a key defense tool wielded by builders, landlords and insurers in litigation. It has also been used to assuage fears of parents following discovery of mold in schools. One point that rarely emerges in these cases: The paper was written by people who regularly are paid experts for the defense side in mold litigation.

The ACOEM doesn't disclose this, nor did its paper. The professional society's president, Tee Guidotti, says no disclosure is needed because the paper represents the consensus of its membership and is a statement from the society, not the individual authors.

The dual roles show how conflicts of interest can color debate on emerging health issues and influence litigation related to it. Mold has been a contentious matter since a Texas jury in 2001 awarded $32.1 million to a family whose home was mold-infested. That award, later reduced, and a couple of mold suits filed by famous people like Ed McMahon and Erin Brockovich helped trigger a surge in mold litigation. Insurers and builders worried it would become a liability disaster for them on the scale of asbestos.

The number of suits hasn't been as big as anticipated. One reason appears to be the insurers' success in getting many states to exclude mold coverage from homeowner's-insurance policies. But also helping turn the tide, lawyers and doctors say, is the ACOEM report. Building groups and the U.S. Chamber of Commerce have cited it to rebut the notion that mold in the home can be toxic.

James Craner, a Nevada doctor who has testified for scores of people who claimed ill effects from mold, says the paper "has been used in every single mold case. The lawyer asks, 'Isn't it true the American College of Occupational and Environmental Medicine concluded that there is no scientific evidence that mold causes any serious health effects?'"

The result, Dr. Craner maintains, is that "a lot people with legitimate environmental health problems are losing their homes and their jobs because of

legal decisions based on this so-called 'evidence-based' statement."

Dr. Craner says a majority of his work is on the plaintiff side and he is paid when he testifies, but he says he currently is an expert for the defense in a case where he concluded the plaintiffs' health issues weren't related to mold.

Two other medical societies have also published statements on mold written, in part, by legal-defense experts. The societies didn't disclose this when they released the papers, although one later published a correction saying two authors served as expert witnesses in mold litigation.

READ MORE

- Read the full text (http://online.wsj.com/public/resources/documents/20070108-Mold.pdf) of Dr. Borak's September 2002 email to the leaders of the American College of Occupational and Environmental Medicine about his struggles in drafting their position paper on mold.
- Read the official position statements of the American College of Occupational and Environmental Medicine (http://www.acoem.org/guidelines.aspx?id=850) and of the American Academy of Allergy, Asthma and Immunology (http://www.aaaai.org/media/resources/academy_statements/position_statements/mold.pdf), as posted on their Web sites.

Mold reproduces through tiny spores. These can float into homes through windows and vent systems or be carried in on clothes or shoes. Indoors, mold grows when moisture is present.

There's debate about how much this matters. Plaintiffs attribute ills ranging from asthma to cognitive problems to inhalation of mold. The Institute of Medicine, a largely federally funded nonprofit, reviewed the research in 2004 and said "studies have demonstrated adverse effects -- including immunotoxic, neurologic, respiratory and dermal responses -- after exposure to specific toxins, bacteria, molds or their products." But it added that the dose required to cause adverse health effects hasn't been determined. The U.S. Centers for Disease Control and Prevention, for its part, says on its Web site that mold can cause wheezing and eye or skin irritation, but a link to more serious conditions "has not been proven."

'Highly Unlikely'

The ACOEM paper goes further. It says not only is there no evidence indoor mold causes serious health effects, but even if mold produced toxic substances, it's "highly unlikely at best" that anyone could inhale enough to cause a problem. The paper reaches this conclusion by extrapolating from animal studies in which rodents' throats were injected with molds.

The paper's authors say their conclusions are validated by the Institute of Medicine's paper. But the author of the Institute paper's mold toxicity chapter, Harriett Ammann, disagrees, and criticizes the ACOEM paper's methodology: "They took hypothetical exposure and hypothetical toxicity and jumped to the conclusion there is nothing there."

Dr. Ammann, a recently retired toxicologist for Washington state's health department, recently helped the plaintiff side in a mold case. She says this was the only time she has done so for pay. In the Fraser lawsuit in New York, after the judge barred testimony that mold caused health problems, Dr. Ammann, on her own and without pay, provided an affidavit filed with the appellate court saying the judge misinterpreted the research.

The ACOEM, a society of more than 5,000 specialists who investigate indoor health hazards and treat patients with related illnesses, first moved to develop a position paper on mold in early 2002. Dean Grove, then the medical society's president, asked the head of its council on scientific affairs, Yale medical professor Jonathan Borak, to set the process in motion.

He turned to a retired deputy director of the National Institute for Occupational Safety and Health -- part of the CDC -- to spearhead the project. Dr. Borak says he wanted someone with "no established background record of litigation related to mold."

For the Defense

The person he chose, Bryan Hardin, says he hadn't worked on any mold lawsuit at that point, though he was a consultant on other matters for GlobalTox Inc., a firm that regularly worked for the defense in mold cases. And Dr. Hardin says he consulted for the defense in a mold case while he was helping write the ACOEM paper.

In a Feb. 27, 2002, email, Dr. Borak told Dr. Hardin: "That position paper would be prepared by you and your GlobalTox colleagues." Dr. Borak says he believes he didn't know at the time that GlobalTox did mold defense work.

A GlobalTox colleague who aided Dr. Hardin was Bruce Kelman, now president of the firm, which recently changed its name to Veritox Inc. Drs. Kelman and Hardin, now principals at the firm and entitled to a share of its profits, were two of the ACOEM paper's three authors. They are paid $375 to $500 an hour for work on mold cases, court records say.

EXPERT WITNESSES

- **The Situation:** Mold defendants rely on medical-society position papers that reject a link to serious ills, but papers were written by scientists who often work for defense side in mold cases.
- **The Debate:** Whether courts get accurate or skewed view of possible health effects of indoor mold.
- **What's at Stake:** Outcome of widespread litigation over mold.

The paper's third author was Andrew Saxon, then chief of clinical immunology and allergy at the medical school of the University of California, Los Angeles. He, too, has served as a defense expert in numerous mold suits. Dr. Saxon says he is paid $510 an hour for his help. If called to testify in court, his rate rises to $720 an hour, according to a deposition he gave.

Until he retired from UCLA in September, money he earned as a legal-defense expert was paid to the university, and he says UCLA then gave him a little less than half of it. Dr. Saxon estimates he generates $250,000 to $500,000 a year from expert defense work, which includes non-mold cases.

The ACOEM knew about mold defense work by the authors of its paper. Dr. Hardin informed the society in a Sept. 23, 2002, document under his letterhead. Labeled "confidential" and "share only with the ACOEM board of directors," it told of his work as a defense expert on one mold case.

The letter said the other two authors, Drs. Saxon and Kelman, "have been retained by both the defense and plaintiff bar in litigation relating to indoor mold." Both say they work mostly for the defense in mold cases.

Internal ACOEM documents indicate that as the paper was being written in August 2002, there was concern within the society that the paper was too friendly to defense interests. Its authors were asked to modify the first draft's tone "because of the concern about possible misinterpretation of 'buzz words' and phrases such as 'belief system,' 'adherents may claim,' 'supposed hypersensitivity,' and 'alleged disorder,'" according to a June 2002 email to Dr. Hardin from the society's communications director. (The email was obtained by a plaintiff's attorney in a mold case, Karen Kahn.)

Dr. Borak, the head of the society's council on scientific affairs, suggested sending a draft for review to one particular mold authority, Michael Hodgson, director of the occupational safety and health program at the U.S. Veterans Health Administration. Dr. Hardin objected. He said it would be "inappropriate to add ad hoc reviewers who are highly visible advocates for a point of view the

draft position paper analyzes and finds lacking." The draft ultimately wasn't sent.

'A Defense Argument'

In September 2002, Dr. Borak emailed colleagues that "I am having quite a challenge in finding an acceptable path for the proposed position paper on mold." He said several reviewers "find the current version, much revised, to still be a defense argument."

The society released a paper two months later, and its authors, as well as ACOEM officials, say it accurately reflects the science on indoor mold exposure. The authors' "views, if prejudicial, were removed," Dr. Borak says. "It went through a dramatic change of top-heavy peer reviews." He says objections come mainly from "activist litigants" who find it "annoying."

Drs. Hardin and Kelman say the paper has been controversial because it challenged "a belief system" that mold can be toxic indoors. "A belief system is built up and there is anger when the science doesn't support that belief system," Dr. Kelman says.

The Manhattan Institute, a conservative think tank, paid Veritox $40,000 to prepare a lay version of the paper. That version said "the notion that 'toxic mold' is an insidious, secret 'killer,' as so many media reports and trial lawyers would claim, is 'junk science' unsupported by actual scientific study." Its authors were the three writers of the longer paper plus a fourth, who also is a principal at Veritox.

Lawyers defending mold suits also cite a position paper from the American Academy of Allergy, Asthma and Immunology. This paper says it concurs with the ACOEM that it is highly unlikely enough mycotoxins could be inhaled to lead to toxic health effects.

Among the academy paper's five authors is Dr. Saxon. Another, Abba Terr, a San Francisco immunologist, has worked as a defense expert in mold cases. The academy published the paper in its Journal of Allergy and Clinical Immunology last February, not citing the mold-defense work of either man. The publication later ran a correction disclosing their litigation work.

The academy's president says officials were aware Dr. Saxon was an expert witness. "We should have published their [disclosure] statements with the

paper," says the official, Thomas Platts-Mills. He says the lapse resulted from a variety of factors, including confusion about whose responsibility the disclosure was.

Unhappy Author

A third author of the academy's paper, Jay Portnoy, chief of allergy, asthma and immunology at the Children's Mercy Hospital in Kansas City, Mo., says he "felt that there was an agenda" -- the effort "seemed very biased toward denying the possibility of there being harmful effects from mold on human health." He says he considered removing his name from the paper, but it was published before he could decide.

Dr. Portnoy says a section he contributed was rewritten by Dr. Saxon to be "a lot more negative." He says the paper wrongly says mold isn't proven to cause allergic rhinitis, with symptoms like wheezing, sore throat and sneezing. Dr. Saxon denies the authors had a bias but says they applied a high standard for proving mold causes a particular effect. He says he didn't skew the content of Dr. Portnoy's section but rewrote it because it was "too diffuse." Dr. Terr in San Francisco didn't return a call seeking comment.

In New York, the Frasers are appealing the refusal of the trial judge, state Supreme Court Justice Shirley Werner Kornreich, to let their expert testify that indoor mold caused their health complaints. The Frasers had moved into the East Side Manhattan apartment in 1996. Their 2002 suit said they repeatedly complained to the co-op's board of dampness and leaks as their health deteriorated.

Their appeal attacks the credibility of mold position papers drafted by scientists who work for defendants. "What you have here is defense experts authoring papers under an official guise," says their attorney, Elizabeth Eilender. Justice Kornreich declined to comment.

Write to David Armstrong at david.armstrong@wsj.com

Corrections & Amplifications:

Harriet Ammann, a toxicologist, says she has been paid as an expert by plaintiffs in three mold cases. This article reports that Dr. Ammann said she had been paid for her work in only one case.

Exhibit 3

Secret Ties to Industry and Conflicting Interests in Cancer Research

Lennart Hardell, MD, PhD,[1,{] Martin J. Walker, MA,[2{] Bo Walhjalt,[3{] Lee S. Friedman, BA, MSc,[4§] and Elihu D. Richter, MD, MPH[5]

Background Recently it was reported that a Swedish professor in environmental health has for decades worked as a consultant for Philip Morris without reporting his employment to his academic employer or declaring conflicts of interest in his research. The potential for distorting the epidemiological assessments of hazard and risk through paid consultants, pretending to be independent, is not exclusive to the tobacco industry.
Methods Documentation is drawn from peer reviewed publications, websites, documents from the Environmental Protection Agency, University reports, Wellcome Library Special Collections and the Washington Post.
Results Some consulting firms employ university researchers for industry work thereby disguising industry links in the income of large departments. If the industry affiliation is concealed by the scientist, biases from conflicting interests in risk assessments cannot be evaluated and dealt with properly. Furthermore, there is reason to suspect that editors and journal staff may suppress publication of scientific results that are adverse to industry owing to internal conflict of interest between editorial integrity and business needs.
Conclusions Examples of these problems from Sweden, UK, and USA are presented. The shortfalls cited in this article illustrate the need for improved transparency, regulations that will help curb abuses as well as instruments for control and enforcement against abuses. Am. J. Ind. Med. 2006.

KEY WORDS: cancer research; conflicts of interest; consulting ethics; industry sponsors

A RECENT DISCLOSURE: RYLANDER AND PHILIP MORRIS

Recently it was revealed that the Swedish professor in environmental health at the Gothenburg University, Dr. Ragnar Rylander, had worked for decades as a contracted consultant for Philip Morris without reporting this outside commission to his employer or declaring conflicts of interest in his research [Diethelm et al., 2005; Editorial, 2006]. His consultancy generated substantial amounts of money both for research and as consultant fees from the tobacco industry. The scientific integrity of his publications has been questioned [Diethelm et al., 2005]. Swedish law requires that public servants, including academic researchers report

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outside commissions, and it is the responsibility of the employer to decide whether the outside commission is acceptable, or if there is an unacceptable conflict of interest. If the commission is considered a case of conflict of interest it should be denied.

For 30 years Rylander kept his commission as a contracted consultant with Philip Morris secret from his employers (the Swedish EPA, the University of Gothenburg and the University of Geneva), while at the same time he discussed all his tobacco related research at the universities with Philip Morris and their lawyers. Industry knew what the universities and the public did not know. His correspondence shed light on this loyalty to Philip Morris [e.g., Rylander, 1987]. When the first systematic description of Rylander's relations with Philip Morris were published in Sweden 2002, he stated: "I have never been a consultant for PM" [Tallmo, 2002]. Two months later in 2002 the contract was made public after being found in the Philip Morris Archives [Philip Morris Incorporated, 1972].

While there is increased scientific and public sensitivity to the scientific validity and public health implications of work funded by the tobacco industry, there is evidence to indicate that other industries such as the chemical industry are still distorting epidemiological research, especially in the field of cancer. Our hypothesis is that the case of Professor Rylander is a seminal event for a far more widespread practice of non-disclosure and concealment of ties to industry, and as well the influence on editorial decisions as to what to publish and not to publish.

EXPONENT, INC., DIOXIN, CANCER, AND ADAMI

In the fall of 2001 a group of Swedish scientists at the Karolinska Institute (KI), Hans-Olov Adami, Anders Ekbom, Magnus Ingelman-Sundberg, Anders Ahlbom, and one researcher in Lund, Lars Hagmar, initiated an attack in a leading Swedish daily newspaper on other researchers who had been reporting on the association between cancer and exposure to various toxic and physical agents [Walhjalt, 2002a,b]. Studies which suggested findings of an association between cellular telephones and brain tumors [Hardell et al., 2001a], dioxin pollutants in mother's milk and the risk for childhood malignancies [Hardell and Dreifaldt, 2001], as well as cancer risks from alcohol [Hardell et al., 2000] and dioxins [Hardell et al., 1995a,b, 2001b; Hardell and Eriksson, 1999]. This work by Hardell et al. was criticized as lacking academic rigor without any regard for research method. Hardell rebutted in a peer-reviewed journal [Hardell, 2004].

Thereafter, one of the authors of the original newspaper article, Professor Hans-Olov Adami, together with Jack Mandel, an epidemiologist working for the U.S. consultancy firm Exponent, Inc., and Dimitrios Trichopoulos, Professor Emeritus of Epidemiology at Harvard, went to the Dioxin 2001 conference in Korea and gave oral presentations. Together they presented the case for the thesis that dioxins are not associated with cancer in humans. The presentations each gave a clean bill of health to dioxin [Adami, 2001; Trichopoulos, 2001; Mandel, 2001a]. Although no new research was presented, statements casting doubt on the carcinogenicity were made as a challenge to the fact that 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) had been classified in 1997 as a human carcinogen of Group I by IARC [IARC, 1997].

Exponent had hired Adami and Trichopoulos and co-ordinated the presentations on behalf of an unnamed client [Mandel, 2001b]. While Mandel appeared as an employee of Exponent, Adami and Trichopoulos only quoted their academic affiliations, which would infer that they were independent researchers rather than consultants hired by Exponent and paid for by some of Exponent's clients. The aim of this re-manufacturing of doubt was the ongoing dioxin review process at the US Environmental Protection Agency.

In another article by Adami et al. [2000] the authors state: "There is persuasive evidence that TCDD at low levels is not carcinogenic to human beings and that it may not be carcinogenic even at high levels." This article was also produced for Exponent. The article, together with another on other endpoints, was delivered to be included in the EPA review, for which the Vice President of Exponent, Dennis Paustenbach, was on the Science Advisory Board. Exponent's activities on dioxin at the time included a number of other consultants from Exponent giving oral and poster presentations which sowed doubt about health effects from dioxins at the Dioxin 2001 conference [Connor and Finley, 2001; Fehling et al., 2001; Hays and Aylward, 2001; Hays et al., 2001; Paustenbach, 2001; Sun et al., 2001].

Paustenbach [2002, 2005] also conducted work during this time for Dow Chemical on dioxin in soils from their production plant in Midland. He recommended a cleanup level nine times above the level stated by Michigan state regulations which would save Dow a lot of money. Dow reached an agreement with the State governor based on Paustenbach's conclusions. The EPA objected to the agreement [EPA, 2002]. Paustenbach was later a member of the panel to set the standards for clean up after Agent Orange negotiated between the US and Vietnam [Memorandum of Understanding, 2002]. Corporate and governmental interests coincided and Exponent was on the market filling the needs.

MONSANTO, ROUNDUP, NON-HODGKIN'S LYMPHOMA, AND ADAMI

Recently litigation on health risks from herbicides in Israel led one of the co-authors of this article to a Monsanto website on Roundup [Monsanto, 2002] which cited Adami.

Via a telephone number on the Monsanto website, we traced "unpublished references" in Monsanto's possession in which Adami and his associate Professor Trichopoulos stated that "errors in exposure assessment, or chance ...are likely explanations for the weak glyphosate/NHL association" [Adami and Trichopoulos, 1999]. This statement, posted on the above web reference, re-emerged, as a word-for word download, without attribution of the source, as a major part of an expert opinion by the Chief Toxicologist of the Israeli Minister of Health to the Israeli Supreme Court. For more details see ED Richter, Expert Opinion, Feb 11 2004; for Physicians for Human Rights vs. Government of Israel [Dallal, 2004].

The Swedish Cancer Society has for a long time funded Adami's appointment as a cancer researcher. Adami's research team has gained substantial amounts in grants from the Swedish Cancer Society over the years. The main source of this money comes from gifts from the Swedish population. The aim of funds held by the Swedish Cancer Society is to make research on different risk factors and improve the possibilities to prevent cancer. Adami's activities, however, seem to have cast doubt on certain environmental cancer risks. The Swedish Cancer Society has made no move to require Professor Adami to publicly disclose his potential conflicts of interest.

MONSANTO, ASBESTOS, HERBICIDES, AND DOLL

It has also been revealed that Professor Sir Richard Doll, a long time epidemiologist for what was until recently the Imperial Cancer Research Fund in England, had failed to disclose his funding from Monsanto [Walker, 2005]. Apart from his relationship with Turner and Newall, the asbestos manufacturers [Tweedale, 2000; Castleman, 2001], the other long-term relationship that Sir Richard Doll had with industry between 1970 and 1990 appears to have been with Monsanto.

During the later part of the 1990s, Sir Richard Doll made depositions as an expert witness on behalf of chemical companies, which were being sued in North America and Italy. Coincidentally, the law firm acting for Dow Chemicals, which took Doll's depositions, Covington and Burling, was previously counsel for the Tobacco Institute and played a decisive role in organizing campaigns for Philip Morris [Covington and Burling, 2005].

Doll presented evidence to rebut claims brought by workers and ex-workers that they had contracted cancer from exposures to vinyl chloride. Doll's statement was only used in the North American case of Ross, in which the plaintiffs, whose deceased husbands had contracted brain tumors after working with vinyl chloride, won massive damages.

Doll [1988] became an expert witness in these cases by virtue of his authorship of the article Effects of Exposure to Vinyl Chloride. The article made no declaration of vested interests or payments in relation to chemical companies. Doll's 1988 review of mainly industry-organized studies reported that there was no significant carcinogenicity associated with vinyl chloride other than in the liver. He gave the seal of approval to the safety of the chemical and its productive process, even though by 1979, a decade earlier, vinyl chloride was classified by IARC as a Group I human carcinogen with target organs liver, brain, lung, and hemato-lymphopoetic system [IARC, 1979].

Doll's article remained the gold standard for more than a decade, and served as the basis for the following statement in 2001, by the American Chemical Council (previously called the Chemical Manufacturers Association). "The world's leading researchers have studied vinyl chloride and brain cancer and concluded that the evidence does not support a link between brain cancer and exposure to vinyl chloride" [American Chemistry Council, 2001]. In fact, in his review, like other researchers, Doll had found an association between brain cancer and working with the production of vinyl chloride but dismissed this association as not significant or unlikely to be caused by occupation. Apart from these broader defences of vinyl chloride production, Doll's paper [Doll, 1988] was specifically responsible for the US Environmental Protection Agency dismissing the significance of non-liver cancers in vinyl-exposed workers, as critically discussed elsewhere [Prince, 2005; Sass et al., 2005].

Doll agreed to write his review after being approached by the ICI Medical Advisor, Brian Bennett [Doll and Bennett, 2005]. Bennett had cleared his suggestion to approach Doll for the work with the US Chemical Manufacturers Association (CMA), the trade organization for chemical manufacture of which Monsanto was an important member.

In 2002 Sir Richard Doll deposited a number of boxes of articles at the Wellcome Institute (see PP/DOL, Sir Richard Doll (b. 1912) Epidemiologist. Wellcome Library for the History and Understanding of Medicine). In these articles there is a letter from the epidemiologist at Monsanto, William Gaffey, renewing Doll's contract to act as a consultant for the company at the billable rate of £1,000 a day. Doll replied to this letter [Doll, 1986]. "I greatly appreciate the offer to extend my consulting agreement and for the increased fee, and I have signed and am returning one contract note." Gaffey was a *mathematician*, brought in by Monsanto specifically to "clean up" the public image of dioxin.

Furthermore, these articles reveal that Bennett and Doll agreed that any article written by Doll would be "peer reviewed" by Julian Peto, Doll's closest colleague and by Geoffrey Paddle and Ted Torkelson (Dow), medical advisers of two chemical companies. The cost of the review was settled at £15,000 plus expenses [Wellcome, 1984, 1986a,b]. One of the first letters which Doll wrote, in March 1986, on beginning the review was to Gaffey, asking for his advice and it was Gaffey who also managed Doll's—at that time

secret—consultative contract with Monsanto [Wellcome, 1986a,b].

In February 1988, Doll sent the finished review of vinyl chloride on Bennett's advice, to the editor of the Scandinavian Journal of Work, Environment and Health, which accepted it for publication [Doll, 1988].

The £15,000 fee for the review was paid for by the CMA, partly by ICI, the biggest producer of vinyl chloride in the UK, and partly by Dow, another big producer of vinyl chloride. However, in the years 1987 and 1988 when Doll was finishing the review he was also separately receiving consultancy funding from Monsanto, also one of the other biggest producers of vinyl chloride in North America and an important member of the CMA. None of this funding was declared in the published article.

In January 2000, Doll was cross-examined by Ross's lawyers on the expert evidence he had given for Dow Chemicals and others. The lawyers cross-examined Doll on his review and the absence of acknowledgements for its funding from the chemical industry. Doll told lawyers that he had written asking Bennett's advice about acknowledging payment for the review from the CMA and Bennett had advised him that there was no need for him to acknowledge the source of his funding. On the matter of his consultancy payments from Monsanto at the time he was writing the review, which involved a Monsanto product, Sir Richard (Doll) said simply that he did not know he should disclose these sources of income [Doll, 2000].

In December 1985, just prior to writing to Gaffey at Monsanto for his advice about his review of vinyl chloride studies, Doll had appeared to add his authority to the campaign that Gaffey was running to counteract the image of dioxin as a highly toxic agent. On December 4, 1985, Doll wrote to Justice Phillip Evatt, who had presided over the Australian Royal Commission that had enquired into the effects of Agent Orange and dioxin on Australian personnel during the Vietnam War [Doll, 1985; Hardell et al., 1998; Hardell, 2004].

The Commission's conclusion was that there was no evidence that exposure to Agent Orange including TCDD was a health hazard. However, it was later revealed that part of this ruling including a review of the scientific evidence, was an almost verbatim account of a Monsanto submission on the issue. As discussed elsewhere [Hardell et al., 1998; Hardell, 2004], the scientific evidence was distorted and manipulated in the Commission's (or rather Monsanto) document.

Doll's unsolicited letter to Evatt, however, supported the Commission's views. In his letter Doll stated:

...relating to 2,4-D and 2,4,5-T (the phenoxy herbicides in question) that there is no reason to suppose that they are carcinogenic in laboratory animals and that even TCDD (dioxin), which has been postulated to be a dangerous contaminant of the herbicides, is at the most, only weakly and inconsistently carcinogenic in animal experiments ...I am sure, however, that it [your review] will be widely quoted and that it will come to be regarded as the definitive work of the subject [Doll, 1985].

Doll's letter also attempted to question the veracity and validity of the work by Dr. Hardell and his colleagues, and for that matter, its very legitimacy as a scientific work as discussed in later publications [Hardell et al., 1998; Hardell and Eriksson, 2003; Hardell, 2004].

"Your Review of Hardell's work, with the additional evidence obtained directly from him at interview, shows that many of his published statements were exaggerated or not supportable and that there were many opportunities for bias to have been introduced in the collection of his data. His conclusions cannot be sustained and in my opinion, his work should no longer be cited as scientific evidence. [Authors italics] [Doll, 1985]."

In spite of receiving copies of articles that revealed the manipulations of scientific facts in the Monsanto submission [Monsanto Australia Limited, 1985] and a rebuttal of the Commission's findings [Axelson, 1986] Doll never changed his position. The questions to be asked are first, whether the now disclosed facts that he was at that time secretly a highly paid Monsanto consultant perhaps influenced his statements. Second, how did Doll's hidden consultancies influence his other work?

MOTOROLA, THE SWEDISH RADIATION PROTECTION AGENCY, INTERNATIONAL EPIDEMIOLOGY INSTITUTE, BOICE, AND MCLAUGHLIN

Another example of industry ties to research, but not one where there was a failure to disclose, involves the potential association between cellular phones and brain tumors. In 2002 the Swedish Radiation Protection Authority (SSI) hired two US epidemiologists to review published epidemiological studies on the relationship between the use of cellular telephones and cancer risk. They were Dr. John D. Boice, Jr. and Dr. Joseph K. McLaughlin from the private company International Epidemiology Institute (IEI). In their review [Boice and McLaughlin, 2002], they claimed that no consistent evidence was observed for increased risk of brain cancer, including meningioma, acoustic neurinoma, ocular melanoma, or salivary gland cancer, and mobile phone use. Featured in their review was an article by Hardell et al. [2002] of an association between cellular telephones and certain brain tumors. The review heavily criticized this article.

However, Boice and McLaughlin were co-authors of some of the studies in their "independent" review. The very positive words by Boice and McLaughlin about their own studies, which showed no association between cellular telephones and certain tumor types, should be viewed while

bearing in mind their own participation in these investigations. Despite the fact that IEI was a co-founder of their studies, cited in the review, Boice and Joseph McLaughlin made no statements of any conflict of interest in the SSI-report.

The Director General of SSI, Lars-Erik Holm, has earlier published several articles with John Boice. Also it appears that the International Epidemiology Institute was at the time of the SSI review involved in a cellular phone and brain tumor litigation in the USA on behalf of the defendants, Motorola [Newman v. Motorola Inc, 2002]. The connection was traced by the fax number on the articles with the referee comments to the journal considering for publication the Hardell et al. article on use of cellular telephones and the association with brain tumors. The information that the article was under review had been communicated to the defendants (Letter from Mr. Tom Watson, defendant lawyer for Motorola, dated January 18, 2002 and referee comments from fax 301 517 4063 International Epidemiology Institute dated 11/19/01), a violation of the confidentiality of the review process. These and other circumstances on this issue have been reviewed by the authors [Hansson Mild et al., 2003; Hardell, 2004].

A number of research projects have taken place at the Karolinska Institute, Stockholm with participation of Boice and McLaughlin, with a funding model through IEI. One of the studies was published in British Medical Journal [Nyrén et al., 1998] with Adami as a co-author. A cohort of Swedish women with breast implants was studied with regard to connective tissue disease. No risk was found. Thanks to strict rules of stating conflicts of interest in the British Medical Journal it can be seen that the project was initiated by IEI, and that the funding from IEI was on behalf of Dow Corning, producer of silicon breast implants.

INDUSTRIAL TIES: THE NEED FOR RULES

We note that relationships between corporations and "independent" researchers appear to be prevalent across most areas of medical research and not be restricted to reviews but also affect original research. In 2001, a study of 1,396 highly ranked scientific and biomedical journals by Krimsky and Rothenberg [2001], reported that only 16% had conflict of interest policies.

A recent study found that one-third of all original research articles published in the New England Journal of Medicine and the Journal of the American Medical Association were funded by for-profit healthcare companies [Friedman and Richter, 2004]. Furthermore, one in four original research articles published in these journals had one or more authors with corporate financial relationships and conflicts of interest. The authors with conflicts of interest were two times more likely to report results supporting their sponsor's products [Friedman and Richter, 2004]. For obvious reasons these numbers are biased. Only those with known conflicts of interests are recognized. Those with hidden ties are not found in the correct column.

There have also been cases in which editors and journal staff have suppressed publication in the peer-reviewed literature [Egilman, 2005; Friedman and Richter, 2005]. In 2004, an editorial questioning the benefits of increased doses of Epogen[1] (epoetin alfa) in patients with renal disease was rejected because it "went beyond what (the) marketing department (was) willing to accommodate." In fact, the executive editor initially accepted the manuscript but was "overruled" by the marketing department, providing a clear example of an internal conflict of interest between editorial integrity and business needs [Vedantam, 2004].

Financial relationships between industry, researchers and academic institutions are becoming increasingly complex [Tuech et al., 2005]. Funding from industrial sources for research itself should be a good thing, because, in theory, it should provide access to resources and information no longer readily available from public sources and can catalyze highly creative interactions to advance knowledge to promote and protect health. But the few examples we give show that it invites abuse when it is secret, concealed, disguised or non-disclosed, and as other research suggests, these examples are not isolated. Conflict of interest in itself is widespread, but its potential for generating misinformation is greatly increased when it is undeclared.

Whatever the rights and wrongs of particular cases there are clear lessons to be drawn from the abuses which have until recently compromised the integrity of epidemiological research on environmental hazard and risk. Unfortunately, powerful industrial interests are undermining independent research on hazard and risk in Europe and North America.

The case studies are troublesome, because they involved some of the world's leading epidemiologists. It is highly likely that there were delays in addressing the carcinogenic risks that these epidemiologists minimized in the interests of their clients. These case studies illustrate the need for rigorous policies and practices to prevent the abuses of this kind by requiring open declaration of direct and indirect support, professional codes of practice that will help curb abuses, enforcement of these codes and evaluation of the efficacy of enforcement.

We call for swift, immediate and forceful policies and action by the independent academic community and, no less important, editors of scientific journals to protect scientific integrity, openness, and fairness. Such policies and actions are needed to ensure credibility and restore the essential role of the medical epidemiologist in protecting the public health.

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Exhibit 4

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Selected science: an industry campaign to undermine an OSHA hexavalent chromium standard

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Abstract

Go to:

While exposure to hexavalent chromium (Cr(VI)) has been associated with increased lung cancer risk for more than 50 years, the chemical is not currently regulated by the U.S. Occupational Safety and Health Administration (OSHA) on the basis of its carcinogenicity. The agency was petitioned in 1993 and sued in 1997 and 2002 to lower the workplace Cr(VI) exposure limit, resulting in a court order to issue a final standard by February 2006. Faced with the threat of stronger regulation, the chromium industry initiated an effort to challenge the scientific evidence supporting a more protective standard. This effort included the use of "product defense" consultants to conduct *post hoc* analyses of a publicly-funded study to challenge results viewed unfavorably by the industry.

The industry also commissioned a study of the mortality experience of workers at four low-exposure chromium plants, but did not make the results available to OSHA in a timely manner, despite multiple agency requests for precisely these sorts of data. The commissioned study found a statistically significant elevation in lung cancer risk among Cr(VI)-exposed workers at levels far below the current standard. This finding changed when the multi-plant cohort was divided into two statistically underpowered components and then published separately. The findings of the first paper published have been used by the chromium industry to attempt to slow OSHA's standard setting process. The second paper was withheld from OSHA until it was accepted for publication in a scientific journal, after the rulemaking record had closed.

Studies funded by private sponsors that seek to influence public regulatory proceedings should be subject to the same access and reporting provisions as those applied to publicly funded science. Parties in regulatory proceedings should be required to disclose whether the studies were performed by researchers who had the right to present their findings without the sponsor's consent or influence, and to certify that all relevant data have been submitted to the public record, whether published or not.

Background

Go to:

In recent years, efforts by major corporations to deflect unwanted scientific findings have been reported

increasingly in the lay and biomedical literature. The tobacco industry, for example, used the attorney-client privilege to shelter scientific studies from disclosure [1-3]; it also funded apparently independent organizations to provide a patina of credibility for its work [1,4]. Pharmaceutical manufacturers have withheld unfavorable clinical trial results [5,6] and have disparaged research that produced unwelcome findings [7,8].

We report a case in the less-scrutinized field of occupational health in which all these elements were combined in a coordinated strategy to challenge the Occupational Safety and Health Administration's (OSHA) action to reduce workers' exposures to the lung carcinogen hexavalent chromium (Cr(VI)).

Cr(VI) is not a newly-identified hazard; the increased risk of lung cancer has been documented in Cr(VI)-exposed workers for more than 50 years [9,10]. Thomas F. Mancuso and Wilhelm C. Hueper, for example, studied the mortality experience of chromium-exposed workers employed between 1931 and 1937 at a Painesville, Ohio facility. Results of their study were published in 1951 [10,11], with updates on the cohorts published by Dr. Mancuso in 1975 [12] and again in 1997 [13], consistently finding an excess risk of lung cancer among exposed workers. Cr(VI) has been classified as a human carcinogen by the National Toxicology Program [14] and the International Agency for Research on Cancer [15]. It is used in chrome plating and in the production of metal alloys and pigments. OSHA estimates that approximately 380,000 U.S. workers are currently exposed to Cr(VI) [16].

At present, OSHA does not regulate Cr(VI) on the basis of its carcinogenicity. The agency's current Permissible Exposure Limit (PEL) of 52 ug/m^3 was originally recommended in 1943 by the American National Standards Institute as a level adequate to prevent nasal perforations in chromium-exposed workers [17]. This 52 ug/m^3 limit was adopted by OSHA in 1971 when the agency was created, without any formal review. In 1976, OSHA announced plans to lower the Cr(VI) standard [18] and in 1994, the OSHA administrator acknowledged that "there is clear evidence that exposure...at the current PEL...can result in an excess risk of lung cancer" [19]. However, until recently, no change was officially proposed and the 52 ug/m^3 PEL remains in effect today.

In 1993, Public Citizen and the Oil, Chemical and Atomic Workers International Union (OCAW) (now part of the United Steelworkers) petitioned OSHA to reduce its PEL from the current level of 52 ug/m^3 to 0.25 ug/m^3, measured as an 8-hour time-weighted average. Two lawsuits ensued, challenging OSHA's "unreasonable delay" in promulgating a stronger standard. Although the chromium industry, through its trade association the Chrome Coalition, had intervened in the lawsuits on OSHA's behalf opposing a change in the PEL, on April 2, 2003, the U.S. Court of Appeals for the Third Circuit ordered the agency to issue a final rule reducing occupational exposure to Cr(VI) by January 18, 2006 [20], later extended to February 28, 2006. In the words of Judge Edward Becker, OSHA's decade-long delay in issuing a Cr(VI) standard "exceeded the bounds of reasonableness" [21].

The industry strategy to forestall OSHA rulemaking

Long before the court ruling, however, the chromium industry had initiated an effort to challenge the scientific evidence supporting any stronger OSHA standard, engaging the services of ChemRisk and Exponent, Inc., two consulting firms that specialize in "litigation support" and "product defense" [22,23]. One industry document noted that "this route [hiring the consultants] is expensive and success is not guaranteed, [but] the longer we wait the more difficult the task becomes." [See additional file 1: File1 to view this document.]

In a meeting with chromium industry representatives in 1996, ChemRisk scientists outlined a strategy that included obtaining and analyzing the raw data from a not-yet-published study of Cr(VI) exposure funded by the Environmental Protection Agency (EPA) in order "to forestall the [OSHA] rulemaking." [See additional file 2: File2 to view this document.] Simultaneously, the industry commissioned new publications that questioned the health effects of low levels of exposure to Cr(VI) [24-26], a central issue in any OSHA regulatory initiative. The industry paid for services provided by ChemRisk and Exponent, Inc. through its trade association's attorneys. This arrangement was selected to "...preserve the confidentiality of information, opinion, and data to the extent provided

for under the attorney-client privilege and attorney work product privilege." [See additional file 3: File3 to view this document], ensuring that material developed through the process could be sequestered from public view. [See additional file 4: File4 to view meeting summary and plan to preserve attorney-client privilege.]

The industry also contracted with a third consulting firm, ENVIRON [27], to study workers who had only been employed in facilities that were either designed with or converted to production processes that resulted in lower levels of Cr(VI) exposure. ENVIRON was hired through a contract with the Industrial Health Foundation (IHF), a descendant of the Air Hygiene Foundation, an organization founded in 1935 in the wake of the 1930's Gauley Bridge occupational silicosis tragedy to provide employers with confidential assessments of industrial hazards [28]. The study protocol entailed combining workers from four plants using newer, lower-exposure processes – two in the U.S. (Castle Hayne, NC, and Corpus Christi, TX) and two in Germany – into a single cohort. ENVIRON's proposal noted that "the relatively small study sizes and short follow-up periods resulted in a limited ability of [previous] studies to clarify the relationship between modern [low-level] occupational chromate exposures and cancer in general, and respiratory cancers in particular." According to the proposal, creating a single cohort with workers from multiple plants was crucial "to improve statistical power and the inferential value of the results" [29].

In August 2000, the EPA study was published. It is the largest, most comprehensive study ever conducted on the effects of workplace Cr(VI) exposure. The study examined a cohort of more than 2,300 workers employed at a chromate production facility in Baltimore, MD, from 1950 to 1974, and followed through 1992. Exposure histories were reconstructed utilizing 70,000 measures of airborne Cr(VI) concentrations; smoking histories for 93% of the cohort were also incorporated into the analyses. Using OSHA's standard assumption of a 45-year working lifetime, the study reported a significantly elevated lung cancer risk of 1.57 among workers whose mean exposure was at levels just above the PEL requested in the Public Citizen-OCAW petition [30].

No sooner had the EPA study been published than the industry-sponsored critiques began. Scientists with the product defense firm Exponent, Inc. created and analyzed the mortality experience of a "simulated cohort" derived by computer from the EPA study's summary data, standard deviations and ranges [31]. In another report, the consultants obtained the raw data from the EPA study through a Freedom of Information Act request and re-analyzed them [32]. Each of these reports challenged the validity of the EPA study's conclusions and was either entered into the record in litigation or submitted to OSHA by the chromium industry, although not published in the peer-reviewed literature. After extensive analysis, most of the issues raised in these critiques were rejected by OSHA [33].

OSHA publishes its proposed rule

On October 4, 2004, OSHA published its court-mandated proposed rule for Cr(VI), including a PEL of 1 ug/m^3 [34]. The agency issued a general request for additional scientific evidence, along with a specific appeal for epidemiological data about the aforementioned cohort in Castle Hayne, where exposure levels were more representative of the concentrations of airborne Cr(VI) found in workplaces today [35]. A mortality study of this group had been published in 1994 [36] and OSHA asked directly, "Are there updated analyses available for [this cohort]?" In addition, OSHA asked, "Are there other cohorts available to look at low exposures?" [37].

Following a three-month comment period, OSHA held 11 days of public hearings [38], at which OSHA reiterated its request for more data and industry repeatedly criticized OSHA for relying on data from high-exposure cohorts [39-44]. In reviewing the hearing transcript, we found no mention by industry representatives or anyone else of any imminent new epidemiological evidence. The public was given until April 20, 2005, to submit additional data and post-hearing comments.

Selected science

Just weeks before the close of the comment period, a study reporting on the mortality experience of workers

employed at the Castle Hayne and Corpus Christi facilities appeared in the *Journal of Occupational and Environmental Medicine* (*JOEM*) [45]. The article had been submitted to *JOEM* in July 2004 and was accepted for publication that October [46], the same month OSHA proposed its rule and specifically asked for information about the Castle Hayne or other cohorts. The analysis has little statistical power (only three lung cancer deaths) and suffers from short follow-up (fewer than half of the workers in the study were followed for twenty years or more, the minimum length of time needed to begin to detect occupational cancer) [47]. Even though they collected data on Cr(VI) exposure, none is presented in the paper and the small sample size precludes logistic regression. Nonetheless, the authors offer the "preliminary conclusion" that "the absence of an elevated lung cancer risk at this time may be a favorable reflection of the post-change [i.e., lower exposure] environment [45]."

Three trade associations made reference to the study in their post-hearing comments [48-50]. For example, the Specialty Steel Industry of North America stated it had "recently" learned of the study:

[W]hile we have not had any opportunity to examine this study...[it] contains potentially incredibly significant data which would allow the development of a dose response relationship based on actual, experienced exposures, as opposed to the modeled exposures upon which OSHA currently relies to set the PEL. Indisputably, this would be much more relevant and appropriate data upon which to establish a risk-based regulatory limit [48].

The Specialty Steel Industry warned that OSHA's failure to consider these results would be "arbitrary and capricious," a legal term, signaling that failure to address these "new" findings would be grounds for a legal challenge. The Society of the Plastics Industry, Inc. (SPI) remarked on the "potentially great significance" [49] of the new ENVIRON study.

Moreover, the comments by these trade associations confirm that they were privy to unpublished details of the ENVIRON analysis. For example, one wrote:

SPI has learned that in the German plants, excess lung cancer mortality was demonstrated only in the highest exposure group, using chromium exposure estimates based on urinary chromium results. It is possible that the data obtained from the German facilities demonstrates that no increase in risk at any but the highest exposure levels to CrVI [49].

The industry thus succeeded in inserting this hearsay material into the record without ever providing the actual study data.

Intrigued by these developments, we conducted an Internet search, using the terms "Industrial Health Foundation" and "Chrome Coalition." To our surprise, we located a notice for a hearing related to the bankruptcy of IHF. In this proceeding, two chromium industry trade associations asserted that files in the possession of IHF actually belonged to the industry, because the IHF was, according to the petitioner, simply a "third-party administrator of the trade association." [See additional file 5: File5 to view this document.] Using the Public Access to Court Electronic Records system [51], we obtained documents filed with the court, some of which have been quoted in this manuscript. These materials also led us to parties in the bankruptcy proceedings who provided additional documents, including ENVIRON's study protocol and the final report of the combined study of the U.S. and German plants.

That report, submitted by ENVIRON to IHF in September 2002 but never by the industry to OSHA and never published in its entirety, provides strong support for the inadequacy of the current standard, and raises questions about whether the proposed OSHA PEL of 1 ug/m^3 is adequately protective. The ENVIRON authors found a significantly elevated risk of lung cancer mortality associated with exposure to Cr(VI) in these newer low-exposure facilities (SMR = 1.66, 95% CI = 1.08–2.46, using a combination of German national rates and U.S. state rates for comparison; SMR = 1.37, 95% CI = 0.89–2.03, using German and U.S. state rates). The investigators developed a series of job exposure matrices and utilized air monitoring data from the U.S. plants and urine monitoring from the German plants to estimate the exposure history of each worker. In order to convert the urinary measurements (*u*g/L)

into air measurements (ug/m^3), we divided by 0.77, the same conversion factor used by the industry [52], and divided by 45, to convert cumulative exposures into mean annual ones.

Logistic regression analyses of the four-plant cohort found increased risk associated with increased cumulative (or lifetime) exposure to Cr(VI). In one analysis, the lung cancer mortality odds ratio among workers with highest annual exposure (≥ 5.8 ug/m^3) was 20.2 (95% CI = 6.2–65.4), compared to the lowest exposure group (< 1.2 ug/m^3) [53]. For the intermediate exposure group (1.2 ug/m^3 – < 5.8 ug/m^3), the odds ratio was 4.9 (95% CI = 1.5–16.0), also in comparison to the lowest exposure group [53]. Thus, the intermediate group includes exposure at levels only slightly higher than the 1 ug/m^3 PEL proposed by OSHA in 2004, and showed elevated lung cancer risk at that level.

The final unpublished four-plant report reiterated the strength of the study design: "This study benefited from the multi-site design that provided a reasonably large cohort of post-change [lower exposure] chromium chemical workers, along with the corresponding increase in statistical power generally lacking in previous studies of post-change cohorts" [52].

The published *JOEM* article, however, reports the mortality experience only of workers at the two U.S. plants studied by the ENVIRON researchers. After submitting the results to their sponsors in 2002, the authors evidently separated the German and U.S. results, despite their repeated emphasis in the protocol on the strength of the combined cohort. Instead of a positive result based on four plants, a negative two-plant study was published. In a response to a letter [54] in the *JOEM*, the authors stated that the German component of the study had not been published because it was rejected by a journal to which it had been submitted, and defended the exclusion of the German data on the ground that different exposure measurements (air vs. urine) were used [55]. This claim is not consistent with the need for large sample size to increase statistical power, as stated in the protocol and the final report. In June 2005, we provided the study protocol [29] and final report of the four-plant study [52] to OSHA [56].

On October 17, 2005, the ENVIRON researchers submitted the German component of the study to OSHA, accompanied by a note saying the paper had been accepted for publication in *JOEM* [57,58]. In this manuscript, the ENVIRON researchers report that "lung cancer risk was elevated only in the highest exposure group (SMR = 2.09 95% CI = 1.08–3.65)" [58].

The authors conducted another logistic regression analysis, but in this new version the estimate of relative risk for workers with high exposure is derived by comparing them to workers in the low and intermediate exposure groups combined. The result of this change is the disappearance of the statistically significant increase in lung cancer mortality risk among the intermediate group that was found in the unpublished final report. Tables 1 and 2, adapted from the unpublished final report [52] and the pre-publication manuscript of the German component of the study submitted by the authors to OSHA [58], respectively, compare the results of the two regression analyses. In addition, while the elevation of the lung cancer SMR in the unpublished final report of the four-plant study was statistically significant, when the cohort was divided into two components, the lung cancer SMR was not statistically significant in either the German or U.S. components.

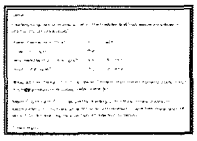

Table 1
Elevated lung cancer mortality risk in intermediate and high exposure groups in original unpublished study†

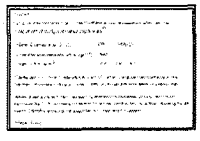

Table 2
Lung cancer mortality risk in intermediate group disappears after German component of study published separately††

Discussion

Go to:

Faced with the threat of stronger OSHA regulation of workplace exposure of Cr(VI), a powerful carcinogen, the chromium industry initiated an effort to challenge the scientific evidence that the agency would likely use to justify a new standard. While criticizing OSHA for relying upon data from high-exposure cohorts, the chromium industry also commissioned a study of the mortality experience of workers at four plants with lower exposures, the results of which confirmed the elevated lung cancer risk in such workers. The consultants presented a final report to their chromium industry sponsors in 2002, but industry never provided OSHA a copy of the full four-plant study. Even when the agency specifically asked for precisely these sorts of data during its 2004–2005 rulemaking proceedings, the chromium industry and the authors remained silent.

For publication, industry-funded scientists divided this study into two components and published them separately. The first paper to be published was a statistically underpowered, negative study, the findings of which are being used by industry to attempt to reduce its regulatory burden. The second paper combined two exposure strata from the final report, resulting in the disappearance of the stratum of particular regulatory interest in which a statistically significant finding was apparent in the unpublished final report. This allowed the industry trade associations to make the misleading assertion that elevated lung cancer mortality risk was only seen among workers with the highest exposure histories.

OSHA's statute instructs its decision makers to use the "best available evidence" [59] in the rulemaking process. The circumstances regarding these studies raise troubling questions about the ability of government to effectively issue rules protecting public health when studies are conducted, controlled and selectively published or provided to the rulemaking agency by the regulated industry [8,60]. The entry of the German study into the OSHA record only after it was accepted for publication, months after the regulatory docket closed and years after data collection was complete, raises an important question for public health research: when regulatory proceedings are underway, should potentially important data be sequestered until the peer review process is complete? Many U.S. regulatory agencies, including the EPA and the Food and Drug Administration (FDA) rely heavily on unpublished studies, submitted by study sponsors, in reaching regulatory decisions. In this case, sponsors withheld data that OSHA requested during an active rulemaking process.

It is now widely recognized that pharmaceutical manufacturers have an obligation to report the existence and results of all clinical trials, although this is often not done satisfactorily [61,62]. The higher standards of practice now being sought in the reporting of pharmaceutical trial results should also be applied in occupational health and safety research. The editors of thirteen leading journals will no longer publish articles based on studies done under contracts in which clinical trial investigators did not have the unfettered right to publish the findings, asserting that such restrictions "erode the fabric of intellectual inquiry that has fostered so much high-quality clinical research" [63]. Parties in regulatory proceedings should be required to disclose whether the studies they submit were performed by researchers who had the right to present or publish their findings without the sponsor's consent or influence [64]. Regulatory agencies should weigh the submitted information accordingly.

Public health is not well served by the unequal treatment of public and private science [65]. Parties submitting scientific analyses and reports to the record should be required to disclose the true sponsorship of the study, including the original source of the sponsor's funding. Parties involved in the rulemaking process should also be required to certify that they have submitted all relevant data to the public record, whether or not those data have undergone peer review. Medical journals are increasingly willing to publish findings even if they have already been made available in another form. Regardless, public health rulemakings should not be based on partial records or limited by scientists' career concerns, particularly when lives hang in the balance.

List of Abbreviations

Go to:

Cr(VI) Hexavalent Chromium

EPA US Environmental Protection Agency

IHF Industrial Health Foundation

JOEM Journal of Occupational and Environmental Medicine

OCAW Oil, Chemical and Atomic Workers International Union

OSHA US Occupational Safety and Health Administration

PEL Permissible Exposure Limit

SMR Standardized Mortality Ratio

ug/m^3 micrograms per cubic meter of air

Competing interests

Go to:

PL is with Public Citizen's Health Research Group, a party in the lawsuit filed against the US Department of Labor to compel OSHA to issue an occupational hexavalent chromium standard. DM and CM declare that they have no competing interests.

Authors' contributions

Go to:

DM, CM and PL researched and wrote the article. All authors read and approved the final manuscript.

Supplementary Material

Go to:

Additional File 1:

Chrome Coalition Ad Hoc PEL Committee. Summary of Chrome Coalition's meeting with ChemRisk on February 13, 1996.

Click here for file(1.0M, pdf)

Additional File 2:

Chrome Coalition Meeting Minutes. Meeting minutes describing the Chrome Coalition's February 13, 1996 meeting with ChemRisk.

Click here for file(1.6M, pdf)

Additional File 3:

Agreement between Collier, Shannon, Rill & Scott, PLLC, ChemRisk and the Industrial Health Foundation signed September 10, 1996. The agreement outlines services to be provided by ChemRisk on behalf of the Chrome Coalition.

Click here for file(3.0M, pdf)

Additional File 4:

Chrome Coalition Meeting Summary, September 12, 2002. Summary of the Chrome Coalition's meeting on

September 12, 2002.

Click here for file(1.5M, pdf)

Additional File 5:

Affidavit of Dr. Joel Barnhart, December 17, 2004, in Re: Industrial Health Foundation, Inc., U.S. Bankruptcy Court for the Western District of Pennsylvania. The affidavit describes, among other things, the Industrial Health Foundation's role with respect to the Chrome Coalition.

Click here for file(910K, pdf)

Acknowledgements

Go to:

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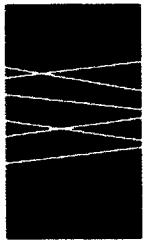
Exhibit 5



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AN EVALUATION OF THE POSSIBLE RISKS FROM ELECTRIC AND MAGNETIC FIELDS (EMFS) FROM POWER LINES, INTERNAL WIRING, ELECTRICAL OCCUPATIONS, AND APPLIANCES

Prepared by
Raymond Richard Neutra, M.D. Dr.P.H.
Vincent DelPizzo, Ph.D. GDE
Geraldine M. Lee, Ph.D.

FINAL REPORT JUNE 2002

[redacted]

CONDITION	REVIE-WER	IARC CLASS	CERTAINTY PHRASE	IRL	DEGREE OF CERTAINTY FOR POLICY ANALYSIS THAT AN AGENT (EMFs) INCREASES DISEASE RISK TO SOME DEGREE
Heart Disease					0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
	1	3	Close tc dividing line	6	
	2	3	Prone not to believe	8	
	3	3	Prone not to believe	3	

21.2 How Different Is This Evaluation From The NIEHS, NRPB and IARC Findings?

As outlined in Table 21.2 below, there are both common points and significant differences between the EMF Program's evaluation and those carried out at about the same time by the NIEHS Working Group for the Federal EMF-RAPID Program (Portier & Wolfe, 1998), (IARC, 2001), and the NRPB (NRPB, 2001a), (NRPB, 2001b) (Note: The NRPB did not use the IARC classification system but expressed their conclusion using common language expressions).

The following table compares these evaluations:

Table 21.2 A Comparison of DHS Reviewers' Degree of Certainty with That of Other Agencies

HEALTH OUTCOME	NIEHS WORKING GROUP	IARC	NRPB	DHS
Childhood leukemia	2B*	2B	Possible	2B to 1
Adult leukemia	2B (lymphocytic)	Inadequate	Inadequate	2B to 1
Adult brain cancer	Inadequate	Inadequate	Inadequate	2B
Miscarriage	Inadequate	Not Considered	Not considered	2B
ALS	Inadequate	Not Considered	Possible but perhaps due to shocks	2B
Childhood brain cancer, breast cancers, other reproductive, Alzheimer's, suicide, sudden cardiac death, sensitivity	Inadequate	Inadequate or Not Considered	No for Parkinson's disease, inadequate for Alzheimer's, other endpoints not yet considered	Inadequate

* Although the majority of scientists assembled to prepare the NIEHS Working Group Report voted for a "possible 2B" classification for these cancers, the lay person's summary submitted by the Director of NIEHS to Congress stated: "ELF-EMF exposure cannot be recognized as entirely safe because of weak scientific evidence that exposure may pose a leukemia hazard." (Final Report NIH Publication 99-4493, May 1999)

Exhibit 6

California Health Department Report

(Released for public discussion April 2001)

An Evaluation of the Possible Risks From Electric and Magnetic Fields (EMFs) From Power Lines, Internal Wiring, Electrical Occupations and Appliances*

Summary and Commentary

by

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*Access at: http://www.dhs.ca.gov/ehib/emf/RiskEvaluation/riskeval.html
or via
http://www.electric-fields.bris.ac.uk "California EMF Health Report"

Conclusions in tabular form

Table 1 below summarises the risk assessment from exposure to magnetic fields given in the California Health Department Report. For a given condition, note that the probabilities of a link include a chance that EMFs have no effect. The table on the following page summarises the criteria used by the Assessors.

Condition	Probability of a link with exposure to power frequency magnetic fields
Cancer	
Childhood Leukaemia	Two of the reviewers said 50 – 80% likely; one reviewer said virtually certain (>98% likely)
Adult leukaemia	Two of the reviewers said 50% to 90% possible One reviewer said 10 – 50% likely
Adult Brain Cancer	50% - 90% likely
Childhood Brain Cancer	10% - 50% likely
Male Breast Cancer	10% - 50% likely
Female Beast Cancer	Two of the reviewers said 10% - 50% likely One reviewer said 50% - 90% likely
All Cancers	Very improbable, 2 – 10% likely
Other conditions	
Miscarriage	50% - 90% likely that exposure could add 5-10% to the baseline risk
Birth Defects	Very improbable, 2 –10% likely
Amyotrophic Lateral Sclerosis (ALS)	50% - 90% likely
Heart Disease	10% - 50% likely
Suicide	10% - 50% likely

Exhibit 7

Magnetic-Field–Induced DNA Strand Breaks in Brain Cells of the Rat

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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-α-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. *Key words:* apoptosis, DNA strand breaks, free radicals, iron, magnetic field, necrosis. *Environ Health Perspect* 112:687–694 (2004). doi:10.1289/ehp.6355 available via *http://dx.doi.org/* [Online 27 January 2004]

Use of electricity exposes people constantly to low-intensity, extremely low-frequency electromagnetic fields, particularly at the power frequencies of 50 and 60 Hz. In previous research (Lai and Singh 1997a),we found that rats acutely exposed to a 60-Hz sinusoidal magnetic field showed an increase in DNA single- and double-strand breaks in their brain cells as measured by the microgel electrophoresis assay. An increase in DNA single-strand breaks was observed after 2 hr of exposure to the magnetic field at flux density of ≥ 0.1 millitesla (mT), whereas an increase in double-strand breaks was observed at ≥ 0.25 mT. Using the microgel electrophoresis assay, Ahuja et al. (1997, 1999), Phillips et al. (1997), Svedenstal et al. (1999a, 1999b), and Zmyslony et al. (2000) have also reported an increase in DNA strand breaks in cells after magnetic field exposure. In studies by Ahuja et al. (1997, 1999), an increase in DNA single-strand breaks in human lymphocytes was observed after 1 hr of exposure to a 50-Hz magnetic field at 0.2–2 mT, whereas in the study by Phillips et al. (1997), an increase in single-strand breaks was observed in human Molt-4 cells after 24 hr of exposure to a 60-Hz magnetic field at 0.1 mT. Svedenstal et al. observed an increase in DNA double-strand breaks in brain cells of mice after 32 days of exposure to magnetic fields of 7.5 μT (Svedenstal et al. 1999a) and after 14 days of exposure at 0.5 mT (Svedenstal et al. 1999b). Zmyslony et al. (2000) reported an increase in single-strand breaks in rat lymphocytes exposed to a 50-Hz magnetic field at 7 mT in the presence of iron cations. More recently, Ivancsits et al. (2002, 2003a, 2003b) reported an increase in DNA single- and double-strand breaks in human fibroblasts intermittently (5 min on/10 min off) exposed to a 50-Hz magnetic field at 1 mT, whereas continuous exposure produced no significant effect. Because the other studies reporting effects of magnetic fields on DNA were carried out under continuous exposure conditions, the results of Ivancsits et al. (2002, 2003a, 2003b) indicate that the interaction of magnetic fields with DNA is quite complicated and apparently depends on many factors. Furthermore, McNamee et al. (2002) reported no significant effect on DNA strand breaks in cerebellar cells of immature mice exposed continuously to a 60-Hz magnetic field at 1 mT for 2 hr. Miyakoshi et al. (2000) reported that a high-intensity (> 50 mT) 50-Hz magnetic field had no significant effect alone, whereas it potentiated X-ray–induced DNA single-strand breaks in human glioma cells. Thus, effects of magnetic fields on DNA may depend on factors such as the mode of exposure, the type of cells studies, and the intensity and duration of exposure.

In the present study, we further investigated the effect and mechanism of interaction of magnetic field exposure on brain cell DNA in the rat. In a previous experiment (Lai and Singh 1997b), we found that pretreating rats with melatonin and a spin-trap compound (*N-tert*-butyl-α-phenylnitrone) blocked the effect of a 60-Hz magnetic field on DNA. Because melatonin and spin-trap compounds are efficient free-radical scavengers, the data suggest that free radicals play a role in the effect of the magnetic field. In another study (Singh and Lai 1998), we found that acute magnetic field exposure induced the formation of DNA–protein and DNA–DNA cross-links in brain cells of rats, which could be the results of free-radical damage involving iron cations (Altman et al. 1995; Lloyd et al. 1997).

In this study, effects of exposure duration and treatments with the vitamin E analog Trolox (Forrest et al. 1994), the nitric oxide synthase inhibitor 7-nitroindazole (Kalisch et al. 1996; Moore and Bland-Ward 1996), and the iron chelator deferiprone (Fredenburg et al. 1996; Kontoghiorghes 1995) were investigated. In addition, incidences of apoptosis and necrosis in brain cells of rats acutely exposed to a 60-Hz magneric field were studied.

Materials and Methods

Animals. Male Sprague-Dawley rats (2–3 months old, 250–300 g), purchased from B & K Laboratory (Bellevue, WA), were used in this research. They were housed for at least 24 hr before an experiment in the room in which they would be exposed to magnetic fields. The laboratory was maintained on a 12/12-hr light/dark cycle (light on 0700–1900 hr), at an ambient temperature of 22°C and a relative humidity of 65%. Animals were provided with food and water *ad libitum* in their home cages and during exposure.

In vivo *magnetic field exposure system.* A Helmholtz coil pair system was used to expose rats to a sinusoidal 60-Hz magnetic field. This exposure system has been described in detail previously (Lai et al. 1993). Briefly, a computer program was used to design this Helmholtz coil pair system, which can produce a magnetic field with minimal heating and field variations over the exposure area. Each coil is made of two sets of 40 turns each of #6 wire wound in rectangular loops, with minimum internal dimensions of 0.86 × 0.543 m.

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During construction, epoxy was layered between loops to glue them together. This minimizes vibration noise when the coils are activated. The coils are wound on frames fabricated from wood and aluminum and are therefore completely shielded against emission of electric fields. They are designed with split windings terminated on multiterminal blocks so that they may be wired in various series or parallel combinations for impedance matching and connecting to multichannel or multifrequency sources. It is wired such that a switch can be used to put the coils "in phase," to generate magnetic fields, or in the "bucking mode," in which the two sets of coil are activated in an antiparallel direction (with the same current as in the in-phase condition) to cancel the fields generated by each other. The bucking mode was used as a control condition in our research to control for the possible effects of heat and vibration generated by the coils. By varying the input current to the coils, exposure fields could be set anywhere from the ambient level to the maximum coil designed magnetic field strength of 5.6 mT. With an exposure level set at 1 mT, the heat dissipation from each of the Helmholtz coils is < 8 W of power. The heat generated is efficiently dissipated because of the large surface area of the coils and good ventilation in the exposure room. The magnetic field during exposure was monitored by input current to the Helmholtz coils and measuring the magnetic flux density with an EMDEX II magnetic field survey meter (Enertech, Campbell, CA). The variation of the magnetic fields within the space between the coils as determined by theoretical calculation and actual measurement was ± 3% of the mean. The ambient magnetic field in our laboratory (i.e., when the power supply to the coils was turned off) was 0.14 μT.

We have two similar exposure systems in two separate rooms in our laboratory. Thus, two exposure conditions could be run simultaneously. During exposure, rats were housed in a plastic cage (length, 45 cm; width, 21 cm; height, 22 cm) fitted with a Styrofoam cover. The cage was placed at the center of the space between the coils. During exposure, animals were provided with food and drinking water. Water was put in a plastic bottle fitted with a glass spout inserted through the Styrofoam cover. Up to three animals were exposed in a cage at a time.

Experimental procedures for effects of magnetic field exposure on DNA strand breaks in brain cells. **Magnetic field exposure at 0.01 mT for 24 and 48 hr.** In this experiment, rats were exposed in the Holmholtz coil system for 24 or 48 hr. Controls were exposed at the bucking mode for the same period of time. Immediately after exposure, one rat at a time was anesthetized by placing it in a covered foam box containing dry ice for 65 sec. (A piece of cardboard was placed on top of the dry ice to prevent its direct contact with the animal.) The rat was then decapitated and its brain was dissected out immediately for DNA strand break assay. To allow time for tissue processing, there was a 5-min time gap between animals.

Effects of drug treatments. In drug treatment experiments, there were four treatment groups in each experiment: magnetic field/drug, bucking/drug, magnetic field/drug vehicle, and bucking/drug vehicle. Animals were exposed for 2 hr to the magnetic field at 0.5 mT or exposed to the bucking mode. At 4 hr postexposure, animals were sacrificed as described above and their brains removed for DNA strand break assay. The 2-hr exposure/4-hr waiting schedule was used in our previous studies (Lai and Singh 1997a, 1997b).

The drug treatment schedules were as follows: for Trolox (Sigma Chemical Co., St. Louis, MO), 100 mg/kg/injection, dissolved in 5% (wt/vol) propylene glycol, injected intraperitoneally at a volume of 2 mL/kg at 24 hr before exposure and immediately after exposure; for deferiprone (CP 20 L1, a gift from R.A. Yokel, College of Pharmacy, University of Kentucky, Lexington, KY), 15 mg/kg/injection, dissolved in physiologic saline, injected intraperitoneally at a volume of 1 mL/kg immediately before and after exposure; for 7-nitroindazole (Sigma), 50 mg/kg/injection, dissolved in 5% (wt/vol) propylene glycol, injected intraperitoneally at a volume of 2 mL/kg at 30 min before exposure and immediately after exposure.

Drug-injection controls were similarly injected with an equal volume of the appropriate vehicle. The drugs used in this study are hydrophobic and could easily pass through the blood–brain barrier.

Assay methods for DNA single- and double-strand breaks. The microgel electrophoresis assay for DNA single- and double-strand breaks in rat brain cells was carried out as described previously (Lai and Singh 1997b). The technique involves making a microgel on a microscopic slide. The microgel consists of a cell suspension imbedded in low-melting-temperature agarose and phosphate-buffered saline (PBS). The cells are then lysed in the microgel in high salt and detergents, treated with proteinase K, and electrophoresed in a highly alkaline condition for single-strand break determinations and in a neutral condition for double-strand break determinations. The DNA is then stained with a fluorescent dye to allow visual measurement of the extent of DNA migration, an index of DNA damage. This method is more sensitive than other available methods in detecting DNA strand breaks. It can detect DNA single-strand breaks induced by 0.01 Gy of γ-rays (Singh et al. 1995) or 0.032 Gy of X rays (Singh et al. 1994), and double-strand breaks induced by 0.125 Gy of X rays (Singh and Stephens 1997) in human lymphocytes.

All chemicals used in the assay were purchased from Sigma unless otherwise noted. Immediately after removal from the skull, a brain was immersed in ice-cold PBS (NaCl, 8.01 g/L; KCl, 0.20 g/L; Na_2HPO_4, 1.15 g/L; KH_2PO_4, 0.20 g/L, pH 7.4) containing 200 μM *N-tert*-butyl-α-phenylnitrone. The brain was quickly washed four times with the PBS to remove most of the red blood cells. A tissue press was used to break up the brain tissue into small pieces (~1 mm^3) in 5 mL ice-cold PBS (Singh 1998). Four more washings with cold PBS removed most of the remaining red blood cells. Finally, in 5 mL PBS, tissue pieces were dispersed into single-cell suspensions using a P-5000 Pipetman pipette (Rainin Instruments, Oakland, CA). This cell suspension consisted of different types of brain cells. Ten microliters of this cell suspension was mixed with 0.2 mL 0.5% agarose (high-resolution 3:1 agarose; Amresco, Solon, OH) maintained at 45°C, and 50 μL of this mixture was pipetted onto a fully frosted slide (Erie Scientific Co., Portsmouth, NH) and immediately covered with a 24 × 50 mm rectangular #1 coverglass (Corning Glass Works, Corning, NY) to make a microgel on the slide. Slides were put in a cold steel tray on ice for 1 min to allow the agarose to gel. The coverglass was removed and 200 μL agarose solution was layered as before. Slides were then immersed in an ice-cold lysing solution (2.5 M NaCl, 1% sodium *N*-lauroyl sarcosinate, 100 mM disodium EDTA, 10 mM Tris base, pH 10) containing 1% Triton X-100.

To measure single-strand DNA breaks, after lysing for 3 hr at 4°C in an ice bath, slides were treated with DNase-free proteinase K (1 mg/mL; Amresco, Solon, OH) in the lysing solution without detergents overnight at 37°C. They were then put on the horizontal slab of an electrophoretic assembly (Hoefer Scientific, San Francisco, CA) modified so that both ends of each electrode are connected to the power supply. One liter of an electrophoresis buffer [300 mM NaOH, 0.1% 8-hydroxyquinoline, 2% dimethyl sulfoxide (DMSO), and 10 mM tetrasodium EDTA, pH 13] was gently poured into the assembly to cover the slides to a height of approximately 6.5 mm above their surface. After allowing 20 min for DNA unwinding, electrophoresis was started (0.4 V/cm, ~250 mA, for 60 min) and the buffer was recirculated.

At the end of the electrophoresis, slides were removed from the electrophoresis apparatus and immersed in an excess amount of neutralization buffer (1 M ammonium acetate in ethanol, consisting of 5 mL of 10 M ammonium acetate in 45 mL absolute ethanol) in a Coplin jar (two slides per jar) for 30 min.

After neutralization, the slides were dehydrated in absolute ethanol in a Coplin jar for 2 hr followed by 5 min in 70% ethanol and then air dried.

For double-strand breaks, microgel preparation and cell lysis were done as described above. Slides were then treated with ribonuclease A (Boehringer Mannheim Corp., Indianapolis, IN; 10 µg/mL in the lysing solution) for 2 hr and then with proteinase K (1 mg/mL in the lysing solution) overnight at 37°C. They were then placed for 20 min in an electrophoretic buffer (100 mM Tris, 300 mM sodium acetate, and acetic acid at pH 9.0), and then electrophoresed for 1 hr at 0.4 V/cm (~100 mA). The slides were neutralized and dehydrated in 1 M ammonium acetate in absolute ethanol and 70% ethanol and then air dried as described above.

Staining and DNA migration measurement procedures were similar for both single- and double-strand breaks. One slide at a time was prestained with 50 µL 5% DMSO in 30 mM NaH_2PO_4 and 5% sucrose, and then stained with 50 µL 1-µM solution of YOYO-1 (stock, 1 mM in DMSO from Molecular Probes, Eugene, OR) and then covered with a 24 × 50 mm coverglass. Slides were examined and analyzed with a Reichert vertical fluorescent microscope (model 2071) equipped with a filter combination for fluorescent isothiocyanate (excitation at 490 nm, emission filter at 515 nm, and dichromic filter at 500 nm). We measured the length of DNA migration by eye with a micrometer mounted in the eyepiece of the microscope. The migration length is defined as the length (in micrometers) from the beginning of the nuclear area to the last three pixels of DNA perpendicular to the direction of migration at the leading edge. It is used as the index of DNA strand breaks.

Two slides were prepared from the brain of each animal: one for assay of DNA single-strand breaks and the other for double-strand breaks. Fifty cells were randomly chosen and scored from each slide. However, cells that showed extensive damage with DNA totally migrated out from the nuclear region were not included in the measurement. These highly damaged cells probably resulted from the tissue and cell processing procedures, and they occurred equally in magnetic-field–exposed and bucking samples. Therefore, from each animal, 50 cells each were scored for single- and double-strand DNA breaks. The average migration length from 50 cells of a slide (an animal) was calculated and used in data analysis.

Effects of magnetic field exposure on apoptosis and necrosis of brain cells. In this experiment, rats were exposed to magnetic field (0.5 mT) for 2 hr or to the bucking mode. The method of Singh (2000) was used to study apoptosis and necrosis. This method has been validated with two other methods of apoptosis measurement (morphologic estimation and DNA ladder pattern) using several known apoptosis inducers (Singh 2000).

At 4 hr postexposure, microgel from brain cells was made and processed as described above for the microgel electrophoresis assay to remove lipid and protein. Instead of electrophoresis, slides were immersed for 10 min in 0.3 M NaOH and 0.2% DMSO to reveal apoptotic and necrotic cells. Then they were immersed in 1 M ammonium acetate in 50% ethanol for 10 min and then in 100% ethanol with 1 mg/mL spermine for 2 hr to fix the DNA in agarose. Slides were then immersed for 5 min in 70% ethanol. Slides were dried at room temperature and, after staining with YOYO-1, observed under a fluorescent microscope for characteristics of apoptosis and necrosis. The percentage of cells undergoing apoptosis and necrosis was scored from each slide.

In general, apoptosis is caused by programmed cleavage of DNA into a unique size of approximately 186 bases and its multiples. After cells are lysed, DNA from apoptotic cells, in alkaline condition, would diffuse in agarose in a wider area than that of normal cells. Because of this diffusion, DNA is lightly stained. Cells in early apoptosis are easily lysed and show a dense, diffuse, lightly stained, and granular DNA. These are easy to observe because of their larger size and diffuse staining characteristics. Cells in late apoptosis show highly condensed chromatin (intensely stained), even after lysis, and diffused DNA around this condensed spot. In general, nuclear DNA outline in apoptotic cells is diffuse and fuzzy. However, necrotic cells appear different from apoptotic cells after lysis and staining. Because of DNA strand breaks at random and at fewer sites, the nuclear DNA outline is sharply defined but occupies significantly larger area compared with normal cells.

The experiment was run under blind condition; that is, the experimenters who prepared the slides and did the DNA strand-break, apoptosis, and necrosis measurements did not know the treatment conditions of the animals from which the slides were prepared.

Data analysis. Data from the DNA strand break assay were analyzed by the one-way analysis of variance (ANOVA), and difference between two groups was evaluated by the Newman-Keuls test. Data of apoptosis and necrosis were analyzed by the Mann-Whitney *U*-test. A difference at $p < 0.05$ was considered statistically significant.

Results

Effects of 24- and 48-hr exposures to a 0.01-mT, 60-Hz magnetic field on DNA single- and double-strand breaks in rat brain cells are shown in Figures 1–4. Figures 1 and 2 show that magnetic field exposure increased single- and double-strand breaks, respectively, in brain cells. In addition, prolonging the duration of exposure from 24 to 48 hr significantly increased cumulative single- and double-strand breaks in cells: Single-strand breaks: $F(3,28) = 28.66$, $p < 0.01$; 24-hr vs. bucking, $p < 0.01$; 48-hr vs. bucking, $p < 0.01$; 24-hr vs. 48-hr, $p < 0.01$. Double-strand breaks: $F(3,28) = 17.91$, $p < 0.01$; 24-hr vs. bucking, $p < 0.01$; 48-hr vs. bucking, $p < 0.01$; 24-hr vs. 48-hr, $p < 0.05$. Figures 3 and 4 show, respectively, the distribution of cells according to their migration lengths of single and double DNA strand break measurements. Increase in cells with higher DNA strand breaks (longer DNA migration) shifts the distribution pattern to the right. The distribution patterns support the conclusion above from the data shown in Figures 1 and 2.

Effects of treatment with Trolox on magnetic-field–induced DNA single- and double-strand breaks are presented in Figures 5 and 6, respectively. ANOVA of the data shows significant treatment effect on both types of breaks: $F(3,28) = 79.61$, $p < 0.001$ for single-strand breaks, and $F(3,28) = 49.59$, $p < 0.001$ for double-strand breaks. Treatment with Trolox blocked the effects of the magnetic field on DNA strand breaks in brain cells.

Effects of deferiprone treatment are shown in Figures 7 and 8. Deferiprone treatment blocked the magnetic-field–induced increases in single- and double-strand breaks in brain cells [ANOVA shows significant treatment effects: $F(3,26) = 33.53$, $p < 0.001$ for single-strand breaks; $F(3,26) = 49.02$, $p < 0.001$ for double-strand breaks].

Similarly, the effects of 7-nitroindazole treatment are shown in Figures 9 and 10. 7-Nitroindazole treatment also blocked magnetic-field–induced increases in single- and double-strand breaks in brain cells [ANOVA shows significant treatment effects: $F(3,26) = 50.52$, $p < 0.001$ for single-strand breaks; $F(3,26) = 22.57$, $p < 0.001$ for double-strand breaks].

Data on apoptosis and necrosis of brain cells of rats after exposure to the 60-Hz magnetic field are shown in Table 1. Both apoptosis and necrosis were significantly increased by magnetic field exposure.

Discussion

Taken together, results from this series of experiments and our previous research show that by prolonging the duration of magnetic field exposure, DNA strand breaks can be observed in brain cells of the rat at a lower flux density. In previous research (Lai and Singh 1997a, 1997b), we found no significant increase in DNA double-strand breaks in brain cells of rats exposed for 2 hr to a 0.1-mT 60-Hz magnetic field. In the present

experiment, a significant increase in double-strand breaks was observed at 0.01 mT after 24 hr of exposure. These data indicate an interaction between intensity and duration of exposure on biologic effects of magnetic fields. More interestingly, a significantly larger increase in DNA single- and double-strand breaks was observed after 48 hr of exposure compared with 24-hr exposure. This suggests a cumulative nature of the effects.

Results from the drug-treatment experiments indicate the following: *a)* Trolox treatment can block the effects of magnetic fields on DNA strand breaks. This further supports the hypothesis that these effects of magnetic fields are mediated by free radicals, because Trolox is a potent free radical scavenger (Forrest et al. 1994). *b)* Nitric oxide may also be involved in the effects of magnetic fields on DNA (nitric oxide is also a free radical and plays important roles in cell functions). *c)* Data from the deferiprone treatment study suggest that iron may play a role in the effects of magnetic fields. This may also support the free radical hypothesis because iron is closely involved in free radical formation (e.g., via the Fenton reaction) in cells.

Relevant to our finding that magnetic fields can cause iron-dependent DNA strand breaks is that iron is present in higher concentration in the nucleus than in the cytoplasm because of the presence of an ATPase-related iron pump on the nuclear membrane (Meneghini 1997). Another study has reported iron atoms intercalated in DNA molecules, and DNA–ferrous iron complexes could enhance hydroxy radical formation from hydrogen peroxide compared with ferrous iron alone (Floyd 1981). These make DNA more vulnerable to iron-catalyzed free radical attack.

Increases in apoptosis and necrosis in brain cells of rats exposed to magnetic fields may also be related to free radical formation. Both hydroxy radical and nitric oxide have been shown to cause apoptotic and necrotic cell death, especially in brain cells (Simonian and Coyle 1996). In addition to the present study, others have shown apoptosis in various other cell types after exposure to extremely low-frequency electromagnetic fields (Blumenthal et al. 1997; Ismael et al. 1998; Phillips et al. 1997; Simko et al. 1998; Singh et al. 1994).

The free radical hypothesis that extremely low-frequency electromagnetic fields increase free radical activity in cells has been proposed by various researchers (Grundler et al. 1992; Reiter 1997). Effects of magnetic fields on cellular kinetics of free radicals (Eveson et al. 2000; Khadir et al. 1999; Roy et al. 1995) and free radical–related cellular processes (Fiorani et al. 1997; Katsir and Parola 1998) have been reported. Free radical–induced damage to DNA could have important effects on health (Beckman and Ames 1997). In addition to DNA damage, free radicals can cause damage in other biologic molecules,

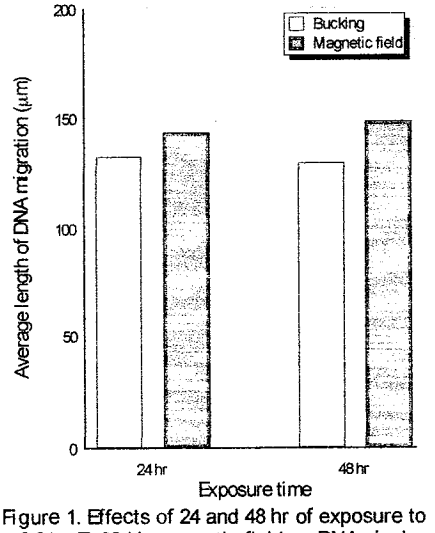

Figure 1. Effects of 24 and 48 hr of exposure to a 0.01-mT, 60-Hz magnetic field on DNA single-strand breaks in brain cells of the rat. n = 8 for each treatment group. Magnetic field significantly different from sham at $p < 0.01$ for both 24- and 48-hr exposure.

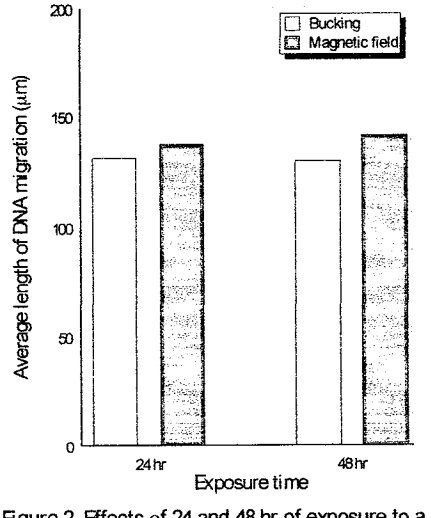

Figure 2. Effects of 24 and 48 hr of exposure to a 0.01-mT, 60-Hz magnetic field on DNA double-strand breaks in brain cells of the rat. n = 8 for each treatment group. Magnetic field significantly different from sham at $p < 0.01$ for both 24- and 48-hr exposure.

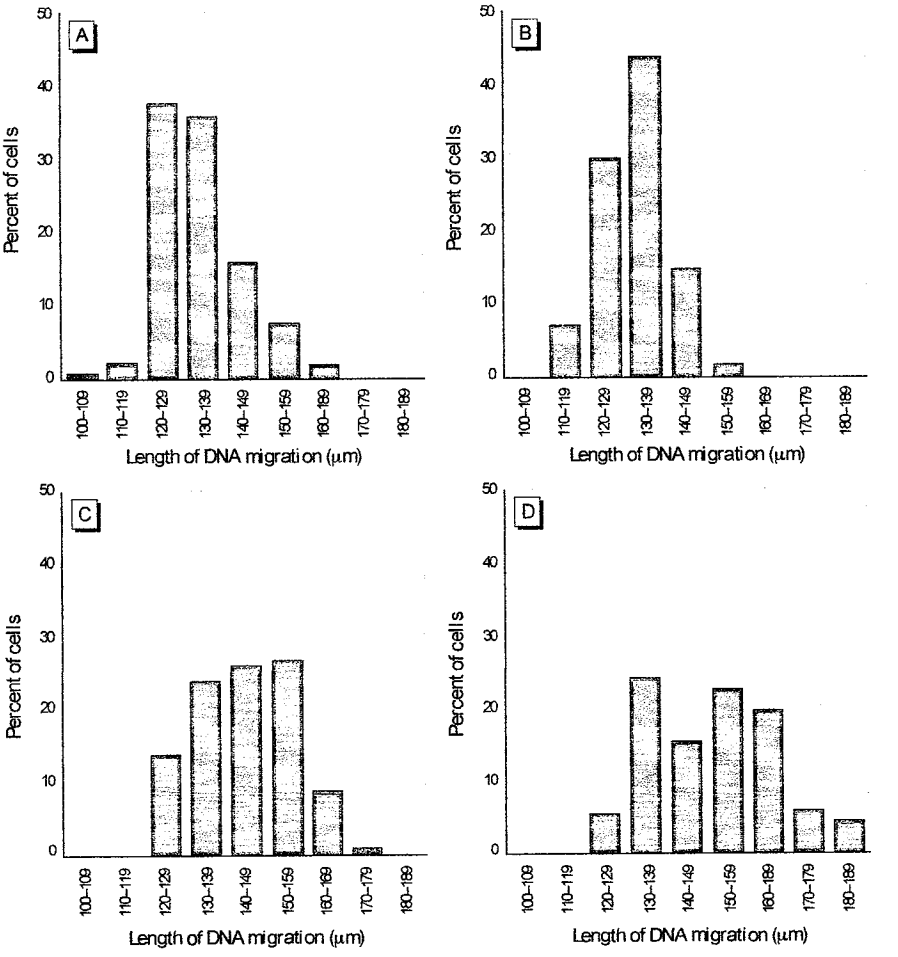

Figure 3. Percentage distribution of cells as a function of DNA migration length (single-strand breaks) of the data shown in Figure 1. (A) Bucking, 24 hr. (B) Bucking, 48 hr. (C) 0.01 mT, 24 hr. (D) 0.01 mT, 48 hr.

such as lipids and proteins, and can profoundly affect cellular homeostasis. In addition, under subtoxic conditions, free radicals are known to play an important role in cellular signal transduction processes (Suzuki et al. 1997). Disturbance in free radical metabolism could affect these biomolecular processes and cell functions.

Data from the present experiments suggest that magnetic-field–induced DNA strand breaks are caused by an iron-mediated free radical process, probably via the Fenton reaction, which converts hydrogen peroxide to the more potent and toxic hydroxy radical (Figure 11). Iron-induced oxidant formation is known to cause DNA strand breaks, DNA–protein cross-links, and activation of protein kinase C; increase the production of heat-shock proteins; and alter calcium homeostasis in cells (Altman et al. 1995; Farber 1994; Mello Filho and Meneghini 1984; Meneghini 1997; Stohs and Bagchi 1995). Other recent experiments have also implicated the involvement of iron/transition metals in the effects of electromagnetic fields. Zmyslony et al. (2000) reported an increase in DNA strand breaks in lymphocytes exposed to a 50-Hz magnetic field in the presence of ferrous chloride in the medium, whereas exposing the cells in the absence of ferrous ion had no significant effect. Further experiments from the same group of researchers (Jajte et al. 2001) showed that the effect was blocked by melatonin, suggesting the involvement of free radicals. An experiment by Lourencini da Silva et al. (2000) also implies that electromagnetic fields can cause damage in DNA plasmids in the presence of a transition metal (tin).

Our data show that inhibition of nitric oxide synthase by 7-nitroindazole can completely block the effects of magnetic fields on DNA. We propose that the effects of magnetic fields manifest through a two-stage process. In the first step, magnetic field exposure affects iron homeostasis in certain cells, leading to an increase in free iron in the cytoplasm and nucleus, which in turn leads to an increase in hydroxy radicals, via the catalytic reaction of the Fenton reaction, which damage DNA, lipids, and proteins. Damage to lipids (lipid peroxidation) in the cellular membrane in turn leads to an increase in calcium leakage from internal storage sites in the cell. This will trigger the second step, an increase in nitric oxide synthesis via the activation of calmodulin-dependent nitric oxide synthase. 7-Nitroindazole is an effective blocker of that enzyme (Kalisch et al. 1996). Involvement of nitric oxide in the biologic effects of magnetic

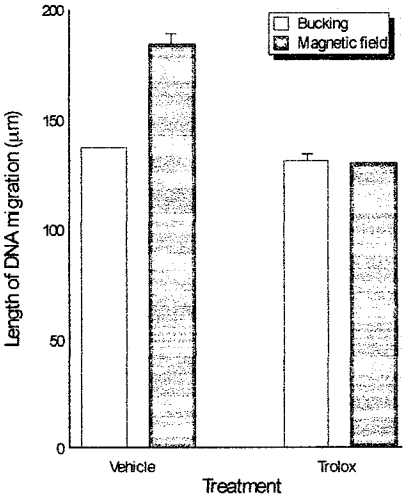

Figure 5. Effect of treatment with Trolox on magnetic-field–induced increase in DNA single-strand breaks in rat brain cells (mean ± SE). Trolox (100 mg/kg) was injected intraperitoneally at 24 hr and immediately before exposure to a magnetic field or the bucking mode. Drug-treatment controls were similarly injected with equal volume of the drug vehicle (propylene glycol). n = 8 for each treatment group. Magnetic field significantly different from sham at $p < 0.01$ in vehicle-treated animals. No significant difference between magnetic field and sham in Trolox-treated animals.

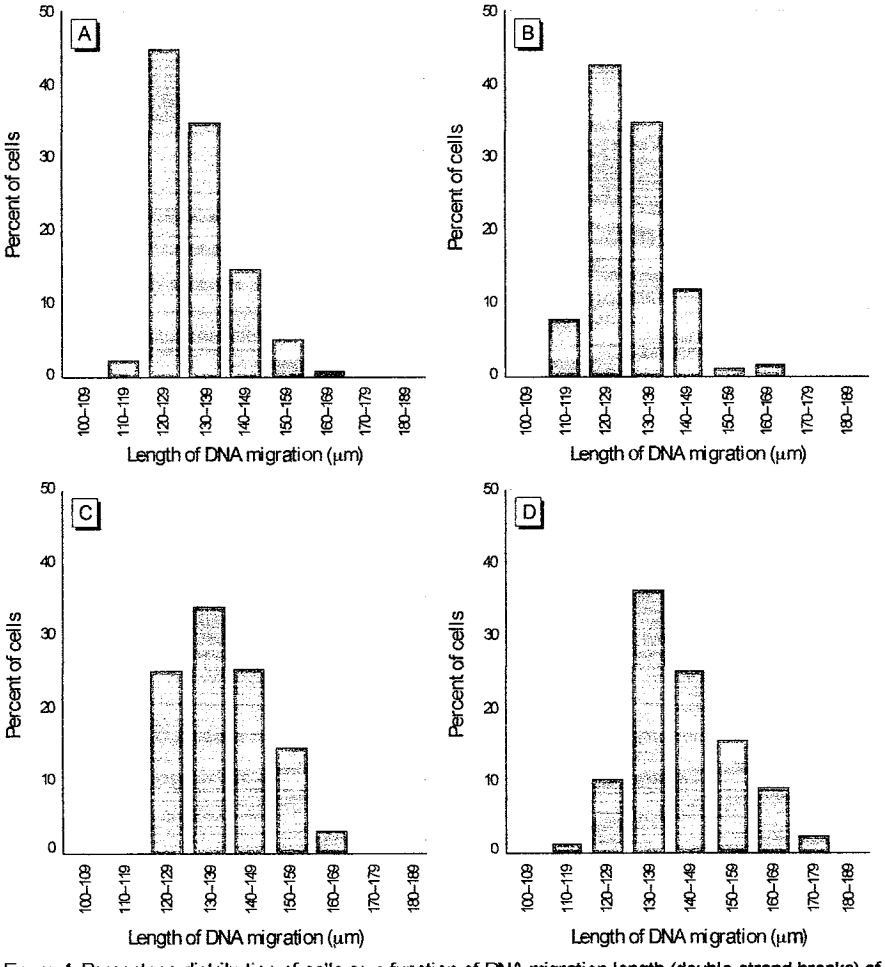

Figure 4. Percentage distribution of cells as a function of DNA migration length (double-strand breaks) of the data shown in Figure 2. (A) Bucking, 24 hr. (B) Bucking, 48 hr. (C) 0.01 mT, 24 hr. (D) 0.01 mT, 48 hr.

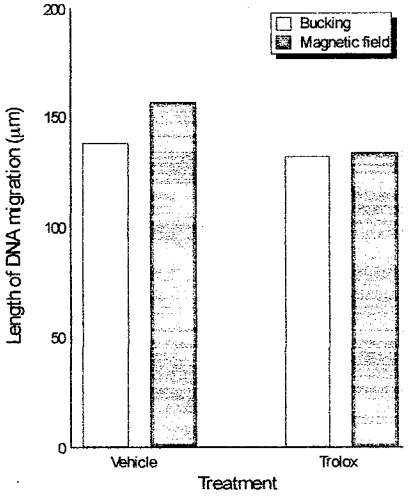

Figure 6. Effect of treatment with Trolox on magnetic-field–induced increase in DNA double-strand breaks in rat brain cells (mean ± SE). Treatment conditions were similar to those described for Figure 5. n = 8 for each treatment group. Magnetic field significantly different from sham at $p < 0.01$ in vehicle-treated animals. No significant difference between magnetic field and sham in Trolox-treated animals.

fields has been proposed by Adey (1997) and Yoshikawa et al. (2000).

In the second stage, DNA and other macromolecular damages are probably caused mainly by nitric oxide. Because the hydroxy radical has only a short distance of action (~40 Å), whereas nitric oxide can diffuse the distance of several cell diameters, the transition from stage 1 to stage 2 changes the magnetic-field–triggered free radical damage from a localized event to a more widespread phenomenon. Nitric oxide can further amplify iron-mediated free radical formation via its effects on iron metabolism (Richardson and Ponka 1997) and release of iron from ferritin (Reif and Simmons 1990). Thus, the effects will amplify. This damage can lead to two possible outcomes: *a*) Exogenous and endogenous cellular antioxidation processes will keep the damage in check by neutralizing free radicals, and eventually the cell will repair itself and survive. However, DNA damage and repair could lead to mutation and increase the chance of carcinogenesis. *b*) If the processes of free radical damage are not checked by cellular antioxidation and repair processes, the cell will die, because free radical peroxidation of lipids is a chain reaction. Both apoptosis and necrosis are possible. Increase in necrosis is probably a consequence of lipid peroxidative damage in cell membranes, especially that of mitochondria, whereas apoptosis is mainly triggered by DNA damage. The outcome of oxidative damage induced by magnetic fields will therefore depend on various factors, including the oxidative status of the cell, capability of endogenous antioxidation enzymes and processes to counteract free radical buildup, availability of exogenous antioxidants, iron homeostasis (a balance of iron influx, storage, and use), the parameters of exposure (e.g., intensity and duration of exposure and possibly the waveform of the magnetic field), and whether the oxidative damage is cumulative. Oxidative damage to DNA and its subsequent misrepair (i.e., mistakes in repairing the damage) are probably cumulative. To add to this, nitric oxide can be either mutagenic or cytotoxic (i.e., causing cell death) depending on intracellular conditions. It has been suggested that nitric oxide is

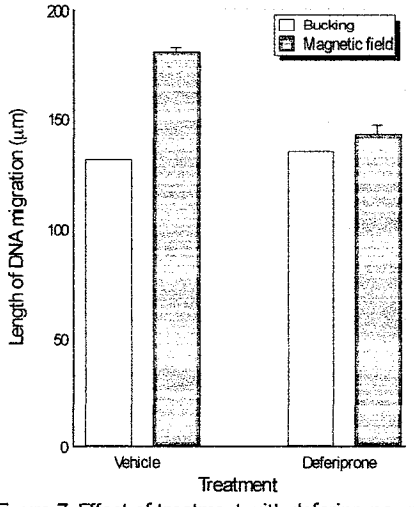

Figure 7. Effect of treatment with deferiprone on magnetic-field–induced increase in DNA single-strand breaks in rat brain cells (mean ± SE). Deferiprone (5 mg/kg) was injected intraperitoneally immediately before and after exposure to a magnetic field or to the bucking mode. Drug-treatment controls were similarly injected with equal volume of the drug vehicle (physiologic saline). n = 8 for vehicle group, 7 for deferiprone treatment group. Magnetic field significantly different from sham at $p < 0.01$ in vehicle-treated animals. No significant difference between magnetic field and sham in deferiprone-treated animals.

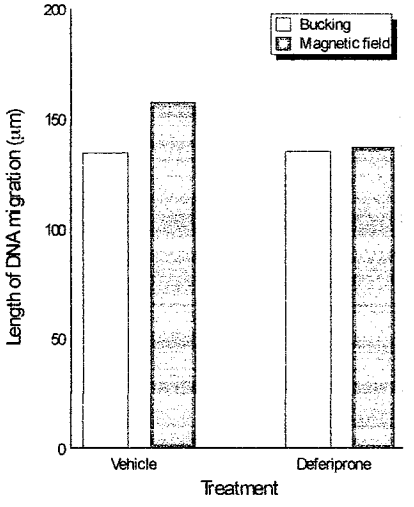

Figure 8. Effect of treatment with deferiprone on magnetic-field–induced increase in DNA double-strand breaks in rat brain cells (mean ± SE). Treatment conditions were similar to those described for Figure 7. n = 8 for vehicle group, 7 for deferiprone treatment group. Magnetic field significantly different from sham at $p < 0.01$ in vehicle-treated animals. No significant difference between magnetic field and sham in deferiprone-treated animals.

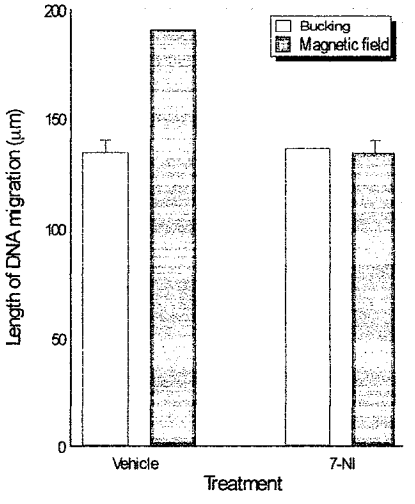

Figure 9. Effect of treatment with 7-nitroindazole (7-NI) on magnetic-field–induced increase in DNA single-strand breaks in rat brain cells (mean ± SE). 7-NI (50 mg/kg) was injected intraperitoneally at 30 min before and immediately after exposure to a magnetic field or to the bucking mode. Drug-treatment controls were similarly injected with equal volume of the drug vehicle (propylene glycol). n = 8 for each treatment group. Magnetic field significantly different from sham at $p<0.01$ in vehicle-treated animals. No significant difference between magnetic field and sham in 7NI-treated animals.

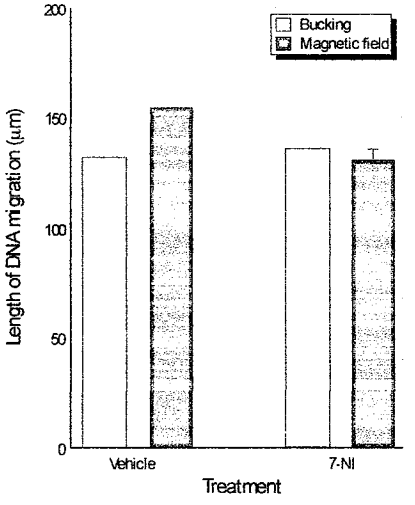

Figure 10. Effect of treatment with 7-nitroindazole (7-NI) on magnetic-field–induced increase in DNA double-strand breaks in rat brain cells (mean ± SE). Treatment conditions were similar to those described for Figure 9. n = 8 for each treatment group. Magnetic field significantly different from sham at $p < 0.01$ in vehicle-treated animals. No significant difference between magnetic field and sham in 7NI-treated animals.

Table 1. Apoptosis and necrosis of brain cells of rats after exposure to magnetic fields.

	Percent	No.	p-Value
Apoptosis			
Magnetic field	0.61	8	
Bucking	0.28	8	<0.025
Necrosis			
Magnetic field	1.88	8	
Bucking	0.99	8	<0.02

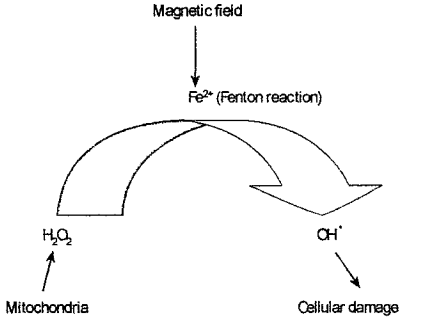

Figure 11. Schematic diagram of mechanism of effect of magnetic fields involving the Fenton reaction and free radicals.

mutagenic when the intracellular level of reduced glutathione is low, but cytotoxic (leading to apoptosis and inhibition of tumor growth) in a thiol-rich cellular environment that favors the formation of toxic nitrosothiols (Felley-Bosco 1998).

Summing up, we propose that magnetic fields initiate an iron-dependent free radical generation process in cells, which can lead to genotoxic changes and/or cell death. From this hypothesis, one can make the following speculation regarding the biologic effects of magnetic fields: Cells with high rates of iron intake (e.g., proliferating cells, cells infected by virus, and cells with high metabolic rates such as brain cells) would be more susceptible to the effects of magnetic fields because hydrogen peroxide, the substrate of the Fenton reaction, is a metabolic product of mitochondria. This may partially explain the negative results of two previous studies investigating the effect of magnetic fields on DNA. Reese et al. (1988) reported no significant effect of a 60-Hz magnetic field (0.1 and 2 mT for 1 hr) on DNA single-strand breaks in Chinese hamster cells. However, during exposure, in order to decrease DNA repair, their cells were kept under iced conditions. In a study by Fairbairn and O'Neill (1994), cells were first suspended in agarose on a slide before exposure to a 50-Hz pulsed magnetic field (peak flux density, 5 mT; pulse duration, 3 msec). Cells suspended in agarose are not in good physiologic conditions. Thus, in both of these studies, cells studied were probably not in a very active metabolic state.

A question is whether the DNA strand breaks induced by magnetic fields in our studies (Lai and Singh 1997a; present results) are biologically significant. The flux densities (0.01–0.5 mT) used in our studies are within the levels that one could encounter in the environment. Household and office levels of extremely low-frequency magnetic fields can vary from 0.01 to 1 μT. Intermittent levels can reach more than 10 μT. Levels near a power transmission line can be 10–30 μT, whereas the magnetic flux density can vary between 0.1 and 1 mT near some electrical appliances (e.g., electric blankets, hair dryers). Much higher levels are expected in occupational exposures (Bernhardt 1985; Gauger 1984; Krause 1986; Tenforde and Kaune 1987).

To compare with the effect of ionizing radiation, we have exposed rats to 2 Gy of X rays and assayed DNA single-strand breaks in their brain cells. A peak increase of 76% was observed at 30 min after exposure (average length of DNA migration in nonexposed controls = 133 ± 2.2 μm; in X-ray–exposed rats = 234 ± 2.2 μm; $n = 3$ in each treatment (unpublished data). It may not be appropriate to compare DNA damage caused by X rays with those by magnetic fields directly, because different mechanisms may be involved. However, from the data presented in Figures 1 and 2, one can infer that the effect of environmental magnetic fields on DNA is relatively small compared with that of 2 Gy of X rays. It seems that cells may respond differently to high and low levels of DNA damage. A recent report by Rothkamm and Lobrich (2003) indicates a lack of DNA double-strand break repair in nondividing human fibroblasts exposed to very low X-ray doses (~1 mGy). Rothkamm and Lobrich speculated that "instead of repairing a DSB [double-strand break] in a particular cell with the risk of causing genetic alterations, it could be beneficial for an organism to remove the damaged cell and replace it by the division of an undamaged neighboring cell." However, in the case of neurons that cannot divide and be replaced, such a response could lead to neurodegenerative diseases.

The human brain contains relatively high amount of nonheme iron, mainly in glial cells and myelin. It has been speculated that iron is used in the production and maintenance of myelin by oligodendrocytes (Francois et al. 1981; Gerber and Connor 1989). Thus, myelinated nerve fibers, such as those of motor neurons, could be more susceptible to damage by magnetic fields. Increased risk of neurodegenerative diseases due to magnetic field exposure could be a result of the death of neurons and glial cells or demyelination. Increased risks of amyotropic lateral sclerosis (Davanipour et al. 1997; Hakansson et al. 2003; Johansen and Olsen 1998; Savitz et al. 1998), Alzheimer's disease (Feychting et al. 2003; Hakansson et al. 2003; Sobel et al. 1995), and Parkinson's disease (Noonan et al. 2002) have been reported in occupational exposure to extremely low-frequency electromagnetic fields.

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Exhibit 8

SECTION 7

The Cellular Stress Response: EMF-DNA Interaction

2012 Supplement

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ABSTRACT

The research on stress proteins stimulated by EMF was reviewed by the author in the BioInitiative Report (2007) as well as in the special issue of Pathophysiology (2009) devoted to EMF. This review emphasizes the more recent research on the mechanism of interaction of EMF with DNA. It appears that the DNA molecule is particularly vulnerable to damage by EMF because of the coiled-coil configuration of the compacted molecule in the nucleus. The unusual structure endows it with the self similarity of a fractal antenna and the resulting sensitivity to a wide range of frequencies. The greater reactivity of DNA with EMF, along with a vulnerability to damage, underscores the urgent need to revise EMF exposure standards in order to protect the public. Recent studies have also exploited the properties of stress proteins to devise therapies for limiting oxidative damage and reducing loss of muscle strength associated with aging.

I. INTRODUCTION

The cellular stress response is a protective reaction of individual cells to potentially harmful stimuli in the environment. It is characterized by the synthesis of a class of proteins referred to as stress proteins. The cellular stress response differs from the more familiar responses of entire organisms to stresses that lead to secretion of cortisol and adrenalin and that result in the activation of various systems throughout the body. The cellular stress response, as the name indicates, is a specific response of individual cells, and stress proteins are the chemical agents that also serve as markers.

The cellular stress response was first described as a reaction to elevated temperature (Ritossa, 1962), which accounts for the proteins initially being called heat shock proteins. Several physical and chemical environmental influences have since been found to evoke the response, and in 1994, Goodman and Blank (1994) were the first to show that the response was stimulated by EMF. In fact, the cells were far more sensitive to EMF than to thermal stimuli, the threshold energy of the EMF stimulus being more than one billion times weaker than an effective thermal stimulus (Blank , Goodman, 1994).

The 'heat shock' response, i.e., hsp synthesis, is activated by a variety of potentially harmful stresses, including physical stimuli like pH and osmotic pressure changes, as well as chemicals such as ethanol and toxic metal ions like Cd^{2+}. The ability of EMF in the power frequency (extremely low frequency, ELF) range (Goodman, Blank, 1998) to evoke this response was followed by reports of similar effects due to radio frequency (RF) fields (de Pomerai et al. 2003) and amplitude modulated RF fields (Czyz et al, 2004).

The finding that EMF evoked the cellular stress response had obvious and important biological implications:

- Because the cellular stress response is a reaction to potentially harmful stimuli in the environment, the cells were asserting that *EMF is potentially harmful* to cells.
- Because EMF stimulated protein synthesis, it meant that *EMF causes the two strands of DNA to come apart* for the protein code to be read and for synthesis to proceed.
- Since *EMF can interact with DNA*, it can cause *errors during replication*, as well as during protein synthesis, and higher energy EMF could be expected to cause *DNA strand breaks*, as has been observed (Lai and Singh, 1995).
- The incremental increase of DNA strand breaks with increases in field strength indicates a *dose-response*, evidence in support of EMF as the responsible agent.

II. CELLULAR STRESS PROTEINS ARE A NEW CLASS OF PROTEINS

Proteins are important components of cells and make up about 50% of the dry weight of most cells. The many different proteins are classified according to their functions, and stress proteins are now recognized as a new class of proteins with functions related to cell protection. Stress proteins join such well-known categories as contractile proteins (e.g. actin, myosin), catalytic proteins or enzymes (e.g. pepsin, amylase), transport proteins

(e.g. ATPases for ions across membranes, hemoglobins for blood gases, cytochromes for electrons), etc. Stress proteins were originally described as being synthesized in response to external stimuli and that is currently the area of greatest interest. However, they are also present constitutively.

Cellular stress proteins are synthesized when cells come in contact with stimuli that cause damage to macromolecules (Kultz, 2005), and the stress proteins aid in the repair and transport of these molecules. Because the first stimulus identified was an increase in temperature, the proteins were called 'heat shock' proteins and designated using the original terminology that starts with 'hsp' (for 'heat shock' protein) and a number equal to the molecular weight in kilodaltons.

The transition from heat shock protein to stress protein should alert (perhaps even alarm) the government agencies responsible for setting EMF safety standards. The thermal stimuli that evoked synthesis of protective proteins were believed to be dangerous for cells, but now we see that non-thermal EMF stimuli cause the same protective reactions in cells. The heat shock response and the EMF stress response both relate to the threshold for biological damage, and we should realize that EMF damage is caused by non-thermal stimuli. Compared to the energy needed to stimulate heat shock, EMF requires but a small fraction of the thermal energy needed to produce the same response (Blank et al., 1992).

The government agencies that assess safety of EMF exposure assume that danger is associated with an increase in temperature, i.e., a thermal criterion. It is clear from the responses of cells that the safety of EMF exposure, as indicated by the synthesis of protective stress proteins, is unrelated to the temperature increase. The cells are very sensitive to EMF, and the protective biological response to EMF occurs long before there is a significant change in temperature. It should be obvious that EMF safety standards are based on false assumptions and must be revised to reflect the scientific evidence. Non-thermal EMF stimuli are potentially harmful.

III. PROTEIN SYNTHESIS

The stress response, like all protein synthesis, indicates that all of the different physical and chemical stimuli that can initiate this response cause the two strands of DNA to come apart for the amino acid code for protein synthesis code to be read. Therefore, the observed stress protein synthesis is evidence that EMF has interacted with the DNA to start this process. The research showing that EMF in both the ELF and RF frequency ranges can also cause DNA strand breaks (Lai, Singh, 1995; 1996; Reflex Report 1994), suggests that the two phenomena are due to the same interaction mechanism, and that there is greater molecular damage with greater EMF energy.

Many research papers and some reviews have been published since the cellular stress response was reported to be stimulated by EMF. In addition to earlier reviews on EMF stimulation of the cellular stress response in the ELF (Goodman, Blank, 1998) and RF (Cotgreave, 2005) ranges, the subject was reviewed in Pathophysiology (Blank, 2009). Also, Calderwood (2007) has edited the volume on cell stress proteins in volume 7 of the series Protein Reviews. A recent (ICEMS, 2010) review on EMF and Bio-Effects includes many papers focused on a variety of possible EMF interaction mechanisms, but does not review the stress response, the stimulation of DNA or biosynthesis.

Section 7 of the Bioinitiative Report summarized both ELF and RF studies, mainly at frequencies 50 Hz, 60 Hz, 900MHz and 1.8 GHz. The citations in that review were not exhaustive, but the different frequencies and many different cells indicated the diversity of results on stimulation of DNA and stress protein synthesis. The many different types of cells that respond to EMF, both *in vivo* and *in vitro*, include epithelial, endothelial and epidermal cells, cardiac muscle cells, fibroblasts, yeast, *E. coli*, developing chick eggs, and dipteran cells.

It is clear that the stress response does not occur in reaction to EMF in all types of cells, and that tissue cultured cells (as opposed to natural cells) are less likely to show an effect of EMF, probably because immortalized cells have been changed significantly to enable them to live indefinitely in unnatural laboratory conditions. Even the same cell line from

two different suppliers can respond differently. Jin et al. (1997) showed that HL60 cells from one supplier reacted to EMF while identically labeled cells from another supplier did not respond. Some cancer cells (e.g., MCF7 breast cancer cells) have responded to EMF (Liburdy et al., 1993; Lin et al., 1998), and Czyz et al. (2004) found that p53-deficient embryonic stem cells showed an increased EMF response, but the wild type did not. Ivanscits et al., 2005) found no genotoxic effects (i.e., DNA damage) in lymphocytes, monocytes and skeletal muscle cells, but did find effects with fibroblasts, melanocytes and rat granulosa cells. Lantow et al. (2006) and Simko et al. (2006) found that blood elements, such as lymphocytes and monocytes did not respond. Obviously, the cellular stress response is widespread but not universal.

IV. MECHANISM OF PROTEIN SYNTHESIS BY EMF

The stress response has provided an opportunity to investigate EMF interaction with DNA, and in particular, how this results in stimulating DNA to start the synthesis of proteins. Because the DNA sequence is known for hsp70, it was possible to study the effects of changes in the DNA sequence on protein synthesis. As a result of these experiments, it was possible to identify two distinct regions in the promoter region of the HSP 70 gene - an EMF sensitive region that was not sensitive to increased temperature, as well as a region sensitive only to temperature. The EMF sensitive domain contains number of nCTCTn myc-binding sites relative to the transcription initiation site and upstream of the temperature sensitive binding sites (Lin et al. 1999; 2001). These electromagnetic response elements (EMREs) are also found on the c-*myc* promoter which also reacts to EMF.

The EMF sensitivity of the DNA sequences, nCTCTn, was demonstrated by transfecting these sequences into CAT and Luciferase reporter genes and stimulating those genes (with EMF) to synthesize CAT and luciferase, respectively (Lin et al., 1999; 2001). Thus, the HSP70 promoter contains different DNA regions that are specifically sensitive to thermal and non-thermal stressors. This biological mechanism is obviously based on direct interaction with specific segments of DNA, and there is reason to believe that EMF can interact similarly with other segments of DNA. In our experiments, induction of

increased levels of hsp70 by EMF is rapid and occurs at extremely low levels of energy input, 14 orders of magnitude lower than with a thermal stimulus (Blank et al. 1994).

V. EMF INTERACTION WITH SIGNALING PATHWAYS

EMF penetrate cells unattenuated and so can interact directly with the DNA in the cell nucleus, as well as with other cell constituents. The above-cited experiments demonstrating the ability of electromagnetic response elements (EMREs) to interact with EMF, after being transferred to another DNA chain, is further support for direct EMF-DNA interaction as the most likely mechanism for EMF initiation of the cellular stress response.

In contrast to EMF, most biological agents are impeded by membranes and require special mechanisms to gain access to the cell interior. Friedman et al, (2007) have demonstrated that, in those situations, the initial step in transmitting extracellular information from the plasma membrane to the nucleus of the cell occurs when NADH oxidase rapidly generates reactive oxygen species (ROS). These ROS stimulate matrix metalloproteinases that allow them to cleave and release heparin binding epidermal growth factor. This secreted factor activates the epidermal growth receptor, which in turn activates the extracellular signal regulated kinase 1\2 (ERK) cascade. The ERK cascade is one of the four mitogen-activated protein kinase (MAPK) signaling cascades that regulate transcriptional activity in response to extracellular stimuli.

Stress protein synthesis can occur by direct interaction of EMF with DNA, as well as by membrane mediated stimulation via chemical signaling. While both mechanisms are possible, it is of interest to note that the body responds directly to physical inputs when there is a need for a rapid response. The body cannot rely upon slowly responding pathways for the synthesis of a relatively large amount of urgently needed protein molecules. The signal pathways function primarily as a mechanism for maintaining homeostasis by minimizing change and responding slowly to stimuli.

VI. INSIGHTS FROM MUSCLE PROTEIN SYNTHESIS

EMF stimulated protein synthesis may appear to be an unnatural mechanism, but it is essentially the same as the natural process in striated muscle. The only difference is that the electrons in DNA are driven by EMF, while in striated muscle, they are driven by the changes in electric (membrane) potential that cause contraction. Striated muscle is a tissue that requires steady protein synthesis to ensure proper function. Protein synthesis is initiated by the same electric currents that stimulate the muscle contractions. Body builders know that one must stimulate muscle contraction in order to increase muscle mass, and biologists have shown that the electric currents that flow across the muscle membranes during contraction pass through the DNA in the muscle nuclei and stimulate protein synthesis.

Muscle nuclei are not spread evenly throughout a muscle fiber, but are located near the muscle membranes that carry the currents. This means that the DNA in the nuclei can be stimulated every time the muscle is stimulated. The estimated magnitude of electric field along the muscle nuclei, ~10V/m, provides a large safety margin in muscle, since fields as low as 3mV/m were found to stimulate biosynthesis in HL60 cells (Blank et al, 1992).

Studies showing effects of EMF on electron transfer reactions in solution suggest that ionic (electric) currents affect electron movements within DNA in much the same way (Blank, 1995). Both electric and EMF (AC magnetic fields) stimulate protein synthesis in HL60 cells and have similar effects on electron transfer in the Na,K-ATPase (Blank and Soo, 2001a; 2001b). This suggests that interaction with DNA, of both electric fields and EMF, initiate stress protein synthesis by a similar mechanism.

Studies on muscle protein synthesis also suggest the possibility of a

frequency code that controls the particular segment of DNA that is activated. Studies have shown that different proteins can be synthesized by changing the frequency of the action potentials that stimulate the process. These experiments were possible because 'fast' and 'slow' muscles contract at different rates because they are composed of different proteins. For this reason it was possible to stimulate muscles at different rates and to study changes in the proteins as a result of changing the frequency of the action potentials (Pette, Vrbova, 1992). The review by Blank (1995) includes many additional experiments that show the importance of the frequency in controlling the segment of the muscle DNA that is affected by the current and translated into protein.

Studies of effects of EMF on well characterized electron transfer reactions, involving cytochrome oxidase, ATP hydrolysis by Na,K-ATPase, and the Belousov–Zhabotinski (BZ) redox reaction, have shown that:

- EMF can accelerate electron transfer rates
- EMF acts as a force that competes with the chemical forces driving a reaction. This means that the effect of EMF varies inversely with the intrinsic reaction rate, and that EMF effects are only seen when intrinsic rates are low. (*N.B. EMF has a greater effect when the system is in a rundown state.*)
- Experimentally determined thresholds are low (~0.5μT).
- Effects vary with frequency, with different optima for the reactions studied: The two enzymes showed broad frequency optima close to the reaction turnover numbers for Na,K-ATPase (60 Hz) and cytochrome oxidase (800 Hz), suggesting that EMF interacted optimally when in synchrony with the molecular kinetics. EMF interactions with DNA in both ELF and RF ranges and do not appear to involve electron transfer reactions with well-defined kinetics.

The effects of EMF on electron transfer reactions were studied in the ELF frequency range, and one would expect differences in the RF range. However, the situation is more

complicated. The effects of EMF on electrons in chemical reactions were detected in the Na,K-ATPase when electric or magnetic fields, each accelerated the reaction only when the enzyme was relatively inactive, i.e., the chemical driving forces were weak. These experiments enabled an estimate of the electron velocity as approximately 10^3 m/s (Blank and Soo, 2001a; 2001b), a velocity similar to that of electrons in DNA. An electron moving at a velocity of 10^3 m/s crosses the enzyme ($\sim 10^{-8}$ m) before the ELF field has had a chance to change. This means that a low frequency effect on fast moving electrons in DNA or in enzymes should be viewed as effectively due to a repeated DC pulse. In the RF range, the pulse train is longer.

VII. DNA IS A FRACTAL ANTENNA

Human DNA is about 2 m long, and the molecule is greatly compacted so that it fits into the nuclei of cells that are microns in diameter.

DNA has a unique double helical structure where two strands of DNA are bound together by hydrogen bonds between pairs of nucleotide bases (one on each strand) and they form a long twisted ribbon with delocalized π electrons that form continuous planar clouds on both surfaces of the ribbon. The result is a structure with two continuous paths that can conduct an electron current along the DNA.

Many studies, initially from the laboratory of Barton at Cal Tech (Hall et al, 1996), have shown that DNA does indeed conduct electrons. As would be expected, the rate of conduction can be influenced by the detailed structure of DNA. Changes, such as hairpin turns and mismatched bases, can lead to the disruption of the ordered double helical structure and anomalies in the rate of electron flow (Arkin et al, 1996; Hall et al, 1997; Lewis et al, 1997; Kelley et al, 1999; Giese, 2002). Electron flow can lead to local charging as well as oxidative damage.

Variations in the rate of electron flow can lead to the accumulation of charge at bottlenecks. The temporary buildup of charge at a site results in strong repulsive forces that can cause a disruption of H-bonds. A net charge can even disrupt the structure of a complex molecule, such as occurs when the four protein chains of hemoglobin

disaggregate in response to a gradual buildup of charge in the hemoglobin tetramer (Blank, 1984; Blank and Soo, 1998). For similar reasons, one would expect disaggregating forces at the DNA site where charge builds up. This would be expected to occur more easily in a compact structure such as DNA in the nucleus.

The tightly coiled DNA in the nucleus uses fractal patterns in order to occupy space efficiently. A fractal is a shape that displays *self-similarity*, where each part of the shape resembles the entire shape. Thus, the double helix is wound into a coil and that coil is wound into a larger coil, and so on. DNA in a cell nucleus is a coiled-coil many times over.

Since the DNA molecule in the nucleus conducts electricity and is organized in a self-similar pattern, it has the two key characteristics of *fractal antennas* when interacting with EMF (Blank, Goodman 2011). Fractal design is desirable for an antenna because it minimizes the overall size, while reacting to a wide range of electromagnetic frequencies. However, these characteristics are not desirable in DNA, because of the many frequencies in the environment that can and do react with DNA. The almost continuous cloud of delocalized electrons along both faces of the 'ribbon' formed by the base pairs provides a conducting path for responding to EMF and makes it more vulnerable to damage. The chemical changes that result from electron transfer reactions, are associated with molecular damage in DNA.

VIII. DNA DAMAGE AND CANCER

Stress proteins are essential for cell protection. They help defend cells against damaging forces like increases in temperature and reductions in oxygen supply that could be life-threatening. Similarly, the body generates stress proteins to strengthen cellular resistance to the effects of EM radiation. However, stress protein synthesis is really only an emergency measure that is designed to be effective in the short term. The response to repeated stimuli diminishes with repeated exposure and this could be dangerous.

Thermotolerance, the ability to tolerate higher temperatures as a result of repeated exposures to high temperature, was originally demonstrated at the molecular level in connection with heat shock. Repeated exposure to increased temperature resulted in a decreased heat shock response. A similar mechanism applies when the cellular stress response is stimulated by EMF, since repeated EMF stimuli result in lower production of stress proteins. This could very well be a mechanism by which repeated exposure to EMF can result in less protection and more damage to molecules like DNA. The lower protection predisposes exposed individuals to an increased risk of mutation and initiation of cancer.

DiCarlo and Litovitz (2008) at Catholic University in Washington, D.C. demonstrated the development of EMF tolerance in an experiment performed on chicken embryos. In those eggs exposed to ELF-radiation of 8 μT for 30 or 60 minutes at a time, twice a day for four days, production of hsp70 in response to oxygen deprivation declined. The same response was noted in those eggs exposed to RF radiation of 3.5 $\mu W/cm^2$ for 30 or 60 minutes, once a day, for four days. The researchers noted that these eggs produced 27% less hsp70 following these exposures, and had correspondingly reduced ability to fend off cell damage (reduced *cytoprotection*). Similar experiments have been carried out with short, repeated exposures (in contrast to extended exposures). There too, the rate of stress protein synthesis is reduced with each repetition. The reduction in stress protein synthesis as a result of continuous exposure to EMF would predispose an individual to the accumulation of DNA damage and the development of cancer.

Cancers are believed to be the long term result of the errors in DNA that occur during the normal functioning of cells. Living cells are continuously growing (making protein) and dividing (making DNA), and errors in synthesis occur. The error rate is a very small but finite, so the vast majority of errors is repaired, but not all. When the error rate is too high, the cell activates apoptosis and destroys itself . However, the small number of errors that is retained accumulates over time as mutations, some of which can affect function. It is particularly bad when mutation inactivates a tumor suppressor gene or a

DNA repair gene and enables creation of an oncogene, since this accelerates the development of a cancer.

Although damage can occur during protein synthesis and cell division, as well as upon exposure to oxidizing chemicals, the probability of developing cancer is increased as a result of damage to DNA structure caused by exposure to EMF (Verschaeve, 2008). EMF induced oxidative damage to DNA has even been reported on exposure to high ELF fields (Yokus et al, 2008).

IX. STRESS RESPONSE: BIOLOGICAL GUIDE TO SAFETY

The cellular stress response is the way the body tells us that it has come in contact with a potentially harmful stimulus. Since cells react to relatively low levels of EMF, both ELF and RF, one would think that the low biological thresholds for a protective reaction to harmful stimuli would provide critical guidance for the authorities seeking to establish meaningful safety standards. By ignoring the information from the cellular stress response, the authorities appear to be saying that they are better judges of what is harmful to cells than the cells themselves.

Research on the cellular stress response has drawn attention to the inadequacy of EMF safety standards. The synthesis of stress proteins at EMF levels that are currently considered safe indicates that ambient exposure levels can influence the molecular processes involved in protein synthesis needed to provide new molecules and replace damaged molecules. The ability of EMF to interfere with normal function and damage the protein and DNA molecules that are being synthesized is definitely a reason to consider this effect for guidance regarding its health implications. The system of safety standards is not at all protective because processes stimulated at non-thermal levels have been overlooked. The standards must be revised.

The authorities have been misguided in assuming that only thermal stimuli could affect chemical bonds and that non-thermal stimuli cannot cause chemical changes. Non-thermal biological mechanisms activated by EMF have been known for some time, and

some experiments have even been aimed specifically at demonstrating unusual changes in biological systems due to non-thermal EMF stimuli. Bohr and Bohr (2000) showed that both a reaction and its reverse, the denaturation and renaturation of β-lactoglobulin, are accelerated by microwave EMF, and de Pomerai et al (2003) showed that microwave radiation causes protein aggregation in the absence of bulk heating. A clear separation of thermal and non-thermal mechanisms in biology was shown by Mashevich et al (2002) in experiments where chromosomal damage in lymphocytes that had been observed under RF was not seen when the cells were exposed to elevated temperatures. The neglect of non-thermal mechanisms by regulators is based on their ignorance of reactions in biological systems. By greatly underestimating the risk of EMF exposure, they continue to endanger the public.

The cellular stress response is activated by a mechanism that involves interaction of EMF with the DNA molecule. This reaction of DNA, and/or the stress proteins that are synthesized, could be used to develop new EMF safety standards (Blank and Goodman, 2012). A biologically-based measure of EMF radiation could replace the misguided energy-based "specific absorption rate" (SAR). (It should be noted that SAR is the safety standard in the radiofrequency (RF) range, but it fails as a standard for predicting cancer risk in the ELF range.) A standard based on stress proteins would have several advantages compared to SAR:

- it is based on a protective cellular mechanism that is stimulated by a variety of potentially harmful environmental agents
- it is stimulated by a wide range of frequencies in the EM spectrum so there would be no need for different standards in different frequency ranges.

Cancers are believed to arise from mutations in DNA, and changes in DNA induced by interaction with EMF could be a better measure of the biologically effective dose. It may be possible to measure the changes by transcriptional alterations and/or translational changes in specific proteins. A biologically-based standard related to stimulation of DNA

could apply over a much wider range of the electromagnetic spectrum and include ionizing radiation.

X. STRESS RESPONSE: GUIDE TO NEW THERAPIES

Since activation of the cellular stress response by EMF was shown to be a protective mechanism, it was only a matter of time before the response would be studied as a potential therapeutic agent. Thermal activation of the stress response has already been shown to be effective in cardiac bypass surgery (Currie et al., 1993; Udelsman et al., 1993; Nitta et al., 1994). Stress protein activation can apparently minimize the oxidative damage of ischemia (low oxygen level in a tissue) reperfusion that occurs when the blood supply is reconnected to the heart after surgery. However, the temperature control required for thermal activation is cumbersome and the technique is not easily applied compared to EMF. A study of non-invasive EMF induction of hsp70, prior to cardiac bypass surgery, has shown that myocardial function can be preserved, and at the same time decrease ischemic injury (George et al, 2008).

EMF activation of stress protein synthesis has a clear advantage over thermal activation. The biological response is not related to the EMF energy, so protective biological responses should occur far below thermal levels. 60 Hz fields were shown to induce elevated levels of hsp70 protein in the absence of elevated temperature (Goodman et al., 1994; Goodman and Blank, 1998; Han et al., 1998; Lin et al., 1998, 1999, 2001; Carmody et al., 2000) in cells including cultured rodent cardiomyocytes (Goodman and Blank, 2002). Also, Di Carlo et al. (1999) and Shallom et al. (2002) confirmed that cardiomyocytes were protected from anoxic damage in EMF exposed chick embryos.

Another potential therapeutic application has come from a study of the stress protein hsp10 in relation to striated muscle function. Kayani et al (2010) at the University of Liverpool found that this stress protein can prevent the age-related deterioration of muscle strength in skeletal muscle of transgenic mice. Hsp10 is often linked with hsp60 in supporting mitochondrial function. In cardiac myocytes this combination protects mitochondrial function as well as preventing cell deaths induced by ischemia-reperfusion.

These results suggest that mitochondrial hsp10 and hsp60 in combination or individually play an important role in maintaining mitochondrial integrity and ability to generate ATP, which are crucial for survival of cardiac myocytes during ischemia/reperfusion.

Research on therapeutic effects using stress proteins is obviously just beginning and we can expect other applications where EMF is used to generate this group of therapeutic agents essentially instantaneously and in situ.

XI. THE ENVIRONMENTAL EMF ISSUE AND CONCLUSIONS

Research has shown that the EMF-activated cellular stress response:

- is an effective protective mechanism for cells exposed to a wide range of EMF frequencies
- thresholds are very low (safety standards must be reduced to limit biological responses)
- mechanism involves direct interaction of EMF with the DNA molecule (claims that there are no known mechanisms of interaction are patently false)
- the coiled-coil structure of DNA in the nucleus makes the molecule react like a fractal antenna to a wide range of frequencies (there is a need for stricter EMF safety standards)
- biologically-based EMF safety standards could be developed from the research on the stress response.

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Exhibit 9

SECTION 6

Genetic Effects of Non-Ionizing Electromagnetic Fields

2014 Supplement

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I. INTRODUCTION

The following is an update of information and abstracts on research papers published since 2006/2007 on the genetic effects of nonionizing electromagnetic fields (EMF) in the radiofrequency (RF) and extremely-low frequency (ELF) ranges. Two static magnetic field papers (Jouni et al. 2012; Wang et al., 2009) are also included. Where additional information is relevant, some earlier papers, or papers not specifically related to genetic effects, are also included with citations contained within the discussion below. A list of abstracts, with summary sentences underlined for reader convenience, can be found at the end of this paper.

Analysis of these recent publications shows that there are more papers reporting effects than no effect.

In summary, the new radiofrequency studies report that 65% of genetic studies show effects and 35% do not show effects. **[Effects = 74 (65%) No Effects = 40 (35%)]**

In summary, the new ELF-EMF studies report that 82% of genetic studies show effects and 18% do not show effects **[Effects= 49 (83%) No Effects= 10 (17%)]**

Appendix A has references and abstracts for the RFR literature. Appendix B has references and abstracts for the ELF-EMF literature.

II. GENOTOXIC EFFECTS OF RADIOFREQUENCY RADIATION (RFR) AND OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS (ELF-EMF) (2007-2014)

The following is an update of information and abstracts on research papers published since 2006/2007 on the genetic effects of nonionizing electromagnetic fields (EMF) in the radiofrequency (RF) and extremely-low frequency (ELF) ranges. Two static magnetic field papers (Jouni et al. 2012; Wang et al., 2009) are also included. Where additional information is relevant, some earlier papers, or papers not specifically related to genetic effects, are also included with citations contained within the discussion below. A list of abstracts, with summary sentences underlined for reader convenience, can be found at the end of this paper.

Analysis of these recent publications shows that there are more papers reporting effects than no effect. With E representing a biological effect, and NE representing no biological effects, the recent literature finds RFR-genetic effects at: E=74 publications (65%); NE=40 publications (35%); and ELF-genetic effects at: E=49 (83%); NE=10 (17%).

Discussion

1. The effects of both RF and ELF fields are very similar. This is surprising because the energies carried by these EMFs are billions of folds different. An explanation for similar genetic effects has been provided by a recent paper by Blank and Goodman (Blank M, Goodman R. DNA is a fractal antenna in electromagnetic fields. Int. J. Radiat. Biol. 87(4):409-415, 2011) in which they stated that '...the wide frequency range of interaction with EMF is the functional characteristic of a fractal antenna, and DNA appears to possess the two structural characteristics of fractal antennas, electronic conduction and self symmetry.' However, similarities in effects between ELF and RF fields have also been reported in studies of other physiological processes, e.g., neurochemical and behavioral effects (Cf. Lai, H., Carino, M.A., Horita, A. and Guy, A.W. Opioid receptor subtypes that mediate a microwave-induced decrease in central cholinergic activity in the rat. Bioelectromagnetics 13:237-246, 1992; Lai, H. and Carino, M.A. Intracerebroventricular injections of mu and delta-opiate receptor antagonists block 60-Hz magnetic field-induced decreases in cholinergic activity in the frontal cortex and hippocampus of the rat. Bioelectromagnetics 19:433-437, 1998; Lai, H., Carino, M.A. and Ushijima, I. Acute exposure to a 60 Hz magnetic field affects rats' performance in the water maze. Bioelectromagnetics 19:117-122, 1998; Wang, B.M. and Lai, H. Acute exposure to pulsed 2450-MHz microwaves affects water maze learning in the rat. Bioelectromagnetics 21:52-56, 2000.) Thus, there is a basic interaction mechanism of biological tissues with electromagnetic fields that is independent of frequency.
Many studies have implicated the involvement of free radical processes in the genetic effects of EMF: ELF-EMF (Butdak et al., 2012; Jouni et al., 2012; Luukkonen et al., 2014; Tiwari et al.,2014); RFR (Agarwal et al., 2009; Atasoy et al., 2012; Burlaka et al., 2013; Campisi et al., 2010; De Iuliis et al., 2009; Esmekaya et al., 2011; Ferreira et al., 2006; Gajski and Garaj-Vrhovac, 2009; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Khalil et al., 2012; Kumar et al., 2010; Liu et al., 2013a,b; Luukkonan et al., 2009; Tomruk et al., 2010; Tkalec et al., 2013; Wu et al., 2008; Xu et al., 2010; Yao et al., 2003). Increase in free radical activity and changes in enzymes involved in cellular oxidative processes are the most consistent effects observed in cells and animals after EMF exposure. However, they are reports indicating that EMF could induce genetic effects without the involvement of free radicals (ELF- Alcaraz et al., 2013; RFR- Ferreira et al., 2006; Furtado-Filho et al., 2013) and increase in free radical after EMF exposure did not lead to genetic effects (Frahm et al., 2006). There are at least a couple of hundred published papers on the effects of EMF exposure on cellular oxidative processes. Many biological effects of EMF can be explained by intracellular changes in oxidative status, including the genetic effects reported in this review.

2. An important observation of the studies is that EMF can interact with other entities and synergistically cause genetic effects. These entities include: ELF-EMF- cisplastin (Buldak et al., 2012; El-Bialy et al., 2013), bleomycin (Cho et al., 2007), gadolinium (Cho et al., 2014); hydrogen peroxide and methyl methane sulfonate (Koyama et al., 2008), menadione (Luukkonan et al., 2011, 2014; Markkanen et al., 2008), ionizing radiation (Mairs et al., 2007; Journi et al., 2012 Yoon et al., 2014); RFR- chemical

mutagens (Baohong et al., 2005), clastogens (Kim et al., 2008), x-rays (Manti et al., 2008), ultraviolet ray (Baohong et al., 2007), aphidicolin (Tiwari et al., 2008), picrotoxin (López-Martín et al., 2009), doxorubicin (Zhijian et al., 2010), and incoherent electromagnetic noise (Wu et al., 2008; Yao et al., 2008). Most of the compounds that interact with EMF are mutagens. This is important because in real life situations, a person is usually exposed to many different environmental factors simultaneously. Synergism of these factors with EMF should be considered more seriously.

3. Several long term/repeated exposure papers are included in this update: ELF-EMF (Borhani et al., 2011; Cuccurazzu et al., 2010; Erdal et al., 2007; Fedrowitz and Loscher, 2012; Mariucci et al., 2010; Panagopoulous et al., 2013; Udroiu et al., 2006), and RFR (Asasoy et al., 2012; Atli Serkeroglu et al., 2013; Burlaka et al., 2013; Chavdoula et al., 2010; Deshmukh et al., 2013; Ferreira et al., 2006; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Lakshmi et al., 2010; Paulraj and Behari, 2006; Tomruk et al., 2010; Yan et al., 2008). These data are important in the understanding of the biological effects of EMF exposure in real life situation, since human environmental EMF exposure is both chronic and intermittent. Within these long-term exposure studies, there are several that investigated the effect of EMF exposure on developing animals (ELF-EMF: Borhani et al., 2011; Cuccurazzu et al., 2010; Panagopoulous et al., 2013; Udroiu et al., 2006, RFR: Burlaka et al., 2013; Ferreira et al., 2006; Guler et al., 2010, 2012; Serkeroglu et al., 2013; Tomruk et al., 2010; Zalata et al., In press). Data of effects of EMF exposure on growth and development of young animals are urgently needed. There are several studies indicating that RFR may affect reproduction, particularly with effects on sperm physiology and DNA (Agarwal et al., 2009; Atasoy et al., 2012; Avendano et al., 2012; Chavdoula et al., 2010; de Iuliis et al., 2009; Liu et al., 2013b; Panagopoulous et al., 2007). Similar effects of ELF-EMF on sperm have also been reported, e.g., 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; Iorio R, Scrimaglio R, Rantucci E, Delle Monache S, Di Gaetano A, Finetti N, Francavilla F, Santucci R, Tettamanti E, Colonna R. A preliminary study of oscillating electromagnetic field effects on human spermatozoon motility. Bioelectromagnetics. 28(1):72-75, 2007; Iorio R, Delle Monache S, Bennato F, Di Bartolomeo C, Scrimaglio R, Cinque B, Colonna RC. Involvement of mitochondrial activity in mediating ELF-EMF stimulatory effect on human sperm motility. Bioelectromagnetics. 32(1):15-27, 2011.

4. Another area that needs more research is the biological effects of low-intensity exposure. This is particularly true for ELF-EMF, since intensities of ELF-EMF in the environment are in microtesla (μT) levels. There are many studies on biological effects of low-intensity RFR (see Table 1 in Levitt, B.B. and Lai, H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays. Environ. Rev. 18:369-395, 2010.) However, most cell and animal studies in ELF-EMF used fields in the millitesla (mT) level. Exceptions are the study of Sarimov et al. (2011) listed below in the reference section and the study of de Bruyn and de Jager (2010) (de Bruyn L and de Jager L. Effect of long-term exposure to a randomly varied 50

Hz power frequency magnetic field on the fertility of the mouse. Electromag. Biol. Med. 29(1-2):52-61, 2010).

5. Two other important findings of these recent studies are that the effects of EMF are shown to be waveform specific and cell-type specific. Regarding waveform specificity, Campisi et al. (2010) reported increases in free radical activity and DNA fragmentation in brain cells after acute exposure to a 50-Hz amplitude-modulated 900-MHz RFR, whereas a continuous-wave 9000-MHz field produced no effect. Franzellitti et al. (2010) showed increased DNA strand breaks in trophoblasts after exposure to a 217-Hz modulated 1.8 GHz-RFR, but a continuous-wave field of the same carrier frequency was without effect. Tkalec et al (2013) reported that AM-modulated (1 KHz sinusoidal) 900-MHz RFR is more potent than non-modulated field in causing DNA damage in coelomocytes of exposed earthworms. Luukkonen et al. (2009) reported a continuous-wave 872-MHz RFR increased chemically-induced DNA strand breaks and free radicals in human neuroblastoma cells, whereas a GSM-modulated 872-MHz field had no significant effect. Zhang et al. (2008) found that gene expression in rat neurons is more sensitive to intermittent than continuous exposure to a 1.8 GHz-RFR. López-Martín et al. (2009) found that GSM and unmodulated RFR caused different effects on c-Fos gene expression in the rat brain. Regarding cell-type specificity, Nylund and Leszczynski (2006) and Remondini et al. (2006) reported different patterns of gene expression in different types of cells after exposure to RFR. Zhao et al. (2007) found than neurons are more sensitive to a 1.9 GHz cell phone radiation than astrocytes. Schwarz et al. (2008) reported DNA strand breaks and micronucleus formation in human fibroblasts, but not in lymphocytes, after exposure to a 1950-MHz UMTS field. Furthermore, Xu et al (2013) found DNA damages in some cell types and not in others after exposure to 1800-MHz RFR. Valbonesi et at. (2014) reported that HSP70 expression and MAPK signaling pathways in PC12 cells were affected by GSM-217 Hz signal and not by CW or GSM-talk signals. In ELF-EM research, Giorgi et al. (2011) found that DNA transposition in E. coli was decreased after exposure to a sinusoidal magnetic field and increased after exposure to a pulsed magnetic field. Kim et al. (2012) described DNA strand breaks in human fibroblasts after exposure to ELF magnetic field. They found that the pattern of changes depended on the eddy current and Lorentz force in the field. Nahab et al. (2007) reported that a square-continuous ELF magnetic field was more effective than sinusoidal-continuous or pulsed field in inducing sister chromatid exchange in human lymphocytes. These findings underscore the complicity of interaction of EMF with biological tissues and may partially explain why effects were observed in some studies and not others. It is essential to understand why and how certain wave-characteristics of an EMF are more effective than other characteristics in causing biological effects, and why certain types of cells are more susceptible to the effect of EMF? That there are different biological effects elicited by different EMF wave characteristics is critical proof for the existence of nonthermal effects.

6. Many biological/health effects have been reported in cells and animals after exposure to EMFs in both the ELF and RF ranges. (Sixty-five percent of the RFR papers and 82% of the ELF-EMF papers in the publication list below reported effects.) It is highly dishonest for a scientist to summarily deny the existence of biological effects of EMF. A

biological effect of EMF can be detrimental to health, but can also be turned into a beneficial means for the treatment of human diseases. Denying any effects hampers the development of electromagnetic treatments for diseases. Examples of possible clinical uses of EMF are: Alzheimer's disease (Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C. Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. J Alzheimers Dis. 19(1):191-210, 2010); Parkinson's disease (Wang Z, Che PL, Du J, Ha B, Yarema KJ. Static magnetic field exposure reproduces cellular effects of the Parkinson's disease drug candidate ZM241385. PLoS One. 5(11):e13883, 2010); bone regeneration (Lee HM, Kwon UH, Kim H, Kim HJ, Kim B, Park JO, Moon ES, Moon SH. Pulsed eltromagnetic field stimulates cellular proliferation in human intervertebral disc cells. Yonsei Med. J. 51(6):954-959, 2010); cancer treatment (Costa FP, de Oliveira AC, Meirelles R, Machado MC, Zanesco T, Surjan R, Chammas MC, de Souza Rocha M, Morgan D, Cantor A, Zimmerman J, Brezovich I, Kuster N, Barbault A, Pasche B. Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields. Br. J. Cancer. 105(5):640-648, 2011), and tissue regeneration (Gaetani R, Ledda M, Barile L, Chimenti I, De Carlo F, Forte E, Ionta V, Giuliani L, D'Emilia E, Frati G, Miraldi F, Pozzi D, Messina E, Grimaldi S, Giacomello A, Lisi A. Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic fields. Cardiovasc. Res. 82(3):411-420, 2009).

7. It must be pointed out that, consistent with previous research, not very much of the cellular and animal genetic research data directly indicate that EMF (both RF and ELF EMF) is a carcinogen. However, the data show that EMF can possibly alter genetic functions and thus it is advisable that one should limit one's exposure to EMF.

CONCLUSIONS: In conclusion, the data did not indicate RF-induced genotoxicity in mice after two years of exposure.

APPENDIX B - ABSTRACTS ON GENETIC EFFECTS OF EXTREMELY-LOW FREQUENCY ELECTROMAGNETIC FIELDS (2007-2014)

Below is a key to abbreviations used throughout the following list of abstracts for recent papers published since 2006 and serve as my comments to help the reader quickly identify the significance of each work. The summary sentences by each author are underlined. The list is divided into RF effects papers, and ELF effects papers.

(E- effect observed; NE- no effect observed) (LE- long term exposure; GT- genotoxic effect, e.g., DNA damage, micronucleus formation, chromosome alterations; GE- gene expression; HU- human study; OX- oxidative effects, i.e., involvement of free radicals and oxidative enzymes; IA- interaction with other factors to cause genetic effects; DE- effects on developing animals; RP- reproduction, e.g., sperm damage; EH- compared with electro-hypersensitive subjects; WS- waveform specific effect, e.g., modulation and frequency; CS- cell type specific effect).

(NE) Albert GC, McNamee JP, Marro L, Bellier PV, Prato FS, Thomas AW. Assessment of genetic damage in peripheral blood of human volunteers exposed (whole-body) to a 200 muT, 60 Hz magnetic field. Int J Radiat Biol. 85(2):144-152, 2009. (GT, IA)

AIM: To investigate the extent of damage in nucleated cells in peripheral blood of healthy human volunteers exposed to a whole-body 60 Hz, 200 microT magnetic field. MATERIALS AND METHODS: In this study, 10 male and 10 female healthy human volunteers received a 4 h whole-body exposure to a 200 microT, 60 Hz magnetic field. In addition, five males and five females were treated in a similar fashion, but were exposed to sham conditions. For each subject, a blood sample was obtained prior to the exposure period and aliquots were used as negative- (pre-exposure) and positive- [1.5 Gray (Gy) (60)Cobalt ((60)Co) gamma-irradiation] controls. At the end of the 4 h exposure period, a second blood sample was obtained. The extent of DNA damage was assessed in peripheral human blood leukocytes from all samples using the alkaline comet assay. To detect possible clastogenic effects, the incidence of micronuclei was assessed in phytohemagglutinin (PHA)-stimulated lymphocytes using the cytokinesis-block micronucleus assay. RESULTS: There was no evidence of either increased DNA damage, as indicated by the alkaline comet assay, or increased incidence of micronuclei (MN) in the magnetic field exposed group. However, an in vitro exposure of 1.5 Gy gamma-irradiation caused a significant increase in both DNA damage and MN induction. CONCLUSIONS: This study found no evidence that an acute, whole-body exposure to a 200 microT, 60 Hz magnetic field for 4 hours could cause DNA damage in human blood.

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(E) Alcaraz M, Olmos E, Alcaraz-Saura M, Achel DG, Castillo J. Effect of long-term 50'Hz magnetic field exposure on the micronucleated polychromatic erythrocytes of mice. Electromagn Biol Med. 2013 Jun 19. [Epub ahead of print] (GT)

Abstract In recent years extremely low-frequency magnetic fields (ELF-EMF) have become widely used in human activities, leading to an increased chance of exposure to ELF-EMF. There are few reports on in vivo mammalian genotoxic effects using micronucleus (MN) assays, which generally have been used as a short-term screening system. We analyzed the possible genotoxic effect induced by long-term exposure (7, 14, 21, 28!d) of a 50!Hz ELM-MF to mice by measuring the increase in frequency of micronucleated polychromatic erythrocyte in their bone marrow (MNPCEs) and we compared it with that induced by 50!cGy of X-rays. Subsequently, we tried to reduce this chromosomal damage by administering four antioxidants substances with radioprotective capacities: dimethyl sulfoxide (DMSO), 6-n-propyl-2-thiouracil (PTU), grape-procyanidins (P) and citrus flavonoids extract (CE). The increase in micronucleated cells was higher in both physical treatments (Control!<!ELF-EMF (p!<!0.01) <X-rays (p!>!0.001)); however, the antioxidant substances only showed a genoprotective capacity against the damage induced by ionizing radiation (Ci!>!PTU!=!DMSO (p!<!0.001) >P!=!CE (p!<!0.001). The 50!Hz ELM-MF increased MNPCEs in mouse bone marrow, expressing a genotoxic capacity. Administration of antioxidant substances with radioprotective capacities known to act through the elimination of free radicals did not diminish the genotoxic effect induced by ELM-MF.

(E) Balamuralikrishnan B, Balachandar V, Kumar SS, Stalin N, Varsha P, Devi SM, Arun M, Manikantan P, Venkatesan C, Sasikala K, Dharwadkar SN. Evaluation of Chromosomal Alteration in Electrical Workers Occupationally Exposed to Low Frequency of Electro Magnetic Field (EMFs) in Coimbatore Population, India. Asian Pac J Cancer Prev. 13(6):2961-2966, 2012. (HU, LE, GT)

Extremely low frequency electromagnetic fields (EMFs) have been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer. An increased number of chromosomal alterations in peripheral lymphocytes are correlated with elevated incidence of cancer. The aim of the present study was to assess occupationally induced chromosomal damage in EMF workers exposed to low levels of radiation. We used conventional metaphase chromosome aberration (CA) analysis and the micronucleus (MN) assay as biological indicators of nonionizing radiation exposure. In the present study totally 70 subjects were selected including 50 exposed and 20 controls. Informed written consent was obtained from all participants and the study was performed in accordance with the Declaration of Helsinki and the approval of the local ethical committee. A higher degree of CA and MN was observed in exposed subjects compared to controls, the frequency of CA being significantly enhanced with long years of exposure (P<0.05). Moreover increase in CA and MN with age was noted in both exposed subjects and controls, but was significantly greater in the former. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers occupationally exposed to EMFs in electric transformer and distribution stations. In conclusion, our findings suggest that EMFs possess genotoxic capability, as measured by CA and MN assays; CA analysis appeared more sensitive than other cytogenetic end-points. It can be concluded that chronic occupational exposure to EMFs may lead to an increased risk of genetic damage among electrical workers.

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(E) Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. Bioelectromagnetics 26:173-184, 2005. (GT, EH)

We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power frequency magnetic field (50 Hz, 15 muT peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. 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. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock.

(E) Borhani N, Rajaei F, Salehi Z, Javadi A. Analysis of DNA fragmentation in mouse embryos exposed to an extremely low-frequency electromagnetic field. Electromagn Biol Med. 30(4):246-252, 2011. (GT, DE, LE)

Effects of extremely low-frequency electromagnetic fields (ELF-EMFs) on DNA damage in biological systems are still a matter of dispute. The aim of the present study was to investigate the possible effect of electromagnetic field exposure on DNA fragmentation in cells (blastomers) of mouse blastocysts. Eighty female NMRI mice were randomly divided into 2 groups of 40 animals each. The control group was left unexposed whereas the animals in the EMF-group were exposed to a 50-Hz EMF at 0.5 mT 4 h per day, 6 days a week for a duration of 2 weeks. After the 8(th) day of exposure, the female mice in both groups were superovulated (with injections of pregnant mare serum gonadotropin and human chorionic gonadotropin) and then mated overnight. At approximately 4 days after mating (102 h after the human chorionic gonadotropin treatment), blastocysts were obtained by flushing the uterus horns. The mean numbers of pregnant mice, blastocysts after flushing, blastomers within the blastocysts, and the DNA fragmentation index following staining in both groups were compared using statistical methods (SPSS, the Chi-square test, the Student's t-test and the Mann-Whitney U-test, $P < 0.05$). The results showed that the mean number of blastocysts after flushing was significantly decreased in the EMF-group compared to that of the control group ($P < 0.03$). The DNA fragmentation index was significantly increased in the EMF-group compared to control (10.53% vs. 7.14%; $P <$

0.001). However, there was no significant difference in the mean numbers of blastomers and numbers of pregnant mice between the EMF-exposed and control group. Our findings indicate that the EMF exposure in preimplantation stage could have detrimental effects on female mouse fertility and embryo development by decreasing the number of blastocysts and increasing the blastocysts DNA fragmentation.

(E) Bułdak RJ, Polaniak R, Bułdak L, Zwirska-Korczala K, Skonieczna M, Monsiol A, Kukla M, Duława-Bułdak A, Birkner E. Short-term exposure to 50'Hz ELF-EMF alters the cisplatin-induced oxidative response in AT478 murine squamous cell carcinoma cells. Bioelectromagnetics. 2012 Apr 25. doi: 10.1002/bem.21732. [Epub ahead of print] (GT, IA, OX)

The aim of this study was to assess the influence of cisplatin and an extremely low frequency electromagnetic field (ELF-EMF) on antioxidant enzyme activity and the lipid peroxidation ratio, as well as the level of DNA damage and reactive oxygen species (ROS) production in AT478 carcinoma cells. Cells were cultured for 24 and 72!h in culture medium with cisplatin. Additionally, the cells were irradiated with 50!Hz/1!mT ELF-EMF for 16!min using a solenoid as a source of the ELF-EMF. The amount of ROS, superoxide dismutase (SOD) isoenzyme activity, glutathione peroxidase (GSH-Px) activity, DNA damage, and malondialdehyde (MDA) levels were assessed. Cells that were exposed to cisplatin exhibited a significant increase in ROS and antioxidant enzyme activity. The addition of ELF-EMF exposure to cisplatin treatment resulted in decreased ROS levels and antioxidant enzyme activity. A significant reduction in MDA concentrations was observed in all of the study groups, with the greatest decrease associated with treatment by both cisplatin and ELF-EMF. Cisplatin induced the most severe DNA damage; however, when cells were also irradiated with ELF-EMF, less DNA damage occurred. Exposure to ELF-EMF alone resulted in an increase in DNA damage compared to control cells. ELF-EMF lessened the effects of oxidative stress and DNA damage that were induced by cisplatin; however, ELF-EMF alone was a mild oxidative stressor and DNA damage inducer. We speculate that ELF-EMF exerts differential effects depending on the exogenous conditions. This information may be of value for appraising the pathophysiologic consequences of exposure to ELF-EMF.

(E) Calabrò E, Condello S, Magazù S, Ientile, R. Static and 50 Hz electromagnetic fields effects on human neuronal-like cells vibration bands in the mid-infrared region. J Electromagnetic Analysis and Applications 3(2) 69-78, 2011. (GT)

Human neuronal-like cells were exposed to static and 50 Hz electromagnetic fields at the intensities of 2 mT and 1 mT, respectively. The effects of exposure were investigated in the mid-infrared region by means of Fourier self deconvolution spectroscopic analysis. After exposure of 3 hours to static and 50 Hz electromagnetic fields, the vibration bands of CH2 methilene group increased significantly after both exposures, suggesting a relative increase of lipid related to conformational changes in the cell membrane due to electromagnetic fields. In addition, PO2- stretching phosphate bands decreased after both exposures, suggesting that alteration in DNA/RNA can be occurred. In particular, exposure of 3 hours to 50 Hz electromagnetic fields produced significant increases in β-sheet contents in amide I, and around the 1740 cm^{-1} band assigned to non-hydrogen-bonded ester carbonyl stretching mode, that can be

related to unfolding processes of proteins structure and cells death. Further exposure up to 18 hours to static magnetic field produced an increase in β-sheet contents as to α-helix components of amide I region, as well.

(E) Celikler S, Aydemir N, Vatan O, Kurtuldu S, Bilaloglu R. A biomonitoring study of genotoxic risk to workers of transformers and distribution line stations. Int J Environ Health Res. 19(6):421-430, 2009. (GT, HU)

A cytogenetic monitoring study was carried out on a group of workers from transformer and distribution line stations in the Bursa province of Turkey, to investigate the genotoxic risk of occupational exposure to extremely low frequency electric (ELF) and magnetic fields (EMF). Cytogenetic analysis, namely chromosomal aberrations (CAs) and micronucleus (MN) tests were performed on a strictly selected group of 55 workers and compared to 17 controls. CA and MN frequencies in electrical workers appeared significantly higher than in controls ($p < 0.001$, 0.05, respectively). The frequency of CA in exposed groups were significantly enhanced with the years of exposure ($p < 0.01$). The effect of smoking on the level of CA and MN was not significant in the control and exposure groups. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers engaged to occupational exposure to ELMF in electric transformer and distribution stations.

(E) Chen GD, Lu DQ, Jiang H, Xu ZP.[Effects of 50 Hz magnetic fields on gene expression in MCF-7 cells]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):15-22, 2008. [Article in Chinese] (GT, GE)

OBJECTIVE: To investigate whether 50 Hz magnetic fields (MF) can change the gene expression profile in MCF-7 cells and to screen MF responsive genes. METHODS: In vitro cultured MCF-7 cells were continuously exposed or sham-exposed to 0.4 mT of 50 Hz MF for 24 hours. Affymetrix Human Genome Genechips (U133A) were applied to analyze gene expression profiles in MF exposed and sham-exposed MCF-7 cells and the data were processed with Genechip data analysis software MAS 5.0 and DMT 3.0. Real-time RT-PCR assay was employed to examine the differentially expressed genes. RESULT: Thirty differentially expressed genes were screened with 100 % consistency change calls in the MF exposed MCF-7 cells. Six independent real-time RT-PCR analyses showed that SCNN1A, METTL3 and GPR137B were slightly but statistically significantly changed in MCF-7 cells after exposure to 50 Hz MF ($P<0.05$), while other analyzed genes exhibited slight up-and down-fluctuations in expressions and no increase or decrease in each gene expression reached statistical significance ($P>0.05$). CONCLUSION: The present study identified three 50 Hz MF responsive genes in MCF-7 cells and the biological consequences of expression changes in these MF responsive genes need to be further investigated.0.4 mT 50 Hz MF exposure for longer duration might induce DNA double-strand breaks in human lens epithelial cells in vitro.

(NE) Chen G, Lu D, Chiang H, Leszczynski D, Xu Z. Using model organism Saccharomyces cerevisiae to evaluate the effects of ELF-MF and RF-EMF exposure on global gene expression. Bioelectromagnetics. 33(7):550-560, 2012. (GE)

The potential health hazard of exposure to electromagnetic fields (EMF) continues to cause public concern. However, the possibility of biological and health effects of exposure to EMF remains controversial and their biophysical mechanisms are unknown. In the present study, we used Saccharomyces cerevisiae to identify genes responding to extremely low frequency magnetic fields (ELF-MF) and to radiofrequency EMF (RF-EMF) exposures. The yeast cells were exposed for 6!h to either 0.4!mT 50!Hz ELF-MF or 1800!MHz RF-EMF at a specific absorption rate of 4.7!W/kg. Gene expression was analyzed by microarray screening and confirmed using real-time reverse transcription-polymerase chain reaction (RT-PCR). We were unable to confirm microarray-detected changes in three of the ELF-MF responsive candidate genes using RT-PCR (P!>!0.05). On the other hand, out of the 40 potential RF-EMF responsive genes, only the expressions of structural maintenance of chromosomes 3 (SMC3) and aquaporin 2 (AQY2 (m)) were confirmed, while three other genes, that is, halotolerance protein 9 (HAL9), yet another kinase 1 (YAK1) and one function-unknown gene (open reading frame: YJL171C), showed opposite changes in expression compared to the microarray data (P!<!0.05). In conclusion, the results of this study suggest that the yeast cells did not alter gene expression in response to 50!Hz ELF-MF and that the response to RF-EMF is limited to only a very small number of genes. The possible biological consequences of the gene expression changes induced by RF-EMF await further investigation.

(E) Cho S, Lee Y, Lee S, Choi YJ, Chung HW. Enhanced cytotoxic and genotoxic effects of gadolinium following ELF-EMF irradiation in human lymphocytes. Drug Chem Toxicol. 2014 Jan 30. [Epub ahead of print] (GT, IA)

Gadolinium (Gd) and its chelated derivatives are widely utilized for various industrial and medical purposes, particularly as a contrast agent for magnetic resonance imaging (MRI). There are many studies of Gd nephrotoxicity and neurotoxicity, whereas research on cyto- and genotoxicity in normal human lymphocytes is scarce. It is important to investigate the effect of extremely low-frequency electromagnetic fields (ELF-EMF) on Gd toxicity, as patients are co-exposed to Gd and ELF-EMF generated by MRI scanners. We investigated the cytotoxicity and genotoixcity of Gd and the possible enhancing effect of ELF-EMF on Gd toxicity in cultured human lymphocytes by performing a micronuclei (MN) assay, trypan blue dye exclusion, single cell gel electrophoresis, and apoptosis analyses using flow cytometry. Isolated lymphocytes were exposed to 0.2-1.2!mM of Gd only or in combination with a 60-Hz ELF-EMF of 0.8-mT field strength. Exposing human lymphocytes to Gd resulted in a concentration- and time-dependent decrease in cell viability and an increase in MN frequency, single strand DNA breakage, apoptotic cell death, and ROS production. ELF-EMF (0.8!mT) exposure also increased cell death, MN frequency, olive tail moment, and apoptosis induced by Gd treatment alone. These results suggest that Gd induces DNA damage and apoptotic cell death in human lymphocytes and that ELF-EMF enhances the cytotoxicity and genotoxicity of Gd.

(E) Cho YH, Jeon HK, Chung HW. Effects of extremely low-frequency electromagnetic fields on delayed chromosomal instability induced by bleomycin in normal human fibroblast cells. J Toxicol Environ Health A. 70(15-16):1252-1258, 2007. (GT, IA)

This study was carried out to examine the interaction of extremely low-frequency electromagnetic fields (ELF-EMF) on delayed chromosomal instability by bleomycin (BLM) in

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human fibroblast cells. A micronucleus-centromere assay using DNA probes for chromosomes 1 and 4 was performed and a 60-Hz ELF-EMF of 0.8 mT field strength was applied either alone or with BLM throughout the culture period. The frequencies of micronuclei (MN) and aneuploidy were analyzed at 28, 88, and 240 h after treatment with BLM. The coexposure of cells to BLM and ELF-EMF led to a significant increase in the frequencies of MN and aneuploidy compared to the cells treated with BLM alone. No difference was observed between field-exposed and sham-exposed control cells. The frequency of MN induced by BLM was increased at 28 h, and further analysis showed a persistent increase up to 240 h, but the new levels were not significantly different from the level at 28 h. BLM increased the frequencies of aneuploidy at 28, 88, and 240 h, and significantly higher frequency of aneuploidy was observed in the cells analyzed at 240 h compared to the cells examined at 28 h. No interaction of ELF-EMF on delayed chromosomal instability by BLM was observed. Our results suggest that ELF-EMF enhances the cytotoxicity of BLM. BLM might induce delayed chromosomal instability, but no effect of ELF-EMF was observed on the BLM-induced delayed chromosomal instability in fibroblast cells.

(E) Collard JF, Lazar C, Nowé A, Hinsenkamp M. Statistical validation of the acceleration of the differentiation at the expense of the proliferation in human epidermal cells exposed to extremely low frequency electric fields. Prog Biophys Mol Biol. 111(1):37-45, 2013. (GE)

An acceleration of differentiation at the expense of proliferation is observed in our previous publications and in the literature after exposure of various biological models to low frequency and low-amplitude electric and electromagnetic fields. This observation is related with a significant modification of genes expression. We observed and compared over time this modification. This study use microarray data obtained on epidermis cultures harvested from human abdominoplasty exposed to ELF electric fields. This protocol is repeated with samples collected on three different healthy patients. The sampling over time allows comparison of the effect of the stimulus at a given time with the evolution of control group. After 4 days, we observed a significant difference of the genes expression between control (D4C) and stimulated (D4S) ($p < 0.05$). On the control between day 4 and 7, we observed another group of genes with significant difference ($p < 0.05$) in their expression. We identify the common genes between these two groups and we select from them those expressing no difference between stimulate at 4 days (D4S) and control after 7 days (D7C). The same analysis was performed with D4S-D4C-D12C and D7S-D7C-D12C. The lists of genes which follow this pattern show acceleration in their expressions under stimulation appearing on control at a later time. In this list, genes such as DKK1, SPRR3, NDRG4, and CHEK1 are involved in cell proliferation or differentiation. Numerous other genes are also playing a function in mitosis, cell cycle or in the DNA replication transcription and translation.[1]

(E) Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, Azzena GB, Grassi C.
Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. Exp Neurol. 226(1):173-182, 2010. (LE, GE, DE)

Throughout life, new neurons are continuously generated in the hippocampus, which is therefore a major site of structural plasticity in the adult brain. We recently demonstrated that extremely low-frequency electromagnetic fields (ELFEFs) promote the neuronal differentiation of neural stem cells in vitro by up-regulating Ca(v)1-channel activity. The aim of the present study was to determine whether 50-Hz/1 mT ELFEF stimulation also affects adult hippocampal neurogenesis in vivo, and if so, to identify the molecular mechanisms underlying this action and its functional impact on synaptic plasticity. ELFEF exposure (1 to 7 h/day for 7 days) significantly enhanced neurogenesis in the dentate gyrus (DG) of adult mice, as documented by increased numbers of cells double-labeled for 5-bromo-deoxyuridine (BrdU) and double cortin. Quantitative RT-PCR analysis of hippocampal extracts revealed significant ELFEF exposure-induced increases in the transcription of pro-neuronal genes (Mash1, NeuroD2, Hes1) and genes encoding Ca(v)1.2 channel α(1C) subunits. Increased expression of NeuroD1, NeuroD2 and Ca(v)1 channels was also documented by Western blot analysis. Immunofluorescence experiments showed that, 30 days after ELFEF stimulation, roughly half of the newly generated immature neurons had survived and become mature dentate granule cells (as shown by their immunoreactivity for both BrdU and NeuN) and were integrated into the granule cell layer of the DG. Electrophysiological experiments demonstrated that the new mature neurons influenced hippocampal synaptic plasticity, as reflected by increased long-term potentiation. Our findings show that ELFEF exposure can be an effective tool for increasing in vivo neurogenesis, and they could lead to the development of novel therapeutic approaches in regenerative medicine.

(E) Di Campli E, Di Bartolomeo S, Grande R, Di Giulio M, Cellini L. Effects of extremely low-frequency electromagnetic fields on Helicobacter pylori biofilm. Curr Microbiol. 60(6):412-418, 2010. (GE)

The aim of this work was to investigate the effects of exposure to extremely low-frequency electromagnetic fields (ELF-EMF) both on biofilm formation and on mature biofilm of Helicobacter pylori. Bacterial cultures and 2-day-old biofilm of H. pylori ATCC 43629 were exposed to ELF-EMF (50 Hz frequency-1 mT intensity) for 2 days to assess their effect on the cell adhesion and on the mature biofilm detachment, respectively. All the exposed cultures and the respective sham exposed controls were studied for: the cell viability status, the cell morphological analysis, the biofilm mass measurement, the genotypic profile, and the luxS and amiA gene expression. The ELF-EMF acted on the bacterial population during the biofilm formation displaying significant differences in cell viability, as well as, in morphotypes measured by the prevalence of spiral forms (58.41%) in respect to the controls (33.14%), whereas, on mature biofilm, no significant differences were found when compared to the controls. The measurement of biofilm cell mass was significantly reduced in exposed cultures in both examined experimental conditions. No changes in DNA patterns were recorded, whereas a modulation in amiA gene expression was detected. An exposure to ELF-EMF of H. pylori biofilm induces phenotypic changes on adhering bacteria and decreases the cell adhesion unbalancing the bacterial population therefore reducing the H. pylori capability to protect itself.

(E) Dominici L, Villarini M, Fatigoni C, Monarca S, Moretti M. Genotoxic hazard evaluation in welders occupationally exposed to extremely low-frequency magnetic fields (ELF-MF). Int J Hyg Environ Health. 215(1):68-75, 2011. (GT, HU)

Electric arc welding is known to involve considerable exposure to extremely low-frequency magnetic fields (ELF-MF). A cytogenetic monitoring study was carried out in a group of welders to investigate the genotoxic risk of occupational exposure to ELF-MF. This study assessed individual occupational exposure to ELF-MF using a personal magnetic-field dosimeter, and the cytogenetic effects were examined by comparing micronuclei (MN) and sister chromatid exchange (SCE) frequencies in the lymphocytes of the exposed workers with those of non-exposed control subjects (blood donors) matched for age and smoking habit. Cytogenetic analyses were carried out on 21 workers enrolled from two different welding companies in Central Italy and compared to 21 controls. Some differences between the groups were observed on analysis of SCE and MN, whereas replication indices in the exposed were found not to differ from the controls. In particular, the exposed group showed a significantly higher frequency of MN (group mean±SEM: 6.10±0.39) compared to the control group (4.45±0.30). Moreover, the increase in MN is associated with a proportional increase in ELF-MF exposure levels with a dose-response relationship. A significant decrease in SCE frequency was observed in exposed subjects (3.73±0.21) compared to controls (4.89±0.12). The hypothesis of a correlation between genotoxic assays and ELF-MF exposure value was partially supported, especially as regards MN assay. Since these results are derived from a small-scale pilot study, a larger scale study should be undertaken.

(E) Du XG, Xu SS, Chen Q, Lu DQ, Xu ZP, Zeng QL. [Effects of 50 Hz magnetic fields on DNA double-strand breaks in human lens epithelial cells]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):9-14, 2008. [Article in Chinese] (GT)

OBJECTIVE: To investigate the effects of 50 Hz magnetic fields (MF) on DNA double-strand breaks in human lens epithelial cells (hLECs). METHODS: The cultured human lens epithelial cells were exposed to 0.4 mT 50 Hz MF for 2 h, 6 h, 12 h, 24 h and 48 h. Cells exposed to 4-nitroquinoline-1-oxide, a DNA damage agent, at a final concentration of 0.1 micromol/L for 1 h were used as positive controls.After exposure, cells were fixed with 4 % paraformaldehyde and for H2AX (gamma H2AX) immunofluorescence measurement. gamma H2AX foci were detected at least 200 cells for each sample. Cells were classified as positive when more than three foci per cell were observed. Mean values of foci per cell and percentage of foci positive cells were adopted as indexes of DNA double-strand breaks. RESULT: The mean value of foci per cell and the percentage of gamma H2AX foci positive cells in 50 Hz MF exposure group for 24 h were (2.93 +/-0.43) and (27.88 +/-2.59)%, respectively, which were significantly higher than those of sham-exposure group [(1.77 +/-0.37) and (19.38+/-2.70)%, P <0.05], and the mean value of foci per cell and the percentage of gamma H2AX foci positive cells in 50 Hz MF exposure group for 48 h were (3.14 +/-0.35) and (31.00 +/-3.44)%, which were significantly higher than those of sham-exposure group (P <0.01). However there was no significant difference between 50 Hz MF exposure groups for 2 h, 6 h, 12 h and sham-exposure group for above two indexes (P >0.05). CONCLUSION: 0.4 mT 50 Hz MF exposure for longer duration might induce DNA double-strand breaks in human lens epithelial cells in vitro.

(E) El-Bialy NS, Rageh MM. Extremely low-frequency magnetic field enhances the therapeutic efficacy of low-dose cisplatin in the treatment of Ehrlich carcinoma. Biomed Res Int. 2013;2013:189352. doi: 10.1155/2013/189352. Epub 2013 Jan 14. (GT, IA)

The present study examines the therapeutic efficacy of the administration of low-dose cisplatin (cis) followed by exposure to extremely low-frequency magnetic field (ELF-MF), with an average intensity of 10 mT, on Ehrlich carcinoma in vivo. The cytotoxic and genotoxic actions of this combination were studied using comet assay, mitotic index (MI), and the induction of micronucleus (MN). Moreover, the inhibition of tumor growth was also measured. Treatment with cisplatin and ELF-MF (group A) increased the number of damaged cells by 54% compared with 41% for mice treated with cisplatin alone (group B), 20% for mice treated by exposure to ELF-MF (group C), and 9% for the control group (group D). Also the mitotic index decreased significantly for all treated groups ($P < 0.001$). The decrement percent for the treated groups (A, B, and C) were 70%, 65%, and 22%, respectively, compared with the control group (D). Additionally, the rate of tumor growth at day 12 was suppressed significantly ($P < 0.001$) for groups A, B, and C with respect to group (D). These results suggest that ELF-MF enhanced the cytotoxic activity of cisplatin and potentiate the benefit of using a combination of low-dose cisplatin and ELF-MF in the treatment of Ehrlich carcinoma.

(E) Erdal N, Gürgül S, Celik A. Cytogenetic effects of extremely low frequency magnetic field on Wistar rat bone marrow. Mutat Res. 630(1-2):69-77, 2007. (GT, LE)

In this study, the genotoxic and cytotoxic potential of extremely low frequency magnetic fields (ELF-MF) was investigated in Wistar rat tibial bone marrow cells, using the chromosomal aberration (CA) and micronucleus (MN) test systems. In addition to these test systems, we also investigated the mitotic index (MI), and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs). Wistar rats were exposed to acute (1 day for 4h) and long-term (4h/day for 45 days) to a horizontal 50Hz, 1mT uniform magnetic field generated by a Helmholtz coil system. Mitomycin C (MMC, 2mg/kg BW) was used as positive control. Results obtained by chromosome analysis do not show any statistically significant differences between the negative control and both acute and long-term ELF-MF exposed samples. When comparing the group mean CA of long-term exposure with the negative control and acute exposure, the group mean of the long-term exposed group was higher, but this was not statistically significant. However, the mean micronucleus frequency of the longer-term exposed group was considerably higher than the negative control and acutely exposed groups. This difference was statistically significant ($p<0.01$). The results of the MI in bone marrow showed that the averages of both A-MF and L-MF groups significantly decreased when compared to those in the negative control ($p<0.001$ and $p<0.01$, respectively). No significant differences were found between the group mean MI of A-MF exposure with L-MF. We found that the average of PCEs/NCEs ratios of A-MF exposed group was significantly lower than the negative control and L-MF exposed groups ($p<0.001$ and $p<0.01$, respectively). In addition, the group mean of the PCEs/NCEs ratios of L-MF was significantly lower than negative control ($p<0.01$). We also found that the MMC treated group showed higher the number of CA and the frequency of MN formation when compared to those in all other each groups (p-values of all each groups <0.01) and also MMC treated group showed lower MI and the PCEs/NCEs ratios when compared to those in all other each groups (p-values of all groups <0.01). These observations indicate the in vivo suspectibility of mammals to the genotoxicity potential of ELF-MF.

(E) Fedrowitz M, Löscher W. Gene expression in the mammary gland tissue of female Fischer 344 and Lewis rats after magnetic field exposure (50 Hz, 100 µT) for 2 weeks. Int J

Radiat Biol. 88(5):425-429, 2012. (GE, LE) See also: Fedrowitz M, Hass R, Löscher W. Effects of 50 Hz magnetic field exposure on the stress marker α-amylase in the rat mammary gland. Int J Radiat Biol. 88(7):556-564, 2012.

PURPOSE: The issue of whether exposure to environmental power-frequency magnetic fields (MF) has impact on breast cancer development still remains equivocal. Previously, we observed rat strain differences in the MF response of breast tissue, so that the genetic background plays a role in MF effects. The present experiment aimed to elucidate candidate genes involved in MF effects by comparison of MF-susceptible Fischer 344 (F344) rats and MF-insensitive Lewis rats. MATERIALS AND METHODS: Female F344 and Lewis rats were exposed to MF (50 Hz, 100 µT) for two weeks, and a whole genome microarray analysis in the mammary gland tissue was performed. RESULTS: A remarkably decreased α-amylase gene expression, decreases in carbonic anhydrase 6 and lactoperoxidase, both relevant for pH regulation, and an increased gene expression of cystatin E/M, a tumor suppressor, were observed in MF-exposed F344, but not in Lewis rats. CONCLUSION: The MF-exposed F344 breast tissue showed alterations in gene expression, which were absent in Lewis and may therefore be involved in the MF-susceptibility of F344. Notably α-amylase might serve as a promising target to study MF effects, because first experiments indicate that MF exposure alters the functionality of this enzyme in breast tissue.

(E) Focke F, Schuermann D, Kuster N, Schär P. DNA fragmentation in human fibroblasts under extremely low frequency electromagnetic field exposure. Mutat Res. 683(1-2):74-83, 2010. (GT)

Extremely low frequency electromagnetic fields (ELF-EMFs) were reported to affect DNA integrity in human cells with evidence based on the Comet assay. These findings were heavily debated for two main reasons; the lack of reproducibility, and the absence of a plausible scientific rationale for how EMFs could damage DNA. Starting out from a replication of the relevant experiments, we performed this study to clarify the existence and explore origin and nature of ELF-EMF induced DNA effects. Our data confirm that intermittent (but not continuous) exposure of human primary fibroblasts to a 50 Hz EMF at a flux density of 1 mT induces a slight but significant increase of DNA fragmentation in the Comet assay, and we provide first evidence for this to be caused by the magnetic rather than the electric field. Moreover, we show that EMF-induced responses in the Comet assay are dependent on cell proliferation, suggesting that processes of DNA replication rather than the DNA itself may be affected. Consistently, the Comet effects correlated with a reduction of actively replicating cells and a concomitant increase of apoptotic cells in exposed cultures, whereas a combined Fpg-Comet test failed to produce evidence for a notable contribution of oxidative DNA base damage. Hence, ELF-EMF induced effects in the Comet assay are reproducible under specific conditions and can be explained by minor disturbances in S-phase processes and occasional triggering of apoptosis rather than by the generation of DNA damage.

(E) Frisch P, Li GC, McLeod K, Laramee CB. Induction of heat shock gene expression in RAT1 primary fibroblast cells by ELF electric fields. Bioelectromagnetics. 34(5):405-413, 2013. (GE)

Recent studies have demonstrated that the Ku70 gene fragment can be placed in the anti-sense orientation under the control of a heat-inducible heat shock protein 70 (HSP70) promoter and activated through heat shock exposure. This results in attenuation of the Ku70 protein expression, inhibiting cellular repair processes, and sensitizing the transfected cells to exposures such as the ionizing radiation exposures used clinically. However, achieving the tissue temperatures necessary to thermally induce the HSP70 response presents significant limitations to the clinical application of this strategy. Previous findings suggest an alternative approach to inducing a heat shock response, specifically through the use of extremely low frequency (ELF) electrical field stimulation. To further pursue this approach, we investigated HSP70 responses in transfected rat primary fibroblast (RAT1) cells exposed to 10!Hz electric fields at intensities of 20-500!V/m. We confirmed that low frequency electric fields can induce HSP70 heat shock expression, with peak responses obtained at 8!h following a 2!h field exposure. However, the approximate threefold increase in expression is substantially lower than that obtained using thermal stimulation, raising questions of the clinical utility of the response.

(E) Giorgi G, Marcantonio P, Bersani F, Gavoçi E, Del Re B. Effect of extremely low frequency magnetic field exposure on DNA transposition in relation to frequency, wave shape and exposure time. Int J Radiat Biol. 87(6):601-608, 2011. (GT, WS)

PURPOSE: To examine the effect of extremely low frequency magnetic field (ELF-MF) exposure on transposon (Tn) mobility in relation to the exposure time, the frequency and the wave shape of the field applied. MATERIALS AND METHODS: Two Escherichia coli model systems were used: (1) Cells unable to express β-galactosidase (LacZ(-)), containing a mini-transposon Tn10 element able to give ability to express β-galactosidase (LacZ(+)) upon its transposition; therefore in these cells transposition activity can be evaluated by analysing LacZ(+) clones; (2) cells carrying Fertility plasmid (F(+)), and a Tn5 element located on the chromosome; therefore in these cells transposition activity can be estimated by a bacterial conjugation assay. Cells were exposed to sinusoidal (SiMF) or pulsed-square wave (PMF) magnetic fields of various frequencies (20, 50, 75!Hz) and for different exposure times (15 and 90!min). RESULTS: Both mini-Tn10 and Tn5 transposition decreased under SiMF and increased under PMF, as compared to sham exposure control. No significant difference was found between frequencies and between exposure times. CONCLUSIONS: ELF-MF exposure affects transposition activity and the effects critically depend on the wave shape of the field, but not on the frequency and the exposure time, at least in the range observed.

(E) Heredia-Rojas JA, Rodríguez de la Fuente AO, Alcocer González JM, Rodríguez-Flores LE, Rodríguez-Padilla C, Santoyo-Stephano MA, Castañeda-Garza E, Taméz-Guerra RS. Effect of 60 Hz magnetic fields on the activation of hsp70 promoter in cultured INER-37 and RMA E7 cells. In Vitro Cell Dev Biol Anim. 46(9):758-63, 2010. (GE)

It has been reported that 50-60 Hz magnetic fields (MF) with flux densities ranging from microtesla to millitesla are able to induce heat shock factor or heat shock proteins in various cells. In this study, we investigated the effect of 60 Hz sinusoidal MF at 8 and 80 μT on the expression of the luciferase gene contained in a plasmid labeled as electromagnetic field-plasmid (pEMF). This gene construct contains the specific sequences previously described for the

induction of hsp70 expression by MF, as well as the reporter for the luciferase gene. The pEMF vector was transfected into INER-37 and RMA E7 cell lines that were later exposed to either MF or thermal shock (TS). Cells that received the MF or TS treatments and their controls were processed according to the luciferase assay system for evaluate luciferase activity. An increased luciferase gene expression was observed in INER-37 cells exposed to MF and TS compared with controls ($p < 0.05$), but MF exposure had no effect on the RMA E7 cell line.

(NE) Huwiler SG, Beyer C, Fröhlich J, Hennecke H, Egli T, Schürmann D, Rehrauer H, Fischer HM. Genome-wide transcription analysis of Escherichia coli in response to extremely low-frequency magnetic fields. Bioelectromagnetics. 2012 Feb 13. doi: 10.1002/bem.21709. [Epub ahead of print] (GE)

The widespread use of electricity raises the question of whether or not 50!Hz (power line frequency in Europe) magnetic fields (MFs) affect organisms. We investigated the transcription of Escherichia coli K-12 MG1655 in response to extremely low-frequency (ELF) MFs. Fields generated by three signal types (sinusoidal continuous, sinusoidal intermittent, and power line intermittent; all at 50!Hz, 1!mT) were applied and gene expression was monitored at the transcript level using an Affymetrix whole-genome microarray. Bacterial cells were grown continuously in a chemostat (dilution rate D!=!0.4!h(-1)) fed with glucose-limited minimal medium and exposed to 50!Hz MFs with a homogenous flux density of 1!mT. For all three types of MFs investigated, neither bacterial growth (determined using optical density) nor culturable counts were affected. Likewise, no statistically significant change (fold-change!>!2, P!≤!0.01) in the expression of 4,358 genes and 714 intergenic regions represented on the gene chip was detected after MF exposure for 2.5!h (1.4 generations) or 15!h (8.7 generations). Moreover, short-term exposure (8!min) to the sinusoidal continuous and power line intermittent signal neither affected bacterial growth nor showed evidence for reliable changes in transcription. In conclusion, our experiments did not indicate that the different tested MFs (50!Hz, 1!mT) affected the transcription of E. coli.

(NE) Jin YB, Kang GY, Lee JS, Choi JI, Lee JW, Hong SC, Myung SH, Lee YS. Effects on micronuclei formation of 60-Hz electromagnetic field exposure with ionizing radiation, hydrogen peroxide, or c-Myc overexpression. Int J Radiat Biol. 88(4):374-380, 2012. (GT, IA)

PURPOSE: Epidemiological studies have demonstrated a possible correlation between exposure to extremely low-frequency magnetic fields (ELF-MF) and cancer. However, this correlation has yet to be definitively confirmed by epidemiological studies. The principal objective of this study was to assess the effects of 60 Hz magnetic fields in a normal cell line system, and particularly in combination with various external factors, via micronucleus (MN) assays. MATERIALS AND METHODS: Mouse embryonic fibroblast NIH3T3 cells and human lung fibroblast WI-38 cells were exposed for 4 h to a 60 Hz, 1 mT uniform magnetic field with or without ionizing radiation (IR, 2 Gy), H(2)O(2) (100 μM) and cellular myelocytomatosis oncogene (c-Myc) activation. RESULTS: The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic effects were observed when ELF-MF was combined with IR, H(2)O(2), and c-Myc

activation. CONCLUSIONS: Our results demonstrate that ELF-MF did not enhance MN frequency by IR, H(2)O(2) and c-Myc activation.

(NE) Jin YB, Choi SH, Lee JS, Kim JK, Lee JW, Hong SC, Myung SH, Lee YS. Absence of DNA damage after 60-Hz electromagnetic field exposure combined with ionizing radiation, hydrogen peroxide, or c-Myc overexpression. Radiat Environ Biophys. 2013 Dec 5. [Epub ahead of print] (GT, IA)

The principal objective of this study was to assess the DNA damage in a normal cell line system after exposure to 60 Hz of extremely low frequency magnetic field (ELF-MF) and particularly in combination with various external factors, via comet assays. NIH3T3 mouse fibroblast cells, WI-38 human lung fibroblast cells, L132 human lung epithelial cells, and MCF10A human mammary gland epithelial cells were exposed for 4 or 16 h to a 60-Hz, 1 mT uniform magnetic field in the presence or absence of ionizing radiation (IR, 1 Gy), H_2O_2 (50 μM), or c-Myc oncogenic activation. The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic or additive effects were observed after 4 or 16 h of pre-exposure to 1 mT ELF-MF or simultaneous exposure to ELF-MF combined with IR, H_2O_2, or c-Myc activation.

(E) Jouni FJ, Abdolmaleki P, Ghanati F. Oxidative stress in broad bean (Vicia faba L.) induced by static magnetic field under natural radioactivity. Mutat Res. 741(1-2):116-121, 2012. (LE, GT, OX, IA)

The investigation was performed to evaluate the influence of the static magnetic field on oxidative stress in Vicia faba cultivated in soil from high background natural radioactivity in Iran. Soil samples were collected from Ramsar, Iran where the annual radiation absorbed dose from background radiation is substantially higher than 20 mSv/year. The soil samples were then divided into 2 separate groups including high and low natural radioactivity. The plants were continuously exposed to static magnetic field of 15 mT for 8 days, each 8h/day. The results showed that in the plants cultivated in soils with high background natural radioactivity and low background natural radioactivity the activity of antioxidant enzymes as well as flavonoid content were lower than those of the control. Treatment of plants with static magnetic field showed similar results in terms of lowering of antioxidant defense system and increase of peroxidation of membrane lipids. Accumulation of ROS also resulted in chromosomal aberration and DNA damage. This phenomenon was more pronounced when a combination of natural radiation and treatment with static magnetic field was applied. The results suggest that exposure to static magnetic field causes accumulation of reactive oxygen species in V. faba and natural radioactivity of soil exaggerates oxidative stress.

(E) Kim J, Ha CS, Lee HJ, Song K. Repetitive exposure to a 60-Hz time-varying magnetic field induces DNA double-strand breaks and apoptosis in human cells. Biochem Biophys Res Commun. 400(4):739-744, 2010. (GT)

We investigated the effects of extremely low frequency time-varying magnetic fields (MFs) on human normal and cancer cells. Whereas a single exposure to a 60-Hz time-varying MF of 6 mT for 30min showed no effect, repetitive exposure decreased cell viability. This decrease was

accompanied by phosphorylation of γ-H2AX, a common DNA double-strand break (DSB) marker, and checkpoint kinase 2 (Chk2), which is critical to the DNA damage checkpoint pathway. In addition, repetitive exposure to a time-varying MF of 6 mT for 30 min every 24 h for 3 days led to p38 activation and induction of apoptosis in cancer and normal cells. Therefore, these results demonstrate that repetitive exposure to MF with extremely low frequency can induce DNA DSBs and apoptosis through p38 activation. These results also suggest the need for further evaluation of the effects of repetitive exposure to environmental time-varying MFs on human health.

(E) Kim J, Yoon Y, Yun S, Park GS, Lee HJ, Song K. Time-varying magnetic fields of 60'Hz at 7'mT induce DNA double-strand breaks and activate DNA damage checkpoints without apoptosis. Bioelectromagnetics. 33(5):383-393, 2012. (GT, WS)

The potential genotoxic effect of a time-varying magnetic field (MF) on human cells was investigated. Upon continuous exposure of human primary fibroblast and cervical cancer cells to a 60!Hz MF at 7!mT for 10-60!min, no significant change in cell viability was observed. However, deoxyribonucleic acid (DNA) double-strand breaks (DSBs) were detected, and the DNA damage checkpoint pathway was activated in these cells without programmed cell death (called apoptosis). The exposure of human cells to a 60!Hz MF did not induce intracellular reactive oxygen species (ROS) production, suggesting that the observed DNA DSBs are not directly caused by ROS. We also compared the position and time dependency of DNA DSBs with numerical simulation of MFs. The Lorentz force and eddy currents in these experiments were numerically calculated to investigate the influence of each factor on DNA DSBs. The DNA DSBs mainly occurred at the central region, where the MF was strongest, after a 30-min exposure. After 90!min, however, the amount of DNA DSBs increased rapidly in the outer regions, where the eddy current and Lorentz force were strong.

(NE) Kirschenlohr H, Ellis P, Hesketh R, Metcalfe J. Gene Expression Profiles in White Blood Cells of Volunteers Exposed to a 50 Hz Electromagnetic Field. Radiat Res. 178(3): 138-149, 2012. (GE, HU)

Consistent and independently replicated laboratory evidence to support a causative relationship between environmental exposure to extremely low-frequency electromagnetic fields (EMFs) at power line frequencies and the associated increase in risk of childhood leukemia has not been obtained. In particular, although gene expression responses have been reported in a wide variety of cells, none has emerged as robust, widely replicated effects. DNA microarrays facilitate comprehensive searches for changes in gene expression without a requirement to select candidate responsive genes. To determine if gene expression changes occur in white blood cells of volunteers exposed to an ELF-EMF, each of 17 pairs of male volunteers age 20-30 was subjected either to a 50 Hz EMF exposure of 62.0 ± 7.1 μT for 2 h or to a sham exposure (0.21 ± 0.05 μT) at the same time (11:00 a.m. to 13:00 p.m.). The alternative regime for each volunteer was repeated on the following day and the two-day sequence was repeated 6 days later, with the exception that a *null* exposure (0.085 ± 0.01 μT) replaced the sham exposure. Five blood samples (10 ml) were collected at 2 h intervals from 9:00 to 17:00 with five additional samples during the exposure and sham or null exposure periods on each study day. RNA samples were pooled for the same time on each study day for the group of 17 volunteers that were subjected to the

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ELF-EMF exposure/sham or null exposure sequence and were analyzed on Illumina microarrays. Time courses for 16 mammalian genes previously reported to be responsive to ELF-EMF exposure, including immediate early genes, stress response, cell proliferation and apoptotic genes were examined in detail. No genes or gene sets showed consistent response profiles to repeated ELF-EMF exposures. A stress response was detected as a transient increase in plasma cortisol at the onset of either exposure or sham exposure on the first study day. The cortisol response diminished progressively on subsequent exposures or sham exposures, and was attributable to mild stress associated with the experimental protocol.

(E) Koyama S, Sakurai T, Nakahara T, Miyakoshi J. Extremely low frequency (ELF) magnetic fields enhance chemically induced formation of apurinic/apyrimidinic (AP) sites in A172 cells. Int J Radiat Biol. 84(1):53-59, 2008. (GT, IA)

PURPOSE: To detect the effects of extremely low frequency (ELF) magnetic fields, the number of apurinic/apyrimidinic (AP) sites in human glioma A172 cells was measured following exposure to ELF magnetic fields. MATERIALS AND METHODS: The cells were exposed to an ELF magnetic field alone, to genotoxic agents (methyl methane sulfonate (MMS) and hydrogen peroxide (H2O2)) alone, or to an ELF magnetic field with the genotoxic agents. After exposure, DNA was extracted, and the number of AP sites was measured. RESULTS: There was no difference in the number of AP sites between cells exposed to an ELF magnetic field and sham controls. With MMS or H2O2 alone, the number of AP sites increased with longer treatment times. Exposure to an ELF magnetic field in combination with the genotoxic agents increased AP-site levels compared with the genotoxic agents alone. CONCLUSIONS: Our results suggest that the number of AP sites induced by MMS or H2O2 is enhanced by exposure to ELF magnetic fields at 5 millitesla (mT). This may occur because such exposure can enhance the activity or lengthen the lifetime of radical pairs.

(E) Lee JW, Kim MS, Kim YJ, Choi YJ, Lee Y, Chung HW. Genotoxic effects of 3 T magnetic resonance imaging in cultured human lymphocytes. Bioelectromagnetics. 32(7):535-542, 2011. (GT)

The clinical and preclinical use of high-field intensity (HF, 3 T and above) magnetic resonance imaging (MRI) scanners have significantly increased in the past few years. However, potential health risks are implied in the MRI and especially HF MRI environment due to high-static magnetic fields, fast gradient magnetic fields, and strong radiofrequency electromagnetic fields. In this study, the genotoxic potential of 3 T clinical MRI scans in cultured human lymphocytes in vitro was investigated by analyzing chromosome aberrations (CA), micronuclei (MN), and single-cell gel electrophoresis. Human lymphocytes were exposed to electromagnetic fields generated during MRI scanning (clinical routine brain examination protocols: three-channel head coil) for 22, 45, 67, and 89 min. We observed a significant increase in the frequency of single-strand DNA breaks following exposure to a 3 T MRI. In addition, the frequency of both CAs and MN in exposed cells increased in a time-dependent manner. The frequencies of MN in lymphocytes exposed to complex electromagnetic fields for 0, 22, 45, 67, and 89 min were 9.67, 11.67, 14.67, 18.00, and 20.33 per 1000 cells, respectively. Similarly, the frequencies of CAs in lymphocytes exposed for 0, 45, 67, and 89 min were 1.33, 2.33, 3.67, and 4.67 per 200 cells,

respectively. These results suggest that exposure to 3 T MRI induces genotoxic effects in human lymphocytes.

(E) Leone L, Fusco S, Mastrodonato A, Piacentini R, Barbati SA, Zaffina S, Pani G, Podda MV, Grassi C. Epigenetic Modulation of Adult Hippocampal Neurogenesis by Extremely Low-Frequency Electromagnetic Fields. Mol Neurobiol. 2014 Feb 16. [Epub ahead of print] (GE)

Throughout life, adult neurogenesis generates new neurons in the dentate gyrus of hippocampus that have a critical role in memory formation. Strategies able to stimulate this endogenous process have raised considerable interest because of their potential use to treat neurological disorders entailing cognitive impairment. We previously reported that mice exposed to extremely low-frequency electromagnetic fields (ELFEFs) showed increased hippocampal neurogenesis. Here, we demonstrate that the ELFEF-dependent enhancement of hippocampal neurogenesis improves spatial learning and memory. To gain insights on the molecular mechanisms underlying ELFEFs' effects, we extended our studies to an in vitro model of neural stem cells (NSCs) isolated from the hippocampi of newborn mice. We found that ELFEFs enhanced proliferation and neuronal differentiation of hippocampal NSCs by regulation of epigenetic mechanisms leading to pro-neuronal gene expression. Upon ELFEF stimulation of NSCs, we observed a significant enhancement of expression of the pro-proliferative gene hairy enhancer of split 1 and the neuronal determination genes NeuroD1 and Neurogenin1. These events were preceded by increased acetylation of H3K9 and binding of the phosphorylated transcription factor cAMP response element-binding protein (CREB) on the regulatory sequence of these genes. Such ELFEF-dependent epigenetic modifications were prevented by the Ca,1-channel blocker nifedipine, and were associated with increased occupancy of CREB-binding protein (CBP) to the same loci within the analyzed promoters. Our results unravel the molecular mechanisms underlying the ELFEFs' ability to improve endogenous neurogenesis, pointing to histone acetylation-related chromatin remodeling as a critical determinant. These findings could pave the way to the development of novel therapeutic approaches in regenerative medicine.

(E) Li SS, Zhang ZY, Yang CJ, Lian HY, Cai P. Gene expression and reproductive abilities of male Drosophila melanogaster subjected to ELF-EMF exposure. Mutat Res. 758(1-2):95-103, 2013. (GE, LE, RP)

Extremely low frequency electromagnetic field (ELF-EMF) exposure is attracting increased attention as a possible disease-inducing factor. The in vivo effects of short-term and long-term ELF-EMF exposure on male Drosophila melanogaster were studied using transcriptomic analysis for preliminary screening and QRT-PCR for further verification. Transcriptomic analysis indicated that 439 genes were up-regulated and 874 genes were down-regulated following short-term exposures and that 514 genes were up-regulated and 1206 genes were down-regulated following long-term exposures (expression >2- or <0.5-fold, respectively). In addition, there are 238 up-regulated genes and 598 down-regulated genes in the intersection of short-term and long-term exposure (expression >2- or <0.5-fold). The DEGs (differentially expressed genes) in D. melanogaster following short-term exposures were involved in metabolic processes, cytoskeletal organization, mitotic spindle organization, cell death, protein modification and proteolysis. Long-term exposure let to changes in expression of genes involved in metabolic

processes, response to stress, mitotic spindle organization, aging, cell death and cellular respiration. In the intersection of short-term and long-term exposure, a series of DEGs were related to apoptosis, aging, immunological stress and reproduction. To check the ELF-EMF effects on reproduction, some experiments on male reproduction ability were performed. Their results indicated that short-term ELF-EMF exposure may decrease the reproductive ability of males, but long-term exposures had no effect on reproductive ability. Down-regulation of ark gene in the exposed males suggests that the decrease in reproductive capacity may be induced by the effects of ELF-EMF exposure on spermatogenesis through the caspase pathway. QRT-PCR analysis confirmed that jra, ark and decay genes were down regulated in males exposed for 1 Generation (1G) and 72 h, which suggests that apoptosis may be inhibited in vivo. ELF-EMF exposure may have accelerated cell senescence, as suggested by the down-regulation of both cat and jra genes and the up-regulation of hsp22 gene. Up-regulation of totA and hsp22 genes during exposure suggests that exposed flies might induce an in vivo immune response to counter the adverse effects encountered during ELF-EMF exposure. Down-regulation of cat genes suggests that the partial oxidative protection system might be restrained, especially during short-term exposures. This study demonstrates the bioeffects of ELF-EMF exposure and provides evidence for understanding the in vivo mechanisms of ELF-EMF exposure on male D. melanogaster.

(E) Lupke M, Frahm J, Lantow M, Maercker C, Remondini D, Bersani F, Simkó M. Gene expression analysis of ELF-MF exposed human monocytes indicating the involvement of the alternative activation pathway. Biochim Biophys Acta. 1763(4):402-12, 2006. (GE)

This study focused on the cell activating capacity of extremely low frequency magnetic fields (ELF-MF) on human umbilical cord blood-derived monocytes. Our results confirm the previous findings of cell activating capacity of ELF-MF (1.0 mT) in human monocytes, which was detected as an increased ROS release. Furthermore, gene expression profiling (whole-genome cDNA array Human Unigene RZPD-2) was performed to achieve a comprehensive view of involved genes during the cell activation process after 45 min ELF-MF exposure. Our results indicate the alteration of 986 genes involved in metabolism, cellular physiological processes, signal transduction and immune response. Significant regulations could be analyzed for 5 genes (expression >2- or <0.5-fold): IL15RA (Interleukin 15 receptor, alpha chain), EPS15R (Epidermal growth factor receptor pathway substrate 15 - like 1), DNMT3A (Hypothetical protein MGC16121), DNMT3A (DNA (cytosine-5) methyltransferase 3 alpha), and one gene with no match to known genes, DKFZP586J1624. Real-time RT-PCR analysis of the kinetic of the expression of IL15RA, and IL10RA during 45 min ELF-MF exposure indicates the regulation of cell activation via the alternative pathway, whereas the delayed gene expression of FOS, IL2RA and the melatonin synthesizing enzyme HIOMT suggests the suppression of inflammatory processes. Accordingly, we suggest that ELF-MF activates human monocytes via the alternative pathway.

(E) Luukkonen J, Liimatainen A, Höytö A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced genotoxic effects in human SH-SY5Y neuroblastoma cells. PLoS One. 2011 Mar 23;6(3):e18021. (GT, IA)

BACKGROUND: Extremely low frequency (ELF) magnetic fields (MF) are generated by power lines and various electric appliances. They have been classified as possibly carcinogenic

by the International Agency for Research on Cancer, but a mechanistic explanation for carcinogenic effects is lacking. A previous study in our laboratory showed that pre-exposure to ELF MF altered cancer-relevant cellular responses (cell cycle arrest, apoptosis) to menadione-induced DNA damage, but it did not include endpoints measuring actual genetic damage. In the present study, we examined whether pre-exposure to ELF MF affects chemically induced DNA damage level, DNA repair rate, or micronucleus frequency in human SH-SY5Y neuroblastoma cells. METHODOLOGY/PRINCIPAL FINDINGS: Exposure to 50 Hz MF was conducted at 100 μT for 24 hours, followed by chemical exposure for 3 hours. The chemicals used for inducing DNA damage and subsequent micronucleus formation were menadione and methyl methanesulphonate (MMS). Pre-treatment with MF enhanced menadione-induced DNA damage, DNA repair rate, and micronucleus formation in human SH-SY5Y neuroblastoma cells. Although the results with MMS indicated similar effects, the differences were not statistically significant. No effects were observed after MF exposure alone. CONCLUSIONS: The results confirm our previous findings showing that pre-exposure to MFs as low as 100 μT alters cellular responses to menadione, and show that increased genotoxicity results from such interaction. The present findings also indicate that complementary data at several chronological points may be critical for understanding the MF effects on DNA damage, repair, and post-repair integrity of the genome.

(E) Luukkonen J, Liimatainen A, Juutilainen J, Naarala J. Induction of genomic instability, oxidative processes, and mitochondrial activity by 50Hz magnetic fields in human SH-SY5Y neuroblastoma cells. Mutat Res. 760:33-41, 2014. (GT, OX, IA)

Epidemiological studies have suggested that exposure to 50Hz magnetic fields (MF) increases the risk of childhood leukemia, but there is no mechanistic explanation for carcinogenic effects. In two previous studies we have observed that a 24-h pre-exposure to MF alters cellular responses to menadione-induced DNA damage. The aim of this study was to investigate the cellular changes that must occur already during the first 24h of exposure to MF, and to explore whether the MF-induced changes in DNA damage response can lead to genomic instability in the progeny of the exposed cells. In order to answer these questions, human SH-SY5Y neuroblastoma cells were exposed to a 50-Hz, 100-μT MF for 24h, followed by 3-h exposure to menadione. The main finding was that MF exposure was associated with increased level of micronuclei, used as an indicator of induced genomic instability, at 8 and 15d after the exposures. Other delayed effects in MF-exposed cells included increased mitochondrial activity at 8d, and increased reactive oxygen species (ROS) production and lipid peroxidation at 15d after the exposures. Oxidative processes (ROS production, reduced glutathione level, and mitochondrial superoxide level) were affected by MF immediately after the exposure. In conclusion, the present results suggest that MF exposure disturbs oxidative balance immediately after the exposure, which might explain our previous findings on MF altered cellular responses to menadione-induced DNA damage. Persistently elevated levels of micronuclei were found in the progeny of MF-exposed cells, indicating induction of genomic instability.

(E) Ma Q, Deng P, Zhu G, Liu C, Zhang L, Zhou Z, Luo X, Li M, Zhong M, Yu Z, Chen C, Zhang Y. Extremely low-frequency electromagnetic fields affect transcript levels of

neuronal differentiation-related genes in embryonic neural stem cells. PLoS One. 2014 Mar 3;9(3):e90041. doi: 10.1371/journal.pone.0090041. eCollection 2014. (GE)

Previous studies have reported that extremely low-frequency electromagnetic fields (ELF-EMF) can affect the processes of brain development, but the underlying mechanism is largely unknown. The proliferation and differentiation of embryonic neural stem cells (eNSCs) is essential for brain development during the gestation period. To date, there is no report about the effects of ELF-EMF on eNSCs. In this paper, we studied the effects of ELF-EMF on the proliferation and differentiation of eNSCs. Primary cultured eNSCs were treated with 50 Hz ELF-EMF; various magnetic intensities and exposure times were applied. Our data showed that there was no significant change in cell proliferation, which was evaluated by cell viability (CCK-8 assay), DNA synthesis (Edu incorporation), average diameter of neurospheres, cell cycle distribution (flow cytometry) and transcript levels of cell cycle related genes (P53, P21 and GADD45 detected by real-time PCR). When eNSCs were induced to differentiation, real-time PCR results showed a down-regulation of Sox2 and up-regulation of Math1, Math3, Ngn1 and Tuj1 mRNA levels after 50 Hz ELF-EMF exposure (2 mT for 3 days), but the percentages of neurons (Tuj1 positive cells) and astrocytes (GFAP positive cells) were not altered when detected by immunofluorescence assay. Although cell proliferation and the percentages of neurons and astrocytes differentiated from eNSCs were not affected by 50 Hz ELF-EMF, the expression of genes regulating neuronal differentiation was altered. In conclusion, our results support that 50 Hz ELF-EMF induce molecular changes during eNSCs differentiation, which might be compensated by post-transcriptional mechanisms to support cellular homeostasis.

(E) Mairs RJ, Hughes K, Fitzsimmons S, Prise KM, Livingstone A, Wilson L, Baig N, Clark AM, Timpson A, Patel G, Folkard M, Angerson WJ, Boyd M. Microsatellite analysis for determination of the mutagenicity of extremely low-frequency electromagnetic fields and ionising radiation in vitro. Mutat Res. 626(1-2):34-41, 2007. (GT, IA)

Extremely low-frequency electromagnetic fields (ELF-EMF) have been reported to induce lesions in DNA and to enhance the mutagenicity of ionising radiation. However, the significance of these findings is uncertain because the determination of the carcinogenic potential of EMFs has largely been based on investigations of large chromosomal aberrations. Using a more sensitive method of detecting DNA damage involving microsatellite sequences, we observed that exposure of UVW human glioma cells to ELF-EMF alone at a field strength of 1 mT (50 Hz) for 12 h gave rise to 0.011 mutations/locus/cell. This was equivalent to a 3.75-fold increase in mutation induction compared with unexposed controls. Furthermore, ELF-EMF increased the mutagenic capacity of 0.3 and 3 Gy gamma-irradiation by factors of 2.6 and 2.75, respectively. These results suggest not only that ELF-EMF is mutagenic as a single agent but also that it can potentiate the mutagenicity of ionising radiation. Treatment with 0.3 Gy induced more than 10 times more mutations per unit dose than irradiation with 3 Gy, indicating hypermutability at low dose.

(E) Mariucci G, Villarini M, Moretti M, Taha E, Conte C, Minelli A, Aristei C, Ambrosini MV.
Brain DNA damage and 70-kDa heat shock protein expression in CD1 mice exposed to extremely low frequency magnetic fields. Int J Radiat Biol. 86(8):701-710, 2010. (GT, LE)

PURPOSE: The question of whether exposure to extremely low frequency magnetic fields (ELF-MF), may contribute to cerebral cancer and neurodegeneration is of current interest. In this study we investigated whether exposure to ELF-MF (50 Hz-1 mT) harms cerebral DNA and induces expression of 70-kDa heat shock protein (hsp70). MATERIALS AND METHODS: CD1 mice were exposed to a MF (50 Hz-1 mT) for 1 or 7 days (15 h/day) and sacrificed either at the end of exposure or after 24 h. Unexposed and sham-exposed mice were used as controls. Mouse brains were dissected into cerebral cortex-striatum, hippocampus and cerebellum to evaluate primary DNA damage and hsp70 gene expression. Food intake, weight gain, and motor activity were also evaluated. RESULTS: An increase in primary DNA damage was detected in all cerebral areas of the exposed mice sacrificed at the end of exposure, as compared to controls. DNA damage, as can be evaluated by the comet assay, appeared to be repaired in mice sacrificed 24 h after a 7-day exposure. Neither a short (15 h) nor long (7 days) MF-exposure induced hsp70 expression, metabolic and behavioural changes. CONCLUSIONS: These results indicate that in vivo ELF-MF induce reversible brain DNA damage while they do not elicit the stress response.

(E) Markkanen A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced DNA damage response in murine L929 cells. Int J Radiat Biol. 84(9):742-751, 2008. (IA)

PURPOSE: Effects on DNA damage response were investigated in murine L929 cells exposed to 50 Hz magnetic fields (MF) with or without ultraviolet B (UVB, wavelength 280-320 nm) radiation or menadione (MQ). MATERIALS AND METHODS: Cells were exposed to MF at 100 or 300 microT combined with MQ (150 microM, 1 hour) or UVB radiation (160 J/m(2)) using various exposure schedules. The samples were stained with propidium iodide (PI) and analysed by flow cytometer for cell cycle stages. Apoptotic cells were defined as sub G(1) events. RESULTS: In cells first exposed to 100 microT MF for 24 h, the response to subsequent MQ treatment was significantly altered so that the proportion of sub G(1) cells was decreased and the proportion of cells in the G(2)/M phase was increased. When a 300 microT MF was used, also the proportion of cells in the G(1) phase was decreased. MF exposures after MQ treatment did not alter responses to MQ. No effects were found from MF exposure alone or from MF combined with UVB radiation. CONCLUSIONS: The results strengthen previous findings suggesting that pre-exposure to MF can alter cellular responses to other agents, and indicate that MF as low as 100 microT has measurable impacts on cancer-relevant cellular processes such as DNA-damage.

(NE) Mizuno K, Narita E, Yamada M, Shinohara N, Miyakoshi J. ELF magnetic fields do not affect cell survival and DNA damage induced by ultraviolet B. Bioelectromagnetics. 35(2):108-115, 2014. (GT, IA)

We investigated whether extremely low frequency (ELF) magnetic field exposure has modification effects on cell survival after ultraviolet B (UV-B) irradiation and on repair process of DNA damage induced by UV-B irradiation in WI38VA13 subcloned 2RA and XP2OS(SV) cells. The ELF magnetic field exposure was conducted using a Helmholtz coil-based system that was designed to generate a sinusoidal magnetic field at 5!mT and 60!Hz. Cell survival was assessed by WST assay after UV-B irradiation at 20-80!J/m(2) , ELF magnetic field exposure for

24!h, followed by incubation for 48!h. DNA damage was assessed by quantification of cyclobutane pyrimidine dimer formation and 6-4 photoproduct formation using ELISA after UV-B irradiation at 20-80!J/m(2) followed by ELF magnetic field exposure for 24!h. No significant changes were observed in cell survival between ELF magnetic field and sham exposures. Similarly, DNA damage induced by UV-B irradiation did not change significantly following ELF magnetic field exposure. Our results suggest that ELF magnetic field exposure at 5!mT does not have modification effect on cell survival after UV-B irradiation and on repair process of DNA damage induced by UV-B irradiation.

(E) 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. (GT, GE)

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.

(NE) Okudan N, Celik I, Salbacak A, Cicekcibasi AE, Buyukmumcu M, Gökbel H. Effects of long-term 50 Hz magnetic field exposure on the micro nucleated polychromatic erythrocyte and blood lymphocyte frequency and argyrophilic nucleolar organizer regions in lymphocytes of mice. Neuro Endocrinol Lett. 31(2):208-214, 2010. (GT)

OBJECTIVES: We aimed to investigate the effects of weak extremely low frequency electromagnetic fields (ELF-EMFs) on the nucleus size, the silver staining nucleolar organizer regions (AgNORs), the frequency of micro nucleated peripheral blood lymphocytes (MPBLs) and the micro nucleated polychromatic erythrocytes (MPCEs).METHODS: One hundred and twenty Swiss albino mice were equally divided into 6 groups. The study groups were exposed to 1, 2, 3, 4 and 5 microT 50 Hz-EMFs for 40 days. Micronucleus number (MN) per PBL was determined. RESULTS: ELF-EMF exposure caused a nonlinear decline of nucleus area. A sharp drop occurred in AgNOR area of 1 microT group, and following it gained an insignificantly higher level than that of the control group. The field did not change mean AgNOR

numbers per nucleus of the groups. Relative AgNOR area had the highest level in 1 microT-exposure group, and the level was quite similar to that of the 5 microT-exposure group. The remaining groups had significantly lower values quite similar to that of the control level. The field exposure at any intensity did not affect significantly the frequency of either MPBLs or MPCEs. The number of MN per PBL in the 4 and 5 microT-exposure groups were significantly higher than those of the lower intensity exposure groups. The males in 4 microT-exposure group displayed the highest MN number per PBL, whereas values changed in a nonlinear manner. CONCLUSIONS: The results of the present study suggest that </=5 microT intensities of 50 Hz EMFs did not cause genotoxic effect on the mouse.

(E) Panagopoulos DJ, Karabarbounis A, Lioliousis C. ELF alternating magnetic field decreases reproduction by DNA damage induction. Cell Biochem Biophys. 67(2):703-16, 2013. (LE, GT, RP)

In the present experiments, the effect of 50-Hz alternating magnetic field on Drosophila melanogaster reproduction was studied. Newly eclosed insects were separated into identical groups of ten males and ten females and exposed to three different intensities of the ELF magnetic field (1, 11, and 21 G) continuously during the first 5 days of their adult lives. The reproductive capacity was assessed by the number of F1 pupae according to a well-defined protocol of ours. The magnetic field was found to decrease reproduction by up to 4.3%. The effect increased with increasing field intensities. The decline in reproductive capacity was found to be due to severe DNA damage (DNA fragmentation) and consequent cell death induction in the reproductive cells as determined by the TUNEL assay applied during early and mid-oogenesis (from germarium to stage 10) where physiological apoptosis does not occur. The increase in DNA damage was more significant than the corresponding decrease in reproductive capacity (up to ~7.5%). The TUNEL-positive signal denoting DNA fragmentation was observed exclusively at the two most sensitive developmental stages of oogenesis: the early and mid-oogenesis checkpoints (i.e. region 2a/2b of the germarium and stages 7-8 just before the onset of vitellogenesis)-in contrast to exposure to microwave radiation of earlier work of ours in which the DNA fragmentation was induced at all developmental stages of early and mid-oogenesis. Moreover, the TUNEL-positive signal was observed in all three types of egg chamber cells, mainly in the nurse and follicle cells and also in the oocyte, in agreement with the microwave exposure of our earlier works. According to previous reports, cell death induction in the oocyte was observed only in the case of microwave exposure and not after exposure to other stress factors as toxic chemicals or food deprivation. Now it is also observed for the first time after ELF magnetic field exposure. Finally, in contrast to microwave exposure of previous experiments of ours in which the germarium checkpoint was found to be more sensitive than stage 7-8, in the magnetic field exposure of the present experiments the mid-oogenesis checkpoint was found to be more sensitive than the germarium.

(E) Rageh MM, El-Gebaly RH, El-Bialy NS. Assessment of genotoxic and cytotoxic hazards in brain and bone marrow cells of newborn rats exposed to extremely low-frequency magnetic field. J Biomed Biotechnol. 2012;2012:716023. (LE, GT, DE, OX)

The present study aimed to evaluate the association between whole body exposure to extremely low frequency magnetic field (ELF-MF) and genotoxic , cytotoxic hazards in brain and bone

(E) Udroiu I, Cristaldi M, Ieradi LA, Bedini A, Giuliani L, Tanzarella C. Clastogenicity and aneuploidy in newborn and adult mice exposed to 50 Hz magnetic fields. Int J Radiat Biol. 82(8):561-567, 2006. (GT, DE, LE)

PURPOSE: To detect possible clastogenic and aneugenic properties of a 50 Hz, 650 muT magnetic field. MATERIALS AND METHODS: The micronucleus test with CREST (Calcinosis, Raynaud's phenomenon, Esophageal dismotility, Sclerodactility, Telangectasia) antibody staining was performed on liver and peripheral blood sampled from newborn mice exposed to an ELF (Extremely Low Frequency) magnetic field during the whole intra-uterine life (21 days), and on bone marrow and peripheral blood sampled from adult mice exposed to the same magnetic field for the same period. RESULTS: Data obtained in newborn mice show a significant increase in micronuclei frequencies. In absolute terms, most of the induced micronuclei were CREST-negative (i.e., formed by a chromosome fragment). However, in relative terms, ELF exposure caused a two-fold increase in CREST-negative micronuclei and a four-fold increase in CREST-positive micronuclei (i.e., formed by a whole chromosome). No significant effect was recorded on exposed adults. CONCLUSIONS: These findings suggest the need for investigation of aneugenic properties of ELF magnetic fields in order to establish a possible relationship to carcinogenesis.

(NE) Verschaeve L, Anthonissen R, Grudniewska M, Wudarski J, Gevaert L, Maes A. Genotoxicity investigation of ELF-magnetic fields in Salmonella typhimurium with the sensitive SOS-based VITOTOX test. Bioelectromagnetics. 32(7):580-584, 2011. (GT, IA)

We performed a genotoxicity investigation of extremely low-frequency (ELF) magnetic fields (MFs, 50 Hz, 100 and 500 μT, 1 and 2 h exposure) alone and in combination with known chemical mutagens using the VITOTOX test. This test is a very sensitive reporter assay of Salmonella typhimurium bacteria based on the SOS response. Our study showed that ELF-MFs do not induce SOS-based mutagenicity in S. typhimurium bacteria and do not show any synergetic effect when combined with chemical mutagens.

(E) Villarini M, Ambrosini MV, Moretti M, Dominici L, Taha E, Piobbico D, Gambelunghe C, Mariucci G. Brain hsp70 expression and DNA damage in mice exposed to extremely low frequency magnetic fields: a dose-response study. Int J Radiat Biol. 89(7):562-570, 2013. (LE, GT)

Purpose: To determine whether a dose-response relationship exists among exposure to extremely low frequency magnetic fields (ELF-MF) at different densities and 70-kDa heat shock protein (hsp70) expression and DNA damage in mouse brain. Materials and Methods: Male CD1 mice were exposed to ELF-MF (50 Hz; 0.1, 0.2, 1 or 2 mT) for 7 days (15 hours/day) and sacrificed either at the end of exposure or after 24 h. Hsp70 expression was determined in cerebral cortex-striatum, hippocampus and cerebellum by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and western blot analysis. Primary DNA damage was evaluated in the same tissues by comet assay. Sham-exposed mice were used as controls. Results: No changes in both hsp70 mRNA and corresponding protein occurred following exposure to ELF-MF, except for a weak increase in the mRNA in hippocampus of exposed mice to 0.1 mT ELF-MF. Only mice exposed to 1 or 2 mT and sacrificed immediately after exposure presented DNA strand

marrow cells of newborn rats. Newborn rats (10 days after delivery) were exposed continuously to 50!Hz, 0.5!mT for 30 days. The control group was treated as the exposed one with the sole difference that the rats were not exposed to magnetic field. Comet assay was used to quantify the level of DNA damage in isolated brain cells. Also bone marrow cells were flushed out to assess micronucleus induction and mitotic index. Spectrophotometric methods were used to measure the level of malondialdehyde (MDA) and the activity of glutathione (GSH) and superoxide dismutase (SOD). The results showed a significant increase in the mean tail moment indicating DNA damage in exposed group ($P < 0.01, 0.001, 0.0001$). Moreover ELF-MF exposure induced a significant ($P < 0.01, 0.001$) four folds increase in the induction of micronucleus and about three folds increase in mitotic index ($P < 0.0001$). Additionally newborn rats exposed to ELF-MF showed significant higher levels of MDA and SOD ($P < 0.05$). Meanwhile ELF-MF failed to alter the activity of GSH. In conclusion, the present study suggests an association between DNA damage and ELF-MF exposure in newborn rats.

(E) Reyes-Guerrero G, Guzmán C, García DE, Camacho-Arroyo I, Vázquez-García M. Extremely low-frequency electromagnetic fields differentially regulate estrogen receptor-alpha and -beta expression in the rat olfactory bulb. Neurosci Lett. 471(2):109-13, 2010. (GE)

Recently, the effects of extremely low-frequency electromagnetic fields (ELF EMF) on biological systems have been extensively investigated. In this report, the influence of ELF EMF on olfactory bulb (OB) estrogen receptor-alpha (ER alpha) mRNA and -beta (ER beta) mRNA expression was studied by RT-PCR in adult female and male rats. Results reveal for the first time that ELF EMF exerted a biphasic effect on female OB ER beta mRNA gene expression, which increased during diestrous and decreased during estrous. We did not observe any influence of ELF EMF on female OB ER alpha mRNA expression. Our data demonstrate a fluctuating pattern of ER-alpha and -beta mRNA expression in the female OB throughout the phases of the estrous cycle in non-ELF EMF-exposed animals. Thus the highest ER alpha expression was observed in diestrous and the lowest in proestrous. The pattern of ER beta mRNA was less variable, the lowest expression was observed in diestrous. ER-alpha mRNA and -beta mRNA expression level in the male OB did not exhibit any variation either in ELF EMF-exposed or non-ELF EMF-exposed animals. In summary, ELF EMF modulate ER beta gene expression in the OB of female adult rats but not in males.

(E) Ruiz-Gómez MJ, Sendra-Portero F, Martínez-Morillo M. Effect of 2.45 mT sinusoidal 50 Hz magnetic field on Saccharomyces cerevisiae strains deficient in DNA strand breaks repair. Int J Radiat Biol. 86(7):602-611, 2010. (GT)

PURPOSE: To investigate whether extremely-low frequency magnetic field (MF) exposure produce alterations in the growth, cell cycle, survival and DNA damage of wild type (wt) and mutant yeast strains. MATERIALS AND METHODS: wt and high affinity DNA binding factor 1 (hdf1), radiation sensitive 52 (rad52), rad52 hdf1 mutant Saccharomyces cerevisiae strains were exposed to 2.45 mT, sinusoidal 50 Hz MF for 96 h. MF was generated by a pair of Helmholtz coils. During this time the growth was monitored by measuring the optical density at 600 nm and cell cycle evolution were analysed by microscopic morphological analysis. Then, yeast survival was assayed by the drop test and DNA was extracted and electrophoresed.

RESULTS: A significant increase in the growth was observed for rad52 strain (P = 0.005, Analysis of Variance [ANOVA]) and close to significance for rad52 hdf1 strain (P = 0.069, ANOVA). In addition, the surviving fraction values obtained for MF-exposed samples were in all cases less than for the controls, being the P value obtained for the whole set of MF-treated strains close to significance (P = 0.066, Student's t-test). In contrast, the cell cycle evolution and the DNA pattern obtained for wt and the mutant strains were not altered after exposure to MF. CONCLUSIONS: The data presented in the current report show that the applied MF (2.45 mT, sinusoidal 50 Hz, 96 h) induces alterations in the growth and survival of S. cerevisiae strains deficient in DNA strand breaks repair. In contrast, the MF treatment does not induce alterations in the cell cycle and does not cause DNA damage.

(E) Sarimov R, Alipov ED, Belyaev IY. Fifty hertz magnetic fields individually affect chromatin conformation in human lymphocytes: dependence on amplitude, temperature, and initial chromatin state. Bioelectromagnetics. 32(7):570-579, 2011. (GT)

Effects of magnetic field (MF) at 50 Hz on chromatin conformation were studied by the method of anomalous viscosity time dependence (AVTD) in human lymphocytes from two healthy donors. MF within the peak amplitude range of 5-20 μT affected chromatin conformation. These MF effects differed significantly between studied donors, and depended on magnetic flux density and initial condensation of chromatin. While the initial state of chromatin was rather stable in one donor during one calendar year of measurements, the initial condensation varied significantly in cells from another donor. Both this variation and the MF effect depended on temperature during exposure. Despite these variations, the general rule was that MF condensed the relaxed chromatin and relaxed the condensed chromatin. Thus, in this study we show that individual effects of 50 Hz MF exposure at peak amplitudes within the range of 5-20 μT may be observed in human lymphocytes in dependence on the initial state of chromatin and temperature.

(E) Tiwari R, Lakshmi NK, Bhargava SC, Ahuja YR. Epinephrine, DNA integrity and oxidative stress in workers exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) at 132'kV substations. Electromagn Biol Med. 2014 Jan 24. [Epub ahead of print] (LE, GT, HU, OX)

There is apprehension about widespread use of electrical and electromagnetic gadgets which are supposed to emit electromagnetic radiations. Reports are controversy. These electromagnetic fields (EMFs) have considerable effect on endocrine system of exposed subjects. This study was focused to assess the possible bioeffects of extremely low-frequency (ELF)-EMFs on epinephrine level, DNA damage and oxidative stress in subjects occupationally exposed to 132!kV high-voltage substations. The blood sample of 142 exposed subjects and 151 non-exposed individuals was analyzed. Plasma epinephrine was measured by enzyme-linked immunosorbent assay, DNA damage was studied by alkaline comet assay along with oxidative stress. Epinephrine levels of sub-groups showed mean concentration of 75.22 !±! 1.46, 64.43 !±! 8.26 and 48.47 !±! 4.97 for high, medium and low exposed groups, respectively. DNA damage ranged between 1.69!μm and 9.91!μm. The oxidative stress levels showed significant increase. The individuals employed in the live-line procedures were found to be vulnerable for EM stress with altered epinephrine concentrations, DNA damage and increased oxidative stress.

breaks higher than controls in all the cerebral areas; such DNA breakage reverted to baseline in the mice sacrificed 24 h after exposure. Conclusions: These data show that high density ELF-MF only induce reversible brain DNA damage while they do not affect hsp70 expression.

(E) Wahab MA, Podd JV, Rapley BI, Rowland RE. Elevated sister chromatid exchange frequencies in dividing human peripheral blood lymphocytes exposed to 50 Hz magnetic fields. Bioelectromagnetics. 28(4):281-288, 2007. (GT, WS)

The in vitro cytomolecular technique, sister chromatid exchange (SCE), was applied to test the clastogenic potentiality of extremely low frequency (ELF) electromagnetic fields (EMFs) on human peripheral blood lymphocytes (HPBLs). SCE frequencies were scored in dividing peripheral blood lymphocytes (PBLs) from six healthy male blood donors in two rounds of experiments, R1 and R2, to determine reproducibility. Lymphocyte cultures in the eight experiments conducted in each round were exposed to 50 Hz sinusoidal (continuous or pulsed) or square (continuous or pulsed) MFs at field strengths of 1 microT or 1 mT for 72 h. A significant increase in the number of SCEs/cell in the grouped experimental conditions compared to the controls was observed in both rounds. The highest SCE frequency in R1 was 10.03 for a square continuous field, and 10.39 for a square continuous field was the second highest frequency in R2. DNA crosslinking at the replication fork is proposed as a model which could explain the mechanistic link between ELF EMF exposure and increased SCE frequency.

(E) Wang Z, Sarje A, Che PL, Yarema KJ. Moderate strength (0.23-0.28 T) static magnetic fields (SMF) modulate signaling and differentiation in human embryonic cells. BMC Genomics. 10:356, 2009. (GE)

BACKGROUND: Compelling evidence exists that magnetic fields modulate living systems. To date, however rigorous studies have focused on identifying the molecular-level biosensor (e.g., radical ion pairs or membranes) or on the behavior of whole animals leaving a gap in understanding how molecular effects are translated into tissue-wide and organism-level responses. This study begins to bridge this gulf by investigating static magnetic fields (SMF) through global mRNA profiling in human embryonic cells coupled with software analysis to identify the affected signaling pathways. RESULTS: Software analysis of gene expression in cells exposed to 0.23-0.28 T SMF showed that nine signaling networks responded to SMF; of these, detailed biochemical validation was performed for the network linked to the inflammatory cytokine IL-6. We found the short-term (<24 h) activation of IL-6 involved the coordinate up-regulation of toll-like receptor-4 (TLR4) with complementary changes to NEU3 and ST3GAL5 that reduced ganglioside GM3 in a manner that augmented the activation of TLR4 and IL-6. Loss of GM3 also provided a plausible mechanism for the attenuation of cellular responses to SMF that occurred over longer exposure periods. Finally, SMF-mediated responses were manifest at the cellular level as morphological changes and biochemical markers indicative of pre-oligodendrocyte differentiation. CONCLUSION: This study provides a framework describing how magnetic exposure is transduced from a plausible molecular biosensor (lipid membranes) to cell-level responses that include differentiation toward neural lineages. In addition, SMF provided a stimulus that uncovered new relationships - that exist even in the absence of magnetic fields - between gangliosides, the time-dependent regulation of IL-6 signaling by these glycosphingolipids, and the fate of embryonic cells.

(NE) 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. (GT)

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.

(E) Yokus B, Akdag MZ, Dasdag S, Cakir DU, Kizil M. Extremely low frequency magnetic fields cause oxidative DNA damage in rats. Int J Radiat Biol. 84(10):789-795, 2008. (GT)

PURPOSE: To detect the genotoxic effects of extremely low frequency (ELF) -magnetic fields (MF) on oxidative DNA base modifications [8-hydroxyguanine (8-OH-Gua), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde)] in rat leucocytes, measured following exposure to ELF-MF. MATERIALS AND METHODS: After exposure to ELF-MF (50 Hz, 100 and 500 microT, for 2 hours/day during 10 months), DNA was extracted, and measurement of DNA lesions was achieved by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). RESULTS: Levels of FapyAde, FapyGua and 8OHdG in DNA were increased by both 100 microT and 500 microT ELF-MF as compared to a cage-control and a sham group; however, statistical significance was observed only in the group exposed to 100 microT. CONCLUSION: This is the first study to report that ELF-MF exposure generates oxidatively induced DNA base modifications which are mutagenic in mammalian cells, such as FapyGua, FapyAde and 8-OH-Gua, in vivo. This may explain previous studies showing DNA damage and genomic instability. These findings support the hypothesis that chronic exposure to 50-Hz MF may be potentially genotoxic. However, the intensity of ELF-MF has an important influence on the extent of DNA damage.

(E) Yoon HE, Lee JS, Myung SH, Lee YS. Increased γ-H2AX by exposure to a 60-Hz magnetic fields combined with ionizing radiation, but not hydrogen peroxide, in non-tumorigenic human cell lines. Int J Radiat Biol. 2014 Jan 28. [Epub ahead of print] (GT, IA)

Purpose: Genotoxic effects have been considered the gold standard to determine if an environmental factor is a carcinogen, but the currently available data for extremely low

frequency time-varying magnetic fields (ELF-MFs) remain controversial. As an environmental stimulus, the effect of ELF-MF on cellular DNA may be subtle. Therefore, a more sensitive method and systematic research strategy are warranted to evaluate genotoxicity. Materials and methods: We investigated the effect of ELF-MFs in combination with ionizing radiation (IR) or H_2O_2 on the DNA damage response of expression of phosphorylated H2AX (γ-H2AX) and production of γ-H2AX foci in non-tumorigenic human cell systems consisting of human lung fibroblast WI38 cells and human lung epithelial L132 cells. Results: Exposure to a 60-Hz, 2 mT ELF-MFs for 6 h produced increased γ-H2AX expression, as well as γ-H2AX foci production, a common DNA double-strand break (DSB) marker. However, exposure to a 1 mT ELF-MFs did not have the same effect. Moreover, 2 mT ELF-MFs exposure potentiated the expression of γ-H2AX and γ-H2AX foci production when combined with IR, but not when combined with H_2O_2. Conclusions: ELF-MFs could affect the DNA damage response and, in combination with different stimuli, provide different effects on γ-H2AX.