



## **SECTION 6**

---

# **Genetic Effects of Non-Ionizing Electromagnetic Fields**

2012 Supplement

**Prof. Henry Lai, PhD (Ret.)**  
**Department of Bioengineering**  
**University of Washington**  
**Seattle, WA USA**

Prepared for the BioInitiative Working Group

October 2012

## 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 63% show effects and 37% do not show effects. **[(Effects = 54 (63%) No Effects = 32 (37%)]**

In summary, the new ELF-EMF studies report that 81% show effects and 19% do not show effects **[(Effects= 35 (81%); No Effects= 8 (19%)]**

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) (2007-2012)

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); RFR (Agarwal et al., 2009; Atasoy et al., 2012; Campisi et al., 2010; De Iuliis et al., 2009; Ferreira et al., 2006; Gajski and Garaj-Vrhovac, 2009; Garaj-Vrhovac et al., 2011; Guler et al., 2010; Kesari and Behari, 2009; Kesari et al., 2010; Khalil et al., 2012; Kumar et al., 2010; Luukkunan et al., 2009; Tomruk et al., 2010; 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. There are at least a couple of hundred published papers implicating that EMF affects 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.

An important observation of the studies is that EMF can interact with other entities and synergistically cause genetic effects. These entities include: ELF-EMF- cisplatin (Buldak et al., 2012), bleomycin (Cho et al., 2007), hydrogen peroxide and methyl methane sulfonate (Koyama et al., 2008), menadione (Luukkunan et al., 2011), ionizing radiation (Mairs et al., 2007; Journi et al., 2012), menadione (Markkanen et al., 2008); RFR- chemical mutagens (Baohong et al., 2005), clastogens (Kim et al., 2008), x-rays (Manti et al., 2008), aphidicolin (Tiwari et al., 2008), picrotoxin (López-Martín et al., 2009), 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.

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; Udroui et al., 2006), and RFR (Asasoy et al., 2012; Chavdoula et al., 2010; Ferreira et al., 2006; Garaj-Vrhovac et al., 2011; Guler et al., 2010; 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; Udroui et al., 2006, RFR: Ferreira et al., 2006; Guler et al., 2010; Tomruk et al., 2010). 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; 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.

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 ( $\mu\text{T}$ ) 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).

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. 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 873-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 that 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. 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 complexity 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.

Many biological/health effects have been reported in cells and animals after exposure to EMFs in both the ELF and RF ranges. (Sixty-three percent of the RFR papers and 81% 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 electromagnetic 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).

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 - RFR ABSTRACTS

### Literature on genotoxic effects of radiofrequency radiation (2007-2012)

Keys: **(E)** - effect observed; **(NE)** -no significant effect observed.

**(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).

#### **SUMMARY -**

**Effects = 54 (63%)**

**No Effects = 32 (37%)**

**(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) 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, Lifan 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) 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  $\mu$ T 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 ( $P < 0.02[53BP1]/0.01[\text{gamma-H2AX}]$ ) 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) 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 2012 Feb 21. [Epub ahead of print] (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.*

**(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, 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. 2012 Apr 9. doi: 10.1002/bem.21724. [Epub ahead of print] (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) 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) 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.

**(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 8043-8050, 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) Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (*Apis mellifera*) 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. 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 ( $26.76 \pm 0.054$  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 concerns 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 cytogenetic 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.

**(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 result in significant differences in chromosomal aberrations as compared to controls.*

**(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. 2012 Mar 13. (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 Radiat Biol 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.*

**(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, Koksall 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 absorption 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 158:126-39, 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  $\mu\text{W}/\text{cm}^2$  with nominal specific absorption rate  $8.0 \times 10^{-4}$  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 Exp Toxicol 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/cm<sup>2</sup>, specific absorption rate (SAR) 0.014 W/kg] and 50 GHz (power density 0.86 microW/cm<sup>2</sup>, SAR 8.0 x10<sup>(-4)</sup> 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) 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 lymphocytes 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 deoxynucleotidyltransferase-UTP 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) Papparini 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.*

**(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, Poullietier 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.* 2012 May 8. doi: 10.1111/j.1601-0825.2012.01946.x. [Epub ahead of print] (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 cytogenetic 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, Scarfi 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 Scarfi, 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 disconcerting, 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.*

**(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.

**(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  $\leq$  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) 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, Pavčić 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/m<sup>2</sup>, 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.

**(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 µg/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/cm<sup>2</sup> or 10 mW/cm<sup>2</sup>, 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  $\gamma$  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  $\gamma$  irradiation exhibited significantly more damage than RF-radiation- and sham-exposed lymphocytes.

**(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 1311:189-96. 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 specific 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) 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) 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 Xue Za Zhi 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 reverse 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.*

**SUMMARY -**

**Effects = 54 (63%)**

**No Effects = 32 (37%)**

## APPENDIX B - ELF-EMF ABSTRACTS

### Literature on neurological effects of extremely-low frequency electromagnetic fields (2007-2012)

(**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).

#### **SUMMARY -**

**Effects= 35 (81%)**

**No Effects= 8 (19%)**

**(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  $\mu$ T, 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)** 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  $\mu$ T 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 blastomeres 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) Buldak RJ, Polaniak R, Buldak L, Zwirska-Korczala K, Skonieczna M, Monsiol A, Kukla M, Dulawa-Bulda 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 CH<sub>2</sub> methylene 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, PO<sub>2</sub>- 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  $\beta$ -sheet contents in amide I, and around the 1740 cm<sup>-1</sup> 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  $\beta$ -sheet contents as to  $\alpha$ -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.* 2012 Apr 9. doi: 10.1002/bem.21724. [Epub ahead of print] **(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 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) 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  $\alpha$ (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 $\pm$ SEM: 6.10 $\pm$ 0.39) compared to the control group (4.45 $\pm$ 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 $\pm$ 0.21) compared to controls (4.89 $\pm$ 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  $\pm$  0.43) and (27.88  $\pm$  2.59)%, respectively, which were significantly higher than those of sham-exposure group [(1.77  $\pm$  0.37) and (19.38  $\pm$  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  $\pm$  0.35) and (31.00  $\pm$  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) 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 susceptibility 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  $\mu$ T) 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  $\alpha$ -amylase in the rat mammary gland. Int J Radiat Biol. 88(7):556-564, 2012.**

**PURPOSE:** The issue of whether exposure to environmental power-frequency magnetic fields (MF) has impact on breast cancer development still remains equivocal. Previously, we observed rat strain differences in the MF response of breast tissue, so that the genetic background plays a role in MF effects. The present experiment aimed to elucidate candidate genes involved in MF effects by comparison of MF-susceptible Fischer 344 (F344) rats and MF-insensitive Lewis rats. **MATERIALS AND METHODS:** Female F344 and Lewis rats were exposed to MF (50 Hz, 100  $\mu$ T) for two weeks, and a whole genome microarray analysis in the mammary gland tissue was performed. **RESULTS:** A remarkably decreased  $\alpha$ -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  $\alpha$ -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) 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  $\beta$ -galactosidase (LacZ(-)), containing a mini-transposon Tn10 element able to give ability to express  $\beta$ -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  $\mu$ T 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  $D = 0.4 \text{ h}^{-1}$ ) 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),  $\text{H}_2\text{O}_2$  (100  $\mu\text{M}$ ) 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,  $\text{H}_2\text{O}_2$ , and c-Myc activation.

**CONCLUSIONS:** *Our results demonstrate that ELF-MF did not enhance MN frequency by IR,  $\text{H}_2\text{O}_2$  and 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  $\gamma\text{-H2AX}$ , 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  $62.0 \pm 7.1 \mu\text{T}$  for 2 h or to a sham exposure ( $0.21 \pm 0.05 \mu\text{T}$ ) 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 ( $0.085 \pm 0.01 \mu\text{T}$ ) 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/aprimidinic (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 (H<sub>2</sub>O<sub>2</sub>)) 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 H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>O<sub>2</sub> 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) 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  $\mu$ T 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  $\mu$ T 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) 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<sup>2</sup>) 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.*

**(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  $\leq 5$  microT intensities of 50 Hz EMFs did not cause genotoxic effect on the mouse.*

**(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):109-113, 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  $\mu$ T 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  $\mu$ T may be observed in human lymphocytes in dependence on the initial state of chromatin and temperature.*

**(E) Udroui 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  $\mu$ T magnetic field. **MATERIALS AND METHODS:** The micronucleus test with CREST (Calcinosis, Raynaud's phenomenon, Esophageal dysmotility, Sclerodactyly, Telangiectasia) 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  $\mu$ T, 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) 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  $\mu$ T 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.

**SUMMARY**    **Effects = 35 (81%)**

**No Effects = 8 (19%)**