**April 1, 2017**

# ELF-EMF Comet Assay Studies

Of 46 total studies: **(E= 34** (**74**%**); NE= 12** (**26**%)

(E = reported effect; NE = reported no significant effect)

|  |  |  |  |
| --- | --- | --- | --- |
|  | **E** | **NE** |  |
| ***in vitro*** | 21 | 10 | 31 |
| ***in vivo*** | 13 | 2 | 15 |
|  | 34(74%) | 12 (26%) | 46 |

(Break-out of VO-in vivo VI = in vitro)

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

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

**(NE) (VI)** [**Albert GC**](http://www.ncbi.nlm.nih.gov/pubmed?term=Albert%20GC%5BAuthor%5D&cauthor=true&cauthor_uid=19280467)**,** [**McNamee JP**](http://www.ncbi.nlm.nih.gov/pubmed?term=McNamee%20JP%5BAuthor%5D&cauthor=true&cauthor_uid=19280467)**,** [**Marro L**](http://www.ncbi.nlm.nih.gov/pubmed?term=Marro%20L%5BAuthor%5D&cauthor=true&cauthor_uid=19280467)**,** [**Bellier PV**](http://www.ncbi.nlm.nih.gov/pubmed?term=Bellier%20PV%5BAuthor%5D&cauthor=true&cauthor_uid=19280467)**,** [**Prato FS**](http://www.ncbi.nlm.nih.gov/pubmed?term=Prato%20FS%5BAuthor%5D&cauthor=true&cauthor_uid=19280467)**,** [**Thomas AW**](http://www.ncbi.nlm.nih.gov/pubmed?term=Thomas%20AW%5BAuthor%5D&cauthor=true&cauthor_uid=19280467)**. Assessment of genetic damage in peripheral blood of human volunteers exposed (whole-body) to a 200 muT, 60 Hz magnetic field.** [**Int J Radiat Biol.**](http://www.ncbi.nlm.nih.gov/pubmed/19280467) **85(2):144-152, 2009.**

**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) (VO)** [**Al-Huqail AA**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Al-Huqail%20AA%5BAuthor%5D&cauthor=true&cauthor_uid=26180815)**,** [**Abdelhaliem E**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Abdelhaliem%20E%5BAuthor%5D&cauthor=true&cauthor_uid=26180815)**. Evaluation of Genetic Variations in Maize Seedlings Exposed to Electric Field Based on Protein and DNA Markers.** [**Biomed Res Int.**](http://www.ncbi.nlm.nih.gov/pubmed/26180815) **2015; 2015:874906.**

The current study analyzed proteins and nuclear DNA of electric fields (ELF) exposed and nonexposed maize seedlings for different exposure periods using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), isozymes, random amplified polymorphic DNA (RAPD), and comet assay, respectively. SDS-PAGE analysis revealed total of 46 polypeptides bands with different molecular weights ranging from 186.20 to 36.00 KDa. It generated distinctive polymorphism value of 84.62%. Leucine-aminopeptidase, peroxidase, and catalase isozymes showed the highest values of polymorphism (100%) based on zymograms number, relative front (R f ), and optical intensity while esterase isozyme generated polymorphism value of 83.33%. Amino acids were analyzed using high-performance liquid chromatography, which revealed the presence of 17 amino acids of variable contents ranging from 22.65% to 28.09%. RAPD revealed that 78 amplified DNA products had highly polymorphism value (95.08%) based on band numbers, with variable sizes ranging from 120 to 992 base pairs and band intensity. Comet assay recorded the highest extent of nuclear DNA damage as percentage of tailed DNA (2.38%) and tail moment unit (5.36) at ELF exposure of maize nuclei for 5 days. The current study concluded that the longer ELF exposing periods had genotoxic stress on macromolecules of maize cells and biomarkers used should be augmented for reliable estimates of genotoxicity after exposure of economic plants to ELF stressors.

**(E) (VI)** [**Cho S**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cho%20S%5BAuthor%5D&cauthor=true&cauthor_uid=24479558)**,** [**Lee Y**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lee%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=24479558)**,** [**Lee S**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lee%20S%5BAuthor%5D&cauthor=true&cauthor_uid=24479558)**,** [**Choi YJ**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Choi%20YJ%5BAuthor%5D&cauthor=true&cauthor_uid=24479558)**,** [**Chung HW**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chung%20HW%5BAuthor%5D&cauthor=true&cauthor_uid=24479558)**. Enhanced cytotoxic and genotoxic effects of gadolinium following ELF-EMF irradiation in human lymphocytes.** [**Drug Chem Toxicol.**](http://www.ncbi.nlm.nih.gov/pubmed/24479558) **37(4):440-447, 2014.**

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

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

The effects of pulsed electric fields of low frequency (50 Hz) on DNA of human lymphocytes were investigated. The influence of additional external factors, such as hydrogen peroxide (H2O2) and gamma-irradiation, as well as the repair efficiency in these lymphocytes, was also evaluated. The comet assay, a very sensitive and rapid method for detecting DNA damage at the single cells level was the method used. A significant amount of damage was observed after exposure to the electric fields, compared to the controls. After 2 h incubation at 37 degrees C, a proportion of damage was repaired. H2O2 and gamma-irradiation increased the damage to lymphocytes exposed to pulsed electric fields according to the dose used, while the amount of the repair was proportional to the damage.

**(E)** **(VI)** [**Duan W**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Duan%20W%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**Liu C**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20C%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**Zhang L**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhang%20L%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**He M**](http://www.ncbi.nlm.nih.gov/pubmed/?term=He%20M%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**Xu S**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Xu%20S%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**Chen C**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chen%20C%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**Pi H**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pi%20H%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**Gao P**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Gao%20P%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**Zhang Y**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhang%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**Zhong M**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhong%20M%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**Yu Z**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Yu%20Z%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**,** [**Zhou Z**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhou%20Z%5BAuthor%5D&cauthor=true&cauthor_uid=25688995)**. Comparison of the Genotoxic Effects Induced by 50 Hz Extremely Low-Frequency Electromagnetic Fields and 1800 MHz Radiofrequency Electromagnetic Fields in GC-2 Cells.** [**Radiat Res.**](http://www.ncbi.nlm.nih.gov/pubmed/25688995?dopt=Abstract) **2015 Feb 17. [Epub ahead of print]**

Extremely low-frequency electromagnetic fields (ELF-EMF) and radiofrequency electromagnetic fields (RF-EMF) have been considered to be possibly carcinogenic to humans. However, their genotoxic effects remain controversial. To make experiments controllable and results comparable, we standardized exposure conditions and explored the potential genotoxicity of 50 Hz ELF-EMF and 1800 MHz RF-EMF. A mouse spermatocyte-derived GC-2 cell line was intermittently (5 min on and 10 min off) exposed to 50 Hz ELF-EMF at an intensity of 1, 2 or 3 mT or to RF-EMF in GSM-Talk mode at the specific absorption rates (SAR) of 1, 2 or 4 W/kg. After exposure for 24 h, we found that neither ELF-EMF nor RF-EMF affected cell viability using Cell Counting Kit-8. Through the use of an alkaline comet assay and immunofluorescence against γ-H2AX foci, we found that ELF-EMF exposure resulted in a significant increase of DNA strand breaks at 3 mT, whereas RF-EMF exposure had insufficient energy to induce such effects. Using a formamidopyrimidine DNA glycosylase (FPG)-modified alkaline comet assay, we observed that RF-EMF exposure significantly induced oxidative DNA base damage at a SAR value of 4 W/kg, whereas ELF-EMF exposure did not. Our results suggest that both ELF-EMF and RF-EMF under the same experimental conditions may produce genotoxicity at relative high intensities, but they create different patterns of DNA damage. Therefore, the potential mechanisms underlying the genotoxicity of different frequency electromagnetic fields may be different.

**(E)** **(VO)** [**El-Bialy NS**](http://www.ncbi.nlm.nih.gov/pubmed?term=El-Bialy%20NS%5BAuthor%5D&cauthor=true&cauthor_uid=23484088)**,** [**Rageh MM**](http://www.ncbi.nlm.nih.gov/pubmed?term=Rageh%20MM%5BAuthor%5D&cauthor=true&cauthor_uid=23484088)**. Extremely low-frequency magnetic field enhances the therapeutic efficacy of low-dose cisplatin in the treatment of Ehrlich carcinoma.** [**Biomed Res Int.**](http://www.ncbi.nlm.nih.gov/pubmed/23484088) **2013;2013:189352. doi: 10.1155/2013/189352. Epub 2013 Jan 14.**

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

**(NE) (VI)** [**Fairbairn DW**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Fairbairn%20DW%5BAuthor%5D&cauthor=true&cauthor_uid=8061573)**,** [**O'Neill KL**](http://www.ncbi.nlm.nih.gov/pubmed/?term=O'Neill%20KL%5BAuthor%5D&cauthor=true&cauthor_uid=8061573)**. The effect of electromagnetic field exposure on the formation of DNA single strand breaks in human cells.** [**Cell Mol Biol (Noisy-le-grand).**](http://www.ncbi.nlm.nih.gov/pubmed/8061573) **40(4):561-567, 1994.**

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

**(E)** **(VI)** [**Focke F**](http://www.ncbi.nlm.nih.gov/pubmed?term=Focke%20F%5BAuthor%5D&cauthor=true&cauthor_uid=19896957)**,** [**Schuermann D**](http://www.ncbi.nlm.nih.gov/pubmed?term=Schuermann%20D%5BAuthor%5D&cauthor=true&cauthor_uid=19896957)**,** [**Kuster N**](http://www.ncbi.nlm.nih.gov/pubmed?term=Kuster%20N%5BAuthor%5D&cauthor=true&cauthor_uid=19896957)**,** [**Schär P**](http://www.ncbi.nlm.nih.gov/pubmed?term=Sch%C3%A4r%20P%5BAuthor%5D&cauthor=true&cauthor_uid=19896957)**. DNA fragmentation in human fibroblasts under extremely low frequency electromagnetic field exposure.**[**Mutat Res.**](http://www.ncbi.nlm.nih.gov/pubmed/19896957) **683(1-2):74-83, 2010.**

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) (VI) 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. [Article in Chinese]**

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

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

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

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

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

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

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

**(E) (VI) Jajte J, Zmyslony M, Palus J, Dziubaltowska E, Rajkowska E. Protective effect of melatonin against in vitro iron ions and 7 mT 50 Hz magnetic field-induced DNA damage in rat lymphocytes. Mutat Res. 483(1-2):57-64, 2001.**

We have previously shown that simultaneous exposure of rat lymphocytes to iron ions and 50Hz magnetic field (MF) caused an increase in the number of cells with DNA strand breaks. Although the mechanism of MF-induced DNA damage is not known, we suppose that it involves free radicals. In the present study, to confirm our hypothesis, we have examined the effect of melatonin, an established free radicals scavenger, on DNA damage in rat peripheral blood lymphocytes exposed in vitro to iron ions and 50Hz MF. The alkaline comet assay was chosen for the assessment of DNA damage. During preincubation, part of the cell samples were supplemented with melatonin (0.5 or 1.0mM). The experiments were performed on the cell samples incubated for 3h in Helmholtz coils at 7mT 50Hz MF. During MF exposure, some samples were treated with ferrous chloride (FeCl2, 10microg/ml), while the rest served as controls. A significant increase in the number of cells with DNA damage was found only after simultaneous exposure of lymphocytes to FeCl2 and 7mT 50Hz MF, compared to the control samples or those incubated with FeCl2 alone. However, when the cells were treated with melatonin and then exposed to iron ions and 50Hz MF, the number of damaged cells was significantly reduced, and the effect depended on the concentration of melatonin. The reduction reached about 50% at 0.5mM and about 100% at 1.0mM. Our results indicate that melatonin provides protection against DNA damage in rat lymphocytes exposed in vitro to iron ions and 50Hz MF (7mT). Therefore, it can be suggested that free radicals may be involved in 50Hz magnetic field and iron ions-induced DNA damage in rat blood lymphocytes. The future experimental studies, in vitro and in vivo, should provide an answer to the question concerning the role of melatonin in the free radical processes in the power frequency magnetic field.

**(NE) (VI)**  [**Jin YB**](http://www.ncbi.nlm.nih.gov/pubmed?term=Jin%20YB%5BAuthor%5D&cauthor=true&cauthor_uid=24305851)**,** [**Choi SH**](http://www.ncbi.nlm.nih.gov/pubmed?term=Choi%20SH%5BAuthor%5D&cauthor=true&cauthor_uid=24305851)**,** [**Lee JS**](http://www.ncbi.nlm.nih.gov/pubmed?term=Lee%20JS%5BAuthor%5D&cauthor=true&cauthor_uid=24305851)**,** [**Kim JK**](http://www.ncbi.nlm.nih.gov/pubmed?term=Kim%20JK%5BAuthor%5D&cauthor=true&cauthor_uid=24305851)**,** [**Lee JW**](http://www.ncbi.nlm.nih.gov/pubmed?term=Lee%20JW%5BAuthor%5D&cauthor=true&cauthor_uid=24305851)**,** [**Hong SC**](http://www.ncbi.nlm.nih.gov/pubmed?term=Hong%20SC%5BAuthor%5D&cauthor=true&cauthor_uid=24305851)**,** [**Myung SH**](http://www.ncbi.nlm.nih.gov/pubmed?term=Myung%20SH%5BAuthor%5D&cauthor=true&cauthor_uid=24305851)**,** [**Lee YS**](http://www.ncbi.nlm.nih.gov/pubmed?term=Lee%20YS%5BAuthor%5D&cauthor=true&cauthor_uid=24305851)**. Absence of DNA damage after 60-Hz electromagnetic field exposure combined with ionizing radiation, hydrogen peroxide, or c-Myc overexpression.** [**Radiat Environ Biophys.**](http://www.ncbi.nlm.nih.gov/pubmed/24305851) **2013 Dec 5. [Epub ahead of print]**

The principal objective of this study was to assess the DNA damage in a normal cell line system after exposure to 60 Hz of extremely low frequency magnetic field (ELF-MF) and particularly in combination with various external factors, via comet assays. NIH3T3 mouse fibroblast cells, WI-38 human lung fibroblast cells, L132 human lung epithelial cells, and MCF10A human mammary gland epithelial cells were exposed for 4 or 16 h to a 60-Hz, 1 mT uniform magnetic field in the presence or absence of ionizing radiation (IR, 1 Gy), H2O2 (50 μM), or c-Myc oncogenic activation. The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic or additive effects were observed after 4 or 16 h of pre-exposure to 1 mT ELF-MF or simultaneous exposure to ELF-MF combined with IR, H2O2, or c-Myc activation.

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

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

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

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

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

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

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

In previous research, we have found an increase in DNA single- and double-strand breaks in brain cells of rats after acute exposure (two hours) to a sinusoidal 60-Hz magnetic field. The present experiment was carried out to investigate whether treatment with melatonin and the spin-trap compound N-tert-butyl-alpha-phenylnitrone (PBN) could block the effect of magnetic fields on brain cell DNA. Rats were injected with melatonin (1 mg/kg, sc) or PBN (100 mg/kg, ip) immediately before and after two hours of exposure to a 60-Hz magnetic field at an intensity of 0.5 mT. We found that both drug treatments blocked the magnetic field-induced DNA single- and double-strand breaks in brain cells, as assayed by a microgel electrophoresis method. Since melatonin and PBN are efficient free radical scavengers, these data suggest that free radicals may play a role in magnetic field-induced DNA damage.

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

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

**(E) (VI)** [**Luukkonen J**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Luukkonen%20J%5BAuthor%5D&cauthor=true&cauthor_uid=27646005)**,** [**Höytö A**](https://www.ncbi.nlm.nih.gov/pubmed/?term=H%C3%B6yt%C3%B6%20A%5BAuthor%5D&cauthor=true&cauthor_uid=27646005)**,** [**Sokka M**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sokka%20M%5BAuthor%5D&cauthor=true&cauthor_uid=27646005)**,** [**Liimatainen A**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Liimatainen%20A%5BAuthor%5D&cauthor=true&cauthor_uid=27646005)**,** [**Syväoja J**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Syv%C3%A4oja%20J%5BAuthor%5D&cauthor=true&cauthor_uid=27646005)**,** [**Juutilainen J**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Juutilainen%20J%5BAuthor%5D&cauthor=true&cauthor_uid=27646005)**,** [**Naarala J**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Naarala%20J%5BAuthor%5D&cauthor=true&cauthor_uid=27646005)**. Modification of p21 level and cell cycle distribution by 50 Hz magnetic fields in human SH-SY5Y neuroblastoma cells.** [**Int J Radiat Biol.**](https://www.ncbi.nlm.nih.gov/pubmed/27646005) **2017 Feb;93(2):240-248, 2017.**

PURPOSE: In our previous studies, exposure to extremely low frequency (ELF) magnetic fields (MF) altered responses to DNA damage caused by menadione. The aim of this study was to evaluate possible ELF MF induced changes in proteins involved in DNA damage responses and in cell cycle distribution. MATERIALS AND METHODS: Based on our previous studies, the exposure protocol included pre-exposure of human SH-SY5Y neuroblastoma cells to a 50 Hz, 100 μT MF for 24 h prior to a 3-h menadione treatment. As DNA damage responses are relatively fast processes, a 1-h menadione treatment was also included in the experiments. The menadione concentrations used were 1, 10, 15, 20, and 25 μM. Immunoblotting was used to assess the levels of DNA damage response-related proteins (γ-H2AX, Chk1, phospho-Chk1, p21, p27, and p53), while the level of DNA damage was assessed by the alkaline Comet assay. Cell cycle distribution was assayed by SYTOX Green staining followed by flow cytometry analysis. RESULTS: The main findings in MF-exposed cells were decreased p21 protein level after the 1-h menadione treatment, as well as increased proportion of cells in the G1 phase and decreased proportion of S phase cells after the 3-h menadione treatment. These effects were detectable also in the absence of menadione. CONCLUSIONS: The results indicate that MF exposure can alter the G1 checkpoint response and that the p21 protein may be involved in early responses to MF exposure.

**(E)** **(VO)** [**Mariucci G**](http://www.ncbi.nlm.nih.gov/pubmed?term=Mariucci%20G%5BAuthor%5D&cauthor=true&cauthor_uid=20569191)**,** [**Villarini M**](http://www.ncbi.nlm.nih.gov/pubmed?term=Villarini%20M%5BAuthor%5D&cauthor=true&cauthor_uid=20569191)**,** [**Moretti M**](http://www.ncbi.nlm.nih.gov/pubmed?term=Moretti%20M%5BAuthor%5D&cauthor=true&cauthor_uid=20569191)**,** [**Taha E**](http://www.ncbi.nlm.nih.gov/pubmed?term=Taha%20E%5BAuthor%5D&cauthor=true&cauthor_uid=20569191)**,** [**Conte C**](http://www.ncbi.nlm.nih.gov/pubmed?term=Conte%20C%5BAuthor%5D&cauthor=true&cauthor_uid=20569191)**,** [**Minelli A**](http://www.ncbi.nlm.nih.gov/pubmed?term=Minelli%20A%5BAuthor%5D&cauthor=true&cauthor_uid=20569191)**,** [**Aristei C**](http://www.ncbi.nlm.nih.gov/pubmed?term=Aristei%20C%5BAuthor%5D&cauthor=true&cauthor_uid=20569191)**,** [**Ambrosini MV**](http://www.ncbi.nlm.nih.gov/pubmed?term=Ambrosini%20MV%5BAuthor%5D&cauthor=true&cauthor_uid=20569191)**. Brain DNA damage and 70-kDa heat shock protein expression in CD1 mice exposed to extremely low frequency magnetic fields.** [**Int J Radiat Biol.**](http://www.ncbi.nlm.nih.gov/pubmed/20569191) **86(8):701-710, 2010.**

**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.

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

Several recent studies have reported that whole-body exposure of rodents to power frequency magnetic fields (MFs) can result in DNA single- and double-strand breaks in the brains of these animals. The current study was undertaken to investigate whether an acute 2h exposure of a 1 mT, 60 Hz MF could elicit DNA damage, and subsequently apoptosis, in the brains of immature (10-day-old) mice. DNA damage was quantitated at 0, 2, 4, and 24h after exposure using the alkaline comet assay. Apoptosis was quantitated in the external granule cell layer (EGCL) of the immature mouse cerebellum at 0 and 24h after exposure to MF by the TdT-mediated dUTP nick-end labeling (TUNEL) assay. Four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. While increased DNA damage was detected by tail ratio at 2h after MF exposure, no supporting evidence of increased DNA damage was detected by the other parameters. In addition, no similar differences were observed using these parameters at any of the other post-exposure times. No increase in apoptosis was observed in the EGCL of MF-exposed mice, when compared to sham mice. Taken together, these results do not support the hypothesis that acute MF exposure causes DNA damage in the cerebellums of immature mice.

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

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

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

We examined the effect of an extremely low-frequency magnetic field (ELFMF) at 5, 50 and 400 mT on DNA strand breaks in human glioma MO54 cells. A DNA damage analysis was performed using the method of alkaline comet assay. The cells were exposed to X-rays alone (5 Gy), ELFMF alone, or X-rays followed by ELFMF at 4 degrees C or on ice. No significant difference in the tail moment was observed between control and ELFMF exposures up to 400 mT. X-ray irradiation increased DNA strand breaks. When cells were exposed to X-rays followed by ELFMF at 50 and 400 mT, the tail moment increased significantly compared with that for X-rays alone. When the exposure of cells was performed at 37 degrees C, no significant change was observed between X-rays alone and X-rays plus 400 mT. We previously observed that exposure to 400 mT ELFMF for 2 h increased X-ray-induced mutations (Miyakoshi et al, Mutat. Res., 349: 109-114, 1996). Additionally, an increase in the mutation by exposure to the ELFMF was observed in cells during DNA-synthesizing phase (Miyakoshi et al., Int. J. Radiat. Biol., 71: 75-79, 1997). From these results, it appears that exposure to the high density ELFMF at more than 50 mT may potentiate X-ray-induced DNA strand breaks.

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

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

**(NE) (VI)** [**Nakayama M**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Nakayama%20M%5BAuthor%5D&cauthor=true&cauthor_uid=27430265)**,** [**Nakamura A**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Nakamura%20A%5BAuthor%5D&cauthor=true&cauthor_uid=27430265)**,** [**Hondou T**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hondou%20T%5BAuthor%5D&cauthor=true&cauthor_uid=27430265)**,** [**Miyata H**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Miyata%20H%5BAuthor%5D&cauthor=true&cauthor_uid=27430265)**. Evaluation of cell viability, DNA single-strand breaks, and nitric oxide production in LPS-stimulated macrophage RAW264 exposed to a 50-Hz magnetic field.** [**Int J Radiat Biol.**](http://www.ncbi.nlm.nih.gov/pubmed/27430265) **2016 Jul 19:1-7. [Epub ahead of print]**

#### PURPOSE: Synergistic effects between cellular oxidative stress and magnetic fields may explain the adverse biological effects of 50/60 Hz magnetic fields. To determine whether this hypothesis holds in macrophage RAW264 cells, we measured DNA single-strand breaks (SSB), cell viability, and nitric oxide (NO) production in cells with or without exposure to 0.5-mT, 50-Hz magnetic fields for 24 h and with or without simultaneous stimulation via the bacterial endotoxin, lipopolysaccharide (LPS). MATERIALS AND METHODS: Macrophages stimulated with 10 ng/ml LPS for 1 h were exposed to or not exposed to a magnetic field and were then subjected to (1) the alkaline comet assay to measure SSBs, (2) trypan-blue exclusion assay for cell viability, and (3) measurements of NO for evaluation of oxidative stress. RESULTS: The 50-Hz magnetic field enhanced DNA SSB and decreased cell viability only in the LPS-stimulated macrophages in which NO production was greatly enhanced. The magnetic field alone did not alter NO production. CONCLUSION: Co-stimulation of the cell with LPS and a 50-Hz magnetic field promoted SSB and lowered cell viability, but these were not mediated by LPS-induced NO production.

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

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, apoptosisrelated, 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) (VO)** [**Rageh MM**](http://www.ncbi.nlm.nih.gov/pubmed?term=Rageh%20MM%5BAuthor%5D&cauthor=true&cauthor_uid=23091355)**,** [**El-Gebaly RH**](http://www.ncbi.nlm.nih.gov/pubmed?term=El-Gebaly%20RH%5BAuthor%5D&cauthor=true&cauthor_uid=23091355)**,** [**El-Bialy NS**](http://www.ncbi.nlm.nih.gov/pubmed?term=El-Bialy%20NS%5BAuthor%5D&cauthor=true&cauthor_uid=23091355)**. Assessment of genotoxic and cytotoxic hazards in brain and bone marrow cells of newborn rats exposed to extremely low-frequency magnetic field.** [**J Biomed Biotechnol.**](http://www.ncbi.nlm.nih.gov/pubmed/23091355) **2012;2012:716023.**

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

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

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

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

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

**(E) (VI)** [**Scassellati Sforzolini G**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Scassellati%20Sforzolini%20G%5BAuthor%5D&cauthor=true&cauthor_uid=15554538)**,** [**Moretti M**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Moretti%20M%5BAuthor%5D&cauthor=true&cauthor_uid=15554538)**,** [**Villarini M**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Villarini%20M%5BAuthor%5D&cauthor=true&cauthor_uid=15554538)**,** [**Fatigoni C**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Fatigoni%20C%5BAuthor%5D&cauthor=true&cauthor_uid=15554538)**,** [**Pasquini R**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pasquini%20R%5BAuthor%5D&cauthor=true&cauthor_uid=15554538)**. [Evaluation of genotoxic and/or co-genotoxic effects in cells exposed in vitro to extremely-low frequency electromagnetic fields].** [**Ann Ig.**](http://www.ncbi.nlm.nih.gov/pubmed/15554538) **16(1-2):321-240, 2004. [Article in Italian]**

During the last two decades, concerns have arisen regarding a possible association between extremely-low frequency (ELF) electromagnetic fields (EMF) exposure and cancer incidence (e.g. childhood acute leukaemia, cancer of the nervous system, and lymphomas). In 1979, Wertheimer and Leeper firstly reported an excess of cancer mortality among children living in homes located near power lines and presumably exposed to elevated magnetic fields. Subsequently, a large number of epidemiological studies investigated the possible association between residential or occupational exposure to ELF-EMF and cancer. Several in vivo and in vitro models have been investigated with the effort to determine a link, if any, between such fields and mutagenesis and to determine the possible mechanism of cancer risk. However, a causal relationship between exposure to ELF-EMF and cancer has been suggested but has not been unequivocally demonstrated. In 1998, following an analysis of the results retrieved in the literature, the U.S. National Institute of Environmental Health Sciences proposed to apply a "possible human carcinogen" category (Group 2B) to ELF-EMF. More recently, in 2002, the same classification for ELF-MF was proposed by the International Agency for Research on Cancer. In this in vitro approach, to test the genotoxic and/or co-genotoxic potency of ELF-MF, we used the alkaline single-cell microgel-electrophoresis (comet) assay and the cytokinesis block micronucleus test. Co-exposure assays were performed in the presence of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 4-nitroquinoline N-oxide (4NQO), benzene, 1,4-benzenediol (1,4-BD), or 1,2,4-benzenetriol (1,2,4-BT). An ELF-MF (50 Hz, 5 mT) was obtained by a system composed of capsulated induction coils. ELF-MF alone was unable to cause direct primary DNA damage. Whereas, an increased extent of DNA damage was observed in cells co-exposed to ELF-MF and MNNG, 1,4-BD, or 1,2,4-BT. An opposite trend was observed in cells treated with 4NQO and co-exposed to ELF-MF. Moreover, the frequency of micronucleated cells in ELF-MF-exposed cells was higher than in control cultures. Our findings suggest that the tested ELF-MF (50 Hz, 5 mT) possess genotoxic (micronucleus test) and co-genotoxic (comet assay) capabilities. The possibility that ELF-MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

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

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

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

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

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

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

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

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

**(E)** **(VO)** [**Tiwari R**](http://www.ncbi.nlm.nih.gov/pubmed?term=Tiwari%20R%5BAuthor%5D&cauthor=true&cauthor_uid=24460415)**,** [**Lakshmi NK**](http://www.ncbi.nlm.nih.gov/pubmed?term=Lakshmi%20NK%5BAuthor%5D&cauthor=true&cauthor_uid=24460415)**,** [**Bhargava SC**](http://www.ncbi.nlm.nih.gov/pubmed?term=Bhargava%20SC%5BAuthor%5D&cauthor=true&cauthor_uid=24460415)**,** [**Ahuja YR**](http://www.ncbi.nlm.nih.gov/pubmed?term=Ahuja%20YR%5BAuthor%5D&cauthor=true&cauthor_uid=24460415)**. Epinephrine, DNA integrity and oxidative stress in workers exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) at 132 kV substations.** [**Electromagn Biol Med.**](http://www.ncbi.nlm.nih.gov/pubmed/24460415) **2014 Jan 24. [Epub ahead of print]**

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

**(E) (VO)** [**Udroiu I**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Udroiu%20I%5BAuthor%5D&cauthor=true&cauthor_uid=26559811)**,** [**Antoccia A**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Antoccia%20A%5BAuthor%5D&cauthor=true&cauthor_uid=26559811)**,** [**Tanzarella C**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Tanzarella%20C%5BAuthor%5D&cauthor=true&cauthor_uid=26559811)**,** [**Giuliani L**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Giuliani%20L%5BAuthor%5D&cauthor=true&cauthor_uid=26559811)**,** [**Pacchierotti F**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Pacchierotti%20F%5BAuthor%5D&cauthor=true&cauthor_uid=26559811)**,** [**Cordelli E**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cordelli%20E%5BAuthor%5D&cauthor=true&cauthor_uid=26559811)**,** [**Eleuteri P**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Eleuteri%20P%5BAuthor%5D&cauthor=true&cauthor_uid=26559811)**,** [**Villani P**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Villani%20P%5BAuthor%5D&cauthor=true&cauthor_uid=26559811)**,** [**Sgura A**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Sgura%20A%5BAuthor%5D&cauthor=true&cauthor_uid=26559811)**. Genotoxicity Induced by Foetal and Infant Exposure to Magnetic Fields and Modulation of Ionising Radiation Effects.** [**PLoS One.**](http://www.ncbi.nlm.nih.gov/pubmed/26559811) **2015 Nov 11;10(11):e0142259. doi: 10.1371/journal.pone.0142259. eCollection 2015.**

#### BACKGROUND: Few studies have investigated the toxicity and genotoxicity of extremely low frequency magnetic fields (ELF-MF) during prenatal and neonatal development. These phases of life are characterized by cell proliferation and differentiation, which might make them sensitive to environmental stressors. Although in vitro evidences suggest that ELF-MF may modify the effects of ionizing radiation, no research has been conducted so far in vivo on the genotoxic effects of ELF-MF combined with X-rays. AIM AND METHODS: Aim of this study was to investigate in somatic and germ cells the effects of chronic ELF-MF exposure from mid gestation until weaning, and any possible modulation produced by ELF-MF exposure on ionizing radiation-induced damage. Mice were exposed to 50 Hz, 65 μT magnetic field, 24 hours/day, for a total of 30 days, starting from 12 days post-conception. Another group was irradiated with 1 Gy X-rays immediately before ELF-MF exposure, other groups were only X-irradiated or sham-exposed. Micronucleus test on blood erythrocytes was performed at multiple times from 1 to 140 days after birth. Additionally, 42 days after birth, genotoxic and cytotoxic effects on male germ cells were assessed by comet assay and flow cytometric analysis. RESULTS: ELF-MF exposure had no teratogenic effect and did not affect survival, growth and development. The micronucleus test indicated that ELF-MF induced a slight genotoxic damage only after the maximum exposure time and that this effect faded away in the months following the end of exposure. ELF-MF had no effects on ionizing radiation (IR)-induced genotoxicity in erythrocytes. Differently, ELF-MF appeared to modulate the response of male germ cells to X-rays with an impact on proliferation/differentiation processes. These results point to the importance of tissue specificity and development on the impact of ELF-MF on the early stages of life and indicate the need of further research on the molecular mechanisms underlying ELF-MF biological effects.

**(E) (VO)** [**Villarini M**](http://www.ncbi.nlm.nih.gov/pubmed?term=Villarini%20M%5BAuthor%5D&cauthor=true&cauthor_uid=23484452)**,** [**Ambrosini MV**](http://www.ncbi.nlm.nih.gov/pubmed?term=Ambrosini%20MV%5BAuthor%5D&cauthor=true&cauthor_uid=23484452)**,** [**Moretti M**](http://www.ncbi.nlm.nih.gov/pubmed?term=Moretti%20M%5BAuthor%5D&cauthor=true&cauthor_uid=23484452)**,** [**Dominici L**](http://www.ncbi.nlm.nih.gov/pubmed?term=Dominici%20L%5BAuthor%5D&cauthor=true&cauthor_uid=23484452)**,** [**Taha E**](http://www.ncbi.nlm.nih.gov/pubmed?term=Taha%20E%5BAuthor%5D&cauthor=true&cauthor_uid=23484452)**,** [**Piobbico D**](http://www.ncbi.nlm.nih.gov/pubmed?term=Piobbico%20D%5BAuthor%5D&cauthor=true&cauthor_uid=23484452)**,** [**Gambelunghe C**](http://www.ncbi.nlm.nih.gov/pubmed?term=Gambelunghe%20C%5BAuthor%5D&cauthor=true&cauthor_uid=23484452)**,** [**Mariucci G**](http://www.ncbi.nlm.nih.gov/pubmed?term=Mariucci%20G%5BAuthor%5D&cauthor=true&cauthor_uid=23484452)**. Brain hsp70 expression and DNA damage in mice exposed to extremely low frequency magnetic fields: a dose-response study.** [**Int J Radiat Biol.**](http://www.ncbi.nlm.nih.gov/pubmed/23484452) **89(7):562-570, 2013.**

Purpose: To determine whether a dose-response relationship exists among exposure to extremely low frequency magnetic fields (ELF-MF) at different densities and 70-kDa heat shock protein (hsp70) expression and DNA damage in mouse brain. Materials and Methods: Male CD1 mice were exposed to ELF-MF (50 Hz; 0.1, 0.2, 1 or 2 mT) for 7 days (15 hours/day) and sacrificed either at the end of exposure or after 24 h. Hsp70 expression was determined in cerebral cortex-striatum, hippocampus and cerebellum by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and western blot analysis. Primary DNA damage was evaluated in the same tissues by comet assay. Sham-exposed mice were used as controls. Results: No changes in both hsp70 mRNA and corresponding protein occurred following exposure to ELF-MF, except for a weak increase in the mRNA in hippocampus of exposed mice to 0.1 mT ELF-MF. Only mice exposed to 1 or 2 mT and sacrificed immediately after exposure presented DNA strand breaks higher than controls in all the cerebral areas; such DNA breakage reverted to baseline in the mice sacrificed 24 h after exposure. Conclusions: These data show that high density ELF-MF only induce reversible brain DNA damage while they do not affect hsp70 expression.

**(E) (VO)**  [**Villarini M**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Villarini%20M%5BAuthor%5D&cauthor=true&cauthor_uid=26152536)**,** [**Dominici L**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Dominici%20L%5BAuthor%5D&cauthor=true&cauthor_uid=26152536)**,** [**Fatigoni C**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Fatigoni%20C%5BAuthor%5D&cauthor=true&cauthor_uid=26152536)**,** [**Levorato S**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Levorato%20S%5BAuthor%5D&cauthor=true&cauthor_uid=26152536)**,** [**Vannini S**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Vannini%20S%5BAuthor%5D&cauthor=true&cauthor_uid=26152536)**,** [**Monarca S**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Monarca%20S%5BAuthor%5D&cauthor=true&cauthor_uid=26152536)**,** [**Moretti M**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Moretti%20M%5BAuthor%5D&cauthor=true&cauthor_uid=26152536)**. Primary DNA damage in welders occupationally exposed to extremely-low-frequency magnetic fields (ELF-MF).** [**Ann Ig.**](http://www.ncbi.nlm.nih.gov/pubmed/26152536) **27(3):511-519, 2015.**

#### BACKGROUND: Electric arc welding is known to involve considerable exposure to extremely-low-frequency magnetic fields (ELF-MF; 50 Hz). The aim of the present study was to evaluate individual exposure to ELF-MF during arc welding and to assess the eventually associated genotoxic hazard by evaluating primary DNA damage. METHODS: The study group comprised 21 electric arc welders (exposed) and 21 non-exposed control subjects (healthy blood donors). Occupational exposure to ELF-MF was measured using personal dosimeters worn during one complete work-shift (7 am to 5 pm). The extent of primary DNA damage was measured in peripheral blood leukocytes with the standard procedure of the alkaline comet assay. RESULTS: Tail length showed to have similar values in welders and controls. Whereas, the data showed a significant decrease for tail intensity (p = 0.01) and tail moment (p = 0.02) counts in exposed subjects compared to controls. CONCLUSIONS: The different results of our present study and published investigations from other research groups reporting positive results in the comet assay might be a result of different chromium and/or nickel (or other metals) exposure levels, which lead to DNA-protein cross-links at lower concentrations and DNA single-strand breakages at higher concentrations. Since these results are derived from a small-scale pilot study, a larger scale study should be undertaken.

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

In the present study, we used human peripheral blood leukocytes from 4 different donors, to investigate in vitro the possible genotoxic and/or co-genotoxic activity of extremely low frequency magnetic fields (ELF-MF) at 3 mT intensity. Two model mutagens were used to study the possible interaction between ELF-MF and xenobiotics: N-methyl-N'nitro-N-nitrosoguanidine (MNNG) and 4-nitroquinoline N-oxide (4NQO). Primary DNA damage was evaluated by the alkaline single-cell microgel-electrophoresis ("comet") assay. Control cells (leukocytes not exposed to ELF-MF, nor treated with genotoxins) from the different blood donors showed a comparable level of basal DNA damage, whereas the contribution of individual susceptibility toward ELF-MF and the tested genotoxic compounds led to differences in the extent of DNA damage observed following exposure to the genotoxins, both in the presence and in the absence of an applied ELF-MF. A 3 mT ELF-MF alone was unable to cause direct primary DNA damage. In leukocytes exposed to ELF-MF and genotoxins, the extent of MNNG-induced DNA damage increased with exposure duration compared to sham-exposed cells. The opposite was observed in cells treated with 4NQO. In this case the extent of 4NQOinduced DNA damage was somewhat reduced in leukocytes exposed to ELF-MF compared to sham-exposed cells. Moreover, in cells exposed to ELF-MF an increased concentration of GSH was always observed, compared to sham-exposed cells. Since following GSH conjugation the genotoxic pattern of MNNG and 4NQO is quite different, an influence of ELF-MF on the activity of the enzyme involved in the synthesis of GSH leading to different activation/deactivation of the model mutagens used was hypothesized to explain the different trends observed in MNNG and 4NQO genotoxic activity in the presence of an applied ELF-MF. The possibility that ELF-MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

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

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

**(E) (VI)** [**Yin C**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Yin%20C%5BAuthor%5D&cauthor=true&cauthor_uid=27470406)**,** [**Luo X**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Luo%20X%5BAuthor%5D&cauthor=true&cauthor_uid=27470406)**,** [**Duan Y**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Duan%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=27470406)**,** [**Duan W**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Duan%20W%5BAuthor%5D&cauthor=true&cauthor_uid=27470406)**,** [**Zhang H**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Zhang%20H%5BAuthor%5D&cauthor=true&cauthor_uid=27470406)**,** [**He Y**](https://www.ncbi.nlm.nih.gov/pubmed/?term=He%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=27470406)**,** [**Sun G**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sun%20G%5BAuthor%5D&cauthor=true&cauthor_uid=27470406)**,** [**Sun X**](https://www.ncbi.nlm.nih.gov/pubmed/?term=Sun%20X%5BAuthor%5D&cauthor=true&cauthor_uid=27470406)**. Neuroprotective effects of lotus seedpod procyanidins on extremely low frequency electromagnetic field-induced neurotoxicity in primary cultured hippocampal neurons.** [**Biomed Pharmacother.**](https://www.ncbi.nlm.nih.gov/pubmed/27470406) **82:628-639, 2016.**

The present study investigated the protective effects of lotus seedpod procyanidins (LSPCs) on extremely low frequency electromagnetic field (ELF-EMF)-induced neurotoxicity in primary cultured rat hippocampal neurons and the underlying molecular mechanism. The results of MTT, morphological observation, superoxide dismutase (SOD) and malondialdehyde (MDA) assays showed that compared with control, incubating neurons under ELF-EMF exposure significantly decreased cell viability and increased the number of apoptotic cells, whereas LSPCs evidently protected the hippocampal neurons against ELF-EMF-induced cell damage. Moreover, a certain concentration of LSPCs inhibited the elevation of intracellular reactive oxygen species (ROS) and Ca(2+) level, as well as prevented the disruption of mitochondrial membrane potential induced by ELF-EMF exposure. In addition, supplementation with LSPCs could alleviate DNA damage, block cell cycle arrest at S phase, and inhibit apoptosis and necrosis of hippocampal neurons under ELF-EMF exposure. Further study demonstrated that LSPCs up-regulated the activations of Bcl-2, Bcl-xl proteins and suppressed the expressions of Bad, Bax proteins caused by ELF-EMF exposure. In conclusion, these findings revealed that LSPCs protected against ELF-EMF-induced neurotoxicity through inhibiting oxidative stress and mitochondrial apoptotic pathway.

**(NE) (VI)** [**Zhu K**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zhu%20K%5BAuthor%5D&cauthor=true&cauthor_uid=27079842)**,** [**Lv Y**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Lv%20Y%5BAuthor%5D&cauthor=true&cauthor_uid=27079842)**,** [**Cheng Q**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Cheng%20Q%5BAuthor%5D&cauthor=true&cauthor_uid=27079842)**,** [**Hua J**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Hua%20J%5BAuthor%5D&cauthor=true&cauthor_uid=27079842)**,** [**Zeng Q**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Zeng%20Q%5BAuthor%5D&cauthor=true&cauthor_uid=27079842)**. Extremely Low Frequency Magnetic Fields Do Not Induce DNA Damage in Human Lens Epithelial Cells In Vitro.** [**Anat Rec (Hoboken).**](http://www.ncbi.nlm.nih.gov/pubmed/27079842) **299(5):688-697, 2016.**

Non-ionizing radiations, e.g., radiofrequency electromagnetic fields, could induce DNA damage and oxidative stress in human lens epithelial cells (LECs) which can be early events in cataractogenesis. Extremely low frequency magnetic fields (ELF MF) as another common form of man-made electromagnetic fields has been considered as suspected human carcinogen by International Agency for Research on Cancer (IARC) and become a focus that people play more and more attentions to. This study aimed to determine whether ELF MF can induce DNA damage in cultured human LECs at a relatively low intensity. Human LECs were exposed or sham-exposed to a 50 Hz ELF MF which produced by a well-designed exposure system at the intensity of 0.4 mT. DNA damage in human LECs was examined by the phosphorylated form of histone variant H2AX (γH2AX) foci formation assay and further explored with western blot, flow cytometry, and alkaline comet assay. Immunofluorescence analysis showed that 0.4 mT ELF MF did not significantly increase γH2AX foci formation in human LECs after 2, 6, 12, 24, or 48 hr exposure. No significant differences had been detected in γH2AX expression level between the ELF MF- and sham-exposure groups, while no obvious chromosomal DNA fragmentation was detected by alkaline comet assay after ELF MF exposure. The results indicate an absence of genotoxicity in ELF MF-exposed human epithelial cells and do not support the hypothesis that environmental ELF MF might be causally led to genomic instability via chromosomal damage response processes. Neither short nor long term continuous exposure to 50 Hz ELF MF at 0.4 mT could induce DNA damage in human lens epithelial cells in vitro.

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

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

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

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