SECTION 4

Evidence for Inadequacy of the Standards

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I. Introduction

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

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

As a result of current international research and scientific discussion on whether the prevailing RF and ELF standards are adequate for protection of public health, there are many recent developments prior to 2007 to provide valuable background on the uncertainty about whether current standards adequately protect the public. Since 2007, there are important new milestone publications that underscore the critical need to update public safety limits. These newer documents calling for review and updating are based on a deluge of new scientific studies reporting effects at non-thermal, low-intensity ELF and RF exposure levels. There is little doubt that bioeffects and adverse health effects are occurring at lower-than-safety limit levels, meaning the existing protections are inadequate.

II. United States Government Accountability Office

The US Government Accountability Office published a report in 2012 urging the US Federal Communications Commission to revisit the outdated safety standards for the exposures from wireless devices. (US GAO, 2012)

The rapid adoption of mobile phones has occurred amidst controversy over whether the technology poses a risk to human health as a result of long-term exposure to RF energy from mobile phone use. FCC and FDA share regulatory responsibilities for mobile phones. GAO was asked to examine several issues related to mobile phone health effects and regulation. Specifically, this report addresses:

> (1) what is known about the health effects of RF energy from mobile phones and what are current research activities,
> (2) how FCC set the RF energy exposure limit for mobile phones, and
> (3) federal agency and industry actions to inform the public about health issues related to mobile phones, among other things.

GAO reviewed scientific research; interviewed experts in fields such as public health and engineering, officials from federal agencies, and representatives of academic institutions, consumer groups, and the mobile phone industry; reviewed mobile phone testing and certification regulations and guidance; and reviewed relevant federal agency websites and mobile phone user manuals.

The Report noted that the FCC's RF energy exposure limit may not reflect the latest research. Redundant and overlapping jurisdiction over the setting of public safety limits is highlighted where the GAO Report notes:

> *"FCC told GAO that it relies on the guidance of federal health and safety agencies when determining the RF energy exposure limit, and to date, none of these agencies have advised FCC to change the limit. However, FCC has not formally asked these agencies for a reassessment. By not formally reassessing it's current limit, FCC cannot ensure it is using a limit that reflects the latest research on RF energy exposure. FCC has also not reassessed it's testing requirements to ensure that they identify the maximum RF energy exposure a user could experience. Some consumers may use mobile phones against the body, which FCC does not currently test, and could result in RF energy exposure higher than the FCC limit." (US GAO, 2012)*

The GAO Report recommends to the FCC that it formally reassess, and, if appropriate, change it's current RF energy exposure limit and mobile phone testing requirements related to likely usage configurations, particularly when phones are held against the body.

FCC noted that a draft document that is now under consideration by the FCC has the potential to address GAO's recommendations. (US GAO, 2012)

III. International Agency for Research on Cancer - World Health Organization Classifies Radiofrequency Radiation as 2B Possible Human Carcinogen

In 2011, a group of 30 researchers, scientists and medical doctors were invited to participate in an assessment of the scientific literature on radiofrequency radiation carcinogenicity in Lyon, France. Under the auspices of IARC, they conducted a comprehensive scientific assessment of RF studies and determined:

> *"In view of the limited evidence in humans and in experimental animals, the Working Group classified RF- EMF as "possibly carcinogenic to humans" (Group 2B). This evaluation was supported by a large majority of Working Group members." (Baan et al, 2011)*
>
> *"(T)he Working Group concluded that the (Interphone Final Report) findings could not be dismissed as reflecting bias alone, and that a causal interpretation between mobile phone RF-EMF exposure and glioma is possible. A similar conclusion was drawn from these two studies for acoustic neuroma, although the case numbers were substantially smaller than for glioma." (Baan et al, 2011)*

It is important to recognize that the IARC RF Working Group did not find the evidence insufficient to classify (Group 3) or not a carcinogen (Group 4). Both of these possible outcomes to the scientific assessment could have rendered a substantially weaker conclusion. Where there has been the necessity of a virtual scientific paradigm shift to accommodate ANY consideration of both ELF-EMF and RFR to the status where legitimate scientific attention is achieved is a notable achievement. There is a very high bar set to show that non-chemical carcinogens warrant IARC carcinogenicity evaluation - it greatly exceeds that necessary for chemicals and other toxins.

IV. World Health Organization *INTERPHONE* Study on Mobile Phone Cancer Risk

In 2010, the World Health Organization released the final results of it's investigation on

cell phones and cancer. (INTERPHONE Study Group, 2010) The ten-year long World Health Organization *INTERPHONE Study* confirms previous reports showing what many experts have warned – that regular use of a cell phone by adults can significantly increase the risk of glioma by 40% with 1640 hours or more of use (this is about one-half hour per day over ten years). Tumors were more likely to occur on the side of the head most used for calling. The risk increases to 96% for adults with ipsilateral cell phone use (when the cell phone is used predominantly on one side of the head). The study appears in the International Journal of Epidemiology. Thirteen teams from countries around the world combined their results. Only the glioma findings were released (final results on acoustic neuroma and parotid tumors are not yet published.

A comprehensive and technically reliable description of the *INTERPHONE* study findings is provided within the International Agency for Research on Cancer, 2011 RF Monograph as part of the publication in Lancet Oncology on IARC's classification of radiofrequency radiation as a 2B Possible Human Carcinogen. Results of the *INTERPHONE* Study were highly scrutinized by IARC, and influenced the classification of RF based on the cell phone-brain cancer findings of *INTERPHONE*.

From Baan et al, 2011:

> *"The INTERPHONE study, a multi-centre case-control study, is the largest investigation so far of mobile phone use and brain tumours, including glioma, acoustic neuroma, and meningioma.The pooled analysis included 2708 glioma cases and 2972controls(participation rates64% and 53%, respectively). Comparing those who ever used mobile phones with those who never did yielded an odds ratio (OR) of 0·81 (95% CI 0·70–0·94). In terms of cumulative call time, ORs were uniformly below or close to unity for all deciles of exposure except the highest decile (>1640 h of use), for which the OR for glioma was 1·40 (95% CI 1·03–1·89). There was suggestion of an increased risk for ipsilateral exposure(on the same side of the head as the tumour) and for tumours in the temporal lobe, where RF exposure is highest. Associations between glioma and cumulative specific energy absorbed at the tumour location were examined in a subset of 553 cases that had estimated RF doses.10 The OR for glioma increased with increasing RF dose for exposures 7 years or more before diagnosis, whereas there was no association with estimated dose for exposures less than 7 years before diagnosis.*
>
> *A Swedish research group did a pooled analysis of two very similar studies of associations between mobile and cordless phone use and glioma, acoustic neuroma, and meningioma.9 The analysis included 1148 glioma cases (ascertained 1997–2003) and 2438 controls,obtained through cancer and population registries,*

respectively. Self-administered mailed questionnaires were followed by telephone interviews to obtain information on the exposures and covariates of interest, including use of mobile and cordless phones (response rates 85% and 84%, respectively). Participants who had used a mobile phone for more than 1 year had an OR for glioma of 1.3 (95% CI 1·1–1·6). The OR increased with increasing time since first use and with total call time, reaching 3.2 (2·0–5·1) for more than 2000 h of use. Ipsilateral use of the mobile phone was associated with higher risk. Similar findings were reported for use of cordless phones.

Although both the INTERPHONE study and the Swedish pooled analysis are susceptible to bias—due to recall error and selection for participation— the Working Group concluded that the findings could not be dismissed as reflecting bias alone, and that a causal interpretation between mobile phone RF-EMF exposure and glioma is possible. A similar conclusion was drawn from these two studies for acoustic neuroma, although the case numbers were substantially smaller than for glioma. Additionally, a study from Japan (11) found some evidence of an increased risk for acoustic neuroma associated with ipsilateral mobile phone use.

(Baan et al, 2011)

No that no increased risk was detected overall. But this is not unexpected. No exposures to carcinogens that cause solid tumors like brain cancer or lung cancers, for example from tobacco and asbestos have ever been shown to significantly increase cancer risk in people with such short duration of exposure. The latency period for brain cancer is 15-30 years.

The final INTERPHONE results support findings of several research groups who have published studies reporting that continuing use of a mobile phone increases risk of brain cancer. We would not expect to see substantially increased brain tumor risk for most cancer-causing agents except in the longer term (10 year and longer) as is the case here in the population of regular cell phone users. Further, the participants included in this study were 30-59 years old, excluding younger and older users. Use of cordless phones was neglected in the analysis. Radiofrequency radiation from some cordless phones can be as high as mobile phones in some countries, so excluding such use would underestimate the risk for brain tumors and other cancers.

For public health experts and members of the public who looked to IARC for further clarification of the scope of this 2B Possible Human Carcinogen designation, Dr. Baan replied to informal queries that:

"Although the key information came from mobile telephone use, the Working Group considered that the three types of exposure entail basically the same type of radiation, and decided to make an overall evaluation on RF-EMF, covering the whole radiofrequency region of the electromagnetic spectrum.

In support of this, information from studies with experimental animals showed that effects on cancer incidence and cancer latency were seen with exposures to different frequencies within the RF region.

So the classification 2B, possibly carcinogenic, holds for all types of radiation within the radiofrequency part of the electromagnetic spectrum, including the radiation emitted by base-station antennas, radio/ TV towers, radar, Wi-Fi, smart meters, etc." (Personal communication of Dr. Robert Baan to Connie Hudson, August 29, 2011)

V. President's Cancer Panel Report of 2010

The United States President's Cancer Panel Report (2010) includes important and unprecedented recognition of non-ionizing radiation as a possible carcinogen deserving of further research and possible public health action. The Report found "the true burden of environmentally induced cancers has been grossly underestimated" and strongly urged action to reduce peoples' widespread exposures to carcinogens. The 240-page report issued for 2008-2009 by a panel of experts that report to the US president indicate that environmental factors are underestimated in cancer prevention. The Report specifically addresses the link between cell phones and cancer. The Panel recommends that people reduce their cell phone exposure, even when absolute proof of harm is not yet available.

Research Recommended by Presidents Cancer Panel

- Resolve controversies regarding the safety or harm of low doses of various forms of radiation in adults and children. Identify circumstances under which low- dose radiation may have a hormetic effect.

- Develop radiation dose and risk estimates that better reflect the current and future U.S. population. Existing dose and risk estimates have been based on adult males; estimates should account for population diversity, including children. In addition, develop medical radiation risk estimates that are not based on acute doses received by atomic bomb survivors.

• Expand research on possible harmful effects of cell phone use, especially in children. Cell phone use still is relatively recent, and studies to date have had mixed findings; most involve users of older equipment. Findings from cohort studies now underway are anticipated, but longer-term studies of individuals using current equipment are needed.

• Conduct additional research on possible links between electromagnetic fields (EMF) and cancer; identify mechanism(s) of EMF carcinogenesis.

• Monitor changing patterns of radiation exposure.

• Raise the priority of and investment in research to develop non-toxic products anD processes.

• Develop, test, and evaluate prevention communication strategies and interventions, especially in high-risk occupations and populations.

(National Cancer Institute, 2010)

VI. World Health Organization Research Agenda for Radiofrequency Fields (2010)

In 2010, the WHO produced a research agenda to address growing scientific questions and public concern about health effects of radiofrequency radiation, particularly with the explosive rise in exposures from new telecommunications technologies. It replaced a 2006 research agenda developed by the International EMF Project.

"Telecommunication technologies based on radiofrequency (RF) transmission, such as radio and television, have been in widespread use for many decades. However, there are numerous new applications for the broadcast and reception of RF waves and the use of RF devices such as mobile phones is now ubiquitous.

The attendant increased public exposure to RF fields has made its effects on human health a topic of concern for scientists and the general public.
(emphasis added)

To respond to these concerns, an important research effort has been mounted over the past decade and many specific questions about potential health effects of RF fields have already been investigated by scientists around the world. Nonetheless, several areas still warrant further investigation and the rapid evolution of technology in this field is raising new questions." (WHO, 2010)

"This Research Agenda is developed ahead of the major hazard/health risk evalu-

ations that the IARC and WHO are due to carry out over the next two years. It focuses on identifying short- and long-term research needs that will enable more complete health risk assessments to be undertaken and communicated more effectively to the public." (WHO, 2010)

Recommendations of the WHO Research Agenda for Radiofrequency Fields are as follows. This section is necessarily extensive to document the advice of experts at WHO by 2010 in recognizing radiofrequency radiation has the potential to result in global health impacts; even if very slow to implement precautionary advice to the European Commission and member countries.

Priority: Epidemiology

High - Prospective cohort studies of children and adolescents with outcomes including behavioural and neurological disorders and cancer

Rationale: As yet, little research has been conducted in children and adolescents and it is still an open question whether children are more susceptible to Rf EMF since the brain continues to develop during childhood and adolescence. also, children are starting to use mobile phones at a younger age. given the existence of large-scale cohort studies of mothers and children with follow-up started during or before pregnancy, an Rf sources component could be added at a reasonably low cost. Billing records for mobile phones are not valid for children, therefore the prospective collection of exposure data is needed. for neuropsychological studies, one challenge is to distinguish the "training" of motor and neu- ropsychological skills caused by the use of a mobile phone from the effects of the Rf field. any future study should try to address this issue. in any case it should be of longitudinal design, thereby allowing the study of several outcomes and changes in technology and the use of mobile phones as well as other sources of Rf eMf exposure, such as wireless laptops.

High - Monitoring of brain tumour incidence trends through well-established population-based cancer registries, if possible combined with population exposure data

Rationale: If there is a substantial risk associated with mobile phone use, it should be observable in data sources of good quality. such time trend analyses can be performed quite quickly and inexpensively. By using modern statistical techniques for analysing popu- lation data it should be possible to link changes in exposure prevalence in the population to the incidence of brain tumours and, if high-quality surveillance data are available, the incidence of other diseases at the population level. given the shortcomings in the exposure assessment and participation of previous studies based on individual data, an ecological study would have benefits that may outweigh its limitations.

Other - case-control studies of neurological diseases provided that objective exposure data and confounder data are available and reasonable participation is achieved

Rationale: Neurological endpoints, such as alzheimer disease and Parkinson disease, may be as biologically plausible as brain cancer and an increased risk would have a major public health impact. This study could give an early warning sign that can be elaborated further in the prospective cohort studies. an analysis of time-trends in neurological disease could also serve as an early warning sign. However, a feasibility study would be necessary in order to determine whether a good quality case-control study could be carried out.

Priority: Human studies

High - further RF EMf provocation studies on children of different ages

Rationale: current research has focused primarily on adolescents; very little is known about possible effects in younger children. longitudinal testing at different ages, for example by studying children already participating in current cohort studies, is recommended. This would allow consideration of the influence of potentially confounding factors such as lifestyle.

High - Provocation studies to identify neurobiological mechanisms underlying possible effects of RF on brain function, including sleep and resting EEG

Rationale: These studies should include validation of these effects using a range of brain imaging methods. They should also include studies investigating possible thresholds and dose-response relationships at higher exposure levels such as those encountered during occupational exposure.

Priority: Animal studies

High - Effects of early-life and prenatal RF exposure on development and behaviour

Rationale: There is still a paucity of information concerning the effects of prenatal and early life exposure to RF EMf on subsequent development and behaviour. Such studies are regarded as important because of the widespread use of mobile phones by children and the increasing exposure to other RF sources such as wireless local area networks (Wlans) and the reported effects of RF EMf on the adult EEG. further study is required which should include partial (head only) exposure to mobile phones at relatively high specific absorption rate (SAR) levels.

High - effects of RF exposure on ageing and neurodegenerative diseases

Rationale: age-related diseases, especially neurodegenerative diseases of the brain such as alzheimer disease and Parkinson disease, are increasingly prevalent and are therefore an important public health issue. Mobile phone use typically involves repeated Rf eMf

exposure of the brain; a recent study has suggested that this type of exposure could affect alzheimer disease in a transgenic mouse model for this condition (arendash et al., 2010). There are a few ongoing studies of possible Rf eMf effects on neurodegenerative diseases but further studies are required to investigate this subject more fully.

Other research needs - Effects of RF exposure on reproductive organs

Rationale: The available data concerning possible effects of Rf eMf from mobile phones on male fertility are inconsistent and their quality and exposure assessments are weak. in vivo studies on fertility should consider effects on both males and females and investigate a range of relevant endpoints including Rf eMf effects on the development and function of the endocrine system.

Priority: Cellular studies

Other - Identify optimal sets of experimental tests to detect cellular response after exposure to new RF technologies and co-exposures of RF EMF with environmental agents

Rationale: a number of in vitro studies investigating the effects of exposure to mobile phone frequencies/signals, or co-exposures of RF EMf with chemical or physical agents, have been published in the last fifteen years. Results obtained have been inconsistent and contradictory, not least because of the use of a large variety of cell types and study approaches. a set of highly sensitive, well-harmonized cellular and molecular methods should be developed in order to screen the toxic potential of new types of RF signals used in new technologies and of co-exposures of RF EMf and environmental agents – especially those suspected to have toxic effects. This research must be multicentred in order to allow the widest possible acceptance and application of this screening tool.

Other - further studies on the influence of genetic background and cell type: possible effects of mobile phone type Rf exposure on a variety of cell types using newer, more sensitive methods less susceptible to artefact and/or bias

Rationale: More rigorous quantitative methods should be employed in the evaluation of positive results that suggest a specific cell type response, e.g. of embryonic cells (Czyz et al., 2004; Franzellitti et al., 2010), raising the possibility that RF impacts specific cell subpopulations or cell types. These studies should include a variety of cell types such as stem cells and cells with altered genetic backgrounds.

Priority: Mechanisms: none

Priority: Dosimetry

High - Assess characteristic RF EMF emissions, exposure scenarios and corresponding exposure levels for new and emerging RF technologies; also for changes in the use of established technologies

Rationale: The work should address the latest developments in areas such as mobile/cordless phones, wireless data networking, asset tracking and identification, wireless transfer of electrical power and body imaging/scanners. it should also consider the possible combined effect of exposure to multiple sources. This will allow exposures from new devices/scenarios to be compared with those that are more familiar and with exposure guidelines for risk communication purposes. This information will also be of value for exposure assessment in epidemiological studies and in the design of biological exposure systems.

High - quantify personal exposures from a range of RF sources and identify the determinants of exposure in the general population

Rationale: The quantification of personal exposure from a range of RF sources will provide valuable information for risk assessment and communication, and for the development of future epidemiological research. it is particularly useful for global exposure assessment in view of the upcoming WHO health risk assessment. The study will also provide baseline data for identification of any changes in the level of exposure and the dominant contributing factors over time. subgroup analyses should be carried out to identify any influence from demographic aspects of the user as well as the microenvironment in which the exposure occurs. exposure metrics should also be considered, especially in combining localized exposures from body-worn devices and whole-body exposures.

Other research needs - Monitoring of personal exposure of Rf workers

Rationale: The exposure patterns of both workers and the general public change continuously, mainly due to the development of new RF technologies. However, workers encounter industrial sources and exposure situations that lead to much higher energy deposition in the body. When epidemiological studies on RF workers are performed, it is imperative to monitor adequately their RF exposure. new instruments are needed to address the lack of adequate measurement tools for evaluating this type of exposure e.g. portable devices suitable for measuring different frequencies and waveforms. in addition, a study of the feasibility of monitoring the personal exposure of RF workers is required for future epidemiological studies. such studies would be facilitated by the production of a job exposure matrix (JeM) for RF workers – in which job designations can be characterized by their exposure. (WHO, 2010)

VII. National Academy of Sciences, National Research Council (2008)

The U.S. Food and Drug Administration (FDA) of the Department of Health and Human Services asked the National Academies to organize a workshop of national and international experts to identify research needs and gaps in knowledge of biological effects and adverse health outcomes of exposure to radiofrequency (RF) energy from wireless communications devices. To accomplish this task, the National Academies appointed a seven member committee to plan the workshop.1

Following the workshop, the committee was asked to issue a report based on the presentations and discussions at the workshop that identified research needs and current gaps in knowledge. The committee's task did not include the evaluation of health effects or the generation of recommendations relating to how the identified research needs should be met.

For the purposes of this report, the committee defines research needs as research that will increase our understanding of the potential adverse effects of RF energy on humans. Research gaps are defined as areas of research where the committee judges that scientific data that have potential value are presently lacking, but that closing of these gaps is either ongoing and results should be awaited before judgments are made on further research needs, or the gaps are not judged by the committee to be of as high a priority with respect to directly addressing health concerns at this time.

1. Committee on Identification of Research Needs Relating to Potential Biological or Adverse Health Effects of Wireless Communications Devices.

These needs and gaps are committee judgments derived from the workshop presentations and discussions, and the report does not necessarily reflect the views of the FDA, individual workshop speakers, or other workshop participants.

The committee judged that important research needs included, in order of appearance in the text, the following:

> • Characterization of exposure to juveniles, children, pregnant women, and fetuses from personal wireless devices and RF fields from base station antennas.
>
> • Characterization of radiated electromagnetic fields for typical multiple- element base station antennas and exposures to affected individuals.
>
> • Characterization of the dosimetry of evolving antenna configurations for cell phones and text messaging devices.
>
> • Prospective epidemiologic cohort studies of children and pregnant women.
>
> • Epidemiologic case-control studies and childhood cancers, including brain cancer.
>
> • Prospective epidemiologic cohort studies of adults in a general population and retrospective cohorts with medium to high occupational exposures.
>
> • Human laboratory studies that focus on possible adverse effects on electroencephalography2 activity and that include a sufficient number of subjects.
>
> • Investigation of the effect of RF electromagnetic fields on neural networks.
>
> • Evaluation of doses occurring on the microscopic level.
>
> • Additional experimental research focused on the identification of potential biophysical and biochemical/molecular mechanisms of RF action.
>
> (NAS-NRC, 2008)

VIII. World Health Organization Draft Framework for Electromagnetic Fields

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

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

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

The role of WHO is advisory only to the countries of Europe but it is an important function and can significantly affect decision-making on public health issues. It provides analysis and recommendations on various topics of health and environment, for consideration by member countries of the EU. Given the EU Article 174 policy requires a precautionary approach to judging health and environmental risks, and given that the charter of WHO is to serve the needs of the EU, one would think it essential that the WHO EMF Program health criteria results should be guided by and tailored to compliance with Article 174. This needs to occur in the assessment of the scientific literature (e.g., not requiring studies to provide scientific proof or causal scientific evidence but paying attention to and acting on the evidence, and the trend of the evidence at hand) and in its environmental health criteria recommendations. If the WHO EMF Program instead chooses to use the definitions of adverse impact and risk based on reacting to nothing short of conclusive scientific evidence, it fails to comply with the over-arching EU principle of health.

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

> "(A)nnoyance or discomforts caused by EMF exposure may not be pathological per se, but, if substantiated, can affect the physical and mental well-being of a

person and the resultant effect may be considered as an adverse health effect. A health effect is thus defined as a biological effect that is detrimental to health or well-being. According to the WHO Constitution, health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity." **www.who.int/peh-emf**

IX. The European Union Treaties Article 174

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

Article 174 (2) [ex Article 130r]

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

2. Community policy on the environment shall aim at a high level of protection taking into account the diversity of situations in the various regions of the Community. It shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should as a priority be rectified at source and that the polluter should pay. In this context, harmonization measures answering environmental protection requirements shall include, where appropriate, as a safeguard clause allowing Member States to take provisional measures, for non-economic environmental reasons, subject to a Community inspection procedure.

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

—available scientific and technical data;
—environmental conditions in the various regions of the Community;

—the potential benefits and costs of action or lack of action;
—the economic and social development of the Community as a whole and the balanced development of its regions.

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

X. WHO ELF Environmental Health Criteria Monograph, June 2007

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

ELF Health Criteria Monograph

12.6 Conclusions

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

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

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

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

"Potential long-term effects"

Much of the scientific research examining long-term risks from ELF magnetic field exposure has focused on childhood leukaemia. In 2002, IARC published a monograph classifying ELF magnetic fields as "possibly carcinogenic to humans. This classification was based on pooled analyses of epidemiological studies demonstrating a consistent

pattern of a two-fold increase in childhood leukaemia associated with average exposure to residential power-frequency magnetic field above 0.3 to 0.4 μT. ***The Task Group concluded that additional studies since then do not alter the status of this classification."*** *(emphasis added)*

"International exposure guidelines"

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

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

> *1) Government and industry should monitor science and promote research programmes to further reduce the uncertainty of the scientific evidence on the health effects of ELF field exposure. Through the ELF risk assessment process, gaps in knowledge have been identified and these form the basis of a new research agenda.*
>
> *2) Member States are encouraged to establish effective and open communication programmes with all stakeholders to enable informed decision-making. These may include improving coordination and consultation among industry, local government, and citizens in the planning process for ELF EMF-emitting facilities.*
>
> *3) When constructing new facilities and designing new equipment, including appliances, low-cost ways of reducing exposures may be explored. Appropriate exposure reduction measures will vary from one country to another. However, policies based on the adoption of arbitrary low exposure limits are not warranted."*

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

XI. World Health Organization Report on Children's Health and Environment

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

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

XII. International Agency for Research on Cancer (IARC)

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

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

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

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

SCENIHR Conclusions on Extremely low frequency fields (ELF fields)

> The previous conclusion that ELF magnetic fields are possibly carcinogenic, chiefly based on childhood leukaemia results, is still valid. There is no generally accepted mechanism to explain how ELF magnetic field exposure may cause leukaemia.
>
> For breast cancer and cardiovascular disease, recent research has indicated that an association is unlikely. For neurodegenerative diseases and brain tumours, the link to ELF fields remains uncertain. A relation between ELF fields and symptoms (sometimes referred to as electromagnetic hypersensitivity) has not been demonstrated.

SCENIHR Conclusions on Radiofrequency Radiation fields (RF fields)

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

SCENIHR Conclusions on Sensitivity of Children

> Concerns about the potential vulnerability of children to RF fields have been

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

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

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

XIV. Health Protection Agency (Formerly the NRPB - United Kingdom)

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

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

> *"The brain and nervous system operate using highly complex patterns of \electrical signals. Therefore, the basic restrictions are designed to limit the*

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

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

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

XV. US Government Radiofrequency Interagency Working Group Guidelines Statement

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

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

The United States government Federal Radiofrequency Interagency Working Group has reviewed the existing ANSI/IEEE RF thermal-based exposure standard upon which the FCC limit is based. This Working Group was made up of representatives from the US government's National Institute for Occupational Safety and Health (NIOSH), the

Federal Communications Commission (FCC), Occupational Health and Safety Administration (OSHA), the Environmental Protection Agency (US EPA), the National Telecommunication and Information Administration, and the US Food and Drug Administration (FDA).

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

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

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

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

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

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

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

XVII. Food and Drug Administration (US FDA)

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

Funding for the project will come from a Cooperative Research and Development Agreement (CRADA) between the Food and Drug Administration's Center for Devices and Radiological Health and the Cellular Telecommunications and Internet Association (CTIA). http://www.fda.gov/cellphones/index.html

XVIII. National Institutes for Health - National Toxicology Program

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

> **"** *Animal experiments are crucial because meaningful data will not be available from epidemiological studies for many years due to the long latency period between exposure to a carcinogen and the diagnosis of a tumor.*
>
> *"There is currently insufficient scientific basis for concluding either that wireless communication technologies are safe or that they pose a risk to millions of users."*

> *"FCC radiofrequency radiation guidelines are based on protection from acute injury from thermal effects of RF exposure* and may not be protective *against any non-thermal effects of chronic exposures."*

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

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

XIX. US Food and Drug Administration

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

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

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

XX. National Academy of Sciences - National Research Council

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

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

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

Page 2-13 of the prepublication report (emphasis added)

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

XXI. The IEEE (United States)

IEEE ICES SCC-28 SC-4 Subcommittee (Radiofrequency/Microwave Radiation)

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

Radiofrequency/Microwave Radiation

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

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

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

XXII. BEMS Supplement 6 (Journal of the Bioelectromagnetics Society)

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

Several clues are contained in the BEMS Supplement 6 to understand how the SC-4 IEEE C.95 revision working group and the ICES could arrive at a decision to not to recommend tighter limits on RF exposure. Not one but two definitions of "adverse effect" are described, one by Osepchuk/Petersen (2003) and another by the working group itself (D'Andrea et al, 2003). Both set a very high bar for demonstration of proof, and both are ignored in the final recommendations by the SC-4 Subcommittee.

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

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

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

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

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

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

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

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

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

The SC-4 definitions require proof that RF has caused organic disease or other cited

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

Sidebar: Quotes from BEMS Supplement 6

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

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

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

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

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

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SECTION 5

Evidence for EMF Transcriptomics and Proteomics Research 2007-2012

2012 Supplement

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I. INTRODUCTION

Daily exposure levels for non-ionizing electromagnetic radiation (NI-EMR) have significantly increased in the last few decades for human populations, and for wildlife, plants, and other living creatures on earth. NI-EMR includes a wide range of frequencies, as low as extremely low frequencies (ELF) magnetic fields deriving from the power lines up to microwave radiofrequencies (MW-RF). Within this range are FM and TV broadcast stations, wireless technology devices (mobile phones and masts, cordless phones, Wi-Fi routers and units).

The exposure to any of these frequencies individually, or in combination, raises concern about potentially harmful effects and is the subject of intensive scientific studies around the world. Such studies include epidemiological, clinical, *in vivo* and*in vitro* studies. The pace of scientific study accelerated after 2010, when the World Health Organization following the ELF agenda of 2007 (WHO, 2007), announced the implementation of the International EMF Project's RF Research Agenda as a "*research topic for measurement surveys to characterize population exposures from all radio frequency (RF) sources with a particular emphasis on new wireless technologies*" (WHO, 2010). The IARC (International Agency for Research on Cancer) under the auspices of the WHO classified RFR as a Possible Human Carcinogen (Group 2B) on 2011 (Baan et al., 2011).

The studies published so far have utilized various model systems and approaches but not in a coordinated manner, although there have been international efforts (i.e., INTERPHONE Final Study; Cardis et al., 2011).

As reviewed by Vlaanderen et al. (2009), OMICS technologies are relatively new biomarker discovery tools that can be applied to study large sets of biological molecules. (The English-language neologism omics informally refers to a field of study in biology ending in -*omics*, such as genomics, proteomics or metabolomics). Their applications in EMF and RFR research have become feasible in recent years due to a spectacular increase in the sensitivity, resolution and throughput of OMICS-based assays (Vlaanderen et al., 2009).

.Although, the number of OMIC techniques is ever expanding, the five most developed OMICS technologies are genotyping, transcriptomics, epigenomics, proteomics and metabolomics.

A number of reports have dealt with possible changes on gene/protein expression, either at an individual gene/protein level or using the high throughput "omics" approaches (T & P -transcriptomics and proteomics respectively) (for reviews see Xu & Chen, 2007; Blankenburg et al., 2009; McNamee & Chauhan, 2009; Mevissen M., 2011; Leszczynski et al., 2012). These T & P approaches have gained ground in the investigation of the possible EMF effects the last decade (Blankenburg et al., 2009), since they can screen the whole genome or proteome and may contribute on the elucidation of EMF mechanisms of action.

Following the work of Xu and Chen who gathered all studies on EMF research using T & P high throughput approaches up to 2006 in the BioInitiative Report (Xu & Chen, 2007), this supplemental chapter on Transcriptomics and Proteomics updates newly published work since that initial review in 2007.

II. EXREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS (ELF-EMFS)

A. Transcriptomics

As explicitly described by M. Mevissen (2011), gene expression profiling is the identification and characterization of the mixture of mRNA that is present in a specific sample. Both the presence of specific forms of mRNA and the levels in which these forms occur are parameters that provide information on gene expression. A gene expression profile provides a quantitative overview of the mRNA transcripts that were present in a sample at the time of collection. Therefore, gene expression profiling can be used to determine which genes are differently expressed as a result of changes in environmental conditions. DNA Microarrays represent an innovative and comprehensive technology that allows researchers to assess the expression level of thousands of genes in a high-throughput fashion and has been exploited in EMF research studies.

Schwenzer et al. (2007) reported effects of static magnetic field on genome expression. Specifically, the researchers evaluated the influence of magnetic resonance imaging (MRI) on gene expression in embryonic human lung fibroblasts (Hel 299). The cells were exposed to the static magnetic field and to a turbo spin-echo sequence of an MR scanner at 3.0 Tesla. An MR group (exposed) and a control group

(sham-exposed) were set up using a special MR-compatible incubation system. The exposure time was two hours. Gene expression profiles were studied using a complementary deoxyribonucleic acid (cDNA) microarray containing 498 known genes involved in transcription, intracellular transport, structure/junction/adhesion or extracellular matrix, signalling, host defence, energetics, metabolism, cell shape, and death. No changes in gene expression were found in either group (exposed or sham-exposed cells) at the end of a two-hour exposure for any of the 498 tested protein genes. The results showed that MRI had no influence on protein–gene expression in eugenic human lung cells in this study.

The same year, Walther et al. (2007) analyzed the effects of BEMER type (combination of electromagnetic field and light therapy) electromagnetic field (BTEMF) on gene expression in human mesenchymal stem cells and chondrocytes. Primary mesenchymal stem cells from bone marrow and the chondrocyte cell line C28I2 were stimulated 5 times at 12-h intervals for 8 min each with BTEMF. RNA from treated and control cells was analyzed for gene expression using the affymetrix chip HG-U133A. A limited number of regulated gene products from both cell types, which control cell metabolism and cell matrix structure, was mainly affected. There was no increased expression though of cancer-related genes. RT-PCR analysis of selected transcripts partly confirmed array data. Results indicate that BTEMF in human mesenchymal stem cells and chondrocytes provide the first indications. A limitation of this study is the single array analysis which was performed. Therefore, as stated by the authors, the results should be regarded as a first hint on BTEMF effects on these cellular systems. Nevertheless, their findings indicate that matrix dynamics and cell metabolism/energy balance are processes that are affected by the electromagnetic field application.

In a follow-up study, using fibroblasts as in the study by Schwenzer et al. (2007), but exposing them to electric fields (EFs), Jennings et al. (2008) tried to elucidate the role of EFs during the course of normal wound healing. Fibroblasts at the wound edge are exposed to electric fields (EFs) ranging from 40 to 200 mV/mm and so various forms of EFs can influence fibroblast migration, proliferation, and protein synthesis and may contribute to fibroblast activation during wound repair. These authors compared gene expression in normal adult dermal fibroblasts exposed to a 100 mV/mm EF for 1 h to non-stimulated controls. Significantly increased expression of 162 transcripts and decreased expression of 302 transcripts was detected using

microarrays, with 126 transcripts above the level of 1.4-fold increase or decrease compared to the controls. Only 11 genes were significantly increased or decreased above the level of 2-fold, compared to controls. Many of these significantly regulated genes were associated with wound repair through the processes of matrix production, cellular signalling, and growth. Activity within specific cellular signalling pathways was noted, including TGF-b, G-proteins, and inhibition of apoptosis. In addition, RT-PCR analysis of the expression of KLF6, FN1, RGS2, and JMJD1C over continued stimulation and at different field strengths suggests that there are specific windows of field characteristics for maximum induction in the expression of these genes. EFs thus appeared to have an important role in controlling fibroblast activity in the process of wound healing. The authors highlight that 2-fold changes have traditionally and somewhat arbitrarily been designated as meaningful changes in gene expression, although there is little quantitative information connecting these values to changes in biological function. Therefore, multiple microarray experiments at different time points and field conditions may have revealed induction of different sets of genes under different experimental conditions. Follow-up studies should include proteomic analysis of altered protein production resulting from altered gene expression, alternative splicing in protein translation, and gene silencing studies to further delineate the mechanisms and locations of interaction between EFs and transcriptional regulators.

Kimura et al. (2008) using magnetic resonance imaging with high intensity static magnetic fields (SMFs) demonstrated in the nematode *Caenorhabditis elegans* that genes involved in motor activity, actin binding, cell adhesion, and cuticles were transiently and specifically induced following exposure to 3 or 5 T SMF in this metazoon experimental model . In addition, transient induction of hsp12 family genes was observed after SMF exposure. The small-heat shock protein gene hsp16 was also induced but to a much lesser extent, and the LacZ-stained population of hsp-16.1::lacZ transgenic worms did not significantly increase after exposure to SMFs with or without a second stressor, mild heat shock. Several genes encoding apoptotic cell-death activators and secreted surface proteins were upregulated after IR, but were not induced by SMFs. Real-time quantitative RT-PCR analyses for 12 of these genes confirmed these expression differences between worms exposed to SMFs and IR. In contrast to IR, exposure to high SMFs did not induce DNA double-strand breaks or germline cell apoptosis during meiosis. These results suggest that the response of *C.*

elegans to high SMFs is unique and capable of adjustment during long exposure, and that this treatment may be less hazardous than other therapeutic tools.

On 2010, Chung et al. conducted a study to investigate the possible effect of 60 Hz circularly polarized magnetic fields (MFs) as promoters of genetically initiated lymphoma in AKR mice. One hundred sixty female animals were divided into four different groups. They were exposed to four different intensities of circularly polarized MFs. Animals received exposure to 60 Hz circularly polarized MF at field strengths (rms-value) of 0 microT (sham control, T1, Group I), 5 microT (T2, Group II), 83.3 microT (T3, Group III), or 500 microT (T4, Group IV), for 21 h/day from the age of 4-6 weeks to the age of 44-46 weeks. There were no exposure-related changes in mean survival time, clinical signs, body weights, hematological values, micronucleus assay, gene expression arrays, analysis of apoptosis, and necropsy findings. Examination at the histopathological level, showed lymphoma in all the groups. The tumor incidence was 31/40(78%), 30/40(75%), 32/40(80%), and 31/40(78%) in sham control, 5, 83.3, and 500 microT groups, respectively. However, there were no differences in the tumor incidence between the sham control (T1) and circularly polarized MF exposure groups (T2-T4). In conclusion, there was no evidence that exposure to 60 Hz circularly polarized MF strengths up to 500 microT promoted lymphoma in AKR mice.

In a very recent attempt 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, Kirschenlohr et al. (2012) tried 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 (approximately 600 mG) 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 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.

Commenting the above data, we note that the overall experimental design seems to lack real life conditions since a) the suspicion refers to childhood leukaemia and not to adults, b) exposure is not supposed to be just 2 hours a day but day long for children living in the vicinity of power lines, c) continuous daily exposure for years is the rationale behind the possibility of ELFs causing or increasing leukaemia.

B. Proteomics

Proteins are the key molecules that participate and regulate nearly all cellular functions. The number of each protein species in a given cell changes over time according to the metabolic and signalling demand and is subject to differential gene expression. Proteomics, is the science that explores by high throughput techniques the so called "protein expression profile" of proteins.

The reports on ELF and proteomics are practically absent in the last 5 years leaving only the old study by Seyyedi et al. (2007) in human fibroblast (using 3 Hz, sinusoidal continuous ELF electromagnetic fields, 3 h duration and 4 mT magnetic field intensity) and one more in 2011 by Sulpizio et al. The first study showed that some protein expressions were affected by radiation after comparing the 2-DE separated proteins from the exposed and sham (control) cells. The two proteins that their expression was reduced about 50% were determined as alpha 1 antitrypsin (A1AT) and Transthyretin (TTR) and has been concluded that application of ELF-EMF in therapeutic aspects may be accompanied by their side effects.

Along the "leukaemia ELF rationale" and in addition a possible ELF link with cancer, cardiovascular, and neurological disorders, Sulpizio et al. (2011) exposed human SH-SY5Y neuroblastoma cells to a 50 Hz, 1 mT (10 Gauss) sinusoidal ELF-MF at three duration schemes, 5 days (T5), 10 days (T10), and 15 days (T15). The effects of ELF-MF on proteome expression and biological behavior were investigated. Through comparative analysis between treated and control samples they identified

nine new proteins after a 15-day treatment. They suggested that the proteins were involved in a cellular defence mechanism and/or in cellular organization and proliferation such as peroxiredoxin isoenzymes (2, 3, and 6), 3-mercaptopyruvate sulfurtransferase, actin cytoplasmatic 2, t-complex protein subunit beta, ropporin-1A, and profilin-2 and spindlin-1. These authors concluded that ELF-MFs exposure altered the proliferative status and other important cell biology-related parameters, such as cell growth pattern, and cytoskeletal organization and that ELF radiation could trigger a shift toward a more invasive phenotype.

III. RADIOFREQUENCY ELECTROMAGNETIC FIELDS (RF-EMFS)

A relatively small number of publications have dealt after 2007 with the effects of RF-EMF on the proteome and transcriptome of cells and even less number with the effects on animals.

A. Transcriptomics

Chauhan et al. (2007a) assessed non-thermal RF-field exposure effects on a variety of biological processes (including apoptosis, cell cycle progression, viability and cytokine production) in a series of human-derived cell lines (TK6, HL60 and Mono-Mac-6). Exponentially growing cells were exposed to intermittent (5 min on, 10 min off) 1.9 GHz pulse-modulated RF fields for 6 h at mean specific absorption rates (SARs) of 0, 1 and 10 W/kg. Concurrent negative (incubator) and positive (heat shock for 1 h at 43 degrees C) controls were included in each experiment. Immediately after the 6-h exposure period and 18 h after exposure, cell pellets were collected and analyzed for cell viability, the incidence of apoptosis, and alterations in cell cycle kinetics. The cell culture supernatants were assessed for the presence of a series of human inflammatory cytokines (TNFA, IL1B, IL6, IL8, IL10, IL12) using a cytometric bead array assay. No detectable changes in cell viability, cell cycle kinetics, incidence of apoptosis, or cytokine expression were observed in any of RF-field-exposed groups in any of the cell lines tested, relative to the sham controls. However, the positive (heat-shock) control samples displayed a significant decrease in cell viability, increase in apoptosis, and alteration in cell cycle kinetics (G(2)/M block). Overall, the researchers found no evidence that non-thermal RF-field exposure could elicit any detectable biological effect in three human-derived cell lines.

Chauhan et al. (2007b) have examined the effect of RF field exposure on the possible expression of late onset genes in U87MG cells after a 24 h RF exposure period. In addition, a human monocyte-derived cell-line (Mono-Mac-6, MM6) was exposed to intermittent (5 min ON, 10 min OFF) RF fields for 6 h and then gene expression was assessed immediately after exposure and at 18 h post exposure. Both cell lines were exposed to 1.9 GHz pulse-modulated RF fields for 6 or 24 h at specific absorption rates (SARs) of 0.1-10.0 W/kg (very high SAR value). In support of their previous results, they found no evidence that nonthermal RF field exposure could alter gene expression in either cultured U87MG or MM6 cells, relative to non irradiated control groups. However, exposure of both cell-lines to heat-shock conditions (43 degrees C for 1 h) caused an alteration in the expression of a number of well-characterized heat-shock proteins.

The same year, Zhao et al. (2007) investigated whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to a working GSM cell phone rated at a frequency of 1900 MHz. Primary cultures were exposed for 2h. Microarray analysis and real-time RT-PCR were applied and showed up-regulation of caspase-2, caspase-6 and Asc gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The effects were specific since up-regulation was not seen for other genes associated with apoptosis, such as caspase-9 in either neurons or astrocytes, or Bax in neurons. The results showed that even relatively short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

In an *in vitro* study focusing on the effects of low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system, Hirose et al. (2007) tested the hypothesis that modulated RF fields act to induce phosphorylation and overexpression of heat shock protein hsp27. The study evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Secondly, the study investigated whether

continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz can induce activation or gene expression of hsp27 and other heat shock proteins (hsps). Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80 and 800 mW/kg for 2-48 h, and CW radiation at 80 mW/kg for 24 h. Human IMR-90 fibroblasts from fetal lungs were exposed to W-CDMA at 80 and 800 mW/kg for 2 or 28 h, and CW at 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the expression levels of phosphorylated hsp27 at serine 82 (hsp27[pS82]) were observed between the test groups exposed to W-CDMA or CW signal and the sham-exposed negative controls, as evaluated immediately after the exposure periods by bead-based multiplex assays. Moreover, no noticeable differences in the gene expression of hsps were observed between the test groups and the negative controls by DNA Chip analysis.

Paparini et al. (2008) found no evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal for 1 h at a whole body SAR of 1.1 W/kg. Gene expression was studied in the whole brain, where the average SAR was 0.2 W/kg, by expression microarrays containing over 22,600 probe sets. Comparison of data from sham and exposed animals showed no significant difference in gene expression modulation. However, when less stringent constraints were adopted to analyze microarray results, 75 genes were found to be modulated following exposure. Forty-two probes showed fold changes ranging from 1.5 to 2.8, whereas 33 were down-regulated from 0.67- to 0.29-fold changes, but these differences in gene expression were not confirmed by real-time PCR. Under these specific limited conditions, no consistent indication of gene expression changes in whole mouse brain was found associated to GSM 1800 MHz exposure. *We could possibly explain the lack of gene expression changes in this, as well in other studies, by the very short exposure duration used of 1 h.*

Nittby et al. (2008) applied Microarray hybridizations on Affymetrix rat2302 chips of RNA extracts from cortex and hippocampus of GSM 1800 exposed rats for just 6 h within TEM cells. Using four exposed and four control animals they found that a large number of genes were altered at hippocampus and cortex. The vast majority were downregulated. Since the genes that were differentially expressed between the two groups were responsible to membrane integral and signal transduction, the authors concluded that the change of their expression might be the cause of their

previous observations of blood-brain-barrier leakage and albumin transport through brain capillaries.

Huang et al. (2008a) monitored cellular and molecular changes in Jurkat human T lymphoma cells after irradiating with 1763 MHz RF radiation in order to test the effect on RF radiation in immune cells. Jurkat T-cells were exposed to RF radiation to assess the effects on cell proliferation, cell cycle progression, DNA damage and gene expression. Cells were exposed to 1763 MHz RF radiation at 10 W/kg specific absorption rate (SAR) and compared to sham exposed cells. RF exposure did not produce significant changes in cell numbers, cell cycle distributions, or levels of DNA damage. In genome-wide analysis of gene expressions, there were no genes changed more than 2-fold upon RF-radiation while ten genes changed from 1.3 to approximately 1.8-fold. Among these ten genes, two cytokine receptor genes such as chemokine (C-X-C motif) receptor 3 (CXCR3) and interleukin 1 receptor, type II (IL1R2) were down-regulated upon RF radiation. These results indicate that the alterations in cell proliferation, cell cycle progression, DNA integrity or global gene expression were not detected upon 1763 MHz RF radiation under 10 W/kg SAR for 24 h to Jurkat T cells.

In a follow-up study Huang et al. (2008b) chose HEI-OC1 immortalized mouse auditory hair cells to characterize the cellular response to 1763 MHz RF exposure, because auditory cells can be exposed to mobile phone frequencies. Cells were exposed to 1763 MHz RF at a 20 W/kg specific absorption rate (SAR) in a code division multiple access (CDMA) exposure chamber for 24 and 48 h to check for changes in cell cycle, DNA damage, stress response, and gene expression. Neither cell cycle changes nor DNA damage were detected in RF-exposed cells. The expression of heat shock proteins (HSP) and the phosphorylation of mitogen-activated protein kinases (MAPK) did not change, either. The researchers tried to identify any alteration in gene expression using microarrays. Using the Applied Biosystems 1700 full genome expression mouse microarray, they found that 29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure. From these results, they could not find any evidence of the induction of cellular responses, including cell cycle distribution, DNA damage, stress response and gene expression, after 1763 MHz RF exposure at an SAR of 20 W/kg (very high value) in HEI-OC1 auditory hair cells.

Concerning plant cell experiments Engelmann et al. (2008) searched for physiological processes of plant cells sensitive to RF fields. They reported significant changes (but not more than 2.5-fold) in transcription of 10 genes in cell suspension cultures of *Arabidopsis thaliana*, which were exposed for 24 h to an RF field protocol representing typical microwave exposition in an urban environment. The changes in transcription of these genes were compared with published microarray datasets and revealed a weak similarity of the microwave to light treatment experiments. Considering the large changes described in published experiments, it is questionable if the small alterations caused by a 24 h continuous microwave exposure would have any impact on the growth and reproduction of whole plants.

Using very low SAR values (0.9–3 mWkg) Dawe et al. (2009) applied microarray technology in the nematode *C. elegans.* They compared five Affymetrix gene arrays of pooled triplicate RNA populations from sham-exposed L4/adult worms against five gene arrays of pooled RNA from microwave-exposed worms (taken from the same source population in each run). No genes showed consistent expression changes across all five comparisons, and all expression changes appeared modest after normalisation (< or =40% up- or down-regulated). The number of statistically significant differences in gene expression (846) was less than the false-positive rate expected by chance (1131). The authors concluded that the pattern of gene expression in L4/adult *C. elegans* is substantially unaffected by low-intensity microwave radiation and that the minor changes observed in this study could well be false positives. As a positive control, they compared RNA samples from N2 worms subjected to a mild heat-shock treatment (30 ^{o}C) against controls at 26 ^{o}C (two gene arrays per condition). As expected, heat-shock genes were strongly up-regulated at 30 ^{o}C, particularly an hsp-70 family member (C12C8.1) and hsp-16.2. Under these heat-shock conditions, they confirmed that an hsp-16.2::GFP transgene was strongly up-regulated, whereas two non-heat-inducible transgenes (daf-16::GFP; cyp-34A9::GFP) showed little change in expression. Preliminary work in our lab has indicated that this model organism is highly resistant to EMF sources including mobile phone, DECT and Wi-Fi radiation exposures, for reasons that are under investigation (Margaritis et al., unpublished).

RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines

under these conditions as reported by Sekijima et al. (2010). These authors investigated the mechanisms by which radiofrequency (RF) fields exert their activity, and the changes in both cell proliferation and the gene expression profile in the human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung) following exposure to 2.1425 GHz continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) RF fields at three field levels. During the incubation phase, cells were exposed at specific absorption rates (SARs) of 80, 250, or 800 mW/kg with both CW and W-CDMA RF fields for up to 96 h. Heat shock treatment was used as the positive control. No significant differences in cell growth or viability were observed between any test group exposed to W-CDMA or CW radiation and the sham-exposed negative controls. Using the Affymetrix Human Genome Array, only a very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression in each experiment. According to the authors the results confirm that low-level exposure to 2.1425 GHz CW and W-CDMA RF fields for up to 96 h did not act as an acute cytotoxicant in either cell proliferation or the gene expression profile. These results suggest that RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines under these conditions.

In order to investigate whether exposure to high-frequency electromagnetic fields (EMF) could induce adverse health effects, Trivino et al. (2012) cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of 900 MHz MW-EMF generated by a transverse electromagnetic (TEM) cell at short and long exposure times and the effect of high-frequency EMF on gene expression has been evaluated. Significant changes in gene expression levels of genes involved in DNA repair, cell cycle arrest, apoptosis, chromosomal organization, and angiogenesis were observed.The authors have identified functional pathways influenced by 900 MHz MW-EMF exposure.

It is worth mentioning, although beyond the frequencies used in cellular communication, that changes were detected using millimeter-waves in 56 genes at 6 h exposure and 58 genes at 24 h exposure in rats as shown by Millenbaugh et al. (2008). The animals were subjected to 35 GHz millimeter waves at a power density of 75 mW/cm^2, to sham exposure and to 42 degrees Centigrade environmental heat. Skin

samples were collected at 6 and 24 h after exposure for Affymetrix Gene Chip analysis. The skin was harvested from a separate group of rats at 3-6 h or 24-48 h after exposure for histopathology analysis. Microscopic findings observed in the dermis of rats exposed to 35 GHz millimeter waves included aggregation of neutrophils in vessels, degeneration of stromal cells, and breakdown of collagen. Changes were detected in 56 genes at 6 h and 58 genes at 24 h in the millimeter-wave-exposed rats. Genes associated with regulation of transcription, protein folding, oxidative stress, immune response, and tissue matrix turnover were affected at both times. At 24 h, more genes related to extracellular matrix structure and chemokine activity were altered. Up-regulation of Hspa1a, Timp1, S100a9, Ccl2 and Angptl4 at 24 h by 35 GHz millimeter-wave exposure was confirmed by real-time RT-PCR. These results obtained from histopathology, microarrays and RT-PCR indicated that prolonged exposure to 35 GHz millimeter waves causes thermally related stress and injury in skin while triggering repair processes involving inflammation and tissue matrix recovery.

B. Proteomics

In a series of publications by Leszczynski's research group, consistently using human endothelial cell lines EA.hy926 and EA.hy926v1, protein expression changes occurred after exposure to 900 MHz.

The potential proteome expression changes by RF on the same cell line EA.hy926 have been further investigated by the same group in a follow-up study (Nylund et al., 2009), where they reported that 1h exposure to GSM 1800 MHz mobile phone radiation (SAR 2.0 W/kg) can also alter this cell line's proteome expression. Sham samples were produced simultaneously in the same conditions but without the radiation exposure. Cells were harvested immediately after 1-hour exposure to the radiation, and proteins were extracted and separated using 2-dimensional electrophoresis (2DE). In total, 10 experimental replicates were generated from both exposed and sham samples. About 900 protein spots were detected in the 2DE-gels using PDQuest software and eight of them were found to be differentially expressed in exposed cells ($p<0.05$, t-test). Three out of these eight proteins were identified using Maldi-ToF mass spectrometry (MS). These proteins were: spermidine synthase (SRM), 78 kDa glucose-regulated protein (55 kDa fragment) (GRP78) and proteasome subunit alpha type 1 (PSA1). Due to the lack of the availability of

commercial antibodies the researchers were able to further examine expression of only GRP78. Using SDSPAGE and western blot method they were not able to confirm the result obtained for GRP78 using 2DE. Additionally, no effects were reported this time for 1800GSM exposure on the expression of vimentin and Hsp27 - proteins that were affected by the 900 MHz GSM exposure in their earlier studies. The authors highlight that the observed discrepancy between the expression changes of GRP78 detected with 1DE and 2DE confirms the importance of validation of the results obtained with 2DE using other methods, e.g. western blot.

Using a higher definition technique, the 2D-DIGE, Leszczynski's group investigated whether GSM1800 radiation can alter the proteome of primary human umbilical vein endothelial cells and primary human brain microvascular endothelial cells (Nylund et al., 2010). The cells were exposed for 1 hour to 1800 MHz GSM mobile phone radiation at an average specific absorption rate of 2.0 W/kg. Following that, cells were harvested immediately and the protein expression patterns of the sham-exposed and radiation-exposed cells were examined using two dimensional difference gel electrophoresis based proteomics (2DE-DIGE). Numerous differences were observed between the proteomes of human umbilical vein endothelial cells and human brain microvascular endothelial cells (both sham-exposed). These differences are most likely representing physiological differences between endothelia in different vascular beds. However, the exposure of both types of primary endothelial cells to mobile phone radiation did not cause any statistically significant changes in protein expression. So, radiation did not provoke any proteome expression changes to these kinds of cells immediately at the end of the exposure and when the false discovery rate correction was applied to analysis. This observation agrees with earlier the earlier study of this group showing that the 1800 MHz GSM radiation exposure had only very limited effect on the proteome of human endothelial cell line EA.hy926, as compared with the effect of 900 MHz GSM radiation.

Another "omics" group exposing human lens epithelial cells detected heat-shock protein (HSP) 70 and heterogeneous nuclear ribonucleoprotein K (hnRNP K) to be upregulated following exposure to GSM 1800 MHz for 2 h (Li et al., 2007). In three separate experiments, HLECs were exposed and sham-exposed (six dishes each) to 1800-MHz GSM-like radiation for 2 h. The specific absorption rates were 1.0, 2.0, or 3.5 W/kg. Immediately after radiation, the proteome was extracted from the HLECs. Immobilized pH gradient two-dimensional polyacrylamide gel electrophoresis (2-DE;

silver staining) and PDQuest 2-DE analysis software were used to separate and analyze the proteome of exposed and sham-exposed HLECs. Four differentially expressed protein spots were selected and identified by using electrospray ionization tandem mass spectrometry (ESI-MS-MS). When the protein profiles of exposed cells were compared with those of sham-exposed cells, four proteins were detected as upregulated. After analysis by ESI-MS-MS and through a database search, heat-shock protein (HSP) 70 and heterogeneous nuclear ribonucleoprotein K (hnRNP K) were determined to be upregulated in the exposed cells.

Since the above *in vitro* effects cannot be easily translated into humans, in 2008, Leszczynski's group performed a pilot study on volunteers (Karinen et al., 2008) and showed that mobile phone radiation might alter protein expression in human skin cells. Small area of forearm's skin in 10 female volunteers was exposed to RF-EMF (specific absorption rate SAR = 1.3 W/kg) and punch biopsies were collected from exposed and non-exposed areas of skin. Proteins extracted from biopsies were separated using 2-DE and protein expression changes were analyzed using PDQuest software. Analysis has identified 8 proteins that were statistically significantly affected (Anova and Wilcoxon tests). Two of the proteins were present in all 10 volunteers. This suggests that protein expression in human skin might be affected by the exposure to RF-EMF. The number of affected proteins was similar to the number of affected proteins observed in this group's earlier *in vitro* studies. This is the first study showing that molecular level changes might take place in human volunteers in response to exposure to RF-EMF, although the overall conclusions were criticized by Leszczynski et al. (2012).

However, such a limited and non systematic number of publications using "omics" approaches does not allow for any conclusions to be drawn concerning the impact of mobile phone emitted radiation upon cell proteome, physiology and function (Nylund et al., 2009), as also pointed out by Vanderstraeten & Verschaeve (2008).

Kim et al. (2010) have monitored changes in protein expression profiles in RF-exposed MCF7 human breast cancer cells using two-dimensional gel electrophoresis. MCF7 cells were exposed to 849 MHz RF radiation for 1 h per day for three consecutive days at specific absorption rates (SARs) of either 2 W/Kg or 10 W/kg. During exposure, the temperature in the exposure chamber was kept in an isothermal condition. Twenty-four hours after the final RF exposure, the protein lysates from MCF cells were prepared and two-dimensional electrophoretic analyses were

conducted. The protein expression profiles of the MCF cells were not significantly altered as the result of RF exposure. None of the protein spots on the two-dimensional electrophoretic gels showed reproducible changes in three independent experiments. To determine effect of RF radiation on protein expression profiles more clearly, three spots showing altered expression without reproducibility were identified using electrospray ionization tandem mass spectrometry analysis and their expressions were examined with RT-PCR and Western blot assays. There was no alteration in their mRNA and protein levels. The authors concluded that it seems unlikely that RF exposure modulates the protein expression profile.

Since oxidative stress is gaining more and more ground as being the initial mechanism of action of EMFs, the review by Gaestel M. (2010) describes the (up to 2010) developments in analysing the influence of RF-EMFs on biological systems by monitoring the cellular stress response as well as overall gene expression. Recent data on the initiation and modulation of the classical cellular stress response by RF-EMFs, comprising expression of heat shock proteins and stimulation of stress-activated protein kinases, are summarised and evaluated. Since isothermic RF-EMF exposure is assumed rather than proven there are clear limitations in using the stress response to describe non-thermal effects of RF-EMFs. In particular, according to the authors further experiments are needed to characterise better the threshold of the thermal heat shock response and the homogeneity of the cellular response in the whole sample for each biological system used. Before then, it is proposed that the absence of the classical stress response can define isothermal experimental conditions and qualifies other biological effects of RF-EMFs detected under these conditions to be of non-thermal origin. To minimise the probability that by making this assumption valuable insights into the nature of biological effects of RF-EMFs could be lost, proteotoxic non-thermal RF-EMF effects should also be monitored by measuring activities of labile intracellular enzymes and/or levels of their metabolites before the threshold for the heat shock response is reached. In addition, non-thermal induction of the stress response via promoter elements distinct from the heat shock element (HSE) should be analysed using HSE-mutated heat shock promoter reporter constructs. Screening for non-thermal RF-EMF effects in the absence of a classical stress response should be performed by transcriptomics and proteomics. It is postulated that due to their high-throughput characteristics, these methods inherently generate false positive results and

require statistical evaluation based on quantitative expression analysis from a sufficient number of independent experiments with identical parameters. In future approaches, positive results must be confirmed by independent quantitative methods and should also be evaluated *in vivo* to prove possible non-thermal effects of RF-EMFs on living beings. If successful, this strategy should contribute to identification of new underlying molecular mechanisms of interaction between RF-EMFs and living beings distinct from absorption of thermal energy.

In the review by Leszczynski et al., (2012) the authors have analyzed all available data up through the end of 2010 and have raised a number of concerns regarding the handling of proteomics technology, such as the different proteome analysis methods used, the low number of replicates, the posttreatment sampling (one or very few time points), the large number of protein analyzed, the huge differences in the dynamic range of protein concentrations in cells or plasma, the variety of posttranslational modifications, the lack of validation of the results with a second method, as well as the various SAR/exposure conditions/duration/frequency dependencies in order to properly evaluate the EMF impact. The authors agree along with Gerner et al. (2010) that protein expression per se may be a reliable way to explain EMF effects. We might add that in terms of protein synthesis dynamics, the quantity of any protein species at a given time point (as detected by proteomics) should take into account the protein stability and turnover (as pointed out by Eden et al., 2011) as well as mRNA stability and maturation/translational-posttranslational control. In a hypothetical scenario that EMFs affect gene activation /deactivation (see Blank & Goodman, 2008), the end effect may not be seen by proteomics, since no net quantity change is taking place immediately but (possibly) a few hours following exposure and (also hypothetically) normal levels come back a few days or weeks later due homeostatic mechanisms.

Our own contribution to the field of RF-EMF induced protein expression changes was performed in mice exposed to mobile phone and wireless DECT base radiation under real-time exposure conditions and analyzing thereafter the proteome of three critical brain regions; hippocampus, cerebellum and frontal lobe (Fragopoulou et al. 2012). Three equally divided groups of Balb/c mice (6 animals/group) were used; the first group was exposed to a typical mobile phone, at a SAR level range of 0.17-0.37 W/kg for 3 h daily for 8 months, the second group was exposed to a wireless DECT

base (Digital Enhanced Cordless Telecommunications Telephone) at a SAR level range of 0.012-0.028 W/kg for 8 h/day for 8 months and the third group comprised the sham-exposed animals. Comparative proteomics analysis revealed that long-term irradiation from both EMF sources significantly altered ($p < 0.05$) the expression of 143 proteins in total (as low as 0.003 fold downregulation up to 114 fold overexpression). Several neural function related proteins (i.e., Glial Fibrillary Acidic Protein (GFAP), Alpha-synuclein, Glia Maturation Factor beta (GMF), and apolipoprotein E (apoE)), heat shock proteins, and cytoskeletal proteins (i.e., Neurofilaments and tropomodulin) are included in this list as well as proteins of the brain metabolism (i.e., Aspartate aminotransferase, Glutamate dehydrogenase) to nearly all brain regions studied. Western blot analysis on selected proteins confirmed the proteomics data. The observed protein expression changes may be related to brain plasticity alterations, indicative of oxidative stress in the nervous system or involved in apoptosis and might potentially explain human health hazards reported so far, such as headaches, sleep disturbance, fatigue, memory deficits, and long-term induction of brain tumors under similar exposure conditions.

As mentioned earlier, beyond the mobile phone frequencies, 35 GHz radiation had effects on gene expression. Similarly, Sypniewska et al. (2010) using proteomics reported that this frequency can also alter the proteome of NR8383 rat macrophages. Two-dimensional polyacrylamide gel electrophoresis, image analysis, and Western blotting were used to analyze approximately 600 protein spots in the cell lysates for changes in protein abundance and levels of 3-nitrotyrosine, a marker of macrophage stimulation. Proteins of interest were identified using peptide mass fingerprinting. Compared to plasma from sham-exposed rats, plasma from environmental heat- or millimeter wave-exposed rats increased the expression of 11 proteins, and levels of 3-nitrotyrosine in seven proteins, in the NR8383 cells. These altered proteins are associated with inflammation, oxidative stress, and energy metabolism. Findings of this study indicate both environmental heat and 35 GHz millimeter wave exposure elicit the release of macrophage-activating mediators into the plasma of rats.

Interestingly, there is a wealth of information regarding proteome and/or transcriptomics studies following exposure to ionizing radiation. In the perspective of similar mechanisms of action between NIR and IR, it is worth mentioning just one study using very low dose ionizing radiation by Pluder et al., 2011. In this study low-

dose radiation induced rapid and time-dependent changes in the cytoplasmic proteome of the human endothelial cell line EA.hy926 (used by Dariusz Leszczynski and his group in their EMF studies). The proteomes were investigated at 4 and 24 h after irradiation at two different dose rates (Co-60 gamma ray total dose 200 mGy; 20 mGy/min and 190 mGy/min) using 2D-DIGE technology. The researchers identified 15 significantly differentially expressed proteins, of which 10 were upregulated and 5 down-regulated, with more than ± 1.5-fold difference compared with unexposed cells. Pathways influenced by the low-dose exposures included the Ran and RhoA pathways, fatty acid metabolism and stress response which are reminiscent of EMF impact studies.

Concerning proteomics techniques, a recent review by Damm et al., (2012) re-evaluates the putative advantages of microwave-assisted tryptic digests compared to conventionally heated protocols performed at the same temperature. An initial investigation of enzyme stability in a temperature range of 37-80°C demonstrated that trypsin activity declines sharply at temperatures above 60°C, regardless if microwave dielectric heating or conventional heating is employed. Tryptic digests of three proteins of different size (bovine serum albumin, cytochrome c and β-casein) were thus performed at 37°C and 50°C using both microwave and conventional heating applying accurate internal fiber-optic probe reaction temperature measurements. The impact of the heating method on protein degradation and peptide fragment generation was analyzed by SDS-PAGE and MALDI-TOF-MS. Time-dependent tryptic digestion of the three proteins and subsequent analysis of the corresponding cleavage products by MALDI-TOF provided virtually identical results for both microwave and conventional heating. In addition, the impact of electromagnetic field strength on the tertiary structure of trypsin and BSA was evaluated by molecular mechanics calculations. These simulations revealed that the applied field in a typical laboratory microwave reactor is 3-4 orders of magnitude too low to induce conformational changes in proteins or enzymes.

IV. SUMMARY

The papers analyzed in this review have dealt with a very difficult research problem, which is EMF effects as measured by the highthroughput techniques of transcriptomics and proteomics. It is a very difficult task because the technical

complexity of the approaches is added to the enormous variations of the exposure details (duration, frequency, pulses, repetition, intensity, peak values, e.t.c). In total there were 29 original articles from 2007. Eight (8) of them were in the ELF frequencies, where the three of them indicate an effect in gene expression, the other three indicate no effect in gene expression and two studies show an effect in protein expression. Regarding radiofrequency studies (RF-EMF) a total of 21 papers were published in this area since 2007. Thirteen (13) dealt with transcriptomics [eight (8) effect- five (5) no effect] and eight (8) in proteomics [six (6) show effect and two (2) show no effect]. So, in total, 66% of the studies reveal an effect of EMF on transcriptome and proteome expression (Table 1).

Table 1

EMF Transcriptomics and Proteomics studies 2007-2012

(E=effect, NE= no effect)

The classification of the studies to the category "Effect – No effect" is based on the general conclusions of each article, although different conditions are used in exposure setup, biological system, duration, approaches. It is also considered as an effect even if a single gene or protein is affected by exposure to EMF.

	Exposed biological model	**Exposure set-up**	**SAR or/and power density or intensity of magnetic field**	**Duration of exposure / Time of sampling**	**Method of analysis**	**Category "Effect-No effect"**	**Comments**	**Reference/ Journal**
ELF –EMF Transcriptomics	**Primary human mesenchymal stem cells from the bone marrow and chondrocytes (cell line C28I2)**	BTEMF (combination of electromagnetic field and light therapy) Coil system	35 μT	Stimulated 5 times at 12-h intervals for 8 min each	Affymetrix GeneChip System, HG-U133A /RT-PCR partially confirmed the data	**E**	A limited number of regulated gene products from both cell types, which control cell metabolism and cell matrix structure,	Walther et al. (2007) *EBM*

							was mainly affected. There was no increased expression though of cancer-related genes	
	Adult human dermal fibroblasts *(scope: wound healing)*	Direct current field	100 mV/mm EF	1 h	Microarrays /RT-PCR validated 4 genes	**E**	Significantly increased expression of 162 transcripts and decreased expression of 302 transcripts was detected (126 transcripts above the level of 1.4-fold, 11 above the level of 2-	Jennings et al. (2008) *Bioelectromagnetics*

						fold)	
Caenorhabditis elegans	Static magnetic field (SMF) Magnetic resonance imaging	3 and 5 T	4 and 24 h	Affymetrix whole-genome array /qRT-PCR confirmed changes	**E**	Genes involved in motor activity, actin binding, cell adhesion, and cuticles, hsp12, hsp16 were transiently and specifically induced following exposure. Several genes encoding apoptotic cell-death activators and secreted surface proteins were	Kimura et al. (2008) *Bioelectromagnetics*

						upregulated after IR, but were not induced by SMFs.	
Embryonic human lung fibroblasts (Hel 299)	MR scanner	3.0 Tesla	2 h	cDNA microarray containing 498 known genes	**NE**		Schwenzer et al. (2007) *Journal of Magnetic Resonance imaging*
AKR mice	60 Hz Circularly polarized MFs	0 microT (sham control, T1, Group I), 5 microT (T2, Group II), 83.3 microT (T3, Group III), or 500 microT (T4, Group IV)	21 h/day from the age of 4-6 weeks to the age of 44-46 weeks	Affymetrix GeneChip Mouse Gene 1.0 ST assay	**NE**		Chung et al. (2010) *Bioelectromagnetics*
White blood cells of volunteers	50 Hz Sinusoidal ELF-MF	62.0 ± 7.1 μT	2 h, repeated on the followi	Illumina microarrays	**NE**		Kirschenlohr et al. (2012) *Radiat Res*

				ng day and the two-day sequence was repeated 6 days later, 5 time points				
Proteomics	**Human fibroblasts**	3 Hz continuous ELF, sinusoidal	4 mT	3 h	2-DE	**E**	Alpha 1 antitrypsin (A1AT) and Transthyretin (TTR) reduced their expression	Seyyedi et al. (2007) *Pak J Biol Sci*
	Human SH-SY5Y neuroblastoma cells	50 Hz Sinusoidal ELF-MF	1 mT	5, 10, 15 days	2-DE /Western blot and immuno - histochemical confirmation	**E**	Nine new proteins involved in cellular defence mechanism and/or in cellular organization	Sulpizio et al. (2011) *J Cell Biochem*

							and proliferation	
RF-EMF Transcriptomics	**Primary cultured neurons and astrocytes**	GSM 1900 MHz Real-life exposure conditions	Not calculated	2 h	Microarray analysis /RT-PCR	**E**	Up-regulation of caspase-2, caspase-6 and Asc_gene expression in neurons and astrocytes (and Bax upregulation in astrocytes)	Zhao et al. (2007) *Neurosci Lett*
	Rat cortex and hippocampus	GSM mobile test phone at 1800 MHz	Whole-body SAR- 13 mW/kg brain SAR- 30 mW/kg	6 h	Microarray hybridizations on Affymetrix rat2302 chips	**E**	Altered gene categories in both cortex and hippocampus: extracellular region, signal transducer activity, intrinsic to	Nittby et al. (2008) *Environmentalist*

						membrane, and integral to membrane	
Jurkat human T lymphoma cells	1763 MHz CDMA exposure chamber	10 W/kg	24 h	Applied Biosystems microarrays	**E**	Ten genes changed from 1.3 to approximately 1.8-fold	Huang et al. (2008a) *Int J Radiat Biol*
HEI-OC1 immortalized mouse auditory hair cells	1763 MHz CDMA exposure chamber	20 W/kg	24 h, 48 h	Applied Biosystems 1700 full genome expression mouse microarray	**E**	29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure	Huang et al. (2008b) *Int J Radiat Biol*
Arabidopsis thaliana	RF field protocol representing typical microwave exposition in an urban environme	2 and 0.75 W/kg	24 h	RNA-extraction, microarray hybridization,and quantitative RT-PCR	**E**	Significant changes (but not more than 2.5-fold) in transcription of 10 genes	Engelmann et al. (2008) *Computational Biology and Chemistry*

	nt						
Rats (skin)	35 GHz mm-waves	75 mW/cm^2	6 h, 24 h	Affymetrix Gene Chip analysis	**E**	Expression changes in 56 genes at 6 h exposure and 58 genes at 24 h exposure	Millenbaugh et al. (2008) *Radiat Res*
Cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM)	900 MHz CW TEM cells	3.5 mW/Kg 3 V/m 1 mW in the cell culture dishes	2 h and 48 h	cDNA-microarray analysis /Western blot confirmation	**E**	DNA repair genes activated from 2 hrs, apoptotic genes overexpressed, cell cycle arrest genes activated. Surprisingly effects with	Trivino et al. (2012) *EBM*

						very low dose	
Human cell lines, A172 (glioblastoma), H4 (neurogliom a), and IMR-90 (fibroblasts from normal fetal lung)	W-CDMA CW 2.1425 GHz	80, 250, or 800 mW/kg	For up to 96 h	Affymetrix Human Genome Array	**E**	A very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression	Sekijima et al. (2010) *J. Radiat. Res*
Human-derived cell lines (TK6, HL60 and Mono-Mac-6)	1.9 GHz pulse-modulated RF fields	0, 1 and 10 W/kg	Intermit tent (5 min ON, 10 min OFF) for 6 h	Cell cycle, apoptosis, viability, cytokines tested at 0 and 18h after exposure	**NE**		Chauhan et al. (2007a) *Rad. Research*

	U87MG cells **Mono-Mac-6, MM6**	1.9 GHz pulse-modulated RF fields	0.1-10.0 W/kg	24 h intermittent (5 min ON, 10 min OFF) for 6 h	Microarrays analysis 18 h after exposure	**NE**		Chauhan et al. (2007b) *Proteomics*
	Human glioblastoma A172 cells **Human IMR-90 fibroblasts**	W-CDMA CW 2.1425 GHz	80 and 800 mW/kg 80 mW/kg 80 and 800 mW/kg 80 mW/kg	2-48 h 24 h 2h, 28h 28h	DNA Chip analysis	**NE**		Hirose et al. (2007) *Bioelectromagnetics*
	Mouse brain	GSM 1800 MHz	Whole body SAR of 1.1 W/kg	1 h	Microarrays containing over	**NE**	75 genes were found to be modulated	Paparini et al. (2008) *Bioelectro-magnetics*

			brain SAR 0.2 W/kg		22,600 probe sets RT-PCR		but since they were not confirmed ⇨ no effect	
Proteomics	***C. elegans***	1.0 GHz, 0.5W power input	0.9–3 mW/kg	1.5h 2.5h	Five Affymetrix gene arrays of pooled triplicate RNA populations from L4/adult worms from each group (sham and exposed)	**NE**	Minor changes in gene expression, probably false positives. Strange intensity window effect, no effect in high dose.	Dawe et al. (2009) *Bioelectromagnetics*
	Human endothelial cell line EA.hy926	GSM 1800 MHz	2.0 W/kg	1h	2-DE /Western blot confirmed selected proteins	**E**		Nylund et al. (2009) *Journal of Proteomics and Bioinformatics*

Human lens epithelial cells	GSM-like 1800-MHz	1.0, 2.0, or 3.5 W/kg.	2 h	2-DE	**E**	hnRNP K and HSP70 upregulated	Li et al. (2007) *Jpn. J. Ophtalmol*
Human skin cells.	Mobile phone GSM 900MHz	1.3 W/kg	1 h	2D in skin punch biopsies	**E**	8 proteins were affected	Karinen et al. (2008) *BMC Genomics*
Plasma from exposed rats causes changes in protein expression and levels of 3-NT in a rat alveolar macrophage cell line.NR8383 macrophages	Generator 35 GHz	Peak incident power density of 75 mW/cm^2	46 min	*in vitro* bioassay and proteomic screening	**E**	Increased the expression of 11 proteins, and levels of 3-nitrotyrosine in seven proteins, in the NR8383 cells. These altered proteins are associated with inflammation, oxidative stress, and energy metabolism	Sypniewska et al. (2010) *Bioelectromagnetics*
Human Jurkat T-	Modulated GSM 1800	2 W/kg	Intermit tent	Autoradiography of 2-	**E**	Rate of protein	Gerner et al. (2010)

cells Primary human diploid fibroblasts Peripheral blood mononuclear cells	MHz		exposure 8h (5min ON 10 min OFF)	DE gel		synthesis in proliferating cells is increased by long-term (8 h) RF-EME, while no effect was detectable in quiescent white blood cells treated in the same manner.	*Int. Arch. Occup. Environ. Health*
Balb/c mice (hippocampus, frontal lobe, cerebellum)	GSM 900 MHz Mobile phone, 1880 MHz Wireless DECT base	0.17-0.37 W/kg 0.012-0.028 W/kg	3 h/day x 8 months 8 h/day x 8 months	2-De /Western blot confirmed selected proteins	**E**	Real-life exposure conditions	Fragopoulou et al. (2012) *EBM*
Human primary umbilical vein endothelial cells and	1800 MHz GSM	2.0 W/kg	1 h	2-DE	**NE**		Nylund et al. (2010) *Proteome Sci*

	primary human brain Microvascular endothelial cells							
	Human breast cancer MCF-7 cells	849 MHz CDMA	2 and 10 W/kg	1 h/day x 3 days	2D, 24 h after exposure, Rt-PCR, Western blot	**NE**		Kim et al. (2010) *J Radiat Res*

V. CONCLUSIONS

It is clear that the effects of EMFs are very difficult to predict in the cells, and that SAR values do not provide any information about the molecular mechanisms likely to take place during exposure. Unlike drugs, EMFs are absorbed in a variety of different, diverse and non-linear ways depending on the "microenvironment" receiving the radiation, the orientation of the molecular targets and their shape, the metabolic state at the moment of exposure, the energy absorbance at the microscale of the cell and the modulation of the waves. On this basis, it is rather difficult to replicate experiments under different conditions and cell systems, which may explain the discrepancy of the results among research groups.

As far as changes in gene expression are concerned, they are observed within specific time duration with and without recovery time. As mentioned in some studies i.e., the same endothelial cell line responded to 1800 MHz intermittent exposure, but not to continuous exposure. Exposure time, exposure pattern and type of biological system (organism, tissue, cell) and experimental techniques may also play a key role in the end effect (Mevissen M., 2011).

In addition, we point out that all "averaging approaches" like proteomics and transcriptomics provide a mean value of changes in a specific protein/gene from all cell types of the tissue examined. The same is true for western blotting, RT-PCR and the entire battery of biochemical/molecular biological techniques. Of course, newly developed high sensitivity proteomics and transcriptomics might be able to analyse small quantities from individual cell types, since cell protein/gene expression changes would be the approach of choice in future experiments utilizing sophisticated state of the art microscopical techniques. Under these conditions, we will be able to understand why one cell type responds to EMF whereas another cell type is not responding, thus leading to a net "no effect" in case the second cell type is outnumbered.

Therefore the issue of examining by proteomics various time points during (or after) exposure is of utmost importance in order to unravel the mechanism(s) of EMF action. Approaches including 2D-autoradiography might be in addition very useful in this direction since the actual protein synthetic profile will be revealed (Gerner et al., 2010). As stated by these authors their findings of an association between metabolic activity and the observed cellular reaction to low intensity RF-EMF may reconcile conflicting results of previous studies. They further postulated that the observed

increased protein synthesis reflects an increased rate of protein turnover stemming from protein folding problems caused by the interference of radiofrequency electromagnetic fields with hydrogen bonds. These observations of course do not directly imply a health risk.

Needless to mention that a combination of all available high throughput techniques in the same system under identical exposure conditions will provide better data, especially if different laboratories replicate the results.

Taking into account that many studies using normal exposure conditions have revealed protein and gene expression changes, health hazards are possible.

It is clear that the existing guidelines are inadequate as pointed out by other studies as well (Fragopoulou et al., 2010). The transcriptomics and proteomics data reviewed here report that 66% of the papers published after 2007 show an effect. This is a clear indication of expression changes of proteins and genes at intensity levels commonly used by the wireless devices. Prudent avoidance of excessive usage of these devices is thus recommended.

Concerning the question of which model system is more suitable for such experiments in order to translate the effects into human EMF hazards, we might agree with Leszczynski's point that human volunteer skin is more suitable, but the major target of interest regarding EMF impacts is the brain which consists of an enormous complexity of nerve cell interactions far away from constituents of skin. Therefore, we argue that the system of choice for omics approaches should be rats or mice (preferably the second due to the possibility of handling transgenic material) as evolutionary very close to humans without neglecting the important work that has been (or will be) done using other biological systems, especially cell cultures.

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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.

APPENDIX A - ABSTRACTS ON GENETIC EFFECTS OF RADIOFREQUENCY AND CELL PHONE RADIATION (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).

(E) Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. Fertil Steril 92 1318-1325, 2009. (GT, RP, OX)

OBJECTIVE: To evaluate effects of cellular phone radiofrequency electromagnetic waves (RF-EMW) during talk mode on unprocessed (neat) ejaculated human semen. DESIGN: Prospective pilot study. SETTING: Center for reproductive medicine laboratory in tertiary hospital setting. SAMPLES: Neat semen samples from normal healthy donors (n = 23) and infertile patients (n = 9). INTERVENTION(S): After liquefaction, neat semen samples were divided into two aliquots. One aliquot (experimental) from each patient was exposed to cellular phone radiation (in talk mode) for 1 h, and the second aliquot (unexposed) served as the control sample under identical conditions. MAIN OUTCOME MEASURE(S): Evaluation of sperm parameters (motility, viability), reactive oxygen species (ROS), total antioxidant capacity (TAC) of semen, ROS-TAC score, and sperm DNA damage. RESULT(S): Samples exposed to RF-EMW showed a significant decrease in sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score. Levels of TAC and DNA damage showed no significant differences from the unexposed group. CONCLUSION(S): Radiofrequency electromagnetic waves emitted from cell phones may lead to oxidative stress in human semen. We speculate that keeping the cell phone in a trouser pocket in talk mode may negatively affect spermatozoa and impair male fertility.

(E) Atasoy HI, Gunal MY, Atasoy P, Elgun S, Bugdayci G. Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices.J Pediatr Urol. 2012 Mar 30. [Epub ahead of print] (GT, OX, LE, RP)

OBJECTIVE: To investigate effects on rat testes of radiofrequency radiation emitted from indoor Wi-Fi Internet access devices using 802.11.g wireless standards. METHODS: Ten Wistar albino male rats were divided into experimental and control groups, with five rats per group. Standard wireless gateways communicating at 2.437 GHz were used as radiofrequency wave sources. The experimental group was exposed to radiofrequency energy for 24 h a day for 20 weeks. The rats were sacrificed at the end of the study. Intracardiac blood was sampled for serum 8-hydroxy-2'-deoxyguanosine levels. Testes were removed and examined histologically and immunohistochemically. Testis tissues were analyzed for malondialdehyde levels and prooxidant-antioxidant enzyme activities. RESULTS: We observed significant increases in serum 8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine staining in the testes of the experimental group indicating DNA damage due to exposure ($p < 0.05$). We also found decreased levels of catalase and glutathione peroxidase activity in the experimental group, which may have been due to radiofrequency effects on enzyme activity ($p < 0.05$). CONCLUSIONS: These findings raise questions about the safety of radiofrequency exposure from Wi-Fi Internet access devices for growing organisms of reproductive age, with a potential effect on both fertility and the integrity of germ cells.

(E) Atlı Şekeroğlu Z, Akar A, Sekeroğlu V. Evaluation of the cytogenotoxic damage in immature and mature rats exposed to 900 MHz radio frequency electromagnetic fields. Int J Radiat Biol. 89(11):985-992, 2013. [Epub ahead of print] (GT, DE, LE)

Abstract Purpose: One of the most important issues regarding radio frequency electromagnetic fields (RF-EMF) is their effect on genetic material. Therefore, we investigated the cytogenotoxic effects of 900 MHz radio frequency electromagnetic fields (RF-EMF) and the effect of a recovery period after exposure to RF-EMF on bone marrow cells of immature and mature rats. Materials and methods: The immature and mature rats in treatment groups were exposed to RF-EMF for 2 h/day for 45 days. Average electrical field values for immature and mature rats were 28.1±4.8 V/m and 20.0±3.2 V/m, respectively. Whole-body specific absorption rate (SAR) values for immature and mature rats were in the range of 0.38-0.78 W/kg, and 0.31-0.52 W/kg during the 45 days, respectively. Two recovery groups were kept for 15 days after RF-EMF exposure. Results: Significant differences were observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCE) in all treatment and recovery groups. The cytogenotoxic damage in immature rats was statistically higher than the mature rats. The recovery period did not reduce the damage to the same extent as the corresponding control groups. Conclusions: The exposure of RF-EMF leads to cytotoxic and genotoxic damage in immature and mature rats. More sensitive studies are required to elucidate the possible carcinogenic risk of EMF exposure in humans, especially children.

(E) Avendaño C, Mata A, Sanchez Sarmiento CA, Doncel GF. Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. FertilSteril 97:39-45, 2012. (GT, RP)

OBJECTIVE: To evaluate the effects of laptop computers connected to local area networks wirelessly (Wi-Fi) on human spermatozoa. DESIGN: Prospective in vitro study. SETTING: Center for reproductive medicine. PATIENT(S): Semen samples from 29 healthy donors. INTERVENTION(S): Motile sperm were selected by swim up. Each sperm suspension was divided into two aliquots. One sperm aliquot (experimental) from each patient was exposed to an

internet-connected laptop by Wi-Fi for 4 hours, whereas the second aliquot (unexposed) was used as control, incubated under identical conditions without being exposed to the laptop. MAIN OUTCOME MEASURE(S): Evaluation of sperm motility, viability, and DNA fragmentation. RESULT(S): Donor sperm samples, mostly normozoospermic, exposed ex vivo during 4 hours to a wireless internet-connected laptop showed a significant decrease in progressive sperm motility and an increase in sperm DNA fragmentation. Levels of dead sperm showed no significant differences between the two groups. CONCLUSION(S): To our knowledge, this is the first study to evaluate the direct impact of laptop use on human spermatozoa. Ex vivo exposure of human spermatozoa to a wireless internet-connected laptop decreased motility and induced DNA fragmentation by a nonthermal effect. We speculate that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility. Further in vitro and in vivo studies are needed to prove this contention.

(E) Baohong Wang, Jiliang H, Lifen J, Deqiang L, Wei Z, Jianlin L, Hongping D. Studying the synergistic damage effects induced by 1.8 GHz radiofrequency field radiation (RFR) with four chemical mutagens on human lymphocyte DNA using comet assay in vitro. Mutat Res 578:149-57, 2005. (GT, IA)

The aim of this investigation was to study the synergistic DNA damage effects in human lymphocytes induced by 1.8GHz radiofrequency field radiation (RFR, SAR of 3W/kg) with four chemical mutagens, i.e. mitomycin C (MMC, DNA crosslinker), bleomycin (BLM, radiomimetic agent), methyl methanesulfonate (MMS, alkylating agent), and 4-nitroquinoline-1-oxide (4NQO, UV-mimetic agent). The DNA damage of lymphocytes exposed to RFR and/or with chemical mutagens was detected at two incubation time (0 or 21h) after treatment with comet assay in vitro. Three combinative exposure ways were used. Cells were exposed to RFR and chemical mutagens for 2 and 3h, respectively. Tail length (TL) and tail moment (TM) were utilized as DNA damage indexes. The results showed no difference of DNA damage indexes between RFR group and control group at 0 and 21h incubation after exposure ($P>0.05$). There were significant difference of DNA damage indexes between MMC group and RFR+MMC co-exposure group at 0 and 21h incubation after treatment ($P<0.01$). Also the significant difference of DNA damage indexes between 4NQO group and RFR+4NQO co-exposure group at 0 and 21h incubation after treatment was observed ($P<0.05$ or $P<0.01$). The DNA damage in RFR+BLM co-exposure groups and RFR+MMS co-exposure groups was not significantly increased, as compared with corresponding BLM and MMS groups ($P>0.05$). The experimental results indicated 1.8GHz RFR (SAR, 3W/kg) for 2h did not induce the human lymphocyte DNA damage effects in vitro, but could enhance the human lymphocyte DNA damage effects induced by MMC and 4NQO. The synergistic DNA damage effects of 1.8GHz RFR with BLM or MMS were not obvious.

(E) Baohong W, Lifen J, Lanjuan L, Jianlin L, Deqiang L, Wei Z, Jiliang H.Evaluating the combinative effects on human lymphocyte DNA damage induced by ultraviolet ray C plus 1.8GHz microwaves using comet assay in vitro. Toxicology. 232(3):311-316, 2007. (GT, IA)

The objective of this study was to observe whether 1.8GHz microwaves (MW) (SAR, 3 W/kg) exposure can influence human lymphocyte DNA damage induced by ultraviolet ray C (UVC). The lymphocytes, which were from three young healthy donors, were exposed to 254 nm UVC at the doses of 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 J m(-2), respectively. The lymphocytes were irradiated by 1.8GHz MW (SAR, 3 W/kg) for 0, 1.5 and 4 h. The combinative exposure of UVC

plus MW was conducted. The treated cells were incubated for 0, 1.5 and 4 h. Finally, comet assay was used to measure DNA damage of above treated lymphocytes. The results indicated that the difference of DNA damage induced between MW group and control group was not significant (P>0.05). The MTLs induced by UVC were 1.71+/-0.09, 2.02+/-0.08, 2.27+/-0.17, 2.27+/-0.06, 2.25+/-0.12, 2.24+/-0.11 microm, respectively, which were significantly higher than that (0.96+/-0.05 microm) of control (P<0.01). MTLs of some sub-groups in combinative exposure groups at 1.5-h incubation were significantly lower that those of corresponding UVC sub-groups (P<0.01 or P<0.05). However, MTLs of some sub-groups in combinative exposure groups at 4-h incubation were significantly higher that those of corresponding UVC sub-groups (P<0.01 or P<0.05). In this experiment it was found that 1.8GHz (SAR, 3 W/kg) MW exposure for 1.5 and 4 h did not enhance significantly human lymphocyte DNA damage, but could reduce and increase DNA damage of human lymphocytes induced by UVC at 1.5-h and 4-h incubation, respectively.

(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) Belyaev IY, Koch CB, Terenius O, Roxstrom-Lindquist K, Malmgren LO, H Sommer W, Salford LG, Persson BR. Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. Bioelectromagnetics 27:295-306, 2006. (GE)

We investigated whether exposure of rat brain to microwaves (MWs) of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression. An exposure installation was used based on a test mobile phone employing a GSM signal at 915 MHz, all standard modulations included, output power level in pulses 2 W, specific absorption rate (SAR) 0.4 mW/g. Rats were exposed or sham exposed to MWs during 2 h. After exposure, cell suspensions were prepared from brain samples, as well as from spleen and thymus. For analysis of gene expression patterns, total RNA was extracted from cerebellum. Changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependencies (AVTD). DNA double strand breaks (DSBs) were analyzed by pulsed-field gel electrophoresis (PFGE). Effects of MW exposure were observed on neither conformation of chromatin nor DNA DSBs. Gene expression profiles were obtained by Affymetrix U34 GeneChips representing 8800 rat genes and analyzed with the Affymetrix Microarray Suite (MAS) 5.0 software. In cerebellum from all exposed animals, 11 genes were upregulated in a range of 1.34-2.74 fold and one gene was downregulated 0.48-fold ($P < .0025$). The induced genes encode proteins with diverse functions including neurotransmitter regulation, blood-brain barrier (BBB), and melatonin production. The data shows that GSM MWs at 915 MHz did not induce PFGE-detectable DNA double stranded breaks or changes in chromatin conformation, but affected expression of genes in rat brain cells

(E) Belyaev IY, Markovà E, Hillert L, Malmgren LO, Persson BR. Microwaves from UMTS/GSM mobile phones induce long-lasting inhibition of 53BP1/gamma-H2AX DNA repair foci in human lymphocytes. Bioelectromagnetics 30:129-41, 2009. (GT, EH)

We have recently described frequency-dependent effects of mobile phone microwaves (MWs) of global system for mobile communication (GSM) on human lymphocytes from persons reporting hypersensitivity to electromagnetic fields and healthy persons. Contrary to GSM, universal global telecommunications system (UMTS) mobile phones emit wide-band MW signals. Hypothetically, UMTS MWs may result in higher biological effects compared to GSM signal because of eventual "effective" frequencies within the wideband. Here, we report for the first time that UMTS MWs affect chromatin and inhibit formation of DNA double-strand breaks co-localizing 53BP1/gamma-H2AX DNA repair foci in human lymphocytes from hypersensitive and healthy persons and confirm that effects of GSM MWs depend on carrier frequency. Remarkably, the effects of MWs on 53BP1/gamma-H2AX foci persisted up to 72 h following exposure of cells, even longer than the stress response following heat shock. The data are in line with the hypothesis that the type of signal, UMTS MWs, may have higher biological efficiency and possibly larger health risk effects compared to GSM radiation emissions. No significant differences in effects between groups of healthy and hypersensitive subjects were observed, except for the effects of UMTS MWs and GSM-915 MHz MWs on the formation of the DNA repair foci, which were different for hypersensitive ($P < 0.02$[53BP1]//0.01[gamma-H2AX]) but not for control subjects ($P > 0.05$). The non-parametric statistics used here did not indicate specificity of the differences revealed between the effects of GSM and UMTS MWs on cells from hypersensitive subjects and more data are needed to study the nature of these differences.

(NE) Bourthoumieu S, Joubert V, Marin B, Collin A, Leveque P, Terro F, Yardin C. Cytogenetic studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using R-banded karyotyping. Radiat Res 174:712-718, 2010. (GT)

It is important to determine the possible effects of exposure to radiofrequency (RF) radiation on the genetic material of cells since damage to the DNA of somatic cells may be linked to cancer development or cell death and damage to germ cells may lead to genetic damage in next and subsequent generations. The objective of this study was to investigate whether exposure to radiofrequency radiation similar to that emitted by mobile phones of second-generation standard Global System for Mobile Communication (GSM) induces genotoxic effects in cultured human cells. The cytogenetic effects of GSM-900 MHz (GSM-900) RF radiation were investigated using R-banded karyotyping after in vitro exposure of human cells (amniotic cells) for 24 h. The average specific absorption rate (SAR) was 0.25 W/kg. The exposures were carried out in wire-patch cells (WPCs) under strictly controlled conditions of temperature. The genotoxic effect was assessed immediately or 24 h after exposure using four different samples. One hundred metaphase cells were analyzed per assay. Positive controls were provided by using bleomycin. We found no direct cytogenetic effects of GSM-900 either 0 h or 24 h after exposure. To the best of our knowledge, our work is the first to study genotoxicity using complete R-banded karyotyping, which allows visualizing all the chromosomal rearrangements, either numerical or structural.

(NE) Bourthoumieu S, Terro F, Leveque P, Collin A, Joubert V, Yardin C. Aneuploidy studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using FISH. Int J Radiat Biol 87:400-408, 2011. (GT)

PURPOSE: Since previous research found an increase in the rate of aneuploidies in human lymphocytes exposed to radiofrequencies, it seems important to perform further studies. The objective of this study was then to investigate whether the exposure to RF (radiofrequency) radiation similar to that emitted by mobile phones of a second generation standard, i.e., Global System for Mobile communication (GSM) may induce aneuploidy in cultured human cells. MATERIALS AND METHODS: The potential induction of genomic instability by GSM-900 MHz radiofrequency (GSM-900) was investigated after in vitro exposure of human amniotic cells for 24 h to average-specific absorption rates (SAR) of 0.25, 1, 2 and 4 W/kg in the temperature range of 36.3-39.7°C. The exposures were carried out in a wire-patch cell (WPC). The rate of aneuploidy of chromosomes 11 and 17 was determined by interphase FISH (Fluorescence In Situ Hybridisation) immediately after independent exposure of three different donors for 24 h. At least 100 interphase cells were analysed per assay. RESULTS: No significant change in the rate of aneuploidy of chromosomes 11 and 17 was found following exposure to GSM-900 for 24 h at average SAR up to 4 W/kg. CONCLUSION: Our study did not show any in vitro aneuploidogenic effect of GSM using FISH and is not in agreement with the results of previous research.

(NE) Bourthoumieu S, Magnaudeix A, Terro F, Leveque P, Collin A, Yardin C. Study of p53 expression and post-transcriptional modifications after GSM-900 radiofrequency exposure of human amniotic cells. Bioelectromagnetics. 2012 Jul 5. doi: 10.1002/bem.21744. [Epub ahead of print] (GE)

The potential effects of radiofrequency (RF) exposure on the genetic material of cells are very important to determine since genome instability of somatic cells may be linked to cancer development. In response to genetic damage, the p53 protein is activated and can induce cell cycle arrest allowing more time for DNA repair or elimination of damaged cells through

apoptosis. The objective of this study was to investigate whether the exposure to RF electromagnetic fields, similar to those emitted by mobile phones of the second generation standard, Global System for Mobile Communications (GSM), may induce expression of the p53 protein and its activation by post-translational modifications in cultured human cells. The potential induction of p53 expression and activation by GSM-900 was investigated after in vitro exposure of human amniotic cells for 24 h to average specific absorption rates (SARs) of 0.25, 1, 2, and 4 W/kg in the temperature range of 36.3-39.7 °C. The exposures were carried out using a wire-patch cell (WPC) under strictly controlled conditions of temperature. Expression and activation of p53 by phosphorylation at serine 15 and 37 were studied using Western blot assay immediately after three independent exposures of cell cultures provided from three different donors. Bleomycin-exposed cells were used as a positive control. According to our results, no significant changes in the expression and activation of the p53 protein by phosphorylation at serine 15 and 37 were found following exposure to GSM-900 for 24 h at average SARs up to 4 W/kg in human embryonic cells.

(E) Burlaka A, Tsybulin O, Sidorik E, Lukin S, Polishuk V, Tsehmistrenko S, Yakymenko I. Overproduction of free radical species in embryonal cells exposed to low intensity radiofrequency radiation. Exp Oncol. 35(3):219-225, 2013. (GT, LE, DE, OX)

Aim: Long-term exposure of humans to low intensity radiofrequency electromagnetic radiation (RF-EMR) leads to a statistically significant increase in tumor incidence. Mechanisms of such the effects are unclear, but features of oxidative stress in living cells under RF-EMR exposure were previously reported. Our study aims to assess a production of initial free radical species, which lead to oxidative stress in the cell. Materials and Methods: Embryos of Japanese quails were exposed in ovo to extremely low intensity RF-EMR of GSM 900 MHz (0.25 μW/cm2) during 158-360 h discontinuously (48 c - ON, 12 c - OFF) before and in the initial stages of development. The levels of superoxide (O2·-), nitrogen oxide (NO·), thiobarbituric acid reactive substances (TBARS), 8-oxo-2'-deoxyguanosine (8-oxo-dG) and antioxidant enzymes' activities were assessed in cells/tissues of 38-h, 5- and 10-day RF-EMR exposed and unexposed embryos. Results: The exposure resulted in a significant persistent overproduction of superoxide and nitrogen oxide in embryo cells during all period of analyses. As a result, significantly increased levels of TBARS and 8-oxo-dG followed by significantly decreased levels of superoxide dismutase and catalase activities were developed in the exposed embryo cells. Conclusion: Exposure of developing quail embryos to extremely low intensity RF-EMR of GSM 900 MHz during at least one hundred and fifty-eight hours leads to a significant overproduction of free radicals/reactive oxygen species and oxidative damage of DNA in embryo cells. These oxidative changes may lead to pathologies up to oncogenic transformation of cells.

(E) Buttiglione M, Roca L, Montemurno E, Vitiello F, Capozzi V, Cibelli G. Radiofrequency radiation (900 MHz) induces Egr-1 gene expression and affects cell-cycle control in human neuroblastoma cells. J Cell Physiol. 213(3):759-767, 2007. (GE)

Many environmental signals, including ionizing radiation and UV rays, induce activation of Egr-1 gene, thus affecting cell growth and apoptosis. The paucity and the controversial knowledge about the effect of electromagnetic fields (EMF) exposure of nerve cells prompted us to investigate the bioeffects of radiofrequency (RF) radiation on SH-SY5Y neuroblastoma cells. The effect of a modulated RF field of 900 MHz, generated by a wire patch cell (WPC) antenna

exposure system on Egr-1 gene expression, was studied as a function of time. Short-term exposures induced a transient increase in Egr-1 mRNA level paralleled with activation of the MAPK subtypes ERK1/2 and SAPK/JNK. The effects of RF radiations on cell growth rate and apoptosis were also studied. Exposure to RF radiation had an anti-proliferative activity in SH-SY5Y cells with a significant effect observed at 24 h. RF radiation impaired cell cycle progression, reaching a significant G2-M arrest. In addition, the appearance of the sub-G1 peak, a hallmark of apoptosis, was highlighted after a 24-h exposure, together with a significant decrease in mRNA levels of Bcl-2 and survivin genes, both interfering with signaling between G2-M arrest and apoptosis. Our results provide evidence that exposure to a 900 MHz-modulated RF radiation affect both Egr-1 gene expression and cell regulatory functions, involving apoptosis inhibitors like Bcl-2 and survivin, thus providing important insights into a potentially broad mechanism for controlling in vitro cell viability.

(E) Cam ST, Seyhan N. Single-strand DNA breaks in human hair root cells exposed to mobile phone radiation. Int J Radiat Biol 88(5):420-424, 2012 (GT, HU)

Purpose: To analyze the short term effects of radiofrequency radiation (RFR) exposure on genomic deoxyribonucleic acid (DNA) of human hair root cells. Subjects and methods: Hair samples were collected from 8 healthy human subjects immediately before and after using a 900-MHz GSM (Global System for Mobile Communications) mobile phone for 15 and 30 minutes. Single-strand DNA breaks of hair root cells from the samples were determined using the 'comet assay'. Results: The data showed that talking on a mobile phone for 15 or 30 minutes significantly increased ($p < .05$) single-strand DNA breaks in cells of hair roots close to the phone. Comparing the 15-min and 30-min data using the paired t-test also showed that significantly more damages resulted after 30 minutes than after 15 minutes of phone use. Conclusions: A short-term exposure (15 and 30 minutes) to RFR (900-MHz) from a mobile phone caused a significant increase in DNA single-strand breaks in human hair root cells located around the ear which is used for the phone calls.

(E) Campisi A, Gulino M, Acquaviva R, Bellia P, Raciti G, Grasso R, Musumeci F, Vanella A, Triglia A. Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. Neurosci Lett 473:52-55. 2010. (GT, OX, WS)

The exposure of primary rat neocortical astroglial cell cultures to acute electromagnetic fields (EMF) in the microwave range was studied. Differentiated astroglial cell cultures at 14 days in vitro were exposed for 5, 10, or 20 min to either 900 MHz continuous waves or 900 MHz waves modulated in amplitude at 50 Hz using a sinusoidal waveform and 100% modulation index. The strength of the electric field (rms value) at the sample position was 10V/m. No change in cellular viability evaluated by MTT test and lactate dehydrogenase release was observed. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated EMF for 20 min. No evident effects were detected when shorter time intervals or continuous waves were used. The irradiation conditions allowed the exclusion of any possible thermal effect. Our data demonstrate, for the first time, that even acute exposure to low intensity EMF induces ROS production and DNA fragmentation in astrocytes in primary cultures, which also represent the principal target of modulated EMF. Our findings also suggest the hypothesis that the effects could be due to hyperstimulation of the glutamate receptors, which play a crucial

role in acute and chronic brain damage. Furthermore, the results show the importance of the amplitude modulation in the interaction between EMF and neocortical astrocytes.

(E) **Cervellati F, Valacchi G, Lunghi L, Fabbri E,** Valbonesi **P, Marci R, Biondi C, Vesce F. 17-β-estradiol counteracts the effects of high frequency electromagnetic fields on trophoblastic connexins and integrins. Oxid Med Cell Longev. 2013;2013:280850. doi: 10.1155/2013/280850. (GE)**

We investigated the effect of high-frequency electromagnetic fields (HF-EMFs) and 17-β-estradiol on connexins (Cxs), integrins (Ints), and estrogen receptor (ER) expression, as well as on ultrastructure of trophoblast-derived HTR-8/SVneo cells. HF-EMF, 17-β-estradiol, and their combination induced an increase of Cx40 and Cx43 mRNA expression. HF-EMF decreased Int alpha1 and β 1 mRNA levels but enhanced Int alpha5 mRNA expression. All the Ints mRNA expressions were increased by 17-β-estradiol and exposure to both stimuli. ER-β mRNA was reduced by HF-EMF but augmented by 17-β-estradiol alone or with HF-EMF. ER-β immunofluorescence showed a cytoplasmic localization in sham and HF-EMF exposed cells which became nuclear after treatment with hormone or both stimuli. Electron microscopy evidenced a loss of cellular contact in exposed cells which appeared counteracted by 17-β-estradiol. We demonstrate that 17-β-estradiol modulates Cxs and Ints as well as ER-β expression induced by HF-EMF, suggesting an influence of both stimuli on trophoblast differentiation and migration.

(NE) Chang SK, Choi JS, Gil HW, Yang JO, Lee EY, Jeon YS, Lee ZW, Lee M, Hong MY, Ho Son T, Hong SY. Genotoxicity evaluation of electromagnetic fields generated by 835-MHz mobile phone frequency band. Eur J Cancer Prev 14:175-179, 2005. (GT, IA) (Some interaction effects with chemicals are reported in this paper.)

It is still unclear whether the exposure to electromagnetic fields (EMFs) generated by mobile phone radiation is directly linked to cancer. We examined the biological effects of an EMF at 835 MHz, the most widely used communication frequency band in Korean CDMA mobile phone networks, on bacterial reverse mutation (Ames assay) and DNA stability (in vitro DNA degradation). In the Ames assay, tester strains alone or combined with positive mutagen were applied in an artificial mobile phone frequency EMF generator with continuous waveform at a specific absorption rate (SAR) of 4 W/kg for 48 h. In the presence of the 835-MHz EMF radiation, incubation with positive mutagen 4-nitroquinoline-1-oxide and cumene hydroxide further increased the mutation rate in Escherichia coli WP2 and TA102, respectively, while the contrary results in Salmonella typhimurium TA98 and TA1535 treated with 4-nitroquinoline-1-oxide and sodium azide, respectively, were shown as antimutagenic. However, these mutagenic or co-mutagenic effects of 835-MHz radiation were not significantly repeated in other relevant strains with same mutation type. In the DNA degradation test, the exposure to 835-MHz EMF did not change the rate of degradation observed using plasmid pBluescriptSK(+) as an indicator. Thus, we suggest that 835-MHz EMF under the conditions of our study neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro.

(NE) Chauhan V, Mariampillai A, Bellier PV, Qutob SS, Gajda GB, Lemay E, Thansandote A, McNamee JP. Gene expression analysis of a human lymphoblastoma cell

line exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Radiat Res. 165(4):424-429, 2006. (GE)

This study was designed to determine whether radiofrequency (RF) fields of the type used for wireless communications could elicit a cellular stress response. As general indicators of a cellular stress response, we monitored changes in proto-oncogene and heat-shock protein expression. Exponentially growing human lymphoblastoma cells (TK6) were exposed to 1.9 GHz pulse-modulated RF fields at average specific absorption rates (SARs) of 1 and 10 W/kg. Perturbations in the expression levels of the proto-oncogenes FOS, JUN and MYC after exposure to sham and RF fields were assessed by real-time RT-PCR. In addition, the transcript levels of the cellular stress proteins HSP27 and inducible HSP70 were also monitored. We demonstrated that transcript levels of these genes in RF-field-exposed cells showed no significant difference in relation to the sham treatment group. However, concurrent positive (heat-shock) control samples displayed a significant elevation in the expression of HSP27, HSP70, FOS and JUN. Conversely, the levels of MYC mRNA were found to decline in the positive (heat-shock) control. In conclusion, our study found no evidence that the 1.9 GHz RF-field exposure caused a general stress response in TK6 cells under our experimental conditions.

(NE) Chauhan V, Mariampillai A, Gajda GB, Thansandote A, McNamee JP. Analysis of proto-oncogene and heat-shock protein gene expression in human derived cell-lines exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Int J Radiat Biol. 82(5):347-354, 2006. (GE)

Purpose: Several studies have reported that radiofrequency (RF) fields, as emitted by mobile phones, may cause changes in gene expression in cultured human cell-lines. The current study was undertaken to evaluate this possibility in two human-derived immune cell-lines.Materials and methods: HL-60 and Mono-Mac-6 (MM6) cells were individually exposed to intermittent (5 min on, 10 min off) 1.9 GHz pulse-modulated RF fields at a average specific absorption rate (SAR) of 1 and 10 W/kg at 37 +/- 0.5 degrees C for 6 h. Concurrent negative and positive (heat-shock for 1 h at 43 degrees C) controls were conducted with each experiment. Immediately following RF field exposure (T = 6 h) and 18 h post-exposure (T = 24 h), cell pellets were collected from each of the culture dishes and analyzed for transcript levels of proto-oncogenes (c-jun, c-myc and c-fos) and the stress-related genes (heat shock proteins (HSP) HSP27 and HSP70B) by quantitative reverse transcriptase polymerase chain reaction (RT-PCR).Results: No significant effects were observed in mRNA expression of HSP27, HSP70, c-jun, c-myc or c-fos between the sham and RF-exposed groups, in either of the two cell-lines. However, the positive (heat-shock) control group displayed a significant elevation in the expression of HSP27, HSP70, c-fos and c-jun in both cell-lines at T = 6 and 24 h, relative to the sham and negative control groups.Conclusion: This study found no evidence that exposure of cells to non-thermalizing levels of 1.9 GHz pulse-modulated RF fields can cause any detectable change in stress-related gene expression.

(NE) Chauhan V, Qutob SS, Lui S, Mariampillai A, Bellier PV, Yauk CL, Douglas GR, Williams A, McNamee JP. Analysis of gene expression in two human-derived cell lines exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. Proteomics. 7(21):3896-3905, 2007. (GE)

There is considerable controversy surrounding the biological effects of radiofrequency (RF) fields, as emitted by mobile phones. Previous work from our laboratory has shown no effect related to the exposure of 1.9 GHz pulse-modulated RF fields on the expression of 22,000 genes in a human glioblastoma-derived cell-line (U87MG) at 6 h following a 4 h RF field exposure period. As a follow-up to this study, we have now examined the effect of RF field exposure on the possible expression of late onset genes in U87MG cells after a 24 h RF exposure period. In addition, a human monocyte-derived cell-line (Mono-Mac-6, MM6) was exposed to intermittent (5 min ON, 10 min OFF) RF fields for 6 h and then gene expression was assessed immediately after exposure and at 18 h postexposure. Both cell lines were exposed to 1.9 GHz pulse-modulated RF fields for 6 or 24 h at specific absorption rates (SARs) of 0.1-10.0 W/kg. In support of our previous results, we found no evidence that nonthermal RF field exposure could alter gene expression in either cultured U87MG or MM6 cells, relative to nonirradiated control groups. However, exposure of both cell-lines to heat-shock conditions (43 degrees C for 1 h) caused an alteration in the expression of a number of well-characterized heat-shock proteins.

(E) Chavdoula ED, Panagopoulos DJ, Margaritis LH. Comparison of biological effects between continuous and intermittent exposure to GSM-900-MHz mobile phone radiation: detection of apoptotic cell-death features. Mutat Res 700:51-61, 2010. (RP, LE, GT)

In the present study we used a 6-min daily exposure of dipteran flies, Drosophila melanogaster, to GSM-900 MHz (Global System for Mobile Telecommunications) mobile phone electromagnetic radiation (EMR), to compare the effects between the continuous and four different intermittent exposures of 6min total duration, and also to test whether intermittent exposure provides any cumulative effects on the insect's reproductive capacity as well as on the induction of apoptotic cell death. According to our previous experiments, a 6-min continuous exposure per day for five days to GSM-900 MHz and DCS-1800 MHz (Digital Cellular System) mobile phone radiation, brought about a large decrease in the insect's reproductive capacity, as defined by the number of F pupae. This decrease was found to be non thermal and correlated with an increased percentage of induced fragmented DNA in the egg chambers' cells at early- and mid-oogenesis. In the present experiments we show that intermittent exposure also decreases the reproductive capacity and alters the actin cytoskeleton network of the egg chambers, another known aspect of cell death that was not investigated in previous experiments, and that the effect is also due to DNA fragmentation. Intermittent exposures with 10-min intervals between exposure sessions proved to be almost equally effective as continuous exposure of the same total duration, whereas longer intervals between the exposures seemed to allow the organism the time required to recover and partly overcome the above-mentioned effects of the GSM exposure.

(E) 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. 1

(E) De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4:e6446, 2009. (GT, OX, RP)

BACKGROUND: In recent times there has been some controversy over the impact of electromagnetic radiation on human health. The significance of mobile phone radiation on male reproduction is a key element of this debate since several studies have suggested a relationship between mobile phone use and semen quality. The potential mechanisms involved have not been established, however, human spermatozoa are known to be particularly vulnerable to oxidative stress by virtue of the abundant availability of substrates for free radical attack and the lack of cytoplasmic space to accommodate antioxidant enzymes. Moreover, the induction of oxidative stress in these cells not only perturbs their capacity for fertilization but also contributes to sperm DNA damage. The latter has, in turn, been linked with poor fertility, an increased incidence of miscarriage and morbidity in the offspring, including childhood cancer. In light of these associations, we have analyzed the influence of RF-EMR on the cell biology of human spermatozoa in vitro. PRINCIPAL FINDINGS: Purified human spermatozoa were exposed to radio-frequency electromagnetic radiation (RF-EMR) tuned to 1.8 GHz and covering a range of specific absorption rates (SAR) from 0.4 W/kg to 27.5 W/kg. In step with increasing SAR, motility and vitality were significantly reduced after RF-EMR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation were significantly elevated ($P<0.001$). Furthermore, we also observed highly significant relationships between SAR, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation after RF-EMR exposure. CONCLUSIONS: RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. These findings have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring.

(E) Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, Ardoino L, Lovisolo GA, Giardino L, Calzà L. Continuous exposure to 900MHz GSM-modulated EMF alters morphological maturation of neural cells. Neurosci Lett. 455(3):173-177, 2009. (GE, DE)

The effects of radiofrequency electromagnetic field (RF-EMF) exposure on neuronal phenotype maturation have been studied in two different in vitro models: murine SN56 cholinergic cell line and rat primary cortical neurons. The samples were exposed at a dose of 1W/kg at 900 MHz GSM modulated. The phenotype analysis was carried out at 48 and 72 h (24 and 48 h of SN56 cell line differentiation) or at 24, 72, 120 h (2, 4 and 6 days in vitro for cortical neurons) of exposure, on live and immunolabeled neurons, and included the morphological study of neurite emission, outgrowth and branching. Moreover, cortical neurons were studied to detect alterations in the expression pattern of cytoskeleton regulating factors, e.g. beta-thymosin, and of early genes, e.g. c-Fos and c-Jun through real-time PCR on mRNA extracted after 24h exposure to EMF. We found that RF-EMF exposure reduced the number of neurites generated by both cell systems, and this alteration correlates to increased expression of beta-thymosin mRNA.

(E) Deshmukh PS, Megha K, Banerjee BD, Ahmed RS, Chandna S, Abegaonkar MP, Tripathi AK. Detection of Low Level Microwave Radiation Induced Deoxyribonucleic Acid Damage Vis-à-vis Genotoxicity in Brain of Fischer Rats. Toxicol Int. 20(1):19-24, 2013. (GT, LE)

BACKGROUND: Non-ionizing radiofrequency radiation has been increasingly used in industry, commerce, medicine and especially in mobile phone technology and has become a matter of serious concern in present time. OBJECTIVE: The present study was designed to investigate the possible deoxyribonucleic acid (DNA) damaging effects of low-level microwave radiation in brain of Fischer rats. MATERIALS AND METHODS: Experiments were performed on male Fischer rats exposed to microwave radiation for 30 days at three different frequencies: 900, 1800 and 2450 MHz. Animals were divided into 4 groups: Group I (Sham exposed): Animals not exposed to microwave radiation but kept under same conditions as that of other groups, Group II: Animals exposed to microwave radiation at frequency 900 MHz at specific absorption rate (SAR) 5.953 × 10(-4) W/kg, Group III: Animals exposed to 1800 MHz at SAR 5.835 × 10(-4) W/kg and Group IV: Animals exposed to 2450 MHz at SAR 6.672 × 10(-4) W/kg. At the end of the exposure period animals were sacrificed immediately and DNA damage in brain tissue was assessed using alkaline comet assay. RESULTS: In the present study, we demonstrated DNA damaging effects of low level microwave radiation in brain. CONCLUSION: We concluded that low SAR microwave radiation exposure at these frequencies may induce DNA strand breaks in brain tissue.

(E) Engelmann JC, Deeken R, Müller T, Nimtz G, Roelfsema MR, Hedrich R. Is gene activity in plant cells affected by UMTS-irradiation? A whole genome approach. Adv Appl Bioinform Chem. 1:71-83, 2008. (GE)

Mobile phone technology makes use of radio frequency (RF) electromagnetic fields transmitted through a dense network of base stations in Europe. Possible harmful effects of RF fields on humans and animals are discussed, but their effect on plants has received little attention. In search for physiological processes of plant cells sensitive to RF fields, cell suspension cultures of Arabidopsis thaliana were exposed for 24 h to a RF field protocol representing typical microwave exposition in an urban environment. mRNA of exposed cultures and controls was used to hybridize Affymetrix-ATH1 whole genome microarrays. Differential expression analysis revealed significant changes in transcription of 10 genes, but they did not exceed a fold change

of 2.5. Besides that 3 of them are dark-inducible, their functions do not point to any known responses of plants to environmental stimuli. The changes in transcription of these genes were compared with published microarray datasets and revealed a weak similarity of the microwave to light treatment experiments. Considering the large changes described in published experiments, it is questionable if the small alterations caused by a 24 h continuous microwave exposure would have any impact on the growth and reproduction of whole plants.

(E) Esmekaya MA, Aytekin E, Ozgur E, Güler G, Ergun MA, Omeroğlu S, Seyhan N. Mutagenic and morphologic impacts of 1.8GHz radiofrequency radiation on human peripheral blood lymphocytes (hPBLs) and possible protective role of pre-treatment with Ginkgo biloba (EGb 761). Sci Total Environ. 410-411:59-64, 2011. (GT, OX)

The mutagenic and morphologic effects of 1.8GHz Global System for Mobile Communications (GSM) modulated RF (radiofrequency) radiation alone and in combination with Ginkgo biloba (EGb 761) pre-treatment in human peripheral blood lymphocytes (hPBLs) were investigated in this study using Sister Chromatid Exchange (SCE) and electron microscopy. Cell viability was assessed with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay. The lymphocyte cultures were exposed to GSM modulated RF radiation at 1.8GHz for 6, 8, 24 and 48h with and without EGb 761. We observed morphological changes in pulse-modulated RF radiated lymphocytes. Longer exposure periods led to destruction of organelle and nucleus structures. Chromatin change and the loss of mitochondrial crista occurred in cells exposed to RF for 8h and 24h and were more pronounced in cells exposed for 48h. Cytoplasmic lysis and destruction of membrane integrity of cells and nuclei were also seen in 48h RF exposed cells. There was a significant increase ($p<0.05$) in SCE frequency in RF exposed lymphocytes compared to sham controls. EGb 761 pre-treatment significantly decreased SCE from RF radiation. RF radiation also inhibited cell viability in a time dependent manner. The inhibitory effects of RF radiation on the growth of lymphoctes were marked in longer exposure periods. EGb 761 pre-treatment significantly increased cell viability in RF+EGb 761 treated groups at 8 and 24h when compared to RF exposed groups alone. The results of our study showed that RF radiation affects cell morphology, increases SCE and inhibits cell proliferation. However, EGb 761 has a protective role against RF induced mutagenity. We concluded that RF radiation induces chromosomal damage in hPBLs but this damage may be reduced by EGb 761 pre-treatment.

(NE) Falzone N, Huyser C, Franken DR, Leszczynski D. Mobile phone radiation does not induce pro-apoptosis effects in human spermatozoa. Radiat Res 174:169-176, 2010. (GT, OX)

Abstract Recent reports suggest that mobile phone radiation may diminish male fertility. However, the effects of this radiation on human spermatozoa are largely unknown. The present study examined effects of the radiation on induction of apoptosis-related properties in human spermatozoa. Ejaculated, density-purified, highly motile human spermatozoa were exposed to mobile phone radiation at specific absorption rates (SARs) of 2.0 and 5.7 W/kg. At various times after exposure, flow cytometry was used to examine caspase 3 activity, externalization of phosphatidylserine (PS), induction of DNA strand breaks, and generation of reactive oxygen species. Mobile phone radiation had no statistically significant effect on any of the parameters

studied. This suggests that the impairment of fertility reported in some studies was not caused by the induction of apoptosis in spermatozoa.

(E) Ferreira AR, Knakievicz T, de Bittencourt Pasquali MA, Gelain DP, Dal-Pizzol F, Fernandez CE, de Almeida de Salles AA, Ferreira HB, Moreira JC. Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring. Life Sci 80: 43-50, 2006. (GT, OX, LE, DE)

Mobile telephones and their base stations are an important ultra high frequency-electromagnetic field (UHF-EMF) source and their utilization is increasing all over the world. Epidemiological studies suggested that low energy UHF-EMF emitted from a cellular telephone may cause biological effects, such as DNA damage and changes on oxidative metabolism. An in vivo mammalian cytogenetic test, the micronucleus (MN) assay, was used to investigate the occurrence of chromosomal damage in erythrocytes from rat offspring exposed to a non-thermal UHF-EMF from a cellular phone during their embryogenesis; the irradiated group showed a significant increase in MN occurrence. In order to investigate if UHF-EMF could also alter oxidative parameters in the peripheral blood and in the liver - an important hematopoietic tissue in rat embryos and newborns - we also measured the activity of antioxidant enzymes, quantified total sulfhydryl content, protein carbonyl groups, thiobarbituric acid-reactive species and total non-enzymatic antioxidant defense. No significant differences were found in any oxidative parameter of offspring blood and liver. The average number of pups in each litter has also not been significantly altered. Our results suggest that, under our experimental conditions, UHF-EMF is able to induce a genotoxic response in hematopoietic tissue during the embryogenesis through an unknown mechanism.

(NE) Finnie JW, Cai Z, Blumbergs PC, Manavis J, Kuchel TR. Expression of the immediate early gene, c-fos, in fetal brain after whole of gestation exposure of pregnant mice to global system for mobile communication microwaves. Pathology. 38(4):333-335, 2006. (GE, DE)

AIMS: To study immediate early gene, c-fos, expression as a marker of neural stress after whole of gestation exposure of the fetal mouse brain to mobile telephone-type radiofrequency fields. METHODS: Using a purpose-designed exposure system at 900 MHz, pregnant mice were given a single, far-field, whole body exposure at a specific absorption rate of 4 W/kg for 60 min/day from day 1 to day 19 of gestation. Pregnant control mice were sham-exposed or freely mobile in a cage without further restraint. Immediately prior to parturition on gestational day 19, fetal heads were collected, fixed in 4% paraformaldehyde and paraffin embedded. Any stress response in the brain was detected by c-fos immunohistochemistry in the cerebral cortex, basal ganglia, thalamus, hippocampus, midbrain, cerebellum and medulla. RESULTS: c-fos expression was of limited, but consistent, neuroanatomical distribution and there was no difference in immunoreactivity between exposed and control brains. CONCLUSION: In this animal model, no stress response was detected in the fetal brain using c-fos immunohistochemistry after whole of gestation exposure to mobile telephony.

(E) Franzellitti S, Valbonesi P, Ciancaglini N, Biondi C, Contin A, Bersani F, Fabbri E. Transient DNA damage induced by high-frequency electromagnetic fields (GSM 1.8 GHz)

in the human trophoblast HTR-8/SVneo cell line evaluated with the alkaline comet assay. Mutat Res 683(1-2):35-42, 2010. (GT, WS)

One of the most controversial issue regarding high-frequency electromagnetic fields (HF-EMF) is their putative capacity to affect DNA integrity. This is of particular concern due to the increasing use of HF-EMF in communication technologies, including mobile phones. Although epidemiological studies report no detrimental effects on human health, the possible disturbance generated by HF-EMF on cell physiology remains controversial. In addition, the question remains as to whether cells are able to compensate their potential effects. We have previously reported that a 1-h exposure to amplitude-modulated 1.8 GHz sinusoidal waves (GSM-217 Hz, SAR=2 W/kg) largely used in mobile telephony did not cause increased levels of primary DNA damage in human trophoblast HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations were considered of interest. In the present work, HTR-8/SVneo cells were exposed for 4, 16 or 24h to 1.8 GHz continuous wave (CW) and different GSM signals, namely GSM-217 Hz and GSM-Talk (intermittent exposure: 5 min field on, 10 min field off). The alkaline comet assay was used to evaluate primary DNA damages and/or strand breaks due to uncompleted repair processes in HF-EMF exposed samples. The amplitude-modulated signals GSM-217 Hz and GSM-Talk induced a significant increase in comet parameters in trophoblast cells after 16 and 24h of exposure, while the un-modulated CW was ineffective. However, alterations were rapidly recovered and the DNA integrity of HF-EMF exposed cells was similar to that of sham-exposed cells within 2h of recovery in the absence irradiation. Our data suggest that HF-EMF with a carrier frequency and modulation scheme typical of the GSM signal may affect the DNA integrity.

(E) Furtado-Filho OV, Borba JB, Dallegrave A, Pizzolato TM, Henriques JA, Moreira JC, Saffi J. Effect of 950 MHz UHF electromagnetic radiation on biomarkers of oxidative damage, metabolism of UFA and antioxidants in the livers of young rats of different ages. Int J Radiat Biol. 2013 Jul 25. [Epub ahead of print] (LE, GT, OX)

Purpose: To assess the effect of 950 MHz ultra-high-frequency electromagnetic radiation (UHF EMR) on biomarkers of oxidative damage, as well as to verify the concentration of unsaturated fatty acids (UFA) and the expression of the catalase in the livers of rats of different ages. Materials and methods: Twelve rats were equally divided into two groups as controls (CR) and exposed (ER), for each age (0, 6, 15 and 30 days). Radiation exposure lasted half an hour per day for up to 51 days (21 days of gestation and 6, 15 or 30 days of life outside the womb). The specific absorption rate (SAR) ranged from 1.3-1.0 W/kg. The damage to lipids, proteins and DNA was verified by thiobarbituric acid reactive substances (TBARS), protein carbonyls and comets, respectively. UFA were determined by gas chromatography with a flame ionization detector. The expression of catalase was by Western blotting. Results: The neonates had low levels of TBARS and concentrations of UFA after exposure. There was no age difference in the accumulation of protein carbonyls for any age. The DNA damage of ER 15 or 30 days was different. The exposed neonates exhibited lower expression of catalase. Conclusions: 950 MHz UHF EMR does not cause oxidative stress (OS), and it is not genotoxic to the livers of neonates or those of 6 and 15 day old rats, but it changes the concentrations of polyunsaturated fatty acid (PUFA) in neonates. For rats of 30 days, no OS, but it is genotoxic to the livers of ER to total body irradiation.

(E) Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (Apismellifera) against 915-MHz microwave radiation-induced DNA damage in wistar rat lymphocytes: in vitro study. Int J Toxicol 28:88-98, 2009. (GT, OX)

The aim of this study is to investigate the radioprotective effect of bee venom against DNA damage induced by 915-MHz microwave radiation (specific absorption rate of 0.6 W/kg) in Wistar rats. Whole blood lymphocytes of Wistar rats are treated with 1 microg/mL bee venom 4 hours prior to and immediately before irradiation. Standard and formamidopyrimidine-DNA glycosylase (Fpg)-modified comet assays are used to assess basal and oxidative DNA damage produced by reactive oxygen species. Bee venom shows a decrease in DNA damage compared with irradiated samples. Parameters of Fpg-modified comet assay are statistically different from controls, making this assay more sensitive and suggesting that oxidative stress is a possible mechanism of DNA damage induction. Bee venom is demonstrated to have a radioprotective effect against basal and oxidative DNA damage. Furthermore, bee venom is not genotoxic and does not produce oxidative damage in the low concentrations used in this study.

(E) Gandhi G, Anita, Genetic damage in mobile phone users: some preliminary findings. Ind J Hum Genet 11:99-104, 2005. (GT, HU)

BACKGROUND: The impact of microwave (MW)/radio frequency radiation (RFR) on important biological parameters is probably more than a simply thermal one. Exposure to radio frequency (RF) signals generated by the use of cellular telephones have increased dramatically and reported to affect physiological, neurological, cognitive and behavioural changes and to induce, initiate and promote carcinogenesis. Genotoxicity of RFR has also been reported in various test systems after in vitro and/or in vivo exposure but none in mobile phone users. AIMS: In the present study, DNA and chromosomal damage investigations were carried out on the peripheral blood lymphocytes of individuals using mobile phones, being exposed to MW frequency ranging from 800 to 2000 MHz. METHODS: DNA damage was assessed using the single cell gel electrophoresis assay and aneugenic and clastogenic damage by the in vivo capillary blood micronucleus test (MNT) in a total of 24 mobile phone users. RESULTS: Mean comet tail length (26.76 ± 0.054 mm; 39.75% of cells damaged) in mobile phone users was highly significant from that in the control group. The in vivo capillary blood MNT also revealed highly significant (0.25) frequency of micronucleated (MNd) cells. CONCLUSIONS: These results highlight a correlation between mobile phone use (exposure to RFR) and genetic damage and require interim public health actions in the wake of widespread use of mobile telephony.

(E) Gandhi G, Singh P. Cytogenetic damage in mobile phone users: preliminary data. Int J Hum Genet 5:259-265, 2005. (GT, HU)

Mobile telephones, sometimes called cellular (cell) phones or handies, are now an integral part of modern life. The mobile phone handsets are low-powered radiofrequency transmitters, emitting maximum powers in the range of 0.2 to 0.6 watts. Scientific concenrns have increased sufficiently over the possible hazard to health from using cell phones. The reported adverse health effects include physiological, behavioural and cognitive changes as well as tumour formation and genetic damage. However findings are controversial and no consensus exists. Genotoxicity has been observed either in lower organisms or in vitro studies. The aim of the present study hence was to detect any cytogenertic damage in mobile phone users by analysing short term peripheral lymphocyte cultures for chromosomal aberrations and the buccal mucosal

cells for micronuclei (aneugenicity and clastogenicity). The results revealed increased number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes indicating the genotoxic response from mobile phone use.

(E) Garaj-Vrhovac V, Gajski G, Pažanin S, Sarolić A, Domijan AM, Flajs D, Peraica M. Assessment of cytogenetic damage and oxidative stress in personnel occupationally exposed to the pulsed microwave radiation of marine radar equipment. Int J Hyg Environ Health. 4(1):59-65, 2011. (GT, HU, OX)

Due to increased usage of microwave radiation, there are concerns of its adverse effect in today's society. Keeping this in view, study was aimed at workers occupationally exposed to pulsed microwave radiation, originating from marine radars. Electromagnetic field strength was measured at assigned marine radar frequencies (3 GHz, 5.5 GHz and 9.4 GHz) and corresponding specific absorption rate values were determined. Parameters of the comet assay and micronucleus test were studied both in the exposed workers and in corresponding unexposed subjects. Differences between mean tail intensity (0.67 vs. 1.22) and moment (0.08 vs. 0.16) as comet assay parameters and micronucleus test parameters (micronuclei, nucleoplasmic bridges and nuclear buds) were statistically significant between the two examined groups, suggesting that cytogenetic alterations occurred after microwave exposure. Concentrations of glutathione and malondialdehyde were measured spectrophotometrically and using high performance liquid chromatography. The glutathione concentration in exposed group was significantly lower than in controls (1.24 vs. 0.53) whereas the concentration of malondialdehyde was significantly higher (1.74 vs. 3.17), indicating oxidative stress. Results suggests that pulsed microwaves from working environment can be the cause of genetic and cell alterations and that oxidative stress can be one of the possible mechanisms of DNA and cell damage.

(E) Guler G, Tomruk A, Ozgur E, Seyhan N.The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. Gen Physiol Biophys 29:59-66, 2010. (GT, OX, LE, DE)

The concerns of people on possible adverse health effects of radiofrequency radiation (RFR) generated from mobile phones as well as their supporting transmitters (base stations) have increased markedly. RFR effect on oversensitive people, such as pregnant women and their developing fetuses, and older people is another source of concern that should be considered. In this study, oxidative DNA damage and lipid peroxidation levels in the brain tissue of pregnant and non-pregnant New Zealand White rabbits and their newborns exposed to RFR were investigated. Thirteen-month-old rabbits were studied in four groups as non-pregnant-control, non-pregnant-RFR exposed, pregnant-control and pregnant-RFR exposed. They were exposed to RFR (1800 MHz GSM; 14 V/m as reference level) for 15 min/day during 7 days. Malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were analyzed. MDA and 8-OHdG levels of non-pregnant and pregnant-RFR exposed animals significantly increased with respect to controls ($p < 0.001$, Mann-Whitney test). No difference was found in the newborns ($p > 0.05$, Mann-Whitney). There exist very few experimental studies on the effects of RFR during pregnancy. It would be beneficial to increase the number of these studies in order to establish international standards for the protection of pregnant women from RFR.

(E) Güler G, Tomruk A, Ozgur E, Sahin D, Sepici A, Altan N, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in female and male infant rabbits. Int J Radiat Biol. 88(4):367-373, 2012. (LE, GT, OX, DE)

PURPOSE: We aimed to design a prolonged radiofrequency (RF) radiation exposure and investigate in an animal model, possible bio-effects of RF radiation on the ongoing developmental stages of children from conception to childhood. **MATERIALS AND METHODS:** A total of 72 New Zealand female and male white rabbits aged one month were used. Females were exposed to RF radiation for 15 min/day during 7 days, whereas males were exposed to the same level of radiation for 15 min/day during 14 days. Thirty-six female and 36 male infant rabbits were randomly divided into four groups: Group I [Intrauterine (IU) exposure (-); Extrauterine (EU) exposure (-)]: Sham exposure which means rabbits were exposed to 1800 MHz Global System for Mobile Telecommunication (GSM)-like RF signals neither in the IU nor in the EU periods. Group II [IU exposure (-); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals when they reached one month of age. Group III [IU exposure (+); EU exposure (-)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals in the IU period (between 15th and 22nd days of the gestational period). Group IV [IU exposure (+); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals both in the IU period (between 15th and 22nd days of the gestational period) and in the EU period when they reached one month of age. Biochemical analysis for lipid peroxidation and DNA damage were carried out in the livers of all rabbits. **RESULTS:** Lipid peroxidation levels in the liver tissues of female and male infant rabbits increased under RF radiation exposure. Liver 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of female rabbits exposed to RF radiation were also found to increase when compared with the levels of non-exposed infants. However, there were no changes in liver 8-OHdG levels of male rabbits under RF exposure. **CONCLUSION:** Consequently, it can be concluded that GSM-like RF radiation may induce biochemical changes by increasing free radical attacks to structural biomolecules in the rabbit as an experimental animal model.

(NE) Gurbuz N, Sirav B, Yuvaci HU, Turhan N, Coskun ZK, Seyhan N. Is there any possible genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 MHz GSM-like modulated radio frequency radiation (RFR)? Electromagn Biol Med. 29(3):98-104, 2010. (LE, GT)

People are exposed to many carcinogenic and mutagenic chemicals in their everyday lives. These include antineoplastic drugs, Polycyclic aromatic hydrocarbons (PAH)s, aromatic amines, nitrosamines, metals, and electromagnetic radiation. Based on the state of knowledge acquired during the last 50 years of research on possible biological effects of electromagnetic fields (EMF), the majority of the scientific community is convinced that exposure to EMF below the existing security limits does not cause a risk to the health of the general public. However, this position is questioned by others, who are of the opinion that the available research data are contradictory or inconsistent and, therefore, unreliable. In this study, we aimed to investigate if there is any effect of 1800 MHz GSM modulated radio frequency radiation (RFR) on the number of micronucleus in exfoliated bladder cells of rat which will be informative about the genotoxic damage. Exposure period was 20 min/day, 5 days/week during a month. Six female Wistar rats were used for two groups: Group I (n=6): controls; Group II (n=6): 1.8 GHz exposed animals.

1800 MHz RFR did not showed a significant MN frequencies in rat bladder cells when compared with the control group (p>0.05). 1800 MHz RFR-exposed animals did not produce any genotoxic effect when compared with the control group (p>0.05). Kinetic studies are important for any biomarker, especially those in which tissue differentiation and maturation processes will heavily influence the time between induction of damage and collection of damaged cells for micronucleus analysis.

(NE) Gurbuz N, Sirav B, Colbay M, Yetkin I, Seyhan N. No genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 and 2100-MHz radio frequency radiation. Electromagn Biol Med. 2013 Nov 27. [Epub ahead of print] (GT, LE)

Abstract In this study, we aimed to investigate the effects of 1800 and 2100 MHz Radio Frequency (RF) radiation on the number of micronucleus (MN) in exfoliated bladder cells of rat which shows the genotoxic damage. Exposure period was 30 min/day, 6 days/week for a month and two months exposure periods. Thirty male wistar albino rats were used for five groups: Group I (n = 6): 1800 MHz RF exposed animals for one month, Group II (n = 6): 2100 MHz RF exposed animals for one month, Group III (n = 6): 2100 MHz RF exposed for two months, Group IV (n = 6): control group for one month, Group V (n = 6): control group for two months. Rats of the control groups were housed in their home cages during the entire experimental period without subjecting to any experimental manipulation. 1800 and 2100 MHz RF exposures did not result in any significant MN frequencies in rat bladder cells with respect to the control groups (p > 0.05). There was no statistically significant difference between 2100 MHz RF exposed groups, either. Further studies are needed to demonstrate if there is any genotoxic effect, micronucleus formation in other tissues of rats.

(NE) Hansteen IL, Lågeide L, Clausen KO, Haugan V, Svendsen M, Eriksen JG, Skiaker R, Hauger E, Vistnes AI, Kure EH. Cytogenetic effects of 18.0 and 16.5 GHz microwave radiation on human lymphocytes in vitro. Anticancer Res 29:2885-2892, 2009. (GT, IA, WS)

BACKGROUND: There are few cell studies on the direct genotoxic effects of microwave radiation. In this study, cytogenetic effects of microwave radiation alone or in combination with mitomycin C (MMC) were investigated. MATERIALS AND METHODS: Lymphocytes from two smoking and four non-smoking donors were exposed for 53 hours in vitro to 1.0 W/m continuous-wave radiation at 18.0 GHz or 10 W/m pulsed-wave at 16.5 GHz, alone or in combination with MMC. DNA synthesis and repair were inhibited in vitro in some cultures. RESULTS: No synergistic effect was observed in cells exposed to combinations of microwave radiation and in vitro exposure to MMC, or to cells pre-exposed in vivo to tobacco smoke. For the 16.5 GHz pulsed exposure, a non-significant trend consisting of an increase in aberration frequencies with microwave radiation was shown for the DNA synthesis and repair inhibited cultures both with and without MMC. CONCLUSION: Neither 18.0 GHz continuous-wave nor 16.5 GHz pulsed-wave exposure to human lymphocytes in vitro induced statistically significant increases in chromosomal aberration frequencies. 16.5 GHz pulsed-wave exposure requires further documentation before a true negative conclusion can be drawn.

(NE) Hansteen IL, Clausen KO, Haugan V, Svendsen M, Svendsen MV, Eriksen JG, Skiaker R, Hauger E, Lågeide L, Vistnes AI, Kure EH. Cytogenetic effects of exposure to

2.3 GHz radiofrequency radiation on human lymphocytes in vitro. Anticancer Res 29:4323-4330, 2009. (GT, IA)

BACKGROUND: No previous in vitro studies have tested radio frequency radiation for at least one full cell cycle in culture. The aim was to test if exposure used in mobile phones and wireless network technologies would induce DNA damage in cultured human lymphocytes with and without a known clastogen. MATERIALS AND METHODS: Lymphocytes from six donors were exposed to 2.3 GHz, 10 W/m continuous waves, or 2.3 GHz, 10 W/m pulsed waves (200 Hz pulse frequency, 50% duty cycle). Mitomycin C was added to half of the cultures. DNA synthesis and repair were inhibited in one experiment. RESULTS: No statistically significant differences were observed between control and exposed cultures. A weak trend for more chromosomal damage with the interaction of pulsed fields with mitomycin C compared to a constant field was observed. CONCLUSION: Exposure during the whole cell cycle in inhibited cultures did not resulted in significant differences in chromosomal aberrations as compared to controls.

(E) Hekmat A, Saboury AA, Moosavi-Movahedi AA. The toxic effects of mobile phone radiofrequency (940MHz) on the structure of calf thymus DNA. Ecotoxicol Environ Saf. 2012 Nov 16. pii: S0147-6513(12)00368-5. doi: 10.1016/j.ecoenv.2012.10.016. [Epub ahead of print] (GT)

Currently, the biological effects of nonionizing electromagnetic fields (EMFs) including radiofrequency (RF) radiation have been the subject of numerous experimental and theoretical studies. The aim of this study is to evaluate the possible biological effects of mobile phone RF (940MHz, 15V/m and SAR=40mW/kg) on the structure of calf thymus DNA (ct DNA) immediately after exposure and 2h after 45min exposure via diverse range of spectroscopic instruments. The UV-vis and circular dichroism (CD) experiments depict that mobile phone EMFs can remarkably cause disturbance on ct DNA structure. In addition, the DNA samples, immediately after exposure and 2h after 45min exposure, are relatively thermally unstable compared to the DNA solution, which was placed in a small shielded box (unexposed ct DNA). Furthermore, the exposed DNA samples (the DNA samples that were exposed to 940MHz EMF) have more fluorescence emission when compared with the unexposed DNA, which may have occurred attributable to expansion of the exposed DNA structure. The results of dynamic light scattering (DLS) and zeta potential experiments demonstrate that RF-EMFs lead to increment in the surface charge and size of DNA. The structure of DNA immediately after exposure is not significantly different from the DNA sample 2h after 45min exposure. In other words, the EMF-induced conformational changes are irreversible. Collectively, our results reveal that 940MHz can alter the structure of DNA. The displacement of electrons in DNA by EMFs may lead to conformational changes of DNA and DNA disaggregation. Results from this study could have an important implication on the health effects of RF-EMFs exposure. In addition, this finding could proffer a novel strategy for the development of next generation of mobile phone.

(NE) Hintzsche H, Stopper H. Micronucleus frequency in buccal mucosa cells of mobile phone users. Toxicol Lett. 193(1):124-130, 2010. (GT, HU)

Mobile phones are being used extensively throughout the world, with more than four billion accounts existing in 2009. This technology applies electromagnetic radiation in the microwave

range. Health effects of this radiation have been subject of debate for a long time, both within the scientific community and within the general public. This study investigated the effect of mobile phone use on genomic instability of the human oral cavity's mucosa cells. 131 Individuals donated buccal mucosa cells extracted by slightly scraping the oral cavity with a cotton swab. Every participant filled out a questionnaire about mobile phone use including duration of weekly use, overall period of exposure and headset usage. 13 Individuals did not use mobile phones at all, 85 reported using the mobile phone for three hours per week or less, and 33 reported use of more than three hours per week. Additionally, information on age, gender, body weight, smoking status, medication and nutrition was retrieved. For staining of the cells a procedure using alpha-tubulin-antibody and chromomycin A(3) was applied. Micronuclei and other markers were evaluated in 1000 cells per individual at the microscope. A second scorer counted another 1000 cells, resulting in 2000 analyzed cells per individual. Mobile phone use did not lead to a significantly increased frequency of micronuclei.

(NE) Hintzsche H, Jastrow C, Kleine-Ostmann T, Schrader T, Stopper H. 900 MHz radiation does not induce micronucleus formation in different cell types. Mutagenesis. 27(4):477-483, 2012 . (GT)

The exposure of the population to non-ionising electromagnetic radiation is still increasing, mainly due to mobile communication. Whether low-intensity electromagnetic fields can cause other effects apart from heating has been a subject of debate. One of the effects, which were proposed to be caused by mobile phone radiation, is the occurrence of mitotic disturbances. The aim of this study was to investigate possible consequences of these mitotic disturbances as manifest genomic damage, i.e. micronucleus induction. Cells were irradiated at a frequency of 900 MHz, which is located in one of the main frequency bands applied for mobile communication. Two cell types were used, HaCaT cells as human cells and A(L) cells (human-hamster hybrid cells), in which mitotic disturbances had been reported to occur. After different post-exposure incubation periods, cells were fixed and micronucleus frequencies were evaluated. Both cell types did not show any genomic damage after exposure. To adapt the protocol for the micronucleus test into the direction of the protocol for mitotic disturbances, the post-exposure incubation period was reduced and exposure time was extended to one cell cycle length. This did not result in any increase of the genomic damage. In conclusion, micronucleus induction was not observed as a consequence of exposure to non-ionising radiation, even though this agent was reported to cause mitotic disturbances under similar experimental conditions.

(NE) Hirose H, Sakuma N, Kaji N, Suhara T, Sekijima M, Nojima T, Miyakoshi J. Phosphorylation and gene expression of p53 are not affected in human cells exposed to 2.1425 GHz band CW or W-CDMA modulated radiation allocated to mobile radio base stations. Bioelectromagnetics 27:494-504, 2006. (GT)

A large-scale in vitro study focusing on low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields induce apoptosis or other cellular stress response that activate p53 or the p53-signaling pathway. First, we evaluated the response of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction by the International Commission on Non-Ionizing

Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and wideband code division multiple access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced apoptosis or any signs of stress. Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg, and CW radiation at 80 mW/kg for 24 or 48 h. Human IMR-90 fibroblasts from fetal lungs were exposed to both W-CDMA and CW radiation at a SAR of 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the percentage of apoptotic cells were observed between the test groups exposed to RF signals and the sham-exposed negative controls, as evaluated by the Annexin V affinity assay. No significant differences in expression levels of phosphorylated p53 at serine 15 or total p53 were observed between the test groups and the negative controls by the bead-based multiplex assay. Moreover, microarray hybridization and real-time RT-PCR analysis showed no noticeable differences in gene expression of the subsequent downstream targets of p53 signaling involved in apoptosis between the test groups and the negative controls. Our results confirm that exposure to low-level RF signals up to 800 mW/kg does not induce p53-dependent apoptosis, DNA damage, or other stress response in human cells.

(NE) Hirose H, Sakuma N, Kaji N, Nakayama K, Inoue K, Sekijima M, Nojima T, Miyakoshi J. Mobile phone base station-emitted radiation does not induce phosphorylation of Hsp27. Bioelectromagnetics 28:99-108, 2007. (GE)

An in vitro study focusing on the effects of low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields act to induce phosphorylation and overexpression of heat shock protein hsp27. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced activation or gene expression of hsp27 and other heat shock proteins (hsps). Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80 and 800 mW/kg for 2-48 h, and CW radiation at 80 mW/kg for 24 h. Human IMR-90 fibroblasts from fetal lungs were exposed to W-CDMA at 80 and 800 mW/kg for 2 or 28 h, and CW at 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the expression levels of phosphorylated hsp27 at serine 82 (hsp27[pS82]) were observed between the test groups exposed to W-CDMA or CW signal and the sham-exposed negative controls, as evaluated immediately after the exposure periods by bead-based multiplex assays. Moreover, no noticeable differences in the gene expression of hsps were observed between the test groups and the negative controls by DNA Chip analysis. Our results confirm that exposure to low-level RF field up to 800 mW/kg does not induce phosphorylation of hsp27 or expression of hsp gene family.

(NE) Huang TQ, Lee MS, Oh E, Zhang BT, Seo JS, Park WY. Molecular responses of Jurkat T-cells to 1763 MHz radiofrequency radiation.Int J RadiatBiol 84:734-741, 2008. (GT, GE)

PURPOSE: The biological effects of exposure to mobile phone emitted radiofrequency (RF) radiation are the subject of intense study, yet the hypothesis that RF exposure is a potential health hazard remains controversial. In this paper, we monitored cellular and molecular changes in Jurkat human T lymphoma cells after irradiating with 1763 MHz RF radiation to understand the effect on RF radiation in immune cells. MATERIALS AND METHODS: Jurkat T-cells were exposed to RF radiation to assess the effects on cell proliferation, cell cycle progression, DNA damage and gene expression. Jurkat cells were exposed to 1763 MHz RF radiation at 10 W/kg specific absorption rate (SAR) and compared to sham exposed cells. RESULTS: RF exposure did not produce significant changes in cell numbers, cell cycle distributions, or levels of DNA damage. In genome-wide analysis of gene expressions, there were no genes changed more than two-fold upon RF-radiation while ten genes change to 1.3 approximately 1.8-fold. Among ten genes, two cytokine receptor genes such as chemokine (C-X-C motif) receptor 3 (CXCR3) and interleukin 1 receptor, type II (IL1R2) were down-regulated upon RF radiation, but they were not directly related to cell proliferation or DNA damage responses. CONCLUSION: These results indicate that the alterations in cell proliferation, cell cycle progression, DNA integrity or global gene expression was not detected upon 1763 MHz RF radiation under 10 W/kg SAR for 24 h to Jurkat T cells.

(NE) Huang TQ, Lee MS, Oh EH, Kalinec F, Zhang BT, Seo JS, Park WY. Characterization of biological effect of 1763 MHz radiofrequency exposure on auditory hair cells.Int J Radiat Biol 84:909-915, 2008. (GT, GE)

Purpose: Radiofrequency (RF) exposure at the frequency of mobile phones has been reported not to induce cellular damage in in vitro and in vivo models. We chose HEI-OC1 immortalized mouse auditory hair cells to characterize the cellular response to 1763 MHz RF exposure, because auditory cells could be exposed to mobile phone frequencies. Materials and methods: Cells were exposed to 1763 MHz RF at a 20 W/kg specific absorption rate (SAR) in a code division multiple access (CDMA) exposure chamber for 24 and 48 h to check for changes in cell cycle, DNA damage, stress response, and gene expression. Results: Neither of cell cycle changes nor DNA damage was detected in RF-exposed cells. The expression of heat shock proteins (HSP) and the phosphorylation of mitogen-activated protein kinases (MAPK) did not change, either. We tried to identify any alteration in gene expression using microarrays. Using the Applied Biosystems 1700 full genome expression mouse microarray, we found that only 29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure. Conclusion: From these results, we could not find any evidence of the induction of cellular responses, including cell cycle distribution, DNA damage, stress response and gene expression, after 1763 MHz RF exposure at an SAR of 20 W/kg in HEI-OC1 auditory hair cells.

(E) Jiang B, Nie J, Zhou Z, Zhang J, Tong J, Cao Y. Adaptive response in mice exposed to 900 MHz radiofrequency fields: primary DNA damage. PLoS One. 7(2):e32040, 2012. (LE, GT, IA)

The phenomenon of adaptive response (AR) in animal and human cells exposed to ionizing radiation is well documented in scientific literature. We have examined whether such AR could be induced in mice exposed to non-ionizing radiofrequency fields (RF) used for wireless communications. Mice were pre-exposed to 900 MHz RF at 120 μW/cm(2) power density for 4 hours/day for 1, 3, 5, 7 and 14 days and then subjected to an acute dose of 3 Gy γ-radiation. The

primary DNA damage in the form of alkali labile base damage and single strand breaks in the DNA of peripheral blood leukocytes was determined using the alkaline comet assay. The results indicated that the extent of damage in mice which were pre-exposed to RF for 1 day and then subjected to γ-radiation was similar and not significantly different from those exposed to γ-radiation alone. However, mice which were pre-exposed to RF for 3, 5, 7 and 14 days showed progressively decreased damage and was significantly different from those exposed to γ-radiation alone. Thus, the data indicated that RF pre-exposure is capable of inducing AR and suggested that the pre-exposure for more than 4 hours for 1 day is necessary to elicit such AR.

(NE) Juutilainen J, Heikkinen P, Soikkeli H, Mäki-Paakkanen J. Micronucleus frequency in erythrocytes of mice after long-term exposure to radiofrequency radiation. Int J Radiat Biol. 83(4):213-220, 2007. (LE, GT)

PURPOSE: The aim of the study was to investigate genotoxicity of long-term exposure to radiofrequency (RF) electromagnetic fields by measuring micronuclei in erythrocytes. The blood samples were collected in two animal studies evaluating possible cocarcinogenic effects of RF fields. METHODS: In study A, female CBA/S mice were exposed for 78 weeks (1.5 h/d, 5 d/week) to either a continuous 902.5 MHz signal similar to that emitted by analog NMT (Nordic Mobile Telephone) phones at a whole-body specific absorption rate (SAR) of 1.5 W/kg, or to a pulsed 902.4 MHz signal similar to that of digital GSM (Global System for Mobile Communications) phones at 0.35 W/kg. A third group was sham-exposed, and a fourth group served as cage controls. All but the cage control animals were exposed to 4 Gy of x-rays during three first weeks of the experiment. In study B, female transgenic mice (line K2) and their nontransgenic littermates were exposed for 52 weeks (1.5 h/d, 5 d/week). Two digital mobile phone signals, GSM and DAMPS (Digital Advanced Mobile Phone System), were used at 0.5 W/kg. All but the cage-control animals were exposed 3 times per week to an ultraviolet radiation dose of 1.2 MED (minimum erythema dose). RESULTS AND CONCLUSIONS: The results did not show any effects of RF fields on micronucleus frequency in polychromatic or normochromatic erythrocytes. The results were consistent in two mouse strains (and in a transgenic variant of the second strain), after 52 or 78 weeks of exposure, at three SAR levels relevant to human exposure from mobile phones, and for three different mobile signals.

(E) Karaca E, Durmaz B, Altug H, Yildiz T, Guducu C, Irgi M, Koksal MG, Ozkinay F, Gunduz C, Cogulu O. The genotoxic effect of radiofrequency waves on mouse brain. J Neurooncol 106:53-58, 2012. (GT, GE)

Erratum: J Neurooncol 2012 May;107:665.

Concerns about the health effects of radiofrequency (RF) waves have been raised because of the gradual increase in usage of cell phones, and there are scientific questions and debates about the safety of those instruments in daily life. The aim of this study is to evaluate the genotoxic effects of RF waves in an experimental brain cell culture model. Brain cell cultures of the mice were exposed to 10.715 GHz with specific absorbtion rate (SAR) 0.725 W/kG signals for 6 h in 3 days at 25°C to check for the changes in the micronucleus (MNi) assay and in the expression of 11 proapoptotic and antiapoptotic genes. It was found that MNi rate increased 11-fold and STAT3 expression decreased 7-fold in the cell cultures which were exposed to RF. Cell phones which spread RF may damage DNA and change gene expression in brain cells.

(E) Kesari KK, Behari J. Fifty-gigahertz Microwave exposure effect of radiations on rat brain. Appl Biochem Biotechnol 158:126-139, 2009. (GT, OX, LE)

The object of this study is to investigate the effects of 50-GHz microwave radiation on the brain of Wistar rats. Male rats of the Wistar strain were used in the study. Animals of 60-day age were divided into two groups-group 1, sham-exposed, and group 2, experimental (microwave-exposed). The rats were housed in a temperature-controlled room (25 degrees C) with constant humidity (40-50%) and received food and water ad libitum. During exposure, rats were placed in Plexiglas cages with drilled ventilation holes and kept in an anechoic chamber. The animals were exposed for 2 h a day for 45 days continuously at a power level of 0.86 muW/cm with nominal specific absorption rate 8.0 x 10(-4) w/kg. After the exposure period, the rats were killed and homogenized, and protein kinase C (PKC), DNA double-strand break, and antioxidant enzyme activity [superoxides dismutase (SOD), catalase, and glutathione peroxidase (GPx)] were estimated in the whole brain. Result shows that the chronic exposure to these radiations causes DNA double-strand break (head and tail length, intensity and tail migration) and a significant decrease in GPx and SOD activity ($p = <0.05$) in brain cells, whereas catalase activity shows significant increase in the exposed group of brain samples as compared with control ($p = <0.001$). In addition to these, PKC decreased significantly in whole brain and hippocampus ($p < 0.05$). All data are expressed as mean +/- standard deviation. We conclude that these radiations can have a significant effect on the whole brain.

(E) Kesari KK, Behari J, Kumar S. Mutagenic response of 2.45 GHz radiation exposure on rat brain. Int J Radiat Biol 86:334-343, 2010. (GT, OX, LE)

Purpose: To investigate the effect of 2.45 GHz microwave radiation on rat brain of male wistar strain. Material and methods: Male rats of wistar strain (35 days old with 130 +/- 10 g body weight) were selected for this study. Animals were divided into two groups: Sham exposed and experimental. Animals were exposed for 2 h a day for 35 days to 2.45 GHz frequency at 0.34 mW/cm power density. The whole body specific absorption rate (SAR) was estimated to be 0.11 W/Kg. Exposure took place in a ventilated Plexiglas cage and kept in anechoic chamber in a far field configuration from the horn antenna. After the completion of exposure period, rats were sacrificed and the whole brain tissue was dissected and used for study of double strand DNA (Deoxyribonucleic acid) breaks by micro gel electrophoresis and the statistical analysis was carried out using comet assay (IV-2 version software). Thereafter, antioxidant enzymes and histone kinase estimation was also performed. Results: A significant increase was observed in comet head ($P < 0.002$), tail length ($P < 0.0002$) and in tail movement ($P < 0.0001$) in exposed brain cells. An analysis of antioxidant enzymes glutathione peroxidase ($P < 0.005$), and superoxide dismutase ($P < 0.006$) showed a decrease while an increase in catalase ($P < 0.006$) was observed. A significant decrease ($P < 0.023$) in histone kinase was also recorded in the exposed group as compared to the control (sham-exposed) ones. One-way analysis of variance (ANOVA) method was adopted for statistical analysis. Conclusion: The study concludes that the chronic exposure to these radiations may cause significant damage to brain, which may be an indication of possible tumour promotion (Behari and Paulraj 2007).

(E) Khalil AM, Gagaa M, Alshamali A. 8-Oxo-7, 8-dihydro-2'-deoxyguanosine as a biomarker of DNA damage by mobile phone radiation. Hum ExpToxicol 31(7):734-740, 2012. (GT, OX)

We examined the effect of exposure to mobile phone 1800 MHz radio frequency radiation (RFR) upon the urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG), one major form of oxidative DNA damage, in adult male Sprague-Dawley rats. Twenty-four rats were used in three independent experiments (RFR exposed and control, 12 rats, each). The animals were exposed to RFR for 2 h from Global System for Mobile Communications (GSM) signal generator with whole-body-specific absorption rate of 1.0 W/kg. Urine samples were collected from the rat while housed in a metabolic cage during the exposure period over a 4-h period at 0.5, 1.0, 2.0 and 4.0 h from the beginning of exposure. In the control group, the signal generator was left in the turn-off position. The creatinine-standardized concentrations of 8-oxodG were measured. With the exception of the urine collected in the last half an hour of exposure, significant elevations were noticed in the levels of 8-oxodG in urine samples from rats exposed to RFR when compared to control animals. Significant differences were seen overall across time points of urine collection with a maximum at 1 h after exposure, suggesting repair of the DNA lesions leading to 8-oxodG formation.

(E) Kim JY, Hong SY, Lee YM, Yu SA, Koh WS, Hong JR, Son T, Chang SK, Lee M.In vitro assessment of clastogenicity of mobile-phone radiation (835 MHz) using the alkaline comet assay and chromosomal aberration test. Environ Toxicol 23:319-327, 2008. (GT, IA)

Recently we demonstrated that 835-MHz radiofrequency radiation electromagnetic fields (RF-EMF) neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro. Here, two kinds of cytogenetic endpoints were further investigated on mammalian cells exposed to 835-MHz RF-EMF (the most widely used communication frequency band in Korean CDMA mobile phone networks) alone and in combination with model clastogens: in vitro alkaline comet assay and in vitro chromosome aberration (CA) test. No direct cytogenetic effect of 835-MHz RF-EMF was found in the in vitro CA test. The combined exposure of the cells to RF-EMF in the presence of ethylmethanesulfonate (EMS) revealed a weak and insignificant cytogenetic effect when compared to cells exposed to EMS alone in CA test. Also, the comet assay results to evaluate the ability of RF-EMF alone to damage DNA were nearly negative, although showing a small increase in tail moment. However, the applied RF-EMF had potentiation effect in comet assay when administered in combination with model clastogens (cyclophosphamide or 4-nitroquinoline 1-oxide). Thus, our results imply that we cannot confidently exclude any possibility of an increased risk of genetic damage, with important implications for the possible health effects of exposure to 835-MHz electromagnetic fields.

(E) Kumar S, Kesari KK, Behari J. Evaluation of genotoxic effects in male Wistar rats following microwave exposure. Indian J Exp Biol 48:586-592, 2010. (GT, OX)

Wistar rats (70 days old) were exposed for 2 h a day for 45 days continuously at 10 GHz [power density 0.214 mW/cm2, specific absorption rate (SAR) 0.014 W/kg] and 50 GHz (power density 0.86 microW/cm2, SAR 8.0 x10(-4) W/kg). Micronuclei (MN), reactive oxygen species (ROS), and antioxidant enzymes activity were estimated in the blood cells and serum. These radiations induce micronuclei formation and significant increase in ROS production. Significant changes in the level of serum glutathione peroxidase, superoxide dismutase and catalase were observed in exposed group as compared with control group. It is concluded that microwave exposure can be affective at genetic level. This may be an indication of tumor promotion, which comes through the overproduction of reactive oxygen species.

(E) Lakshmi NK, Tiwari R, Bhargava SC, Ahuja YR. Investigations on DNA damage and frequency of micronuclei in occupational exposure to electromagnetic fields (EMFs) emitted from video display terminals (VDTs). Gen MolBiol 33, 154-158, 2010. (GT, HU, LE)

The potential effect of electromagnetic fields (EMFs) emitted from video display terminals (VDTs) to elicit biological response is a major concern for the public. The software professionals are subjected to cumulative EMFs in their occupational environments. This study was undertaken to evaluate DNA damage and incidences of micronuclei in such professionals. To the best of our knowledge, the present study is the first attempt to carry out cytogenetic investigations on assessing bioeffects in personal computer users. The study subjects (n = 138) included software professionals using VDTs for more than 2 years with age, gender, socioeconomic status matched controls (n = 151). DNA damage and frequency of micronuclei were evaluated using alkaline comet assay and cytochalasin blocked micronucleus assay respectively. Overall DNA damage and incidence of micronuclei showed no significant differences between the exposed and control subjects. With exposure characteristics, such as total duration (years) and frequency of use (minutes/day) sub-groups were assessed for such parameters. Although cumulative frequency of use showed no significant changes in the DNA integrity of the classified sub-groups, the long-term users (> 10 years) showed higher induction of DNA damage and increased frequency of micronuclei and micro nucleated cells.

(E) Liu C, Duan W, Xu S, Chen C, He M, Zhang L, Yu Z, Zhou Z. Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line. Toxicol Lett 218(1): 2-9, 2013a. (GT, OX, RP)

Whether exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from mobile phones can induce DNA damage in male germ cells remains unclear. In this study, we conducted a 24 h intermittent exposure (5 min on and 10 min off) of a mouse spermatocyte-derived GC-2 cell line to 1800 MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at specific absorption rates (SAR) of 1 W/kg, 2 W/kg or 4 W/kg. Subsequently, through the use of formamidopyrimidine DNA glycosylase (FPG) in a modified comet assay, we determined that the extent of DNA migration was significantly increased at a SAR of 4 W/kg. Flow cytometry analysis demonstrated that levels of the DNA adduct 8-oxoguanine (8-oxoG) were also increased at a SAR of 4 W/kg. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS); these phenomena were mitigated by co-treatment with the antioxidant α-tocopherol. However, no detectable DNA strand breakage was observed by the alkaline comet assay. Taking together, these findings may imply the novel possibility that RF-EMR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells.

(E) Liu C, Gao P, Xu SC, Wang Y, Chen CH, He MD, Yu ZP, Zhang L, Zhou Z. Mobile phone radiation induces mode-dependent DNA damage in a mouse spermatocyte-derived cell line: a protective role of melatonin. Int J Radiat Biol. 2013b Aug 19. [Epub ahead of print] (GT, OX, RP)

Purpose: To evaluate whether exposure to mobile phone radiation (MPR) can induce DNA damage in male germ cells. Materials and methods: A mouse spermatocyte-derived GC-2 cell line was exposed to a commercial mobile phone handset once every 20 minutes in standby,

listen, dialed or dialing modes for 24 h. DNA damage was determined using an alkaline comet assay. Results: The levels of DNA damage were significantly increased following exposure to MPR in the listen, dialed and dialing modes. Moreover, there were significantly higher increases in the dialed and dialing modes than in the listen mode. Interestingly, these results were consistent with the radiation intensities of these modes. However, the DNA damage effects of MPR in the dialing mode were efficiently attenuated by melatonin pretreatment. Conclusions: These results regarding mode-dependent DNA damage have important implications for the safety of inappropriate mobile phone use by males of reproductive age and also suggest a simple preventive measure, keeping our body from mobile phones as far away as possible, not only during conversations but during "dialed" and "dialing" operation modes as well. Since the "dialed" mode is actually part of the standby mode, mobile phones should be kept at a safe distance from our body even during standby operation. Furthermore, the protective role of melatonin suggests that it may be a promising pharmacological candidate for preventing mobile phone use-related reproductive impairments.

(E) Lixia S, Yao K, Kaijun W, Deqiang L, Huajun H, Xiangwei G, Baohong W, Wei Z, Jianling L, Wei W. Effects of 1.8GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells. Mutat Res 602(1-2):135-42, 2006. (GT, GE)

To investigate the DNA damage, expression of heat shock protein 70 (Hsp70) and cell proliferation of human lens epithelial cells (hLEC) after exposure to the 1.8GHz radiofrequency field (RF) of a global system for mobile communications (GSM). An Xc-1800 RF exposure system was used to employ a GSM signal at 1.8GHz (217Hz amplitude-modulated) with the output power in the specific absorption rate (SAR) of 1, 2 and 3W/kg. After 2h exposure to RF, the DNA damage of hLEC was accessed by comet assay at five different incubation times: 0, 30, 60, 120 and 240min, respectively. Western blot and RT-PCR were used to determine the expression of Hsp70 in hLECs after RF exposure. The proliferation rate of cells was evaluated by bromodeoxyuridine incorporation on days 0, 1 and 4 after exposure. The results show that the difference of DNA-breaks between the exposed and sham-exposed (control) groups induced by 1 and 2W/kg irradiation were not significant at any incubation time point ($P>0.05$). The DNA damage caused by 3W/kg irradiation was significantly increased at the times of 0 and 30min after exposure ($P<0.05$), a phenomenon that could not be seen at the time points of 60, 120 or 240min ($P>0.05$). Detectable mRNA as well as protein expression of Hsp70 was found in all groups. Exposure at SARs of 2 and 3W/kg for 2h exhibited significantly increased Hsp70 protein expression ($P<0.05$), while no change in Hsp70 mRNA expression could be found in any of the groups ($P>0.05$). No difference of the cell proliferation rate between the sham-exposed and exposed cells was found at any exposure dose tested ($P>0.05$). The results indicate that exposure to non-thermal dosages of RF for wireless communications can induce no or repairable DNA damage and the increased Hsp70 protein expression in hLECs occurred without change in the cell proliferation rate. The non-thermal stress response of Hsp70 protein increase to RF exposure might be involved in protecting hLEC from DNA damage and maintaining the cellular capacity for proliferation.

(E) López-Martín E, Bregains J, Relova-Quinteiro JL, Cadarso-Suárez C, Jorge-Barreiro FJ, Ares-Pena FJ. The action of pulse-modulated GSM radiation increases regional changes in brain activity and c-Fos expression in cortical and subcortical areas in a rat

model of picrotoxin-induced seizure proneness. J Neurosci Res. 87(6):1484-1499, 2009. (AS, GE, WS, IA)

The action of the pulse-modulated GSM radiofrequency of mobile phones has been suggested as a physical phenomenon that might have biological effects on the mammalian central nervous system. In the present study, GSM-exposed picrotoxin-pretreated rats showed differences in clinical and EEG signs, and in c-Fos expression in the brain, with respect to picrotoxin-treated rats exposed to an equivalent dose of unmodulated radiation. Neither radiation treatment caused tissue heating, so thermal effects can be ruled out. The most marked effects of GSM radiation on c-Fos expression in picrotoxin-treated rats were observed in limbic structures, olfactory cortex areas and subcortical areas, the dentate gyrus, and the central lateral nucleus of the thalamic intralaminar nucleus group. Nonpicrotoxin-treated animals exposed to unmodulated radiation showed the highest levels of neuronal c-Fos expression in cortical areas. These results suggest a specific effect of the pulse modulation of GSM radiation on brain activity of a picrotoxin-induced seizure-proneness rat model and indicate that this mobile-phone-type radiation might induce regional changes in previous preexcitability conditions of neuronal activation.

(E) Luukkonen J, Hakulinen P, Mäki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872MHz radiofrequency radiation. Mutat Res 662:54-58, 2009. (GT, OX, WS)

The objective of the study was to investigate effects of 872 MHz radiofrequency (RF) radiation on intracellular reactive oxygen species (ROS) production and DNA damage at a relatively high SAR value (5W/kg). The experiments also involved combined exposure to RF radiation and menadione, a chemical inducing intracellular ROS production and DNA damage. The production of ROS was measured using the fluorescent probe dichlorofluorescein and DNA damage was evaluated by the Comet assay. Human SH-SY5Y neuroblastoma cells were exposed to RF radiation for 1h with or without menadione. Control cultures were sham exposed. Both continuous waves (CW) and a pulsed signal similar to that used in global system for mobile communications (GSM) mobile phones were used. Exposure to the CW RF radiation increased DNA breakage ($p<0.01$) in comparison to the cells exposed only to menadione. Comparison of the same groups also showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60 min after the end of exposure ($p<0.05$ and $p<0.01$, respectively). No effects of the GSM signal were seen on either ROS production or DNA damage. The results of the present study suggest that 872MHz CW RF radiation at 5W/kg might enhance chemically induced ROS production and thus cause secondary DNA damage. However, there is no known mechanism that would explain such effects from CW RF radiation but not from GSM modulated RF radiation at identical SAR.

(NE) Luukkonen J, Juutilainen J, Naarala J. Combined effects of 872 MHz radiofrequency radiation and ferrous chloride on reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells. Bioelectromagnetics 31:417-424, 2010. (GT, OX)

The aim of the present study was to investigate possible cooperative effects of radiofrequency (RF) radiation and ferrous chloride (FeCl) on reactive oxygen species (ROS) production and

DNA damage. In order to test intracellular ROS production as a possible underlying mechanism of DNA damage, we applied the fluorescent probe DCFH-DA. Integrity of DNA was quantified by alkaline comet assay. The exposures to 872 MHz RF radiation were conducted at a specific absorption rate (SAR) of 5 W/kg using continuous waves (CW) or a modulated signal similar to that used in Global System for Mobile Communications (GSM) phones. Four groups were included: Sham exposure (control), RF radiation, Chemical treatment, Chemical treatment, and RF radiation. In the ROS production experiments, human neuroblastoma (SH-SY5Y) cells were exposed to RF radiation and 10 microg/ml FeCl for 1 h. In the comet assay experiments, the exposure time was 3 h and an additional chemical (0.015% diethyl maleate) was used to make DNA damage level observable. The chemical treatments resulted in statistically significant responses, but no effects from either CW or modulated RF radiation were observed on ROS production, DNA damage or cell viability.

(NE) Maes A, Van Gorp U, Verschaeve L. Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation. Mutagenesis 21:139-42, 2006. (GT, IA)

Nowadays, virtually everybody is exposed to radiofrequency radiation (RFR) from mobile phone base station antennas or other sources. At least according to some scientists, this exposure can have detrimental health effects. We investigated cytogenetic effects in peripheral blood lymphocytes from subjects who were professionally exposed to mobile phone electromagnetic fields in an attempt to demonstrate possible RFR-induced genetic effects. These subjects can be considered well suited for this purpose as their RFR exposure is 'normal' though rather high, and definitely higher than that of the 'general population'. The alkaline comet assay, sister chromatid exchange (SCE) and chromosome aberration tests revealed no evidence of RFR-induced genetic effects. Blood cells were also exposed to the well known chemical mutagen mitomycin C in order to investigate possible combined effects of RFR and the chemical. No cooperative action was found between the electromagnetic field exposure and the mutagen using either the comet assay or SCE test.

(E) Manti L, Braselmann H, Calabrese ML, Massa R, Pugliese M, Scampoli P, Sicignano G, Grossi G. Effects of modulated microwave radiation at cellular telephone frequency (1.95 GHz) on X-ray-induced chromosome aberrations in human lymphocytes in vitro. Radiat Res 169:575-583, 2008. (GT, IA)

The case for a DNA-damaging action produced by radiofrequency (RF) signals remains controversial despite extensive research. With the advent of the Universal Mobile Telecommunication System (UMTS) the number of RF-radiation-exposed individuals is likely to escalate. Since the epigenetic effects of RF radiation are poorly understood and since the potential modifications of repair efficiency after exposure to known cytotoxic agents such as ionizing radiation have been investigated infrequently thus far, we studied the influence of UMTS exposure on the yield of chromosome aberrations induced by X rays. Human peripheral blood lymphocytes were exposed in vitro to a UMTS signal (frequency carrier of 1.95 GHz) for 24 h at 0.5 and 2.0 W/kg specific absorption rate (SAR) using a previously characterized waveguide system. The frequency of chromosome aberrations was measured on metaphase spreads from cells given 4 Gy of X rays immediately before RF radiation or sham exposures by fluorescence in situ hybridization. Unirradiated controls were RF-radiation- or sham-exposed. No significant variations due to the UMTS exposure were found in the fraction of aberrant cells. However, the frequency of exchanges per cell was affected by the SAR, showing a small but

statistically significant increase of 0.11 exchange per cell compared to 0 W/kg SAR. We conclude that, although the 1.95 GHz signal (UMTS modulated) does not exacerbate the yield of aberrant cells caused by ionizing radiation, the overall burden of X-ray-induced chromosomal damage per cell in first-mitosis lymphocytes may be enhanced at 2.0 W/kg SAR. Hence the SAR may either influence the repair of X-ray-induced DNA breaks or alter the cell death pathways of the damage response.

(E) Mazor R, Korenstein-Ilan A, Barbul A, Eshet Y, Shahadi A, Jerby E, Korenstein R. Increased levels of numerical chromosome aberrations after in vitro exposure of human peripheral blood lymphocytes to radiofrequency electromagnetic fields for 72 hours. Radiat Res. 169(1):28-37, 2008. (GT)

We investigated the effects of 72 h in vitro exposure of 10 human lymphocyte samples to radiofrequency electromagnetic fields (800 MHz, continuous wave) on genomic instability. The lymphyocytes were exposed in a specially designed waveguide resonator at specific absorption rates (SARs) of 2.9 and 4.1 W/kg in a temperature range of 36-37 degrees C. The induced aneuploidy of chromosomes 1, 10, 11 and 17 was determined by interphase FISH using semi-automated image analysis. We observed increased levels of aneuploidy depending on the chromosome studied as well as on the level of exposure. In chromosomes 1 and 10, there was increased aneuploidy at the higher SAR, while for chromosomes 11 and 17, the increases were observed only for the lower SAR. Multisomy (chromosomal gains) appeared to be the primary contributor to the increased aneuploidy. The effect of temperature on the level of aneuploidy was examined over the range of 33.5-40 degrees C for 72 h with no statistically significant difference in the level of aneuploidy compared to 37 degrees C. These findings suggest the possible existence of an athermal effect of RF radiation that causes increased levels of aneuploidy. These results contribute to the assessment of potential health risks after continuous chronic exposure to RF radiation at SARs close to the current levels set by ICNIRP guidelines.

(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.

(E) Nittby H, Widegren B, Krogh M, Grafström G, Berlin H, Rehn G, Eberhardt JL, Malmgren L, Persson BRR, Salford L. Exposure to radiation from global system for mobile communications at 1,800 MHz significantly changes gene expression in rat hippocampus and cortex. Environmentalist 28(4), 458-465, 2008. (GE)

We have earlier shown that radio frequency electromagnetic fields can cause significant leakage of albumin through the blood–brain barrier of exposed rats as compared to non-exposed rats, and also significant neuronal damage in rat brains several weeks after a 2 h exposure to a mobile phone, at 915 MHz with a global system for mobile communications (GSM) frequency modulation, at whole-body specific absorption rate values (SAR) of 200, 20, 2, and 0.2 mW/kg. We have now studied whether 6 h of exposure to the radiation from a GSM mobile test phone at 1,800 MHz (at a whole-body SAR-value of 13 mW/kg, corresponding to a brain SAR-value of 30 mW/kg) has an effect upon the gene expression pattern in rat brain cortex and hippocampus—areas where we have observed albumin leakage from capillaries into neurons and neuronal damage. Microarray analysis of 31,099 rat genes, including splicing variants, was performed in cortex and hippocampus of 8 Fischer 344 rats, 4 animals exposed to global system for mobile communications electromagnetic fields for 6 h in an anechoic chamber, one rat at a time, and 4 controls kept as long in the same anechoic chamber without exposure, also in this case one rat at a time. Gene ontology analysis (using the gene ontology categories biological processes, molecular functions, and cell components) of the differentially expressed genes of the exposed animals versus the control group revealed the following highly significant altered gene categories in both cortex and hippocampus: extracellular region, signal transducer activity, intrinsic to membrane, and integral to membrane. The fact that most of these categories are connected with membrane functions may have a relation to our earlier observation of albumin transport through brain capillaries.

(E) Nylund R, Leszczynski D. Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome- and proteome-dependent. Proteomics 6:4769-4780, 2006. (GE, CS)

We have examined in vitro cell response to mobile phone radiation (900 MHz GSM signal) using two variants of human endothelial cell line: EA.hy926 and EA.hy926v1. Gene expression changes were examined in three experiments using cDNA Expression Arrays and protein expression changes were examined in ten experiments using 2-DE and PDQuest software. Obtained results show that gene and protein expression were altered, in both examined cell lines, in response to one hour mobile phone radiation exposure at an average specific absorption rate of 2.8 W/kg. However, the same genes and proteins were differently affected by the exposure in each of the cell lines. This suggests that the cell response to mobile phone radiation might be genome- and proteome-dependent. Therefore, it is likely that different types of cells and from different species might respond differently to mobile phone radiation or might have different sensitivity to this weak stimulus. Our findings might also explain, at least in part, the origin of discrepancies in replication studies between different laboratories.

(E) Panagopoulos DJ, Chavdoula ED, Nezis IP, Margaritis LH. Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation. Mutat Res 626:69-78, 2007. (GT, RP)

In the present study, the TUNEL (Terminal deoxynucleotidetransferasedUTP Nick End Labeling) assay - a well known technique widely used for detecting fragmented DNA in various types of cells - was used to detect cell death (DNA fragmentation) in a biological model, the early and mid stages of oogenesis of the insect Drosophila melanogaster. The flies were exposed in vivo to either GSM 900-MHz (Global System for Mobile telecommunications) or DCS 1800-MHz (Digital Cellular System) radiation from a common digital mobile phone, for few minutes per day during the first 6 days of their adult life. The exposure conditions were similar to those to which a mobile phone user is exposed, and were determined according to previous studies of ours [D.J Panagopoulos, A. Karabarbounis, L.H. Margaritis, Effect of GSM 900-MHz mobile phone radiation on the reproductive capacity of D. melanogaster, Electromagn. Biol Med 23 (2004) 29-43; D.J Panagopoulos, N. Messini, A. Karabarbounis, A.L. Philippetis, L.H. Margaritis, Radio frequency electromagnetic radiation within "safety levels" alters the physiological function of insects, in: P. Kostarakis, P. Stavroulakis (Eds.), Proceedings of the Millennium International Workshop on Biological Effects of Electromagnetic Fields, Heraklion, Crete, Greece, October 17-20, 2000, pp. 169-175, ISBN: 960-86733-0-5; D.J Panagopoulos, L.H. Margaritis, Effects of electromagnetic fields on the reproductive capacity of D. melanogaster, in: P. Stavroulakis (Ed.), Biological Effects of Electromagnetic Fields, Springer, 2003, pp. 545-578], which had shown a large decrease in the oviposition of the same insect caused by GSM radiation. Our present results suggest that the decrease in oviposition previously reported, is due to degeneration of large numbers of egg chambers after DNA fragmentation of their constituent cells, induced by both types of mobile telephony radiation. Induced cell death is recorded for the first time, in all types of cells constituting an egg chamber (follicle cells, nurse cells and the oocyte) and in all stages of the early and mid-oogenesis, from germarium to stage 10, during which programmed cell death does not physiologically occur. Germarium and stages 7-8 were found to be the most sensitive developmental stages also in response to electromagnetic stress induced by the GSM and DCS fields and, moreover, germarium was found to be even more sensitive than stages 7-8.

(NE) Paparini A, Rossi P, Gianfranceschi G, Brugaletta V, Falsaperla R, De Luca P, Romano Spica V. No evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal. Bioelectromagnetics. 29(4):312-323, 2008. (GE)

To analyze possible effects of microwaves on gene expression, mice were exposed to global system for mobile communication (GSM) 1800 MHz signal for 1 h at a whole body SAR of 1.1 W/kg. Gene expression was studied in the whole brain, where the average SAR was 0.2 W/kg, by expression microarrays containing over 22,600 probe sets. Comparison of data from sham and exposed animals showed no significant difference in gene expression modulation. However, when less stringent constraints were adopted to analyze microarray results, 75 genes were found to be modulated following exposure. Forty-two probes showed fold changes ranging from 1.5 to 2.8, whereas 33 were down-regulated from 0.67- to 0.29-fold changes, but these differences in gene expression were not confirmed by real-time PCR. Under these specific limited conditions, no consistent indication of gene expression modulation in whole mouse brain was found associated to GSM 1800 MHz exposure.

(E) Paulraj R, Behari J. Single strand DNA breaks in rat brain cells exposed to microwave radiation. Mutat Res 596:76-80, 2006. (GT, LE)

This investigation concerns with the effect of low intensity microwave (2.45 and 16.5GHz, SAR 1.0 and 2.01W/kg, respectively) radiation on developing rat brain. Wistar rats (35 days old, male, six rats in each group) were selected for this study. These animals were exposed for 35 days at the above mentioned frequencies separately in two different exposure systems. After the exposure period, the rats were sacrificed and the whole brain tissue was dissected and used for study of single strand DNA breaks by micro gel electrophoresis (comet assay). Single strand DNA breaks were measured as tail length of comet. Fifty cells from each slide and two slides per animal were observed. One-way ANOVA method was adopted for statistical analysis. This study shows that the chronic exposure to these radiations cause statistically significant ($p<0.001$) increase in DNA single strand breaks in brain cells of rat.

(E) Pesnya DS, Romanovsky AV. Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the Allium cepa test. Mutat Res. 2012 Oct 8. pii: S1383-5718(12)00291-4. doi: 10.1016/j.mrgentox.2012.08.010. [Epub ahead of print] (GT)

The goal of this study was to compare the cytotoxic and genotoxic effects of plutonium-239 alpha particles and GSM 900 modulated mobile phone radiation in the Allium cepa test. Three groups of bulbs were exposed to mobile phone radiation during 0 (sham), 3 and 9hours. A positive control group was treated during 20 min with plutonium-239 alpha-radiation. Mitotic abnormalities, chromosome aberrations, micronuclei and mitotic index were analyzed. Exposure to alpha-radiation from plutonium-239 and exposure to modulated radiation from mobile phone during 3 and 9h significantly increased the mitotic index. GSM 900 mobile phone radiation as well as alpha-radiation from plutonium-239 induced both clastogenic and aneugenic effects. However, the aneugenic activity of mobile phone radiation was more pronounced. After 9 hours of exposure to mobile phone radiation, polyploid cells, three-groups metaphases, amitoses and some unspecified abnormalities were detected, which were not registered in the other experimental groups. Importantly, GSM 900 mobile phone radiation increased the mitotic index, the frequency of mitotic and chromosome abnormalities, and the micronucleus frequency in a time-dependent manner. Due to its sensitivity, the Allium cepa test can be recommended as a useful cytogenetic assay to assess cytotoxic and genotoxic effects of radiofrequency electromagnetic fields.

(NE) Qutob SS, Chauhan V, Bellier PV, Yauk CL, Douglas GR, Berndt L, Williams A, Gajda GB, Lemay E, Thansandote A, McNamee JP. Microarray gene expression profiling of a human glioblastoma cell line exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. Radiat Res 165:636-644, 2006. (GE)

The widespread use of mobile phones has led to public concerns about the health effects associated with exposure to radiofrequency (RF) fields. The paramount concern of most persons relates to the potential of these fields to cause cancer. Unlike ionizing radiation, RF fields used for mobile telecommunications (800-1900 MHz) do not possess sufficient energy to directly damage DNA. Most rodent bioassay and in vitro genotoxicity/mutation studies have reported that RF fields at non-thermal levels have no direct mutagenic, genotoxic or carcinogenic effects.

However, some evidence has suggested that RF fields may cause detectable postexposure changes in gene expression. Therefore, the purpose of this study was to assess the ability of exposure to a 1.9 GHz pulse-modulated RF field for 4 h at specific absorption rates (SARs) of 0.1, 1.0 and 10.0 W/kg to affect global gene expression in U87MG glioblastoma cells. We found no evidence that non-thermal RF fields can affect gene expression in cultured U87MG cells relative to the nonirradiated control groups, whereas exposure to heat shock at 43 degrees C for 1 h up-regulated a number of typical stress-responsive genes in the positive control group. Future studies will assess the effect of RF fields on other cell lines and on gene expression in the mouse brain after in vivo exposure.

(E) Remondini D, Nylund R, Reivinen J, Poulletier de Gannes F, Veyret B, Lagroye I, Haro E, Trillo MA, Capri M, Franceschi C, Schlatterer K, Gminski R, Fitzner R, Tauber R, Schuderer J, Kuster N, Leszczynski D, Bersani F, Maercker C. Gene expression changes in human cells after exposure to mobile phone microwaves. Proteomics 6:4745-4754, 2006. (GE, CS)

Possible biological effects of mobile phone microwaves were investigated in vitro. In this study, which was part of the 5FP EU project REFLEX (Risk Evaluation of Potential Environmental Hazards From Low-Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods), six human cell types, immortalized cell lines and primary cells, were exposed to 900 and 1800 MHz. RNA was isolated from exposed and sham-exposed cells and labeled for transcriptome analysis on whole-genome cDNA arrays. The results were evaluated statistically using bioinformatics techniques and examined for biological relevance with the help of different databases. NB69 neuroblastoma cells, T lymphocytes, and CHME5 microglial cells did not show significant changes in gene expression. In EA.hy926 endothelial cells, U937 lymphoblastoma cells, and HL-60 leukemia cells we found between 12 and 34 up- or down-regulated genes. Analysis of the affected gene families does not point towards a stress response. However, following microwave exposure, some but not all human cells might react with an increase in expression of genes encoding ribosomal proteins and therefore up-regulating the cellular metabolism.

(NE) Ros-Llor I, Sanchez-Siles M, Camacho-Alonso F, Lopez-Jornet P. Effect of mobile phones on micronucleus frequency in human exfoliated oral mucosal cells. Oral Dis. 18:786-792, 2012. (GT)

Objective: In the last two decades, the use of mobile phones has increased enormously all over the world. The controversy regarding whether radiofrequency (RF) fields exert effects upon biological systems is a concern for the general population. An evaluation is made of DNA damage and cytokinetic defects, proliferative potential, and cell death because of RF radiation emitted by mobile phones in healthy young users. Study design: This cohort study was carried out in 50 Caucasian mobile phone users. We collected two cell samples from each subject (a total of 100 cell samples), corresponding to the right and left cheek mucosa, respectively. Case histories and personal information were assessed, including age, gender, body height and weight, history of cancer, smoking and alcohol consumption, exposure to chemical carcinogens or radiation, and dietary habits. Sampling comprised cell collection from both cheeks with a cytobrush, centrifugation, slide preparation, fixation, and staining, followed by fluorescent microscopic analysis. A total of 2000 exfoliated cells were screened for nuclear abnormalities,

especially micronucleus. Results: No statistically significant changes were recorded in relation to age, gender, body mass index, or smoking status. A comparison of the results vs the control area according to the side of the face on which the mobile phone was placed, and in relation to the duration of exposure (years) to mobile phone radiation in the total 100 samples, yielded no significant differences. Conclusions: No genotoxic effects because of RF exposure were observed in relation to any of the study parameters.

(NE) Sakuma N, Komatsubara Y, Takeda H, Hirose H, Sekijima M, Nojima T, Miyakoshi J. DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. Bioelectromagnetics 27:51-57, 2006. (CT)

We conducted a large-scale in vitro study focused on the effects of low level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system in order to test the hypothesis that modulated RF fields may act as a DNA damaging agent. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced different levels of DNA damage. Human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs were exposed to mobile communication frequency radiation to investigate whether such exposure produced DNA strand breaks in cell culture. A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg and CW radiation at 80 mW/kg for 2 and 24 h, while IMR-90 cells were exposed to both W-CDMA and CW radiations at a SAR of 80 mW/kg for the same time periods. Under the same RF field exposure conditions, no significant differences in the DNA strand breaks were observed between the test groups exposed to W-CDMA or CW radiation and the sham exposed negative controls, as evaluated immediately after the exposure periods by alkaline comet assays. Our results confirm that low level exposures do not act as a genotoxicant up to a SAR of 800 mW/kg.

(NE) Sakurai T, Kiyokawa T, Narita E, Suzuki Y, Taki M, Miyakoshi J. Analysis of gene expression in a human-derived glial cell line exposed to 2.45 GHz continuous radiofrequency electromagnetic fields. J Radiat Res. 52(2):185-192, 2011. (GE)

The increasing use of mobile phones has aroused public concern regarding the potential health risks of radiofrequency (RF) fields. We investigated the effects of exposure to RF fields (2.45 GHz, continuous wave) at specific absorption rate (SAR) of 1, 5, and 10 W/kg for 1, 4, and 24 h on gene expression in a normal human glial cell line, SVGp12, using DNA microarray. Microarray analysis revealed 23 assigned gene spots and 5 non-assigned gene spots as prospective altered gene spots. Twenty-two genes out of the 23 assigned gene spots were further analyzed by reverse transcription-polymerase chain reaction to validate the results of microarray, and no significant alterations in gene expression were observed. Under the experimental conditions used in this study, we found no evidence that exposure to RF fields affected gene expression in SVGp12 cells.

(NE) Sannino A, Di Costanzo G, Brescia F, Sarti M, Zeni O, Juutilainen J, Scarfì MR. Human fibroblasts and 900 MHz radiofrequency radiation: evaluation of DNA damage after exposure and co-exposure to 3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-furanone (MX). Radiat Res 171:743-751, 2009. (NT, IA)

Abstract Sannino, A., Di Costanzo, G., Brescia, F., Sarti, M., Zeni, O., Juutilainen, J and Scarfì, M. R. Human Fibroblasts and 900 MHz Radiofrequency Radiation: Evaluation of DNA Damage after Exposure and Co-exposure to 3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-furanone (MX). Radiat Res 171, 743-751 (2009). The aim of this study was to investigate DNA damage in human dermal fibroblasts from a healthy subject and from a subject affected by Turner's syndrome that were exposed for 24 h to radiofrequency (RF) radiation at 900 MHz. The RF-radiation exposure was carried out alone or in combination with 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a well-known environmental mutagen and carcinogen produced during the chlorination of drinking water. Turner's syndrome fibroblasts were also exposed for a shorter time (1 h). A signal similar to that emitted by Global System for Mobile Communications (GSM) mobile phones was used at a specific absorption rate of 1 W/kg under strictly controlled conditions of temperature and dosimetry. To evaluate DNA damage after RF-radiation exposure alone, the alkaline comet assay and the cytokinesis-block micronucleus assay were used. In the combined-exposure experiments, MX was given at a concentration of 25 microM for 1 h immediately after the RF-radiation exposure, and the effects were evaluated by the alkaline comet assay. The results revealed no genotoxic and cytotoxic effects from RF radiation alone in either cell line. As expected, MX treatment induced an increase in DNA migration in the comet assay, but no enhancement of the MX-induced DNA damage was observed in the cells exposed to RF radiation.

(E) Schwarz C, Kratochvil E, Pilger A, Kuster N, Adlkofer F, Rüdiger HW. Radiofrequency electromagnetic fields (UMTS, 1,950 MHz) induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes. Int Arch Occup Environ Health 81:755-767, 2008. (GT, CS)

OBJECTIVE: Universal Mobile Telecommunication System (UMTS) was recently introduced as the third generation mobile communication standard in Europe. This was done without any information on biological effects and genotoxic properties of these particular high-frequency electromagnetic fields. This is discomforting, because genotoxic effects of the second generation standard Global System for Mobile Communication have been reported after exposure of human cells in vitro. METHODS: Human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures were exposed to 1,950 MHz UMTS below the specific absorption rate (SAR) safety limit of 2 W/kg. The alkaline comet assay and the micronucleus assay were used to ascertain dose and time-dependent genotoxic effects. Five hundred cells per slide were visually evaluated in the comet assay and comet tail factor (CTF) was calculated. In the micronucleus assay 1,000 binucleated cells were evaluated per assay. The origin of the micronuclei was determined by fluorescence labeled anticentromere antibodies. All evaluations were performed under blinded conditions. RESULTS: UMTS exposure increased the CTF and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way. Incubation for 24 h at a SAR of 0.05 W/kg generated a statistically significant rise in both CTF and MN ($P = 0.02$). At a SAR of 0.1 W/kg the CTF was significantly

increased after 8 h of incubation (P = 0.02), the number of MN after 12 h (P = 0.02). No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with Phytohemagglutinin. CONCLUSION: UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

(E) Sekeroğlu V, Akar A, Sekeroğlu ZA. Cytotoxic and genotoxic effects of high-frequency electromagnetic fields (GSM 1800 MHz) on immature and mature rats. Ecotoxicol Environ Saf. 80:140-144, 2012. (LE, GT, DE)

We investigated the cytogenotoxic effects of high frequency electromagnetic fields (HF-EMF) for 45 day and the effect of a recovery period of 15 day after exposure to EMF on bone marrow cells of immature and mature rats. The animals in treatment groups were exposed to 1800 MHz EMF at SAR of 0.37 W/kg and 0.49 W/kg for 2h/day for 45 day. Two recovery groups were kept for a recovery period of 15 day without EMF after exposure to HF-EMF. Two control groups for both immature and mature rats were also included. Significant differences were also observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCEs) in all treatment groups. The cytogenotoxic damage was more remarkable in immature rats and, the recovery period did not improve this damage in immature rats. Because much higher and irreversible cytogenotoxic damage was observed in immature rats than in mature rats, further studies are needed to understand effects of EMF on DNA damage and DNA repair, and to determine safe limits for environment and human, especially for children.

(NE) Sekijima M, Takeda H, Yasunaga K, Sakuma N, Hirose H, Nojima T, Miyakoshi J. 2-GHz band CW and W-CDMA modulated radiofrequency fields have no significant effect on cell proliferation and gene expression profile in human cells. J Radiat Res. 51(3):277-284, 2010. (GE)

We investigated the mechanisms by which radiofrequency (RF) fields exert their activity, and the changes in both cell proliferation and the gene expression profile in the human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung) following exposure to 2.1425 GHz continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) RF fields at three field levels. During the incubation phase, cells were exposed at the specific absorption rates (SARs) of 80, 250, or 800 mW/kg with both CW and W-CDMA RF fields for up to 96 h. Heat shock treatment was used as the positive control. No significant differences in cell growth or viability were observed between any test group exposed to W-CDMA or CW radiation and the sham-exposed negative controls. Using the Affymetrix Human Genome Array, only a very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression in each experiment. The results confirm that low-level exposure to 2.1425 GHz CW and W-CDMA RF fields for up to 96 h did not act as an acute cytotoxicant in either cell proliferation or the gene expression profile. These results suggest that RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines under these conditions.

(E) Souza LD, Cerqueira ED, Meireles JR. Assessment of nuclear abnormalities in exfoliated cells from the oral epithelium of mobile phone users. Electromagn Biol Med. 2013 May 28. [Epub ahead of print] (GE, HU)

Abstract Transmission and reception of mobile telephony signals take place through electromagnetic wave radiation, or electromagnetic radiofrequency fields, between the mobile terminal and the radio base station. Based on reports in the literature on adverse effects from exposure to this type of radiation, the objective of this study was to evaluate the genotoxic and cytotoxic potential of such exposure, by means of the micronucleus test on exfoliated cells from the oral epithelium. The sample included 45 individuals distributed in 3 groups according to the amount of time in hours per week (t) spent using mobile phones: group I, $t > 5$ h; group II, $t > 1$ h and ≤ 5 h; and group III, $t \leq 1$ h. Cells from the oral mucosa were analyzed to assess the numbers of micronuclei, broken egg structures and degenerative nuclear abnormalities indicative of apoptosis (condensed chromatin, karyorrhexis and pyknosis) or necrosis (karyolysis in addition to these changes). The occurrences of micronuclei and degenerative nuclear abnormalities did not differ between the groups, but the number of broken egg (structures that may be associated with gene amplification) was significantly greater in the individuals in group I ($p < 0.05$).

(NE) Speit G, Schütz P, Hoffmann H. Genotoxic effects of exposure to radiofrequency electromagnetic fields (RF-EMF) in cultured mammalian cells are not independently reproducible. Mutat Res. 626(1-2):42-47, 2007. (GT)

Conflicting results have been published regarding the induction of genotoxic effects by exposure to radiofrequency electromagnetic fields (RF-EMF). Using the comet assay, the micronucleus test and the chromosome aberration test with human fibroblasts (ES1 cells), the EU-funded "REFLEX" project (Risk Evaluation of Potential Environmental Hazards From Low Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods) reported clearly positive effects for various exposure conditions. Because of the ongoing discussion on the biological significance of the effects observed, it was the aim of the present study to independently repeat the results using the same cells, the same equipment and the same exposure conditions. We therefore exposed ES1 cells to RF-EMF (1800 MHz; SAR 2 W/kg, continuous wave with intermittent exposure) for different time periods and then performed the alkaline (pH>13) comet assay and the micronucleus test (MNT). For both tests, clearly negative results were obtained in independently repeated experiments. We also performed these experiments with V79 cells, a sensitive Chinese hamster cell line that is frequently used in genotoxicity testing, and also did not measure any genotoxic effect in the comet assay and the MNT. Appropriate measures of quality control were considered to exclude variations in the test performance, failure of the RF-EMF exposure or an evaluation bias. The reasons for the difference between the results reported by the REFLEX project and our experiments remain unclear.

(NE) Stronati L, Testa A, Moquet J, Edwards A, Cordelli E, Villani P, Marino C, Fresegna AM, Appolloni M, Lloyd D. 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes. Int J Radiat Biol 82:339-346, 2006. (GT, IA)

Purpose: The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with x-rays was investigated in vitro using several assays on human lymphocytes. The chosen specific absorption rate (SAR) values are near the upper limit of actual energy absorption in localized tissue when persons use some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen.Methods: Blood specimens from 14 donors were exposed continuously for 24 h to a

Global System for Mobile Communications (GSM) basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in vitro cell cycling.Results: By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did not modify any measured effects of the x-radiation. Conclusions: This study has used several standard in vitro tests for chromosomal and DNA damage in Go human lymphocytes exposed in vitro to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic per se or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed.

(E) Sun LX, Yao K, He JL, Lu DQ, Wang KJ, Li HW.[Effect of acute exposure to microwave from mobile phone on DNA damage and repair of cultured human lens epithelial cells in vitro.] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing ZaZhi. 24:465-467, 2006. [Article in Chinese] (GT)

OBJECTIVE: To investigate the DNA damage of human lens epithelial cells (LECs) caused by acute exposure to low-power 217 Hz modulated 1.8 GHz microwave radiation and DNA repair. METHODS: Cultured LECs were exposed to 217 Hz modulated 1.8 GHz microwave radiation at SAR (specific absorption rate) of 0, 1, 2, 3 and 4 W/kg for 2 hours in an sXc-1800 incubator and irradiate system. The DNA single strand breaks were detected with comet assay in sham-irradiated cells and irradiated cells incubated for varying periods: 0, 30, 60, 120 and 240 min after irradiation. Images of comets were digitized and analyzed using an Imagine-pro plus software, and the indexes used in this study were tail length (TL) and tail moment (TM). RESULTS: The difference in DNA-breaks between the exposure and sham exposure groups induced by 1 and 2 W/kg irradiation was not significant at every detect time ($P > 0.05$). As for the dosage of 3 and 4 W/kg there was difference in both groups immediately after irradiation ($P < 0.01$). At the time of 30 min after irradiation the difference went on at both group ($P < 0.01$). However, the difference disappeared after one hour's incubation in 3 W/kg group ($P > 0.05$), and existed in 4 W/kg group. CONCLUSION: No or repairable DNA damage was observed after 2 hour irradiation of 1.8 GHz microwave on LECs when SAR </= 3 W/kg. The DNA damages caused by 4 W/kg irradiation were irreversible.

(E) Tiwari R, Lakshmi NK, Surender V, Rajesh AD, Bhargava SC, Ahuja YR. Combinative exposure effect of radio frequency signals from CDMA mobile phones and aphidicolin on DNA integrity. Electromagn Biol Med 27:418-425, 2008. (GT, IA)

The aim of present study is to assess DNA integrity on the effect of exposure to a radio frequency (RF) signal from Code Division Multiple Access (CDMA) mobile phones. Whole blood samples from six healthy male individuals were exposed for RF signals from a CDMA mobile phone for 1 h. Alkaline comet assay was performed to assess the DNA damage. The combinative exposure effect of the RF signals and APC at two concentrations on DNA integrity was studied. DNA repair efficiency of the samples was also studied after 2 h of exposure. The

RF signals and APC (0.2 microg/ml) alone or in synergism did not have any significant DNA damage as compared to sham exposed. However, univariate analysis showed that DNA damage was significantly different among combinative exposure of RF signals and APC at 0.2 microg/ml ($p < 0.05$) and at 2 microg/ml ($p < 0.02$). APC at 2 microg/ml concentration also showed significant damage levels ($p < 0.05$) when compared to sham exposed. DNA repair efficiency also varied in a significant way in combinative exposure sets ($p < 0.05$). From these results, it appears that the repair inhibitor APC enhances DNA breaks at 2 microg/ml concentration and that the damage is possibly repairable. Thus, it can be inferred that the in vitro exposure to RF signals induces reversible DNA damage in synergism with APC.

(E) Tkalec M, Stambuk A, Srut M, Malarić K, Klobučar GI. Oxidative and genotoxic effects of 900MHz electromagnetic fields in the earthworm Eisenia fetida. Ecotoxicol Environ Saf. 90:7-12, 2013. (GT, OX, WS)

Accumulating evidence suggests that exposure to radiofrequency electromagnetic field (RF-EMF) can have various biological effects. In this study the oxidative and genotoxic effects were investigated in earthworms Eisenia fetida exposed in vivo to RF-EMF at the mobile phone frequency (900MHz). Earthworms were exposed to the homogeneous RF-EMF at field levels of 10, 23, 41 and 120Vm(-1) for a period of 2h using a Gigahertz Transversal Electromagnetic (GTEM) cell. At the field level of 23Vm(-1) the effect of longer exposure (4h) and field modulation (80% AM 1kHz sinusoidal) was investigated as well. All exposure treatments induced significant genotoxic effect in earthworms coelomocytes detected by the Comet assay, demonstrating DNA damaging capacity of 900MHz electromagnetic radiation. Field modulation additionally increased the genotoxic effect. Moreover, our results indicated the induction of antioxidant stress response in terms of enhanced catalase and glutathione reductase activity as a result of the RF-EMF exposure, and demonstrated the generation of lipid and protein oxidative damage. Antioxidant responses and the potential of RF-EMF to induce damage to lipids, proteins and DNA differed depending on the field level applied, modulation of the field and duration of E. fetida exposure to 900MHz electromagnetic radiation. Nature of detected DNA lesions and oxidative stress as the mechanism of action for the induction of DNA damage are discussed.

(E) Tomruk A, Guler G, Dincel AS. The influence of 1800 MHz GSM-like signals on hepatic oxidative DNA and lipid damage in nonpregnant, pregnant, and newly born rabbits. Cell Biochem Biophys 56:39-47, 2010. (GT, OX, DE, LE)

The aim of our study is to evaluate the possible biological effects of whole-body 1800 MHz GSM-like radiofrequency (RF) radiation exposure on liver oxidative DNA damage and lipid peroxidation levels in nonpregnant, pregnant New Zealand White rabbits, and in their newly borns. Eighteen nonpregnant and pregnant rabbits were used and randomly divided into four groups which were composed of nine rabbits: (i) Group I (nonpregnant control), (ii) Group II (nonpregnant-RF exposed), (iii) Group III (pregnant control), (iv) Group IV (pregnant-RF exposed). Newborns of the pregnant rabbits were also divided into two groups: (v) Group V (newborns of Group III) and (vi) Group VI (newborns of Group III). 1800 MHz GSM-like RF radiation whole-body exposure (15 min/day for a week) was applied to Group II and Group IV. No significant differences were found in liver 8 OHdG/10 dG levels of exposure groups (Group II and Group IV) compared to controls (Group I and Group III). However, in Group II and Group IV malondialdehyde (MDA) and ferrous oxidation in xylenol orange (FOX) levels were

increased compared to Group I (P < 0.05, Mann-Whitney). No significant differences were found in liver tissue of 8 OHdG/10 dG and MDA levels between Group VI and Group V (P > 0.05, Mann-Whitney) while liver FOX levels were found significantly increased in Group VI with respect to Group V (P < 0.05, Mann-Whitney). Consequently, the whole-body 1800 MHz GSM-like RF radiation exposure may lead to oxidative destruction as being indicators of subsequent reactions that occur to form oxygen toxicity in tissues.

(E) Trivino Pardo JC, Grimaldi S, Taranta M, Naldi I, Cinti C. Microwave electromagnetic field regulates gene expression in T-lymphoblastoid leukemia CCRF-CEM cell line exposed to 900 MHz. Electromagn Biol Med. 31(1):1-18, 2012. (GE)

Electric, magnetic, and electromagnetic fields are ubiquitous in our society, and concerns have been expressed regarding possible adverse effects of these exposures. Research on Extremely Low-Frequency (ELF) magnetic fields has been performed for more than two decades, and the methodology and quality of studies have improved over time. Studies have consistently shown increased risk for childhood leukemia associated with ELF magnetic fields. There are still inadequate data for other outcomes. More recently, focus has shifted toward Radio Frequencies (RF) exposures from mobile telephony. There are no persuasive data suggesting a health risk, but this research field is still immature with regard to the quantity and quality of available data. This technology is constantly changing and there is a need for continued research on this issue. To investigate whether exposure to high-frequency electromagnetic fields (EMF) could induce adverse health effects, we cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of 900 MHz MW-EMF generated by a transverse electromagnetic (TEM) cell at short and long exposure times. We evaluated the effect of high-frequency EMF on gene expression and we identified functional pathways influenced by 900 MHz MW-EMF exposure.

(E) Trosić I, Pavicić I, Milković-Kraus S, Mladinić M, Zeljezić D. Effect of electromagnetic radiofrequency radiation on the rats' brain, liver and kidney cells measured by comet assay. Coll Antropol 35:1259-1264, 2011. (GT)

The goal of study was to evaluate DNA damage in rat's renal, liver and brain cells after in vivo exposure to radiofrequency/microwave (Rf/Mw) radiation of cellular phone frequencies range. To determine DNA damage, a single cell gel electrophoresis/comet assay was used. Wistar rats (male, 12 week old, approximate body weight 350 g) (N = 9) were exposed to the carrier frequency of 915 MHz with Global System Mobile signal modulation (GSM), power density of 2.4 W/m2, whole body average specific absorption rate SAR of 0.6 W/kg. The animals were irradiated for one hour/day, seven days/week during two weeks period. The exposure set-up was Gigahertz Transversal Electromagnetic Mode Cell (GTEM--cell). Sham irradiated controls (N = 9) were apart of the study. The body temperature was measured before and after exposure. There were no differences in temperature in between control and treated animals. Comet assay parameters such as the tail length and tail intensity were evaluated. In comparison with tail length in controls (13.5 +/- 0.7 microm), the tail was slightly elongated in brain cells of irradiated animals (14.0 +/- 0.3 microm). The tail length obtained for liver (14.5 +/- 0.3 microm) and kidney (13.9 +/- 0.5 microm) homogenates notably differs in comparison with matched sham controls (13.6 +/- 0.3 microm) and (12.9 +/- 0.9 microm). Differences in tail intensity between control and exposed animals were not significant. The results of this study suggest that, under the experimental conditions applied, repeated 915 MHz irradiation could be a cause of DNA breaks

in renal and liver cells, but not affect the cell genome at the higher extent compared to the basal damage.

(NE) Valbonesi P, Franzellitti S, Piano A, Contin A, Biondi C, Fabbri E. Evaluation of HSP70 Expression and DNA damage in cells of a human trophoblast cell line exposed to 1.8 GHz amplitude-modulated radiofrequency fields. Radiat Res 169:270-279, 2008. (GT, GE)

The aim of this study was to determine whether high-frequency electromagnetic fields (EMFs) could induce cellular effects. The human trophoblast cell line HTR-8/SVneo was used as a model to evaluate the expression of proteins (HSP70 and HSC70) and genes (HSP70A, B, C and HSC70) of the HSP70 family and the primary DNA damage response after nonthermal exposure to pulse-modulated 1817 MHz sinusoidal waves (GSM-217 Hz; 1 h; SAR of 2 W/kg). HSP70 expression was significantly enhanced by heat, which was applied as the prototypical stimulus. The HSP70A, B and C transcripts were differentially expressed under basal conditions, and they were all significantly induced above basal levels by thermal stress. Conversely, HSC70 protein and gene expression was not influenced by heat. Exposing HTR-8/SVneo cells to high-frequency EMFs did not change either HSP70 or HSC70 protein or gene expression. A significant increase in DNA strand breaks was caused by exposure to HO, which was used as a positive stimulus; however, no effect was observed after exposure of cells to high-frequency EMFs. Overall, no evidence was found that a 1-h exposure to GSM-217 Hz induced a HSP70-mediated stress response or primary DNA damage in HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations are needed.

(E) Valbonesi P, Franzellitti S, Bersani F, Contin A, Fabbri E. Effects of the exposure to intermittent 1.8 GHz radio frequency electromagnetic fields on HSP70 expression and MAPK signaling pathways in PC12 cells. Int J Radiat Biol. 2014 Feb 11. [Epub ahead of print] (GE, WS)

Purpose: We previously reported effects on heat shock protein 70 (HSP70) mRNA expression, a cytoprotective protein induced under stressful condition, in human trophoblast cells exposed to amplitude-modulated Global System for Mobile Communication (GSM) signals. In the present work the same experimental conditions were applied to the rat PC12 cells, in order to assess the stress responses mediated by HSP70 and by the Mitogen Activated Protein Kinases (MAPK) in neuronal-like cells, an interesting model to study possible effects of mobile phone frequencies exposure. Materials and methods: HSP70 gene expression level was evaluated by reverse transcriptase polymerase chain reaction, HSP70 protein expression and MAPK phosphorylation were assessed by Western blotting. PC12 cells were exposed for 4, 16 or 24 h to 1.8 GHz continuous wave signal (CW, carrier frequency without modulation) or to two different GSM modulation schemes, GSM-217Hz and GSM-Talk (which generates temporal changes between two different GSM signals, active during talking or listening phases respectively, thus simulating a typical conversation). Specific adsorption rate (SAR) was 2 W/kg. Results: After PC12 cells exposure to the GSM-217Hz signal for 16 or 24 h, HSP70 transcription significantly increased, whereas no effect was observed in cells exposed to the CW or GSM-Talk signals. HSP70 protein expression and three different MAPK signaling pathways were not affected by the exposure to any of the three different 1.8 GHz signals. Conclusion: The positive effect on HSP70 mRNA expression, observed only in cells exposed to the GSM-217Hz signal, is a repeatable response

previously reported in human trophoblast cells and now confirmed in PC12 cells. Further investigations towards a possible role of 1.8 GHz signal modulation are therefore advisable.

(NE) Verschaeve L, Heikkinen P, Verheyen G, Van Gorp U, Boonen F, Vander Plaetse F, Maes A, Kumlin T, Maki-Paakkanen J, Puranen L, Juutilainen J. Investigation of co-genotoxic effects of radiofrequency electromagnetic fields in vivo. Radiat Res 165:598-607, 2006. (GT, LE, IA)

We investigated the possible combined genotoxic effects of radiofrequency (RF) electromagnetic fields (900 MHz, amplitude modulated at 217 Hz, mobile phone signal) with the drinking water mutagen and carcinogen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). Female rats were exposed to RF fields for a period of 2 years for 2 h per day, 5 days per week at average whole-body specific absorption rates of 0.3 or 0.9 W/kg. MX was given in the drinking water at a concentration of 19 mug/ml. Blood samples were taken at 3, 6 and 24 months of exposure and brain and liver samples were taken at the end of the study (24 months). DNA damage was assessed in all samples using the alkaline comet assay, and micronuclei were determined in erythrocytes. We did not find significant genotoxic activity of MX in blood and liver cells. However, MX induced DNA damage in rat brain. Co-exposures to MX and RF radiation did not significantly increase the response of blood, liver and brain cells compared to MX exposure only. In conclusion, this 2-year animal study involving long-term exposures to RF radiation and MX did not provide any evidence for enhanced genotoxicity in rats exposed to RF radiation.

(NE) Vijayalaxmi. Cytogenetic studies in human blood lymphocytes exposed in vitro to 2.45 GHz or 8.2 GHz radiofrequency radiation. Radiat Res 166, 532–538, 2006. (GT)

Peripheral blood samples collected from healthy human volunteers were exposed in vitro to 2.45 GHz or 8.2 GHz pulsed-wave radiofrequency (RF) radiation. The net forward power, average power density, mean specific absorption rate, and the temperature maintained during the 2-h exposure of the cells to 2.45 GHz or 8.2 GHz were, respectively, 21 W or 60 W, 5 mW/cm2 or 10 mW/cm2, 2.13 W/kg or 20.71 W/kg, and 36.9 ± 0.1°C or 37.5 ± 0.2°C. Aliquots of the same blood samples that were either sham-exposed or exposed in vitro to an acute dose of 1.5 Gy γ radiation were used as unexposed and positive controls, respectively. Cultured lymphocytes were examined to determine the extent of cytogenetic damage assessed from the incidence of chromosomal aberrations and micronuclei. Under the conditions used to perform the experiments, the levels of damage in RF-radiation-exposed and sham-exposed lymphocytes were not significantly different. Also, there were no significant differences in the response of unstimulated lymphocytes and lymphocytes stimulated with phytohemagglutinin when exposed to 8.2 GHz RF radiation. In contrast, the positive control cells that had been subjected to γ irradiation exhibited significantly more damage than RF-radiation- and sham-exposed lymphocytes.

(NE) Waldmann P, Bohnenberger S, Greinert R, Hermann-Then B, Heselich A, Klug SJ, Koenig J, Kuhr K, Kuster N, Merker M, Murbach M, Pollet D, Schadenboeck W, Scheidemann-Wesp U, Schwab B, Volkmer B, Weyer V, Blettner M. Influence of GSM Signals on Human Peripheral Lymphocytes: Study of Genotoxicity. Radiat Res. 2013 Jan 14. [Epub ahead of print] (GT)

Exposure to radiofrequency (RF) electromagnetic fields (EMF) is continuously increasing worldwide. Yet, conflicting results of a possible genotoxic effect of RF EMF continue to be discussed. In the present study, a possible genotoxic effect of RF EMF (GSM, 1,800 MHz) in human lymphocytes was investigated by a collaboration of six independent institutes (institutes a, b, c, d, e, h). Peripheral blood of 20 healthy, nonsmoking volunteers of two age groups (10 volunteers 16-20 years old and 10 volunteers 50-65 years old) was taken, stimulated and intermittently exposed to three specific absorption rates (SARs) of RF EMF (0.2 W/kg, 2 W/kg, 10 W/kg) and sham for 28 h (institute a). The exposures were performed in a setup with strictly controlled conditions of temperature and dose, and randomly and automatically determined waveguide SARs, which were designed and periodically maintained by ITIS (institute h). Four genotoxicity tests with different end points were conducted (institute a): chromosome aberration test (five types of structural aberrations), micronucleus test, sister chromatid exchange test and the alkaline comet assay (Olive tail moment and % DNA). To demonstrate the validity of the study, positive controls were implemented. The genotoxicity end points were evaluated independently by three laboratories blind to SAR information (institute c = laboratory 1; institute d = laboratory 2; institute e = laboratory 3). Statistical analysis was carried out by institute b. Methods of primary statistical analysis and rules to adjust for multiple testing were specified in a statistical analysis plan based on a data review before unblinding. A linear trend test based on a linear mixed model was used for outcomes of comet assay and exact permutation test for linear trend for all other outcomes. It was ascertained that only outcomes with a significant SAR trend found by at least two of three analyzing laboratories indicated a substantiated suspicion of an exposure effect. On the basis of these specifications, none of the nine end points tested for SAR trend showed a significant and reproducible exposure effect. Highly significant differences between sham exposures and positive controls were detected by each analyzing laboratory, thus validating the study. In conclusion, the results show no evidence of a genotoxic effect induced by RF EMF (GSM, 1,800 MHz).

(E) Wu W, Yao K, Wang KJ, Lu DQ, He JL, Xu LH, Sun WJ. [Blocking 1800 MHz mobile phone radiation-induced reactive oxygen species production and DNA damage in lens epithelial cells by noise magnetic fields.]Zhejiang Da XueXueBao Yi Xue Ban 37:34-38, 2008. [Article in Chinese] (GT, IA, OX)

OBJECTIVE: To investigate whether the exposure to the electromagnetic noise can block reactive oxygen species (ROS) production and DNA damage of lens epithelial cells induced by 1800 MHz mobile phone radiation. METHODS: The DCFH-DA method and comet assay were used respectively to detect the intracellular ROS and DNA damage of cultured human lens epithelial cells induced by 4 W/kg 1800 MHz mobile phone radiation or/and 2microT electromagnetic noise for 24 h intermittently. RESULT: 1800 MHz mobile phone radiation at 4 W/kg for 24 h increased intracellular ROS and DNA damage significantly ($P<0.05$). However, the ROS level and DNA damage of mobile phone radiation plus noise group were not significant enhanced ($P>0.05$) as compared to sham exposure group. Conclusion: Electromagnetic noise can block intracellular ROS production and DNA damage of human lens epithelial cells induced by 1800 MHz mobile phone radiation.

(E) Xu S, Zhong M, Zhang L, Zhou Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Yu Z. Exposure to 1800 MHz radiofrequency radiation induces

oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res 1311:189-196. 2010. (GT, OX)

Increasing evidence indicates that oxidative stress may be involved in the adverse effects of radiofrequency (RF) radiation on the brain. Because mitochondrial DNA (mtDNA) defects are closely associated with various nervous system diseases and mtDNA is highly susceptible to oxidative stress, the purpose of this study was to determine whether radiofrequency radiation can cause oxidative damage to mtDNA. In this study, we exposed primary cultured cortical neurons to pulsed RF electromagnetic fields at a frequency of 1800 MHz modulated by 217 Hz at an average special absorption rate (SAR) of 2 W/kg. At 24h after exposure, we found that RF radiation induced a significant increase in the levels of 8-hydroxyguanine (8-OHdG), a common biomarker of DNA oxidative damage, in the mitochondria of neurons. Consistent with this finding, the copy number of mtDNA and the levels of mitochondrial RNA (mtRNA) transcripts showed an obvious reduction after RF exposure. Each of these mtDNA disturbances could be reversed by pretreatment with melatonin, which is known to be an efficient in the brain. Together, these results suggested that 1800 MHz RF radiation could cause oxidative damage to mtDNA in primary cultured neurons. Oxidative damage to mtDNA may account for the neurotoxicity of RF radiation in the brain.

(E) Xu S, Chen G, Chen C, Sun C, Zhang D, Murbach M, Kuster N, Zeng Q, Xu Z. Cell Type-Dependent Induction of DNA Damage by 1800 MHz Radiofrequency Electromagnetic Fields Does Not Result in Significant Cellular Dysfunctions. PLoS One. 8(1):e54906, 2013. (GT, CS)

BACKGROUND: Although IARC clarifies radiofrequency electromagnetic fields (RF-EMF) as possible human carcinogen, the debate on its health impact continues due to the inconsistent results. Genotoxic effect has been considered as a golden standard to determine if an environmental factor is a carcinogen, but the currently available data for RF-EMF remain controversial. As an environmental stimulus, the effect of RF-EMF on cellular DNA may be subtle. Therefore, more sensitive method and systematic research strategy are warranted to evaluate its genotoxicity. **OBJECTIVES:** To determine whether RF-EMF does induce DNA damage and if the effect is cell-type dependent by adopting a more sensitive method γH2AX foci formation; and to investigate the biological consequences if RF-EMF does increase γH2AX foci formation. **METHODS:** Six different types of cells were intermittently exposed to GSM 1800 MHz RF-EMF at a specific absorption rate of 3.0 W/kg for 1 h or 24 h, then subjected to immunostaining with anti-γH2AX antibody. The biological consequences in γH2AX-elevated cell type were further explored with comet and TUNEL assays, flow cytometry, and cell growth assay. **RESULTS:** Exposure to RF-EMF for 24 h significantly induced γH2AX foci formation in Chinese hamster lung cells and Human skin fibroblasts (HSFs), but not the other cells. However, RF-EMF-elevated γH2AX foci formation in HSF cells did not result in detectable DNA fragmentation, sustainable cell cycle arrest, cell proliferation or viability change. RF-EMF exposure slightly but not significantly increased the cellular ROS level. **CONCLUSIONS:** RF-EMF induces DNA damage in a cell type-dependent manner, but the elevated γH2AX foci formation in HSF cells does not result in significant cellular dysfunctions.

(NE) Yadav AS, Sharma MK. Increased frequency of micronucleated exfoliated cells among humans exposed in vivo to mobile telephone radiations. Mutat Res.650(2):175-180, 2008. (LE, GT, HU)

The health concerns have been raised following the enormous increase in the use of wireless mobile telephones throughout the world. This investigation had been taken, with the motive to find out whether mobile phone radiations cause any in vivo effects on the frequency of micronucleated exfoliated cells in the exposed subjects. A total of 109 subjects including 85 regular mobile phone users (exposed) and 24 non-users (controls) had participated in this study. Exfoliated cells were obtained by swabbing the buccal-mucosa from exposed as well as sex-age-matched controls. One thousand exfoliated cells were screened from each individual for nuclear anomalies including micronuclei (MN), karyolysis (KL), karyorrhexis (KH), broken egg (BE) and binucleated (BN) cells. The average daily duration of exposure to mobile phone radiations is 61.26 min with an overall average duration of exposure in term of years is 2.35 years in exposed subjects along with the 9.84+/-0.745 micronucleated cells (MNCs) and 10.72+/-0.889 total micronuclei (TMN) as compared to zero duration of exposure along with average 3.75+/-0.774 MNC and 4.00+/-0.808 TMN in controls. The means are significantly different in case of MNC and TMN at 0.01% level of significance. The mean of KL in controls is 13.17+/-2.750 and in exposed subjects is 13.06+/-1.793. The value of means of KH in exposed subjects (1.84+/-0.432) is slightly higher than in controls (1.42+/-0.737). Mean frequency of broken egg is found to be more in exposed subjects (0.65+/-0.276) as compared to controls (0.50+/-0.217). Frequency of presence of more than one nucleus in a cell (binucleated) is also higher in exposed (2.72+/-0.374) in comparison to controls (0.67+/-0.231). Although there is a slight increase in mean frequency of KH, BE and BN in exposed subjects but the difference is not found statistically significant. Correlation between 0-1, 1-2, 2-3 and 3-4 years of exposure and the frequency of MNC and TMN has been calculated and found to be positively correlated.

(E) Yan JG, Agresti M, Zhang LL, Yan Y, Matloub HS. Upregulation of specific mRNA levels in rat brain after cell phone exposure. Electromagn Biol Med. 27(2):147-154, 2008. (LE, GE)

Adult Sprague-Dawley rats were exposed to regular cell phones for 6 h per day for 126 days (18 weeks). RT-PCR was used to investigate the changes in levels of mRNA synthesis of several injury-associated proteins. Calcium ATPase, Neural Cell Adhesion Molecule, Neural Growth Factor, and Vascular Endothelial Growth Factor were evaluated. The results showed statistically significant mRNA up-regulation of these proteins in the brains of rats exposed to cell phone radiation. These results indicate that relative chronic exposure to cell phone microwave radiation may result in cumulative injuries that could eventually lead to clinically significant neurological damage.

(E) Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, Ye J, Sun L. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol Vis 14:964-969, 2008. (GT, IA, OX)

PURPOSE: The goal of this study was to investigate whether superposing of electromagnetic noise could block or attenuate DNA damage and intracellular reactive oxygen species (ROS) increase of cultured human lens epithelial cells (HLECs) induced by acute exposure to 1.8 GHz

radiofrequency field (RF) of the Global System for Mobile Communications (GSM). METHODS: An sXc-1800 RF exposure system was used to produce a GSM signal at 1.8 GHz (217 Hz amplitude-modulated) with the specific absorption rate (SAR) of 1, 2, 3, and 4 W/kg. After 2 h of intermittent exposure, the ROS level was assessed by the fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DNA damage to HLECs was examined by alkaline comet assay and the phosphorylated form of histone variant H2AX (gammaH2AX) foci formation assay. RESULTS: After exposure to 1.8 GHz RF for 2 h, HLECs exhibited significant intracellular ROS increase in the 2, 3, and 4 W/kg groups. RF radiation at the SAR of 3 W/kg and 4 W/kg could induce significant DNA damage, examined by alkaline comet assay, which was used to detect mainly single strand breaks (SSBs), while no statistical difference in double strand breaks (DSBs), evaluated by gammaH2AX foci, was found between RF exposure (SAR: 3 and 4 W/kg) and sham exposure groups. When RF was superposed with 2 muT electromagnetic noise could block RF-induced ROS increase and DNA damage. CONCLUSIONS: DNA damage induced by 1.8 GHz radiofrequency field for 2 h, which was mainly SSBs, may be associated with the increased ROS production. Electromagnetic noise could block RF-induced ROS formation and DNA damage.

(NE) Yildirim MS, Yildirim A, Zamani AG, Okudan N. Effect of mobile phone station on micronucleus frequency and chromosomal aberrations in human blood cells. Genet Couns. 21(2):243-251, 2010. (HU, LE, GT)

The use of mobile telephones has rapidly increased worldwide as well as the number of mobile phone base stations that lead to rise low level radiofrequency emissions which may in turn have possible harm for human health. The national radiation protection board has published the known effects of radio waves exposure on humans living close to mobile phone base stations. However, several studies have claimed that the base station has detrimental effects on different tissues. In this study, we aimed to evaluate the effects of mobile phone base stations on the micronucleus (MN) frequency and chromosomal aberrations on blood in people who were living around mobile phone base stations and healthy controls. Frequency of MN and chromosomal aberrations in study and control groups was 8.96 +/- 3.51 and 6.97 +/- 1.52 (p: 0.16); 0.36 +/- 0.31 and 0.75 +/- 0.61 (p: 0.07), respectively. Our results show that there was not a significant difference of MN frequency and chromosomal aberrations between the two study groups. The results claim that cellular phones and their base stations do not produce important carcinogenic changes.

(E) Zalata, A., A. Z. El-Samanoudy, D. Shaalan, Y. El-Baiomy, and T. Mostafa. In vitro effect of cell phone radiation on motility, DNA fragmentation and clusterin gene expression of sperm. Int J Fertil Steril, In Press. Published online ahead of print. (GT, GE, RP)

Background: Use of cellular phones that emits radiofrequency electromagnetic field (RF-EMF) has been increased exponentially and became a part of everyday life. This study aimed to investigate the effects of RF-EMF radiation emitted from cellular phones on sperm motility variables, sperm DNA fragmentation and clusterin (CLU) gene expression. Materials and Methods: 124 semen samples were grouped into; normozoospermia (N, n=26), asthenozoospermia (A, n=32), asthenoteratozoospermia (AT, n=31) and oligoasthenoteratozoospermia (OAT, n=35). Semen samples were divided into two aliquots; samples not exposed to cell phone and samples exposed to cell phone radiation (850 MHz, maximum power < 1 watt; SAR 1.46 W/kg at 10 cm distance) for 1 hr. Before and immediately

after exposure both aliquots were subjected to assessment of sperm motility, acrosin activity, sperm DNA fragmentation and CLU gene expression. Statistical differences were analyzed using paired t-student test for comparisons where P< 0.05 was set as significant. Results: There was significant decrease in sperm motility, sperm linear velocity, sperm linearity index, sperm acrosin activity and significant increase in sperm DNA fragmentation percent, CLU gene expression and CLU protein levels in the exposed semen samples to RF-EMF compared with non- exposed samples in OAT > AT > A > N groups (P<0.05).
Conclusions: Cell phone emissions have a negative impact on exposed sperm motility indices, sperm acrosin activity, sperm DNA fragmentation and CLU gene expression especially in OAT cases.

(NE) Zeni O, Schiavoni A, Perrotta A, Forigo D, Deplano M, Scarfi MR. Evaluation of genotoxic effects in human leukocytes after in vitro exposure to 1950 MHz UMTS radiofrequency field. Bioelectromagnetics 29:177-184, 2008. (GT)

In the present study the third generation wireless technology of the Universal Mobile Telecommunication System (UMTS) signal was investigated for the induction of genotoxic effects in human leukocytes. Peripheral blood from six healthy donors was used and, for each donor, intermittent exposures (6 min RF on, 2 h RF off) at the frequency of 1950 MHz were conducted at a specific absorption rate of 2.2 W/kg. The exposures were performed in a transverse electro magnetic (TEM) cell hosted in an incubator under strictly controlled conditions of temperature and dosimetry. Following long duration intermittent RF exposures (from 24 to 68 h) in different stages of the cell cycle, micronucleus formation was evaluated by applying the cytokinesis block micronucleus assay, which also provides information on cell division kinetics. Primary DNA damage (strand breaks/alkali labile sites) was also investigated following 24 h of intermittent RF exposures, by applying the alkaline single cell gel electrophoresis (SCG)/comet assay. Positive controls were included by treating cell cultures with Mitomycin-C and methylmethanesulfonate for micronucleus and comet assays, respectively. The results obtained indicate that intermittent exposures of human lymphocytes in different stages of cell cycle do not induce either an increase in micronucleated cells, or change in cell cycle kinetics; moreover, 24 h intermittent exposures also fail to affect DNA structure of human leukocytes soon after the exposures, likely indicating that repairable DNA damage was not induced.

(E) Zhang DY, Xu ZP, Chiang H, Lu DQ, Zeng QL. [Effects of GSM 1800 MHz radiofrequency electromagnetic fields on DNA damage in Chinese hamster lung cells.] Zhonghua Yu Fang Yi XueZaZhi 40:149-152, 2006. [Article in Chinese] (GT)

OBJECTIVE: To study the effects of GSM 1800 MHz radiofrequency electromagnetic fields (RF EMF) on DNA damage in Chinese hamster lung (CHL) cells. METHODS: The cells were intermittently exposed or sham-exposed to GSM 1800 MHz RF EMF (5 minutes on/10 minutes off) at a special absorption rate (SAR) of 3.0 W/kg for 1 hour or 24 hours. Meanwhile, cells exposed to 2-acetaminofluorene, a DNA damage agent, at a final concentration of 20 mg/L for 2 hours were used as positive control. After exposure, cells were fixed by using 4% paraformaldehyde and processed for phosphorylated form of H2AX (gammaH2AX) immunofluorescence measurement. The primary antibody used for immunofluorescence was mouse monoclonal antibody against gammaH2AX and the secondary antibody was fluorescein

isothiocyanate (FITC)-conjugated goat anti-mouse IgG. Nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI). The gammaH2AX foci and nuclei were visualized with an Olympus AX70 fluorescent microscope. Image Pro-Plus software was used to count the gammaH2AX foci in each cell. For each exposure condition, at least 50 cells were selected to detect gammaH2AX foci. Cells were classified as positive when more than five foci were detected. The percentage of gammaH2AX foci positive cells was adopted as the index of DNA damage. RESULTS: The percentage of gammaH2AX foci positive cell of 1800 MHz RF EMF exposure for 24 hours (37.9 +/- 8.6)% or 2-acetylaminofluorene exposure (50.9 +/- 9.4)% was significantly higher compared with the sham-exposure (28.0 +/- 8.4)%. However, there was no significant difference between the sham-exposure and RF EMF exposure for 1 hour (31.8 +/- 8.7)%. CONCLUSION: 1800 MHz RF EMF (SAR, 3.0 W/kg) for 24 hours might induce DNA damage in CHL cells.

(E) Zhang SZ, Yao GD, Lu DQ, Chiang H, Xu ZP. [Effect of 1.8 GHz radiofrequency electromagnetic fields on gene expression of rat neurons]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 26(8):449-452, 2008. [Article in Chinese] (GE, WS)

OBJECTIVE: To investigate the changes of gene expression in rat neuron induced by 1.8 GHz radiofrequency electromagnetic fields (RF EMF) to screen for RF EMF-responsive genes and the effect of different exposure times and modes on the gene expression in neuron. METHODS: Total RNA was extracted immediately and purified from the primary culture of neurons after intermittent exposed or sham-exposed to a frequency of 1.8 GHz RF EMF for 24 hours at an average special absorption rate (SAR) of 2 W/kg. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron. Differentially expressed genes (Egr-1, Mbp and Plp) were further confirmed by semi-quantitative revere transcription polymerase chain reaction (RT PCR). The expression levels of Egr-1, Mbp and Plp were observed at different exposure times (6, 24 h) and modes (intermittent and continuous exposure). RESULTS: Among 1200 candidate genes, 24 up-regulated and 10 down-regulated genes were found by using Affymetrix microarray suite software 5.0 which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. Under 24 h and 6 h intermittent exposure, Egr-1 and Plp in experiment groups showed statistic significance ($P < 0.05$) compared with the control groups, while expression of Mbp did not change significantly ($P > 0.05$). After 24 h continuous exposure, Egr-1 and Mbp in experiment groups showed statistic significance ($P < 0.05$) compared with the control group, while expression of Plp did not change significantly ($P > 0.05$). Under the same exposure mode 6 h, expression of all the 3 genes did not change significantly. Different times (6, 24 h) and modes (intermittent and continuous exposure) of exposure exerted remarkable different influences on the expression of Egr-1, Mbp, Plp genes ($P < 0.01$). CONCLUSION: The changes of many genes transcription were involved in the effect of 1.8 GHz RF EMF on rat neurons; Down-regulation of Egr-1 and up-regulation of Mbp, Plp indicated the negative effects of RF EMF on neurons; The effect of RF intermittent exposure on gene expression was more obvious than that of continuous exposure; The effect of 24 h RF exposure (both intermittent and continuous) on gene expression was more obvious than that of 6 h (both intermittent and continuous).

(E) Zhao R, Zhang S, Xu Z, Ju L, Lu D, Yao G. Studying gene expression profile of rat neuron exposed to 1800MHz radiofrequency electromagnetic fields with cDNA microassay. Toxicology 235:167-175, 2007. (GE)

A widespread use of mobile phone (MP) evokes a growing concern for their possible adverse effects on human, especially the brain. Gene expression is a unique way of characterizing how cells and organism adapt to changes in the external environment, so the aim of this investigation was to determine whether 1800 MHz radiofrequency electromagnetic fields (RF EMF) can influence the gene expression of neuron. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron after exposed to the pulsed RF EMF at a frequency of 1800 MHz modulated by 217 Hz which is commonly used in MP. Among 1200 candidate genes, 24 up-regulated genes and 10 down-regulated genes were identified after 24-h intermittent exposure at an average special absorption rate (SAR) of 2 W/kg, which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. The results were further confirmed by quantitative real-time polymerase chain reaction (RT PCR). The present results indicated that the gene expression of rat neuron could be altered by exposure to RF EMF under our experimental conditions.

(E) Zhao TY, Zou SP, Knapp PE. Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosci Lett. 412(1):34-38, 2007. (GE, CS)

The health effects of cell phone radiation exposure are a growing public concern. This study investigated whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to a working Global System for Mobile Communication (GSM) cell phone rated at a frequency of 1900MHz. Primary cultures were exposed to cell phone emissions for 2h. We used array analysis and real-time RT-PCR to show up-regulation of caspase-2, caspase-6 and Asc (apoptosis associated speck-like protein containing a card) gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The effects are specific since up-regulation was not seen for other genes associated with apoptosis, such as caspase-9 in either neurons or astrocytes, or Bax in neurons. The results show that even relatively short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

(E) Zhijian C, Xiaoxue L, Yezhen L, Shijie C, Lifen J, Jianlin L, Deqiang L, Jiliang H. Impact of 1.8-GHz radiofrequency radiation (RFR) on DNA damage and repair induced by doxorubicin in human B-cell lymphoblastoid cells. Mutat Res. 695(1-2):16-21, 2010. (GT, IA)

In the present in vitro study, a comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR, SAR of 2W/kg) can influence DNA repair in human B-cell lymphoblastoid cells exposed to doxorubicin (DOX) at the doses of 0microg/ml, 0.05microg/ml, 0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml. The combinative exposures to RFR with DOX were divided into five categories. DNA damage was detected at 0h, 6h, 12h, 18h and 24h after exposure to DOX via the comet assay, and the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage. The results demonstrated that (1) RFR could not directly induce DNA damage of human B-cell lymphoblastoid cells; (2) DOX could significantly induce DNA damage of human B-cell lymphoblastoid cells with the dose-effect

relationship, and there were special repair characteristics of DNA damage induced by DOX; (3) E-E-E type (exposure to RFR for 2h, then simultaneous exposure to RFR and DOX, and exposure to RFR for 6h, 12h, 18h and 24h after exposure to DOX) combinative exposure could obviously influence DNA repair at 6h and 12h after exposure to DOX for four DOX doses (0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml) in human B-cell lymphoblastoid cells.

(NE) Zhijian C, Xiaoxue L, Yezhen L, Deqiang L, Shijie C, Lifen J, Jianlin L, Jiliang H. Influence of 1.8-GHz (GSM) radiofrequency radiation (RFR) on DNA damage and repair induced by X-rays in human leukocytes in vitro. Mutat Res. 677(1-2):100-104, 2009. (GT, IA)

In the present study, the in vitro comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR) can influence DNA repair in human leukocytes exposed to X-rays. The specific energy absorption rate (SAR) of 2 W/kg (the current European safety limit) was applied. The leukocytes from four young healthy donors were intermittently exposed to RFR for 24 h (fields on for 5 min, fields off for 10 min), and then irradiated with X-rays at doses of 0.25, 0.5, 1.0 and 2.0 Gy. DNA damage to human leukocytes was detected using the comet assay at 0, 15, 45, 90, 150 and 240 min after exposure to X-rays. Using the comet assay, the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage; the DNA repair percentage (DRP) served as the indicator of the DNA repair speed. The results demonstrated that (1) the DNA repair speeds of human leukocytes after X-ray exposure exhibited individual differences among the four donors; (2) the intermittent exposures of 1.8-GHz RFR at the SAR of 2 W/kg for 24 h did not directly induce DNA damage or exhibit synergistic effects with X-rays on human leukocytes.

(NE) Ziemann C, Brockmeyer H, Reddy SB, Vijayalaxmi, Prihoda TJ, Kuster N, Tillmann T, Dasenbrock C. Absence of genotoxic potential of 902 MHz (GSM) and 1747 MHz (DCS) wireless communication signals: In vivo two-year bioassay in B6C3F1 mice. Int J Radiat Biol. 85(5):454-464, 2009. (GT, LE)

PURPOSE: The aim of the present investigation was to determine the incidence of micronuclei in peripheral blood erythrocytes of B6C3F1 mice that had been chronically exposed to radiofrequencies (RF) used for mobile communication. MATERIALS AND METHODS: 'Ferris wheels' were used to expose tube-restrained male and female mice to simulated environmental RF signals of the Global System for Mobile Communications (GSM, 902 MHz) or Digital Cellular System (DCS, 1747 MHz). RF signals were applied to the mice for 2 hours/day on 5 days/week for two years, at maximal whole-body-averaged specific absorption rates of 0.4, 1.3, and 4.0 W/kg body weight. Concurrent sham-exposed mice, cage controls, and positive controls injected with mitomycin C were included in this investigation. At necropsy, peripheral blood smears were prepared, and coded slides were stained using May-Grunwald-Giemsa or acridine orange. The incidence of micronuclei was recorded for each mouse in 2000 polychromatic and 2000 normochromatic erythrocytes. RESULTS: There were no significant differences in the frequency of micronuclei between RF-exposed, sham-exposed, and cage control mice, irrespective of the staining/counting method used. Micronuclei were, however, significantly increased in polychromatic erythrocytes of the positive control mice.

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.

(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.

(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

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 observedrat 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, $P \leq 0.01$) 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

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_v1-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

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.

(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

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.

SECTION 7

The Cellular Stress Response: EMF-DNA Interaction

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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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