

## Extremely-Low Frequency EMF Free Radical Studies Updated March 2014

**110 papers: Effect= 97 (88%); No Effect=13 (12%)**

**(E) \*Akan Z, Aksu B, Tulunay A, Bilsel S, Inhan-Garip A. Extremely low-frequency electromagnetic fields affect the immune response of monocyte-derived macrophages to pathogens. Bioelectromagnetics. 31(8):603-612, 2010.**

This study aimed to determine the effect of extremely low-frequency electromagnetic fields (ELF-EMF) on the physiological response of phagocytes to an infectious agent. THP-1 cells (human monocytic leukemia cell line) were cultured and 50 Hz, 1 mT EMF was applied for 4-6 h to cells induced with *Staphylococcus aureus* or interferon gamma/lipopolysaccharide (IF $\gamma$ /LPS). Alterations in nitric oxide (NO), inducible nitric oxide synthase (iNOS) levels, heat shock protein 70 levels (hsp70), cGMP levels, caspase-9 activation, and the growth rate of *S. aureus* were determined. The growth curve of exposed bacteria was lower than the control. Field application increased NO levels. The increase was more prominent for *S. aureus*-induced cells and appeared earlier than the increase in cells without field application. However, a slight decrease was observed in iNOS levels. Increased cGMP levels in response to field application were closely correlated with increased NO levels. ELF-EMF alone caused increased hsp70 levels in a time-dependent manner. When cells were induced with *S. aureus* or IF $\gamma$ /LPS, field application produced higher levels of hsp70. ELF-EMF suppressed caspase-9 activation by a small extent. These data confirm that ELF-EMF affects bacterial growth and the response of the immune system to bacterial challenges, suggesting that ELF-EMF could be exploited for beneficial uses.

**(E) \*Akdag MZ, Dasdag S, Cakir DU, Yokus B, Kizil G, Kizil M. Do 100- and 500- $\mu$ T ELF magnetic fields alter beta-amyloid protein, protein carbonyl and malondialdehyde in rat brains? Electromagn Biol Med. 2013 Jan 16. [Epub ahead of print]**

Several studies still state that presently accepted safety standards for extremely low-frequency magnetic fields (ELF-MFs) do not provide adequate protection, and therefore the standards are still open to question. To help resolve this question, the aim of this study was to illuminate the interaction between biomolecules and ELF-MFs by investigating the effect of ELF-MFs on beta-amyloid protein (BAP), protein carbonyl (PC) and malondialdehyde (MDA) in rat brain. For this study, 30 adult male Sprague-Dawley rats were used, which were divided into two experimental groups and a sham exposed group. Rats in two experimental groups were exposed to 100- and 500- $\mu$ T ELF-MFs (50 Hz) for 2 h/day for 10 months, which are the generally accepted safety standards for public and occupational exposures. The same procedures were applied to the rats in the sham group, but with the generator turned off. The results of this study showed that neither ELF-MFs used in this study altered BAP level significantly ( $p > 0.05$ ). However, PC and MDA levels were increased by the exposure to 100- and 500- $\mu$ T ELF-MFs ( $p < 0.0001$ ). In conclusion, both PC and MDA levels were altered by long-term exposure to either 100 or 500  $\mu$ T ELF-MF. However, many further and more comprehensive studies will be required to elucidate the interaction mechanisms between ELF-MFs exposure and living organisms.

**(E) Akdag MZ, Bilgin MH, Dasdag S, Tumer C. Alteration of nitric oxide production in rats exposed to a prolonged, extremely low-frequency magnetic field. Electromagn Biol Med. 26(2):99-106, 2007.**

The purpose of this study is to investigate the possible effect of an extremely low-frequency magnetic field (ELF-MF) on nitric oxide (NO) level. In this study, 27 male Sprague-Dawley rats were used. The rats were divided into three groups: two experimental and one control (sham-exposed). The first and second experimental group (n = 10) were exposed to 100 microT and 500 microT ELF-MF during 10 months, 2 h a day, respectively, and the third (n = 7) group was treated like an experimental group except for ELF-MF exposure in methacrylate boxes. After ELF-MF and sham exposure, serum nitrite levels were measured by Griess reaction. A significant reduction was observed in nitrite levels among the first and second experimental groups of rats and sham-exposed rats after exposure for 10 months, 2 h a day, to ELF-MF of 100 and 500 microT ( $p < 0.01$ ). These results suggest that prolonged ELF-MF exposure at intensities of exposure limits, determined by ICNIRP for public and occupational, may reduce NO production probably affected by NO generation pathways.

**(E) Akdag MZ, Dasdag S, Ulukaya E, Uzunlar AK, Kurt MA, Taşkın A. Effects of Extremely Low-Frequency Magnetic Field on Caspase Activities and Oxidative Stress Values in Rat Brain. Biol Trace Elem Res. 138(1):238-249, 2010.**

This study was aimed to investigate the effect of extremely low-frequency magnetic field (ELF-MF) on apoptosis and oxidative stress values in the brain of rat. Rats were exposed to 100 and 500 microT ELF-MF, which are the safety standards of public and occupational exposure for 2 h/day for 10 months. Brain tissues were immunohistochemically stained for the active (cleaved) caspase-3 in order to measure the apoptotic index by a semi-quantitative scoring system. In addition, the levels of catalase (CAT), malondialdehyde (MDA), myeloperoxidase (MPO), total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were measured in rat brain. Final score of apoptosis and MPO activity were not significantly different between the groups. CAT activity decreased in both exposure groups ( $p < 0.05$ ), while TAC was found to be lower in ELF 500 group than those in ELF-100 and sham groups ( $p < 0.05$ ). MDA, TOS, and OSI values were found to be higher in ELF-500 group than those in ELF-100 and sham groups ( $p < 0.05$ ). In conclusion, apoptosis was not changed by long-term ELF-MF exposure, while both 100 and 500 microT ELF-MF exposure induced toxic effect in the rat brain by increasing oxidative stress and diminishing antioxidant defense system.

**(NE) \*Akdag MZ, Dasdag S, Uzunlar AK, Ulukaya E, Oral AY, Celik N, Akşen F. Can safe and long-term exposure to extremely low frequency (50 Hz) magnetic fields affect apoptosis, reproduction, and oxidative stress? Int J Radiat Biol. 2013 Jun 20. [Epub ahead of print]**

Abstract Purpose: The purpose of this study was to determine whether 50 Hz Extremely Low Frequency-Magnetic Fields (ELF-MFs) affects apoptotic processes, oxidative damage, and reproductive characteristics such as sperm count and morphology in rat testes. Materials and Methods: 30 male Sprague-Dawley rats were used in the present study, which were divided into three groups (sham group, n: 10, and two experimental groups, n: 10 for each group). Rats in the experimental group were exposed to 100 and 500  $\mu$ T ELF-MF (2h/day, 7 days/week, for 10 months) corresponding to exposure levels that are considered safe for humans.. Same experimental procedures were applied to the sham group, but the ELF generator was turned off. Tissues from the testes were immunohistochemically stained for active (cleaved) caspase-3 in order to measure the apoptotic index by a semi-quantitative scoring system. The levels of catalase

(CAT), malondialdehyde (MDA), myeloperoxidase (MPO), total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were also measured. Additionally, epididymal sperm count and sperm morphology was evaluated. Results: There were no significant differences in the reproductive and oxidative stress parameters between the sham group and the exposed groups ( $p>0.05$ ). While no difference was observed between the final apoptosis score of the sham and the 100  $\mu\text{T}$  ELF-MF group ( $p>0.05$ ), the final apoptosis score was higher in the 500  $\mu\text{T}$  ELF-MF exposure group than in the sham group ( $p<0.05$ ). Conclusion: Long-term exposure to 100  $\mu\text{T}$  and 500  $\mu\text{T}$  ELF-MF did not affect oxidative or antioxidative processes, lipid peroxidation, or reproductive components such as sperm count and morphology in testes tissue of rats. However, long-term exposure to 500  $\mu\text{T}$  ELF-MF did affect active-caspase-3 activity, which is a well-known apoptotic indicator

**(E) \*Akpınar D, Oztürk N, Ozen S, Agar A, Yargıoğlu P. The effect of different strengths of extremely low-frequency electric fields on antioxidant status, lipid peroxidation, and visual evoked potentials. Electromagn Biol Med. 31(4):436-448, 2012.**

The aim of the study was to investigate the effects of extremely low-frequency electric field (ELF EF) on visual evoked potential (VEP), thiobarbituric acid reactive substances (TBARS), total antioxidant status (TAS), total oxidant status (TOS), and oxidant stress index (OSI). Thirty female Wistar rats, aged 3 months, were divided into three equal groups: Control (C), the group exposed to EF at 12 kV/m strength (E12), and the group exposed to EF at 18 kV/m strength (E18). Electric field was applied to the E12 and E18 groups for 14 days (1 h/day). Brain and retina TBARS, TOS, and OSI were significantly increased in the E12 and E18 groups with respect to the control group. Also, TBARS levels were significantly increased in the E18 group compared with the E12 group. Electric fields significantly decreased TAS levels in both brain and retina in E12 and E18 groups with respect to the control group. All VEP components were significantly prolonged in rats exposed to electric fields compared to control group. In addition, all latencies of VEP components were increased in the E18 group with respect to the E12 group. It is conceivable to suggest that EF-induced lipid peroxidation may play an important role in changes of VEP parameters.

**(E) Aksen F, Akdag MZ, Ketani A, Yokus B, Kaya A, Dasdag S. Effect of 50-Hz 1-mT magnetic field on the uterus and ovaries of rats (electron microscopy evaluation). Med Sci Monit. 12(6):BR215-220, 2006.**

BACKGROUND: The aim of this study was to investigate the effect of extremely low frequency magnetic fields (ELFMF) on the uterus and ovary of rats. MATERIAL/METHODS: Forty-eight female Wistar albino rats were divided into two groups, one for 50 and the other for 100 days of exposure. Each group was further divided into two groups, one sham exposed ( $n=12$ ) and the other the experimental group ( $n=12$ ). The experimental rats were exposed to 50-Hz 1-mT ELFMF for three hours/day for 50 or 100 days. The sham groups of rats were kept under the same circumstances without applying ELFMF. Electron microscopic examination was performed to evaluate the ovaries and uterus. RESULTS: Ultrastructural dissolution, decrease in cell organelles, cavities in cells, heterochromative appearance, and typical structural loss of the nucleus were observed in germinal epithelial cells of the rat ovaries in the 50-days ELFMF exposure group. Ultrastructural alterations in germinal epithelium and tunica albuginea of ovaries, irregularity in nucleus and nucleolus, increase in lipid vacuoles of cell cytoplasm and reduction in organelles were observed in rat ovaries in the 100-days ELFMF exposure group. Similar alterations were observed in uterus. Malondialdehyde concentration (MDA) of the ovaries and uterus increased in rats of the two exposure groups ( $p<0.001$ ). CONCLUSIONS: The results of the study showed

that 50 and 100 days of exposure to a 1-mT ELF-MF can cause alterations at the cellular level and in MDA concentration.

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

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

**(E) \*Ayşe IG, Zafer A, Sule O, Işıl IT, Kalkan T. Differentiation of K562 cells under ELF-EMF applied at different time courses. Electromagn Biol Med. 29(3):122-130, 2010.**

The time-course of ELF-EMF application to biological systems is thought to be an important parameter determining the physiological outcome. This study investigated the effect of ELF-EMF on the differentiation of K562 cells at different time courses. ELF-EMF (50 Hz, 5 mT, 1 h) was applied at two different time-courses; first at the onset of hemin induction for 1 h, and second, daily 1 h for four days. While single exposure to ELF-EMF resulted in a decrease in differentiation, ELF-EMF applied everyday for 1 h caused an increase in differentiation. The effect of co-stressors, magnesium, and heat-shock was also determined and similar results were obtained. ELF-EMF increased ROS levels in K562 cells not treated with hemin, however did not change ROS levels of hemin treated cells indicating that ROS was not the cause. Overall, these results imply that the time-course of application is an important parameter determining the physiological response of cells to ELF-EMF.

**(E) Bawin SM, Satmary WM, Jones RA, Adey WR, Zimmerman G. Extremely-low-frequency magnetic fields disrupt rhythmic slow activity in rat hippocampal slices. Bioelectromagnetics. 17(5):388-395, 1996.**

Several studies have indicated that weak, extremely-low-frequency (ELF; 1-100 Hz) magnetic fields affect brain electrical activity and memory processes in man and laboratory animals. Our studies sought to determine whether ELF magnetic fields could couple directly with brain tissue and affect neuronal activity in vitro. We used rat hippocampal slices to study field effects on a specific brain activity known as rhythmic slow activity (RSA), or theta rhythm, which occurs in 7-15 s bursts in the hippocampus during memory functions. RSA, which, in vivo, is a cholinergic

activity, is induced in hippocampal slices by perfusion of the tissue with carbachol, a stable analog of acetylcholine. We previously demonstrated that the free radical nitric oxide (NO), synthesized in carbachol-treated hippocampal slices, lengthened and destabilized the intervals between successive RSA episodes. Here, we investigate the possibility that sinusoidal ELF magnetic fields could trigger the NO-dependent perturbation of the rate of occurrence of the RSA episodes. Carbachol-treated slices were exposed for 10 min epochs to 1 or 60 Hz magnetic fields with field intensities of 5.6, 56, or 560 microT (rms), or they were sham exposed. All exposures took place in the presence of an ambient DC field of 45 microT, with an angle of -66 degrees from the horizontal plane. Sinusoidal 1 Hz fields at 56 and 560 microT, but not at 5.6 microT, triggered the irreversible destabilization of RSA intervals. Fields at 60 Hz resulted in similar, but not statistically significant, trends. Fields had no effects on RSA when NO synthesis was pharmacologically inhibited. However, field effects could take place when extracellular NO, diffusing from its cell of origin to the extracellular space, was chelated by hemoglobin. These results suggest that ELF magnetic fields exert a strong influence on NO systems in the brain; therefore, they could modulate the functional state of a variety of neuronal ensembles.

**(E) Bediz CS, Baltaci AK, Mogulkoc R, Oztekin E. Zinc supplementation ameliorates electromagnetic field-induced lipid peroxidation in the rat brain. *Tohoku J Exp Med.* 208(2):133-140, 2006.**

Extremely low-frequency (0-300 Hz) electromagnetic fields (EMFs) generated by power lines, wiring and home appliances are ubiquitous in our environment. All populations are now exposed to EMF, and exposure to EMF may pose health risks. Some of the adverse health effects of EMF exposure are lipid peroxidation and cell damage in various tissues. This study has investigated the effects of EMF exposure and zinc administration on lipid peroxidation in the rat brain. Twenty-four male Sprague-Dawley rats were randomly allocated to three groups; they were maintained untreated for 6 months (control, n = 8), exposed to low-frequency (50 Hz) EMF for 5 minutes every other day for 6 months (n = 8), or exposed to EMF and received zinc sulfate daily (3 mg/kg/day) intraperitoneally (n = 8). We measured plasma levels of zinc and thiobarbituric acid reactive substances (TBARS), and levels of reduced glutathione (GSH) in erythrocytes. TBARS and GSH levels were also determined in the brain tissues. TBARS levels in the plasma and brain tissues were higher in EMF-exposed rats with or without zinc supplementation, than those in controls (p < 0.001). In addition, TBARS levels were significantly lower in the zinc-supplemented rats than those in the EMF-exposed rats (p < 0.001). GSH levels were significantly decreased in the brain and erythrocytes of the EMF-exposed rats (p < 0.01), and were highest in the zinc-supplemented rats (p < 0.001). Plasma zinc was significantly lower in the EMF-exposed rats than those in controls (p < 0.001), while it was highest in the zinc-supplemented rats (p < 0.001). The present study suggests that long-term exposure to low-frequency EMF increases lipid peroxidation in the brain, which may be ameliorated by zinc supplementation.

**(E) Belova NA, Potselueva MM, Skrebnitskaia LK, Znobishcheva AV, Lednev VV. [Effects of weak magnetic fields on the production of reactive oxygen species in peritoneal neutrophils in mice]. *Biofizika.* 55(4):657-663, 2010. [Article in Russian]**

The influence of weak magnetic fields of different types on the rate of the formation of reactive oxygen species in mouse peritoneal neutrophils has been studied. It was found that the exposure of neutrophils activated by phorbol 12-myristate 13-acetate to the magnetic field tuned to the parametric resonance for Ca<sup>2+</sup> ions leads to a decrease in the rate of the reactive oxygen species (ROS) generation by 23%. Conversely, the generation of ROS in neutrophils exposed to the same field but stimulated by the bacterial peptide FMLP (N-formyl-L-methionyl-L-leucyl-L-

phenylalanine) increased by about 21%. Pulsed magnetic fields also changed the rate of ROS generation in phorbol-stimulated neutrophils by about 20%, but the sign of the effects observed in this case was opposite to those induced by the magnetic field tuned to the parametric resonance for Ca<sup>2+</sup> ions.

**(E) \*Bonnafous P, Vernhes M, Teissié J, Gabriel B. The generation of reactive-oxygen species associated with long-lasting pulse-induced electropermeabilisation of mammalian cells is based on a non-destructive alteration of the plasma membrane. Biochim Biophys Acta. 1461(1):123-134, 1999.**

Chinese hamster ovary (CHO) cells in suspension were subjected to pulsed electric fields suitable for electrically mediated gene transfer (pulse duration longer than 1 ms). Using the chemiluminescence probe lucigenin, we showed that a generation of reactive-oxygen species (oxidative jump) was present when the cells were electropermeabilised using millisecond pulses. The oxidative jump yield was controlled by the extent of alterations allowing permeabilisation within the electrically affected cell area, but showed a saturating dependence on the pulse duration over 1 ms. Cell electropulsation induced reversible and irreversible alterations of the membrane assembly. The oxidative stress was only present when the membrane permeabilisation was reversible. Irreversible electrical membrane disruption inhibited the oxidative jump. The oxidative jump was not a simple feedback effect of membrane electropermeabilisation. It strongly controlled long-term cell survival. This had to be associated with the cell-damaging action of reactive-oxygen species. However, for millisecond-cumulated pulse duration, an accumulation of a large number of short pulses (microsecond) was extremely lethal for cells, while no correlation with an increased oxidative jump was found. Cell responses, such as the production of free radicals, were present during electropermeabilisation of living cells and controlled partially the long-term behaviour of the pulsed cell.

**(E) Buczyński A, Pacholski K, Dziejczak-Buczyńska M, Henrykowska G, Jerominko A. The assessment of oxygen metabolism selected parameters of blood platelets exposed to low frequency magnetic radiation in cars--in vitro studies. Rocz Akad Med Białymst. 50 Suppl 1:23-25, 2005.**

PURPOSE: The aim of the study was to determine how free radicals generation in blood platelets exposed to electromagnetic field (EMF) occurring in cars affects the process of these morphotic elements cell membranes phospholipid peroxidation. MATERIAL AND METHODS: The suspension of human blood platelets was exposed to EMF of proper characteristics in a specially arranged research stand. After 30, 60 and 90 min exposure of the platelet specimen to EMF, free radicals generation was measured with chemiluminescence and malondialdehyde concentration according to Placer et al. method. The obtained results were compared with the control values. RESULTS: The increase of free radicals generation was observed after 30 and 90 min exposure of platelets to magnetic field. Malondialdehyde reached the highest values also after 30 and 90 min exposure of the platelets to EMF as compared to the control. CONCLUSIONS: The increase in oxygen reactive species generation under the effect of exogenic magnetic radiation as well as proportional intensification of the peroxidation process determined on the basis of malondialdehyde concentration (the marker of this phenomenon) point to the platelet sensitivity to the investigated environmental factor.

**(E) \*Buldak RJ, Polaniak R, Buldak L, Zwirska-Korczala K, Skonieczna M, Monsiol A, Kukla M, Dulawa-Buldak 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*. 33(8):641-651, 2012.**

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) Calota V, Dragoiu S, Meghea A, Giurginca M. Effects of prooxidants on human serum exposed to 50 Hz magnetic fields. *Electromagn Biol Med*.26(2):135-140, 2007.**

The purpose of this article is to evaluate magnetic field effects (50 Hz, different magnetic intensities) on the chemiluminescence intensity of human serum. We find that 1 and 2 h of exposure increased the chemiluminescence emission. The addition to the serum of prooxidants FeCl<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> in different concentrations increased the chemiluminescence intensity even more.

**(E) Canseven AG, Coskun S, Seyhan N. Effects of various extremely low frequency magnetic fields on the free radical processes, natural antioxidant system and respiratory burst system activities in the heart and liver tissues. *Indian J Biochem Biophys*. 45(5):326-331, 2008.**

Magnetic fields (MFs) can affect biological systems by increasing the release of free radicals that are able to alter cell defense systems and breakdown tissue homeostasis. In the present study, the effects of extremely low frequency (ELF) electromagnetic fields (EMF) were investigated on free radical levels, natural antioxidant systems and respiratory burst system activities in heart and liver tissues of guinea pigs exposed to 50 Hz MFs of 1, 2 and 3 mT for 4 h/day and 8 h/day for 5 days by measuring malondialdehyde (MDA), nitric oxide (NO), glutathione (GSH) levels and myeloperoxidase (MPO) activity. A total of sixty-two male guinea pigs, 10-12 weeks old were studied in seven groups as control and exposure groups: Group I (control), II (1 mT, 4 h/day), III (1 mT, 8 h/day), IV (2 mT, 4 h/day), V (2 mT, 8 h/day), VI (3 mT, 4 h/day), and VII (3 mT, 8 h/day). Controls were kept under the same conditions without any exposure to MF. MDA levels increased in liver in groups II and IV, but decreased in group VII for both liver and heart tissues. NO<sub>x</sub> levels declined in heart in groups II and III and in liver in groups III, V, and VI, but

increased in liver in group VII. GSH levels increased in heart in groups II, IV, V, and in liver in groups V and VI and VI, but decreased in groups II and IV in liver. MPO activity decreased in liver in groups III, IV, VI and VII with respect to controls and in heart tissues in groups II, III and IV; however, there was a significant increase MPO activity in heart in group VII. From the results, it can be concluded that the intensity and exposure duration of MFs are among the effective conditions on the formation of free radicals and behaviour of antioxidant enzymes.

**(NE) Cantoni O, Sestili P, Fiorani M, Dachà M. The effect of 50 Hz sinusoidal electric and/or magnetic fields on the rate of repair of DNA single/double strand breaks in oxidatively injured cells. Biochem Mol Biol Int. 37(4):681-689, 1995.**

Exposure of cultured mammalian cells to 50 Hz electric (0.2-20 kV/m), magnetic (0.002-2 G), or combined electric and magnetic fields did not affect the rate of repair of DNA single strand breaks (SSB) induced by hydrogen peroxide. The same lack of effect was observed on the repair of both DNA SSBs and DNA double strand breaks (DSBs) in cells treated with the cocktail hydrogen peroxide/L-Histidine. These results indicate that exposure to electric and/or magnetic fields does not affect the machinery involved in the repair of DNA lesions in oxidatively injured cells thus suggesting that it is unlikely that field exposure might induce changes in the response of the cells to the tumor promoting or carcinogenic effects elicited by reactive oxygen species.

**(E) Cheun BS, Yi SH, Baik KY, Lim JK, Yoo JS, Shin HW, Soh KS. Biophoton emission of MDCK cell with hydrogen peroxide and 60 Hz AC magnetic field. J Environ Biol. 28(4):735-740, 2007.**

We studied biophoton characteristics of Madin-Darby canine kidney (MDCK) cells under the influence of H<sub>2</sub>O<sub>2</sub> by employing a photomultiplier tube (PMT) and a fluorescence microscope. H<sub>2</sub>O<sub>2</sub> was used for producing reactive oxygen species (ROS) in the measurement. Images from a fluorescence microscope show an increase of photon intensity emitted from the sample due to H<sub>2</sub>O<sub>2</sub>. By using a PMT we measured quantitative change in biophoton emission with application of H<sub>2</sub>O<sub>2</sub> to the MDCK cell culture, found that the increase of the biophoton is dependent upon the amount of H<sub>2</sub>O<sub>2</sub>. The agreement between the results of the PMT and the fluorescence microscope suggests the possibility of quantitative measurement of the influence of ROS on living tissue or cell. In addition we applied a 60 Hz AC magnetic field on the cells to investigate the change in reaction between MDCK cell and ROS. It showed that a decay of chemiluminescence intensity has taken a different path following exposure to the magnetic field. As a result, the PMT measurement might be considered as a useful tool for studying biochemical characteristics in relation to ROS.

**(E)\*Chu LY, Lee JH, Nam YS, Lee YJ, Park WH, Lee BC, Kim D, Chung YH, Jeong JH. Extremely low frequency magnetic field induces oxidative stress in mouse cerebellum. Gen Physiol Biophys. 30(4):415-421, 2011.**

We have investigated whether extremely low frequency magnetic field (ELF-MF) induces lipid peroxidation and reactive oxygen species in mouse cerebellum. After exposure to 60 Hz ELF-MF at 2.3 mT intensity for 3 hours, there was a significant increase in malondialdehyde level and hydroxyl radical. ELF-MF significantly induced concomitant increase in superoxide dismutase without alteration in glutathione peroxidase activity. While glutathione contents were not altered, ascorbic acid levels were significantly decreased by ELF-MF exposure. These results indicate that ELF-MF may induce oxidative stress in mouse cerebellum. However, the mechanism remains further to be characterized.

**(E) Ciejka EB, Goraca A. The influence of low-frequency magnetic field on plasma antioxidant capacity and heart rate. Wiad Lek. 62(2):81-86, 2009.**

INTRODUCTION: Low-frequency magnetic field is widely applied as magnetotherapy in physiotherapeutic treatment. Recognition of positive and negative effects of the magnetic field has been the subject of numerous studies. Experimental studies concern, among others, the effect of this field on the heart rate and plasma antioxidant capacity. The aim of the study was to check whether a time-variable magnetic field of constant frequency and induction affects the heart rate and plasma antioxidant capacity. MATERIAL AND METHODS: The tests were performed on Sprague-Dawley rats exposed to the magnetic field of the following parameters: frequency - 40 Hz, induction - 7 mT, time of exposure - 30 and 60 minutes. The measurements of ECG and plasma antioxidant capacity expressed in the number of reduced iron ions were performed on experimental animals: before, after a single exposure and after 14 days of exposure. RESULTS: A significant decrease of the heart rate was observed after 14 days of exposure. A variable magnetic field of the parameters: frequency - 40 Hz, induction - 7 mT and exposure time of 14 days caused an increase of the organism antioxidant defence, whereas a variable magnetic field of the frequency of 40 Hz, induction - 7 mT and exposure time 60 minutes for 14 days caused a significant decrease of the organism antioxidant defence. CONCLUSIONS: The exposure time affects heart rate, plasma antioxidant capacity and the organism defense ability against free radicals.

**(E) Ciejka E, Kleniewska P, Skibska B, Goraca A. Effects of extremely low frequency magnetic field on oxidative balance in brain of rats. J Physiol Pharmacol. 62(6):657-661, 2011.**

Extremely low frequency magnetic field (ELF-MF) may result in oxidative DNA damage and lipid peroxidation with an ultimate effect on a number of systemic disturbances and cell death. The aim of the study is to assess the effect of ELF-MF parameters most frequently used in magnetotherapy on reactive oxygen species generation (ROS) in brain tissue of experimental animals depending on the time of exposure to this field. The research material included adult male Sprague-Dawley rats, aged 3-4 months. The animals were divided into 3 groups: I - control (shame) group; II - exposed to the following parameters of the magnetic field: 7 mT, 40 Hz, 30 min/day, 10 days; III - exposed to the ELF-MF parameters of 7 mT, 40 Hz, 60 min/day, 10 days. The selected parameters of oxidative stress: thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), total free sulphhydryl groups (-SH groups) and protein in brain homogenates were measured after the exposure of rats to the magnetic field. ELF-MF parameters of 7 mT, 40 Hz, 30 min/day for 10 days caused a significant increase in lipid peroxidation and insignificant increase in H<sub>2</sub>O<sub>2</sub> and free -SH groups. The same ELF-MF parameters but applied for 60 min/day caused a significant increase in free -SH groups and protein concentration in the brain homogenates indicating the adaptive mechanism. The study has shown that ELF-MF applied for 30 min/day for 10 days can affect free radical generation in the brain. Prolongation of the exposure to ELF-MF (60/min/day) caused adaptation to this field. The effect of ELF-MF irradiation on oxidative stress parameters depends on the time of animal exposure to magnetic field.

**(E) Ciejka E, Skibska B, Kleniewska P, Goraca A. [Influence of low frequency magnetic field on chosen parameters of oxidative stress in rat's muscles]. Pol Merkur Lekarski. 29(174):361-364, 2010. [Article in Polish]**

Free radicals are atoms, molecules or their fragments, which excess leads to the development of the oxidative stress, which is caused of many neoplastic, neurodegenerative, inflammatory diseases and aging the organism. The main of exogenous sources of free radicals are among others: industrial pollution, tobacco smoke, ionizing radiation, ultrasound and magnetic field. The low magnetic field is applied in the physician therapy. The aim of this study was to evaluate the influence of low magnetic field on the parameters of oxidative stress in rat's muscles.

**MATERIALS AND METHODS:** Thirty male rats, weight of 280-300 g were randomly divided into three experimental groups: control I and treatment II and III (ELFMF-exposed), each containing seven animals. Animals in treat group II were exposed to 40 Hz, 7 mT for 0.5 h/day for 14 days (this kind of the ELFMF is mostly use in magnetotherapy) while, group III was exposed to 40 Hz, 7 mT for 1 h/day for 14 days. Control rats were in separate room without exposing to ELFMF. Immediately after the last exposure, the part of muscles was taken under pentobarbital anaesthesia. The effects of exposure to ELFMF on oxidative states were assessed on the measurements of concentration of -SH group, H<sub>2</sub>O<sub>2</sub>, and the concentration of proteins in muscles homogenates. **RESULTS:** Exposure to ELFMF: 40 Hz, 7 mT, 30 and 60 min/day used for 2 weeks caused significant increase in -SH group concentration and decrease of the protein concentration in the muscles homogenates. **CONCLUSION:** Low magnetic field used in magnetotherapy causes the significant changes of the generating the reactive forms of oxygen in the muscles which depend on the parameters of low magnetic field.

**(E) Coşkun S, Balabanli B, Canseven A, Seyhan N. Effects of continuous and intermittent magnetic fields on oxidative parameters in vivo. Neurochem Res. 34(2):238-243, 2009.**

Continuous and intermittent 50 Hz, 1.5 mT magnetic field with the exposure period of 4 h/day for 4 days was used to investigate its possible effect on adult guinea pigs. Tissues and plasma specimens were assessed by biochemical parameters. Malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO) levels and myeloperoxidase activity (MPO) were examined in plasma, liver and brain tissues. All parameters were determined by spectrophotometer. While intermittent magnetic field was effective on plasma lipid peroxidation, continuous magnetic field was found to be effective on plasma MPO activity and NO levels. Augmentation of lipid peroxidation was also observed in liver tissue both intermittent and continuous magnetic field exposures. These results indicate that both the intermittent and continuous magnetic field exposures affect various tissues in a distinct manner because of having different tissue antioxidant status and responses.

**(E)\*Cui Y, Ge Z, Rizak JD, Zhai C, Zhou Z, Gong S, Che Y. Deficits in water maze performance and oxidative stress in the hippocampus and striatum induced by extremely low frequency magnetic field exposure. PLoS One. 7(5):e32196, 2012.**

The exposures to extremely low frequency magnetic field (ELF-MF) in our environment have dramatically increased. Epidemiological studies suggest that there is a possible association between ELF-MF exposure and increased risks of cardiovascular disease, cancers and neurodegenerative disorders. Animal studies show that ELF-MF exposure may interfere with the activity of brain cells, generate behavioral and cognitive disturbances, and produce deficits in attention, perception and spatial learning. Although, many research efforts have been focused on the interaction between ELF-MF exposure and the central nervous system, the mechanism of interaction is still unknown. In this study, we examined the effects of ELF-MF exposure on

learning in mice using two water maze tasks and on some parameters indicative of oxidative stress in the hippocampus and striatum. We found that ELF-MF exposure (1 mT, 50 Hz) induced serious oxidative stress in the hippocampus and striatum and impaired hippocampal-dependent spatial learning and striatum-dependent habit learning. This study provides evidence for the association between the impairment of learning and the oxidative stress in hippocampus and striatum induced by ELF-MF exposure.

**(NE) De Mattei M, Pasello M, Pellati A, Stabellini G, Massari L, Gemmati D, Caruso A. Effects of electromagnetic fields on proteoglycan metabolism of bovine articular cartilage explants. Connect Tissue Res. 44(3-4):154-159, 2003.**

Electromagnetic field (EMF) exposure has been proposed for the treatment of osteoarthritis. In this study, we investigated the effects of EMF (75 Hz, 2,3 mT) on proteoglycan (PG) metabolism of bovine articular cartilage explants cultured in vitro, both under basal conditions and in the presence of interleukin-1beta (IL-1beta) in the culture medium. Proteoglycan synthesis and the residual PG tissue content resulted significantly higher in EMF-exposed explants than in controls, whereas no effect was observed on PG release and nitric oxide (NO) production. IL-1beta induced both a reduction in PG synthesis and an increase in PG release, related to a strong stimulation of NO production, which resulted in a net loss of tissue PG content. In IL-1beta-treated explants, EMF increased PG synthesis, whereas in spite of a slight stimulation of NO production EMF did not modify PG release. This resulted in the residual PG tissue content being maintained at the control level. In both experimental conditions, the effects of EMF were associated with an increase in lactate production. The results of our study show that EMFs are able to promote anabolic activities and PG synthesis in bovine articular cartilage explants. This effect also is maintained in the presence of IL-1beta, thus counteracting the catabolic activity of the cytokine. Altogether, these data suggest that EMF exposure exerts a chondroprotective effect on articular cartilage in vitro.

**(E) \*De Nicola M, Cordisco S, Cerella C, Albertini MC, D'Alessio M, Accorsi A, Bergamaschi A, Magrini A, Ghibelli L. Magnetic fields protect from apoptosis via redox alteration. Ann N Y Acad Sci. 1090:59-68, 2006.**

Magnetic fields (MFs) are receiving much attention in basic research due to their emerging ability to alter intracellular signaling. We show here that static MFs with intensity of 6 mT significantly alter the intracellular redox balance of U937 cells. A strong increase of reactive oxygen species (ROS) and a decrease of glutathione (GSH) intracellular levels were found after 2 h of MF exposure and maintained thereafter. We found that also other types of MFs, such as extremely-low-frequency (ELF) MFs affect intracellular GSH starting from a threshold at 0.09 mT. We previously reported that static MFs in the intensity range of 0.3-60 mT reduce apoptosis induced by damaging agents (Fanelli et al., 1998). Here, we show that ELF-MFs are also able to protect U937 from apoptosis. Interestingly, this ability is limited to the ELF intensities able to alter redox equilibrium, indicating a link between MF's antiapoptotic effect and the MF alteration of intracellular redox balance. This suggests that MF-produced redox alterations may be part of the signaling pathway leading to apoptosis antagonism. Thus, we tested whether MFs may still exert an antiapoptotic action in cells where the redox state was artificially altered in both directions, that is, by creating an oxidative (via GSH depletion with BSO) or a reducing (with DTT) cellular environment. In both instances, MFs fail to affect apoptosis. Thus, a correct intracellular redox state is required in order for MFs to exert their antiapoptotic effect.

**(E) \*Deng Y, Zhang Y, Jia S, Liu J, Liu Y, Xu W, Liu L. Effects of Aluminum and Extremely Low Frequency Electromagnetic Radiation on Oxidative Stress and Memory in Brain of Mice. Biol Trace Elem Res. 2013 Oct 26. [Epub ahead of print]**

This study was aimed to investigate the effect of aluminum and extremely low-frequency magnetic fields (ELF-MF) on oxidative stress and memory of SPF Kunming mice. Sixty male SPF Kunming mice were divided randomly into four groups: control group, ELF-MF group (2 mT, 4 h/day), load aluminum group (200 mg aluminum/kg, 0.1 ml/10 g), and ELF-MF + aluminum group (2 mT, 4 h/day, 200 mg aluminum/kg). After 8 weeks of treatment, the mice of three experiment groups (ELF-MF group, load aluminum group, and ELF-MF + aluminum group) exhibited firstly the learning memory impairment, appearing that the escaping latency to the platform was prolonged and percentage in the platform quadrant was reduced in the Morris water maze (MWM) task. Secondly are the pathologic abnormalities including neuronal cell loss and overexpression of phosphorylated tau protein in the hippocampus and cerebral cortex. On the other hand, the markers of oxidative stress were determined in mice brain and serum. The results showed a statistically significant decrease in superoxide dismutase activity and increase in the levels of malondialdehyde in the ELF-MF group ( $P < 0.05$  or  $P < 0.01$ ), load aluminum group ( $P < 0.01$ ), and ELF-MF + aluminum group ( $P < 0.01$ ). However, the treatment with ELF-MF + aluminum induced no more damage than ELF-MF and aluminum did, respectively. In conclusion, both aluminum and ELF-MF could impact on learning memory and pro-oxidative function in Kunming mice. However, there was no evidence of any association between ELF-MF exposure with aluminum loading.

**(E) Di Loreto S, Falone S, Caracciolo V, Sebastiani P, D'Alessandro A, Mirabilio A, Zimmitti V, Amicarelli F. Fifty hertz extremely low-frequency magnetic field exposure elicits redox and trophic response in rat-cortical neurons. J Cell Physiol. 219(2):334-343, 2009.**

Large research activity has raised around the mechanisms of interaction between extremely low-frequency magnetic fields (ELF-MFs) and biological systems. ELF-MFs may interfere with chemical reactions involving reactive oxygen species (ROS), thus facilitating oxidative damages in living cells. Cortical neurons are particularly susceptible to oxidative stressors and are also highly dependent on the specific factors and proteins governing neuronal development, activity and survival. The aim of the present work was to investigate the effects of exposures to two different 50 Hz sinusoidal ELF-MFs intensities (0.1 and 1 mT) in maturing rat cortical neurons' major anti-oxidative enzymatic and non-enzymatic cellular protection systems, membrane peroxidative damage, as well as growth factor, and cytokine expression pattern. Briefly, our results showed that ELF-MFs affected positively the cell viability and concomitantly reduced the levels of apoptotic death in rat neuronal primary cultures, with no significant effects on the main anti-oxidative defences. Interestingly, linear regression analysis suggested a positive correlation between reduced glutathione (GSH) and ROS levels in 1 mT MF-exposed cells. On this basis, our hypothesis is that GSH could play an important role in the antioxidant defence towards the ELF-MF-induced redox challenge. Moreover, the GSH-based cellular response was achieved together with a brain-derived neurotrophic factor over-expression as well as with the interleukin 1beta-dependent regulation of pro-survival signaling pathways after ELF-MF exposure.

**Ding GR, Nakahara T, Hirose H, Koyama S, Takashima Y, Miyakoshi J. Extremely low frequency magnetic fields and the promotion of H<sub>2</sub>O<sub>2</sub>-induced cell death in HL-60 cells. *Int J Radiat Biol.* 80(4):317-324, 2004.**

**PURPOSE:** To test whether exposure to an extremely low frequency magnetic field (60 Hz, 5 mT) affects hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-induced cell death in human leukaemia HL-60 cells. **MATERIALS AND METHODS:** Cells were treated with H<sub>2</sub>O<sub>2</sub> with or without exposure to an extremely low frequency magnetic fields. Viable cells, apoptotic and necrotic cells were determined by annexin V flow cytometry assay. The levels of apoptosis-related proteins (caspase-3, caspase-7, Bcl-2 and Bax) and poly(ADP-ribose) polymerase were detected using Western blotting. **RESULTS:** Simultaneous treatment with exposure to the magnetic field and H<sub>2</sub>O<sub>2</sub> (85 or 100 microM) for 24 h increased the number of apoptotic and necrotic cells significantly, and significantly decreased the number of viable cells compared with cells treated with H<sub>2</sub>O<sub>2</sub> alone. The protein levels of Bax and Bcl-2 showed no differences between H<sub>2</sub>O<sub>2</sub>-treated cells and those treated with both H<sub>2</sub>O<sub>2</sub> and an extremely low frequency magnetic field. Exposure to the magnetic field also had no effect on H<sub>2</sub>O<sub>2</sub>-induced caspase-3 activation. However, the protein levels of active caspase-7 in cells simultaneously exposed to an extremely low frequency magnetic field and H<sub>2</sub>O<sub>2</sub> for 2 and 8 h was higher than that of H<sub>2</sub>O<sub>2</sub> treatment alone. In addition, simultaneous exposure to an extremely low frequency magnetic field and H<sub>2</sub>O<sub>2</sub> caused poly(ADP-ribose) polymerase cleavage and induced early inactivation at 2 h, while H<sub>2</sub>O<sub>2</sub> treatment alone did not produce this effect until 4 h. **CONCLUSIONS:** The data suggest that although the magnetic field itself cannot induce apoptosis and necrosis, it exerts a promoting effect on H<sub>2</sub>O<sub>2</sub>-induced cell death, and it demonstrates that caspase-7 as well as poly(ADP-ribose) polymerase might be involved in this process.

**(E) \*Duan Y, Wang Z, Zhang H, He Y, Lu R, Zhang R, Sun G, Sun X. The preventive effect of lotus seedpod procyanidins on cognitive impairment and oxidative damage induced by extremely low frequency electromagnetic field exposure. *Food Funct.* 2013 Jun 14. [Epub ahead of print]**

The present study investigated the effects of lotus seedpod procyanidins (LSPCs) administered by oral gavage on the cognitive deficits and oxidative damage of mice at extremely low frequency electromagnetic field (ELF-EMF) exposure (50 Hz, 8 mT, 28 days). The results showed that 90 mg kg<sup>-1</sup> LSPCs treatment significantly increased body weight compared with the ELF-EMF group at ELF-EMF exposure and effectively maintained liver index, thymus index, kidney index and spleen index close to normal. A water maze test indicated that learning and memory abilities of the ELF-EMF group deteriorated significantly with ELF-EMF exposure when compared with the control group, but the ELF-EMF + LSPCs90 group had remarkably improved learning and memory abilities compared with the ELF-EMF group. Malondialdehyde (MDA), reactive oxygen species (ROS), nitric oxide (NO) and nitric oxide synthase (NOS) mostly exhibited significant increases, while the activities of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) decreased significantly under ELF-EMF exposure in the ELF-EMF group. LSPCs (especially 60, 90 mg kg<sup>-1</sup>) administration decreased MDA, ROS, NO content and lowered NOS activity in LSPCs treatment groups. Furthermore, LSPCs (60, 90 mg kg<sup>-1</sup>) treatment significantly augmented GPx, CAT, SOD activity in the hippocampus and serum. Pathological observation showed that number of pyramidal cells of the CA1 and CA3 regions of the hippocampus of the LSPCs treatment groups was significantly greater than the ELF-EMF group. All the data suggested that the LSPCs can effectively prevent learning and memory damage and oxidative damage caused by the ELF-EMF, most likely through the ability of LSPCs to scavenge oxygen free radicals and to stimulate antioxidant enzyme activity.

**(E) Emre M, Cetiner S, Zencir S, Unlukurt I, Kahraman I, Topcu Z. Oxidative stress and apoptosis in relation to exposure to magnetic field. Cell Biochem Biophys. 59(2):71-77, 2011.**

We investigated the effect of extremely low-frequency electromagnetic field (ELF-EMF) with pulse trains exposure on lipid peroxidation, and, hence, oxidative stress in the rat liver tissue. The parameters that we measured were the levels of plasma alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase as well as plasma albumin, bilirubin, and total protein levels in 30 adult male Wistar rats exposed to ELF. We also determined the percentage of apoptotic and necrotic cells of the kidney extracts from the animals by flow cytometry method. Apoptotic cell death was further characterized by monitoring DNA degradation using gel electrophoresis. The results showed an increase in the levels of oxidative stress indicators, and the flow cytometric data suggested a possible relationship between the exposure to magnetic field and the cell death. We showed significantly lower necrotic cell percentages in experimental animals compared to either unexposed or sham control groups. However, DNA ladder analyses did not differentiate between the groups. Our results were discussed in relation to the response of biological systems to EMF.

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**(E) Erdal N, Gürgül S, Tamer L, Ayaz L. Effects of long-term exposure of extremely low frequency magnetic field on oxidative/nitrosative stress in rat liver. J Radiat Res (Tokyo). 49(2):181-187, 2008.**

Thirty-two adult Wistar-Albino female and male rats were used to investigate the long-term (45 days) effects of extremely low frequency magnetic field (ELF-MF; 50Hz, 1mT, 4h/day) exposure on oxidative/nitrosative stress in liver tissues of rats. The rats were divided randomly into four groups: female control (FC; n = 8) and MF-exposed female rats (F-MF; n = 8); male control (MC; n = 8) and MF-exposed male rats (M-MF; n = 8). Liver tissue from each animal was harvested and utilized for malondialdehyde (MDA) and 3-nitrotyrosine (3-NT) detection. MDA levels were measured by MDA-TBA method, while the 3-NT levels were determined by the HPLC-UV system. There were no significant differences between the MDA levels of the control (FC; MC) and MF-exposed (F-MF; M-MF) rats ( $P > 0.05$ ). In the F-MF rats, 3-NT levels were significantly increased when compared to those of the FC rats ( $P < 0.05$ ). There were no significant differences between the 3-NT levels of the MC and M-MF rats. In conclusion, our study suggests that the long-term ELF-MF exposure may enhance the oxidative/nitrosative stress in liver tissue of the female rats and could have a deteriorative effect on cellular proteins rather than lipids by enhancing 3-NT formation.

**(E) Falone S, Mirabilio A, Carbone MC, Zimmitti V, Di Loreto S, Mariggì MA, Mancinelli R, Di Ilio C, Amicarelli F. Chronic exposure to 50 Hz magnetic fields causes a significant weakening of antioxidant defence systems in aged rat brain. Int J Biochem Cell Biol. 40(12):2762-2770, 2008.**

Several studies suggest that extremely low-frequency magnetic fields (ELF-MFs) may enhance the free radical endogenous production. It is also well known that one of the unavoidable consequences of ageing is an overall oxidative stress-based decline in several physiological functions and in the general resistance to stressors. On the basis of these assumptions, the aim of this study was to establish whether the ageing process can increase susceptibility towards widely present ELF-MF-mediated pro-oxidative challenges. To this end, female Sprague-Dawley rats were continuously exposed to a sinusoidal 50 Hz, 0.1 mT magnetic field for 10 days. Treatment-induced changes in the major antioxidant protection systems and in the neurotrophic support were investigated, as a function of the age of the subjects. All analyses were performed in brain

cortices, due to the high susceptibility of neuronal cells to oxidative injury. Our results indicated that ELF-MF exposure significantly affects anti-oxidative capability, both in young and aged animals, although in opposite ways. Indeed, exposed young individuals enhanced their neurotrophic signalling and anti-oxidative enzymatic defence against a possible ELF-MF-mediated increase in oxygen radical species. In contrast, aged subjects were not capable of increasing their defences in response to ELF-MF treatment but, on the contrary, they underwent a significant decrease in the major antioxidant enzymatic activities. In conclusion, our data seem to suggest that the exposure to ELF-MFs may act as a risk factor for the occurrence of oxidative stress-based nervous system pathologies associated with ageing.

**(E) Fernie KJ, Bird DM. Evidence of oxidative stress in American kestrels exposed to electromagnetic fields. Environ Res. 86(2):198-207, 2001.**

Exposure to electromagnetic fields (EMFs) alters melatonin, behavior, growth, and reproduction of captive American kestrels (*Falco sparverius*), particularly of males. EMF exposure is a "possible" human carcinogen and associated with some neurodegenerative diseases. Oxidative stress contributes to cancer, neurodegenerative diseases, and immune disorders. We tested whether EMF exposure elicits an avian immune response and alters oxidative stress levels. Captive male kestrels were bred under control or EMF conditions equivalent to those experienced by wild kestrels. Short-term EMF exposure (one breeding season) suppressed plasma total proteins, hematocrits, and carotenoids in the first half of the breeding season. It also suppressed erythrocyte cells and lymphocyte proportions, but elevated granulosa proportions at the end of the breeding season. Long-term EMF exposure (two breeding seasons) suppressed hematocrits in the first half of the reproductive period too. Results indicate that only short-term EMF birds experience an immune response, particularly during the early half of the breeding season. The elevation of granulocytes, and the suppression of carotenoids, total proteins, and previously melatonin in the same kestrels, signifies that the short-term EMF male kestrels had higher levels of oxidative stress, due to an immune response and/or EMF exposure. Long-term EMF exposure may be linked to higher levels of oxidative stress through EMF exposure only.

**(E) Fiorani M, Biagiarelli B, Vetrano F, Guidi G, Dachà M, Stocchi V. In vitro effects of 50 Hz magnetic fields on oxidatively damaged rabbit red blood cells. Bioelectromagnetics. 18(2):125-131, 1997.**

The aim of this study was to investigate the effects of 50 Hz magnetic fields (0.2-0.5 mT) on rabbit red blood cells (RBCs) that were exposed simultaneously to the action of an oxygen radical-generating system, Fe(II)/ascorbate. Previous data obtained in our laboratory showed at the exposure of rabbit erythrocytes or reticulocytes to Fe(II)/ascorbate hexokinase inactivation, whereas the other glycolytic enzymes do not show any decay. We also observed depletion of reduced glutathione (GSH) content with a concomitant intracellular and extracellular increase in oxidized glutathione (GSSG) and a decrease in energy charge. In this work we investigated whether 50 Hz magnetic fields could influence the intracellular impairments that occur when erythrocytes or reticulocytes are exposed to this oxidant system, namely, inactivation of hexokinase activity, GSH depletion, a change in energy charge, and hemoglobin oxidation. The results obtained indicate that a 0.5 mT magnetic field had no effect on intact RBCs, whereas it increased the damage with Fe(II)/ascorbate to a 0.5 mT magnetic field induced a significant further decay in hexokinase activity (about 20%) as well as a twofold increase in methemoglobin production compared with RBCs that were exposed to the oxidant system alone. Although further studies will be needed to determine the physiological implications of these data, the results

reported in this study demonstrate that the effects of the magnetic fields investigated are able to potentiate the cellular damage induced in vitro by oxidizing agents.

**(E) Fitzsimmons RJ, Gordon SL, Kronberg J, Ganey T, Pilla AA. A pulsing electric field (PEF) increases human chondrocyte proliferation through a transduction pathway involving nitric oxide signaling. J Orthop Res. 26(6):854-859, 2008.**

A potential treatment modality for joint pain due to cartilage degradation is electromagnetic fields (EMF) that can be delivered, noninvasively, to chondrocytes buried within cartilage. A pulsed EMF in clinical use for recalcitrant bone fracture healing has been modified to be delivered as a pulsed electric field (PEF) through capacitive coupling. It was the objective of this study to determine whether the PEF signal could have a direct effect on chondrocytes in vitro. This study shows that a 30-min PEF treatment can increase DNA content of chondrocyte monolayer by approximately 150% at 72 h poststimulus. Studies intended to explore the biological mechanism showed this PEF signal increased nitric oxide measured in culture medium and cGMP measured in cell extract within the 30-min exposure period. Increasing calcium in the culture media or adding the calcium ionophore A23187, without PEF treatment, also significantly increased short-term nitric oxide production. The inhibitor W7, which blocks calcium/calmodulin, prevented the PEF-stimulated increase in both nitric oxide and cGMP. The inhibitor L-NAME, which blocks nitric oxide synthase, prevented the PEF-stimulated increase in nitric oxide, cGMP, and DNA content. An inhibitor of guanylate cyclase (LY83583) blocked the PEF-stimulated increase in cGMP and DNA content. A nitric oxide donor, when present for only 30 min, increased DNA content 72 h later. Taken together, these results suggest the transduction pathway for PEF-stimulated chondrocyte proliferation involves nitric oxide and the production of nitric oxide may be the result of a cascade that involves calcium, calmodulin, and cGMP production.

**(E) \*Frahm J, Mattsson MO, Simkó M. Exposure to ELF magnetic fields modulate redox related protein expression in mouse macrophages. Toxicol Lett. 192(3):330-336, 2010.**

The interaction of extremely low frequency (ELF) magnetic fields (MF) with cells can induce alterations in various cell physiological processes. Here, we present evidence that exposure of mouse macrophages to 50 Hz, 1.0 mT MF lead to immune cell activation seen as increased production of reactive oxygen species (ROS), and also to modulation on the expression level of important proteins acting in redox regulatory processes and thus explaining the noted changes in ROS levels seen after exposure. The MF exposure caused slight and transient decreases after short term exposures (2h or less) of clathrin, adaptin, PI3-kinase, protein kinase B (PKB) and PP2A, whereas longer exposures had no effect. The levels of the NAD(P)H oxidase subunit gp91phox oscillated between increased and normal levels compared to controls. The stress proteins Hsp70 and Hsp110 exhibited increased levels at certain time points, but not generally. The effects of MF on protein levels are different from the effects exerted by 12-O-tetradecanolyphobol-13-acetate (TPA) or LPS, although all three factors cause increases in ROS release. This suggests that ELF MF interacts with other cellular constituents than these chemicals, although induced pathways at least partially converge.

**(E) Frahm J, Lantow M, Lupke M, Weiss DG, Simkó M. Alteration in cellular functions in mouse macrophages after exposure to 50 Hz magnetic fields. J Cell Biochem. 99(1):168-177, 2006.**

The aim of the present study is to investigate whether extremely low frequency electromagnetic fields (ELF-EMF) affect certain cellular functions and immunologic parameters of mouse

macrophages. In this study, the influence of 50 Hz magnetic fields (MF) at 1.0 mT was investigated on the phagocytic activity and on the interleukin-1beta (IL-1beta) production in differentiated macrophages. MF-exposure led to an increased phagocytic activity after 45 min, shown as a 1.6-fold increased uptake of latex beads in MF-exposed cells compared to controls. We also demonstrate an increased IL-1beta release in macrophages after 24 h exposure (1.0 mT MF). Time-dependent IL-1beta formation was significantly increased already after 4 h and reached a maximum of 12.3-fold increase after 24 h compared to controls. Another aspect of this study was to examine the genotoxic capacity of 1.0 mT MF by analyzing the micronucleus (MN) formation in long-term (12, 24, and 48 h) exposed macrophages. Our data show no significant differences in MN formation or irregular mitotic activities in exposed cells. Furthermore, the effects of different flux densities (ranging from 0.05 up to 1.0 mT for 45 min) of 50 Hz MF was tested on free radical formation as an endpoint of cell activation in mouse macrophage precursor cells. All tested flux densities significantly stimulated the formation of free radicals. Here, we demonstrate the capacity of ELF-EMF to stimulate physiological cell functions in mouse macrophages shown by the significantly elevated phagocytic activity, free radical release, and IL-1beta production suggesting the cell activation capacity of ELF-EMF in the absence of any genotoxic effects.

**(E) Garip AI, Akan Z. Effect of ELF-EMF on number of apoptotic cells; correlation with reactive oxygen species and HSP. Acta Biol Hung. 61(2):158-167, 2010.**

It is by now accepted that extremely low frequency electromagnetic fields ELF-EMF (0-300 Hz) affect biological systems although the mechanism has not been elucidated yet. In this study the effect of ELF-EMF on the number of apoptotic cells of K562 human leukemia cell line induced or not with oxidative stress and the correlation with heat-shock protein 70 (hsp70) levels was investigated. One sample was treated with H<sub>2</sub>O<sub>2</sub> while the other was left untreated. ELF-EMF (1 mT, 50 Hz) was applied for 3 hours. ELF-EMF alone caused a decrease in the number of apoptotic cells and a slight increase in viability. However, it increased the number of apoptotic cells. In cells treated with H<sub>2</sub>O<sub>2</sub>, hsp70 and reactive oxygen species (ROS) levels were increased by ELF-EMF. These results show that the effect of ELF-EMF on biological systems depends on the status of the cell: while in cells not exposed to oxidative stress it is able to decrease the number of apoptotic cells by inducing an increase in hsp levels, it increases the number of apoptotic cells in oxidative stress-induced cells.

**(E) \*Ghodbane S, Amara S, Arnaud J, Garrel C, Faure H, Favier A, Sakly M, Abdelmelek H. Effect of selenium pre-treatment on plasma antioxidant vitamins A (retinol) and E ( $\alpha$ -tocopherol) in static magnetic field-exposed rats. Toxicol Ind Health. 27(10):949-955, 2011.**

In the present study, we evaluate the effect of the co-exposure to static magnetic field (SMF) and selenium (Se) on the antioxidant vitamins A and E levels and some other parameters of oxidative stress in rat. Sub-acute exposure of male adult rats to a uniform SMF (128 mT, 1 h/day during 5 consecutive days) increased plasma activity of glutathione peroxidase (+35%) but decreased  $\alpha$ -tocopherol (-67%) and retinol levels (-41%). SMF exposure failed to alter the plasmatic thiobarbituric acid-reactive species (TBARs), total thiol groups and selenium concentrations. Sub-chronic administration of Se (Na<sub>2</sub>SeO<sub>3</sub>, 0.2 mg/L, for 30 consecutive days, per os) ameliorated the antioxidant capacities in SMF-treated rats. Our investigation demonstrated that sub-acute exposure to SMF induced oxidative stress, which may be prevented by a pretreatment with selenium.

**(E) \*Glinka M, Sieroń A, Birkner E, Cieślak G. Influence of extremely low-frequency magnetic field on the activity of antioxidant enzymes during skin wound healing in rats. Electromagn Biol Med. 2013 Jan 16. [Epub ahead of print]**

The aim of this study was to evaluate the activity of the antioxidant enzymes mitochondrial and cytosolic superoxide dismutase (EC 1.15.1.1), glutathione peroxidase (POX, EC 1.11.1.9) and glutathione S-transferase (EC 3.1.2.7), as well as the concentration of malone dialdehyde (MDA), as an indicator of lipid peroxidation rate in the liver tissue homogenates and blood serum of male rats exposed to extremely low-frequency magnetic field (ELF-MF) in order to improve the healing process of an experimental cut wound on the back of each animal. The exposure to ELF-MF with frequency 40 Hz and magnetic flux density 10 mT induced an increase in POX serum activity and a decrease in MDA contents in the liver tissue, which suggests the inhibition of phospholipid peroxidation and subsequent stabilization of cellular membranes, as a result of ELF-MF action. Based on the results obtained, it seems that ELF-MF could be a useful supplement in the complex treatment of prolonged wound healing, due to the activation of endogenous enzymatic antioxidant system.

**(E) Goraca A, Ciejka E, Piechota A. Effects of extremely low frequency magnetic field on the parameters of oxidative stress in heart. J Physiol Pharmacol. 61(3):333-338, 2010.**

Increasing production of free radicals in organisms is one of the putative mechanisms by which a extremely low frequency magnetic field (ELF-MF) may affect biological systems. The present study was designated to assess if ELF-MF applied in the magnetotherapy, affects generation of reactive oxygen species (ROS) in heart tissue and antioxidant capacity of plasma according to its working time. The experiments were performed on 3 groups of animals: group I - control; group II - exposed to 40 Hz, 7 mT, 30 min/day for 14 days (this field is commonly applied in magnetotherapy); group III - exposed to 40 Hz, 7 mT, 60 min/day for 14 days. Control rats were housed in a separate room without exposure to ELF-MF. Immediately after the last exposure, blood was taken from the tail vein and hearts were removed under anesthesia. The effect of the exposure to ELF-MF on oxidative stress was assessed on the basis of the measurements of thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), total free sulphhydryl groups (-SH groups) and reduced glutathione (GSH) concentrations in heart homogenates. The total antioxidant capacity of plasma was measured using ferric reducing ability method (FRAP). Exposure to ELF-MF (40 Hz, 7 mT, 30 min/day for 2 weeks) did not significantly alter tissue TBARS, H<sub>2</sub>O<sub>2</sub>, total free -SH groups, reduced glutathione (GSH) and total antioxidant capacity of plasma. By contrast, ELF-MF with the same frequency and induction but used for 60 min/day for 14 days caused significant increase in TBARS and H<sub>2</sub>O<sub>2</sub> concentration (P<0.01) and decrease in the concentration of GSH (P<0.05) and total free -SH groups in heart homogenates. Moreover, exposure of rats to ELF-MF (40 Hz, 7 mT, 60 min/day for 2 weeks) resulted in the decrease of plasma antioxidant capacity. Our results indicate that effects of ELF-MF on ROS generation in the heart tissue and antioxidant capacity of plasma depend on its working time.

**(E) Grigor'ev IuG, Mikhaïlov VF, Ivanov AA, Mal'tsev VN, Ulanova AM, Stavrakova NM, Nikolaeva IA, Grigor'ev OA. [Autoimmune processes after long-term low-level exposure to electromagnetic fields (the results of an experiment). Part 4. Manifestation of oxidative intracellular stress-reaction after long-term non-thermal EMF exposure of rats] Radiats Biol Radioecol. 50(1):22-27, 2010. [Article in Russian]**

This paper presents the results of the study of the effects of long-term low-level exposure of rats to microwaves. Rats were exposed in far field to 2450 MHz continuous wave fields providing an incident power density at the cages of 500 microW/cm<sup>2</sup> for 7 hours daily for a total of 30 days resulting in a whole-body SAR of 0.16 +/- 0.04 W/kg. Three groups ("EMF-exposure", "sham-exposure" and cage-control) were formed, each consisting of 16 rats. Circulating antibodies (IgA, IgG and IgM) directed against 16 chemical substances were evaluated in coded serum from each group of rats by enzyme multiplied analysis (ELISA test). An increased amount of compounds resulting from interaction of amino acids with nitric oxide (NO) or its derivatives (NO<sub>2</sub>-Tyrosine, NO-Arginine, NO-Cysteine + NO-Bovine Serum Albumin, NJ-Methionine + NO-Asparagine + No-Histidine, NO-BTrypnohan + NJ-Tyrosin), fatty acids with small chains, hydroxylated fatty acids, palmitic/myristic/oleic acid, AZE (product of oxidation of fatty acids) was found in blood serum from EMF-exposed rats. As a rule, antibodies to conjugated antigens were seen for IgM, rarely seen for IgG and were completely absent for IgA. The levels of antibodies were higher on day 7 after the exposure compared to those on day 14 after the exposure.

**(E) Güler G, Türközer Z, Ozgur E, Tomruk A, Seyhan N, Karasu C. Protein oxidation under extremely low frequency electric field in guinea pigs. Effect of N-acetyl-L-cysteine treatment. Gen Physiol Biophys. 28(1):47-55, 2009.**

Modern age exposes humans to an increasing level of electromagnetic activity in their environment due to overhead power lines and transformers around residential areas. Studies have shown that treatment with antioxidants can suppress the oxidative damage induced by electromagnetic fields in various frequencies of the non-ionizing radiation band. In this study, we detected protein carbonyl content (PCO), advanced oxidation protein products (AOPP) in liver and 3-nitrotyrosine (3-NT) levels in plasma of guinea pigs in order to investigate the effects of N-acetyl-L-cysteine (NAC) administration on oxidative protein damage induced by power frequency electric (E) field (50 Hz, 12 kV/m, 7 days/8 h/day). We also analyzed hepatic hydroxyproline level to study protein synthesis. According to the findings of the present study, no statistically significant changes occurred in PCO, AOPP and 3-NT levels of the guinea pigs that were exposed to the E field with respect to the control group. However, liver hydroxyproline level was significantly diminished in the E field exposure group compared to the control and PCO, hydroxyproline and 3-NT levels changed significantly in the NAC-administrated groups.

**(NE) Güler G, Türközer Z, Ozgur E, Seyhan N. Antioxidants alleviate electric field-induced effects on lung tissue based on assays of heme oxygenase-1, protein carbonyl content, malondialdehyde, nitric oxide, and hydroxyproline. Sci Total Environ. 407(4):1326-1332, 2009.**

In order to test whether antioxidants have beneficiary effects on electric field induced damage, we determined the pulmonary levels of heme oxygenase-1 (HO-1), protein carbonyl content (PCO), malondialdehyde (MDA), nitric oxide (NO) and hydroxyproline (HP) under extremely low frequency (ELF) electric (E) field exposure (50 Hz, 12 kV/m, 7 days/for 8 h/day). While PCO levels significantly increased ( $p < 0.05$ ), insignificant changes ( $p > 0.05$ ) were observed in HO-1, MDA, NO and HP levels for electric field exposure groups compared to the control group. We have not observed any significant change in these parameters on the electric field group compared to the group where NAC and EGCG were separately applied along with electric field. However, during our previous studies, we have concluded that NAC and EGCG are potent antioxidants and we believe that new studies should be established by way of setting up different experimental conditions.

**(E) Guler G, Turkozer Z, Tomruk A, Seyhan N. The protective effects of N-acetyl-L-cysteine and epigallocatechin-3-gallate on electric field-induced hepatic oxidative stress. Int J Radiat Biol. 84(8):669-680, 2008.**

PURPOSE: To investigate the effects of 12 kV/m electric (E) field sourced by power lines on oxidative and nitrosative stress, and antioxidant status. Furthermore, the study aimed to examine the protective effects of N-Acetyl-L-cysteine (NAC) and epigallocatechin-gallate (EGCG) in the liver tissues of guinea pigs against the possible detriments of electromagnetic field exposure. MATERIALS AND METHODS: Guinea pigs were exposed to 50 Hz 12 kV/m E-field. NAC and EGCG were administered intraperitoneally. Malonaldehyde (MDA), a product of lipid peroxidation (LPO), and nitric oxide derivatives (nitrate (NO(3)), nitrite (NO(2)), total level of nitric oxide (NO(x))) were estimated as biomarkers of oxidative and nitrosative stress, respectively. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and myeloperoxidase (MPO) were evaluated as endogenous antioxidant enzymes in liver tissues of the guinea pigs. RESULTS: The results of our study indicated a significant increase in the levels of oxidant products (MDA, NO(3), NO(2), NO(x)), and a significant decrease in antioxidant enzyme (SOD, GSH-Px and MPO) activities. We also found that the individual or plus application of NAC and EGCG resulted in the reduction of oxidative stress prior to E field application. CONCLUSION: To conclude, extremely low frequency (ELF) electric field has potential harmful effects on the living organisms by enhancing the free radical production. NAC and EGCG might have hepatoprotective effects in ELF-E field induced oxidative and nitrosative stress.

**(E) Hajnorouzi A, Vaezzadeh M, Ghanati F, Jamnezhad H, Nahidian B. Growth promotion and a decrease of oxidative stress in maize seedlings by a combination of geomagnetic and weak electromagnetic fields. J Plant Physiol. 168(10):1123-1128, 2011.**

In the present study, we hypothesized that an appropriate combination of a geomagnetic field (as a static field) and an alternative magnetic field may result in the promotion of maize seedling growth by an alleviation of an excess production of reactive oxygen species. First, we determined the applicable range of frequencies by theoretical calculations, and a combined magnetic field was designed. The seeds were germinated in the magnetic field for 4 days, and the seedlings were allowed to grow in a nutrient solution for another 4 days. The magnetic field-treated maize seeds produced seedlings with a faster growth rate than the control seeds. The activity of superoxide dismutase in the magnetic field-treated seedlings was lower, while the total antioxidant capacity of these seedlings was higher than that of the control group. The maintenance of membrane integrity and a decrease of iron content in the magnetic field-treated seedlings suggest that a combination of both static and alternative magnetic fields promotes the growth of the plants by lowering iron absorption, a reduction in the Fenton chemistry, and lowering the risk of oxidative burst.

**(NE) Harakawa S, Inoue N, Hori T, Tochio K, Kariya T, Takahashi K, Doge F, Suzuki H, Nagasawa H. Effects of a 50 Hz electric field on plasma lipid peroxide level and antioxidant activity in rats. Bioelectromagnetics. 26(7):589-594, 2005.**

The effects of exposure to extremely low frequency electric fields (ELF EFs) on plasma lipid peroxide levels and antioxidant activity (AOA) in Sprague-Dawley rats were studied. The test was based on comparisons among rats treated with a combination of the oxidizing agent, 2,2'-azobis(2-aminopropane) dihydrochloride (AAPH) and 50 Hz EF of 17.5 kV/m intensity for 15 min per day for 7 days, AAPH alone, EF alone or no treatment. EF significantly decreased the plasma peroxide level in rats treated with AAPH, similar to treatment by ascorbic acid or the

superoxide dismutase. Ascorbic acid increased AOA; however, EF and superoxide dismutase did not change AOA compared with sham exposure in stressed rats. No influence on the lipid peroxide level and AOA in unstressed rats was observed with EF exposure alone. Although the administration of AAPH decreased AOA, this decrease did not change when EF was added. These data indicate that the ELF EF used in this study influenced the lipid peroxide level in an oxidatively stressed rat.

**(E) Hashish AH, El-Missiry MA, Abdelkader HI, Abou-Saleh RH. Assessment of biological changes of continuous whole body exposure to static magnetic field and extremely low frequency electromagnetic fields in mice. *Ecotoxicol Environ Saf.* 71(3):895-902. 2008.**

The question whether static magnetic fields (SMFs) and extremely low frequency electromagnetic fields (ELF-EMF) cause biological effects is of special interest. We investigated the effects of continuous whole body exposure to both fields for 30 days on some liver and blood parameters in mice. Two exposure systems were designed; the first produced a gradient SMF while the second generated uniform 50Hz ELF-EMF. The results showed a gradual body weight loss when mice were exposed to either field. This is coupled with a significant decrease ( $P < 0.05$ ) in the levels of glucose, total protein and the activity of alkaline phosphatase in serum. A significant increase in lactate dehydrogenase activity was demonstrated in serum and liver paralleled with a significant elevation in hepatic gamma-glutamyl transferase activity. The glutathione-S-transferase activity and lipid peroxidation level in the liver were significantly increased while a significant decrease in hepatic glutathione content was recorded. A significant decrease in the counts of monocytes, platelets, peripheral lymphocytes as well as splenic total, T and B lymphocytes levels was observed for SMF and ELF-EMF exposed groups. The granulocytes percentage was significantly increased. The results indicate that there is a relation between the exposure to SMF or ELF-EMF and the oxidative stress through distressing redox balance leading to physiological disturbances.

**(E) Henrykowska G, Jankowski W, Pacholski K, Lewicka M, Smigielski J, Dziedziczak-Buczyńska M, Buczyński A. The effect of 50 hz magnetic field of different shape on oxygen metabolism in blood platelets: in vitro studies. *Int J Occup Med Environ Health.* 22(3):269-276, 2009.**

**OBJECTIVES:** The aim of the study was to assess the influence that the shape of low frequency magnetic field may have on catalase and superoxide dismutase activity, malondialdehyde concentration and free radicals generation in human blood platelets. **MATERIALS AND METHODS:** The suspension of human blood platelets was exposed for 15 min to 50 Hz magnetic field of different shape, and flux density of 10 mT. **RESULTS:** The determinations of free radicals, malondialdehyde and catalase showed increased values compared with the initial level, regardless of the shape of the magnetic field applied. In contrast, superoxide dismutase activity was lower than at the onset of the experiment. **CONCLUSIONS:** The findings indicate that the oxidative stress resulting from exposure to 50 Hz magnetic field of 10 mT induction may produce a number of adverse effects within the cell and thus may lead to systemic disturbances in the human body.

**(NE) \*Hong MN, Han NK, Lee HC, Ko YK, Chi SG, Lee YS, Gimm YM, Myung SH, Lee JS. Extremely low frequency magnetic fields do not elicit oxidative stress in MCF10A cells. Radiat Res. 53(1):79-86, 2012.**

The aim of this study was to determine whether extremely low frequency magnetic fields (ELF-MF) could affect intracellular reactive oxygen species (ROS) levels and antioxidant enzyme activity. After MCF10A human breast epithelial cells were exposed to 1 mT of 60 Hz ELF-MF for 4 hours, intracellular ROS level, superoxide dismutase (SOD) activity, and reduced to oxidized glutathione (GSH/GSSG) ratio were measured. The cells exposed to ELF-MF did not evidence statistically significant changes in the above-mentioned biological parameters as compared to either the incubator controls or sham-exposed cells. By way of contrast, the IR-exposed cells exhibited marked changes in ROS level, SOD activity, and GSH/GSSG ratio. When we assessed morphological changes and senescence-associated beta-galactosidase (SA- $\beta$ -Gal) activity, only the IR-exposed cells were positive. According to our results, it could be concluded that ELF-MF has no effect on intracellular ROS level, SOD activity, and GSH/GSSG ratio under our exposure condition.

**(E) Jajte J, Zmysłony M. [The role of melatonin in the molecular mechanism of weak, static and extremely low frequency (50 Hz) magnetic fields (ELF)] Med Pr. 51(1):51-57, 2000. [Article in Polish]**

Melatonin is a neurohormone produced by the pineal gland. It has been recently found that it is also an antioxidant and a free radical scavenger. Melatonin was documented to be a direct trap of hydroxyl and peroxy radicals. Therefore, this hormone could protect cells, tissues and organs against oxidative (free radicals) damage (DNA, protein, lipids). It has been suggested that noxious effects of ELF exposure (cancer or immunological disturbances) could be due to increased the concentration of free radicals induced by magnetic field. This is also leading to a hypothesis that melatonin suppression (by electromagnetic fields) in humans may increase the probability of mutagenic and carcinogenic risks. The future experiments, in vitro and in vivo, should provide an answer to the question on what is the real role of melatonin in the molecular (free radicals) mechanisms of weak magnetic fields.

**(E) Jajte J, Zmysłony 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 pre-incubation, part of the cell samples were supplemented with melatonin (0.5 or 1.0mM). The experiments were performed on the cell samples incubated for 3h in Helmholtz coils at 7mT 50Hz MF. During MF exposure, some samples were treated with ferrous chloride (FeCl<sub>2</sub>, 10microg/ml), while the rest served as controls. A significant increase in the number of cells with DNA damage was found only after simultaneous exposure of lymphocytes to FeCl<sub>2</sub> and 7mT 50Hz MF, compared to the control samples or those incubated with FeCl<sub>2</sub> 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.

**(E) Jelenković A, Janać B, Pesić V, Jovanović DM, Vasiljević I, Prolić Z. Effects of extremely low-frequency magnetic field in the brain of rats. Brain Res Bull. 68(5):355-360, 2006.**

An extremely low-frequency magnetic field (50 Hz, 0.5 mT) was used to investigate its possible effect on the brain of adult male Wistar rats following a 7-day exposure. The control rats were sham-exposed. Superoxide dismutase activities and production of superoxide radicals, lipid peroxidation, and nitric oxide were examined in the frontal cortex, striatum, basal forebrain, hippocampus, brainstem, and cerebellum. Significantly increased superoxide radical contents were registered in all the structures examined. Production of nitric oxide, which can oppose superoxide radical activities, was significantly increased in some structures: the frontal cortex, basal forebrain, hippocampus, and brainstem. Augmentation of lipid peroxydation was also observed, with significance only in the basal forebrain and frontal cortex, in spite of the significantly increased superoxide dismutase activities and nitric oxide production in the basal forebrain, and increased production of nitric oxide in the frontal cortex. The results obtained indicate that a 7-day exposure to extremely low-frequency magnetic field can be harmful to the brain, especially to the basal forebrain and frontal cortex due to development of lipid peroxidation. Also, high production of superoxide anion in all regions may compromise nitric oxide signaling processes, due to nitric oxide consumption in the reaction with the superoxide radical.

**(E) Jeong JH, Kum C, Choi HJ, Park ES, Sohn UD. Extremely low frequency magnetic field induces hyperalgesia in mice modulated by nitric oxide synthesis. Life Sci. 78(13):1407-1412, 2006.**

We investigated an effect of extremely low frequency magnetic field (ELF-MF, 60 Hz) on hyperalgesia using hot plate test. The level of nitric oxide (NO) and the expression of nitric oxide synthase (NOS) were measured to determine if ELF-MF is engaged in NO mediated pain mechanism. Additionally, the involvement of Ca<sup>2+</sup>-dependent NO pathway in ELF-MF induced hyperalgesia was evaluated by blocking Ca<sup>2+</sup> sources with NMDA receptor antagonist and Ca<sup>2+</sup> channel blocker. The exposure of mice to ELF-MF lowered pain threshold and elevated NO synthesis in brain and spinal cord. An NOS inhibitor blocked these effects of ELF-MF with attenuating the reduction of pain threshold and the rise of NO level in brain and spine by the exposure of ELF-MF. The hyperalgesic effects of ELF-MF were also blocked by a Ca<sup>2+</sup> channel blocker, nimodipine, but not by a NMDA receptor antagonist, MK-801. The expression of Ca<sup>2+</sup>-dependent nNOS and eNOS and Ca<sup>2+</sup>-independent iNOS were not changed by ELF-MF. These results indicated that the exposure of ELF-MF might cause Ca<sup>2+</sup>-dependent NOS activation, which then induces hyperalgesia with the increase in NO synthesis. In conclusion, ELF-MF may produce hyperalgesia by modulating NO synthesis via Ca<sup>2+</sup>-dependent NOS.

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

The principal objective of this study was to assess the DNA damage in a normal cell line system after exposure to 60 Hz of extremely low frequency magnetic field (ELF-MF) and particularly in combination with various external factors, via comet assays. NIH3T3 mouse fibroblast cells, WI-38 human lung fibroblast cells, L132 human lung epithelial cells, and MCF10A human mammary gland epithelial cells were exposed for 4 or 16 h to a 60-Hz, 1 mT uniform magnetic field in the presence or absence of ionizing radiation (IR, 1 Gy), H<sub>2</sub>O<sub>2</sub> (50 μM), or c-Myc oncogenic activation. The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic or additive effects were observed after 4 or 16 h of pre-exposure to 1 mT ELF-MF or simultaneous exposure to ELF-MF combined with IR, H<sub>2</sub>O<sub>2</sub>, or c-Myc activation.

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

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) Kavaliers M, Choleric E, Prato FS, Ossenkopp K. Evidence for the involvement of nitric oxide and nitric oxide synthase in the modulation of opioid-induced antinociception and the inhibitory effects of exposure to 60-Hz magnetic fields in the land snail. Brain Res. 809(1):50-57, 1998.**

The attenuation of opioid peptide-mediated antinociception is a well-established effect of extremely low frequency (ELF) electromagnetic fields with alterations in calcium channel function and/or calcium ion flux and protein kinase C activity being implicated in the mediation of these effects. The present study was designed to examine the effects of nitric oxide (NO) and calcium ion/calmodulin-dependent nitric oxide synthase (NOS) on opioid-induced antinociception and their involvement in mediating the inhibitory effects of exposure to ELF magnetic fields. We observed that enkephalinase (SCH 34826)-induced, and likely enkephalin-mediated, antinociception in the land snail, *Cepaea nemoralis*, as measured by the enhanced latency of a foot withdrawal response to a thermal (40 degreesC) stimulus, was reduced by the

NO releasing agent, S-nitro-N-acetylpenicillamide (SNP), and enhanced by the NO synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME). Exposure of snails to an ELF magnetic field (15 min, 60 Hz, 141 microT peak) also reduced the enkephalinase-induced antinociception. The inhibitory effects of the 60-Hz magnetic field were significantly reduced by the NO synthase inhibitor, L-NAME, and significantly enhanced by the NO releasing agent, SNP, at dosages which by themselves had no evident effects on nociceptive sensitivity. These results suggest that: (1) NO and NO synthase have antagonistic effects on opioid-induced analgesia in the snail, *Cepaea* and (2) the inhibitory effects of ELF magnetic fields on opioid analgesia involve alteration in NO and NO synthase activity.

**(E) Khadir R, Morgan JL, Murray JJ. Effects of 60 Hz magnetic field exposure on polymorphonuclear leukocyte activation. *Biochim Biophys Acta.* 1472(1-2):359-367, 1999.**

We have investigated the effects of a sinusoidal 60 Hz magnetic field on free radical (superoxide anion) production, degranulation (beta-glucuronidase and lysozyme release) and viability in human neutrophils (PMNs). Experiments were performed blindly in very controlled conditions to examine the effects of a magnetic field in resting PMNs and in PMNs stimulated with a tumor promoter: phorbol 12-myristate 13-acetate (PMA). Exposure of unstimulated human PMNs to a 60 Hz magnetic field did not affect the functions examined. In contrast, exposure of PMNs to a 22 milliTesla (mT), 60 Hz magnetic field induced significant increases in superoxide anion (O<sub>2</sub>-) production (26.5%) and in beta-glucuronidase release (53%) when the cells were incubated with a suboptimal stimulating dose of PMA. Release of lysozyme and lactate dehydrogenase was unchanged by the magnetic field, whether the cells were stimulated or not. A 60 Hz magnetic field did not have any effect on O<sub>2</sub>- generation by a cell-free system xanthine/xanthine oxidase, suggesting that a magnetic field could upregulate common cellular events (signal transduction) leading to O<sub>2</sub>- generation and beta-glucuronidase release. In conclusion, exposure of PMNs to a 22 mT, 60 Hz magnetic field potentiates the effect of PMA on O<sub>2</sub>- generation and beta-glucuronidase release. This effect could be the result of an alteration in the intracellular signaling.

**(E) Koh EK, Ryu BK, Jeong DY, Bang IS, Nam MH, Chae KS. A 60-Hz sinusoidal magnetic field induces apoptosis of prostate cancer cells through reactive oxygen species. *Int J Radiat Biol.* 2008 Nov;84(11):945-955, 2008.**

**PURPOSE:** To explore the effects of power frequency magnetic fields (MF) on cell growth in prostate cancer, DU145, PC3, and LNCaP cells were examined in vitro. **MATERIALS AND METHODS:** The cells were exposed to various intensities and durations of 60-Hz sinusoidal MF in combination with various serum concentrations in the media. To analyze MF effects on cell growth, cell counting, trypan blue exclusion assay, Western blot analysis, flow cytometry, enzyme-linked immunosorbent assay (ELISA), semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), fluorescence microscopy, and spectrofluorometry were used. **RESULTS:** MF exposure induced significant cell growth inhibition and apoptosis in an intensity- and time-dependent manner, in which cell cycle arrest, cleaved Caspase-3, and reactive oxygen species (ROS) increased. Pretreatment with a Caspase-3 inhibitor or antioxidant, N-acetyl-L-cysteine (NAC), significantly attenuated MF-induced cell growth inhibition and cell death. Media replacement experiments failed to show any notable change in the MF effects. **CONCLUSIONS:** These results demonstrate 60-Hz sinusoidal MF-activated cell growth inhibition of prostate cancer in vitro. Apoptosis together with cell cycle arrest were the dominant causes of the MF-elicited cell growth inhibition, mediated by MF-induced ROS. These results suggest that a possibility of using 60-Hz MF in radiation therapy of prostate cancer could usefully be investigated.

**(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.**

PURPOSE: To detect the effects of extremely low frequency (ELF) magnetic fields, the number of apurinic/apyrimidinic (AP) sites in human glioma A172 cells was measured following exposure to ELF magnetic fields. MATERIALS AND METHODS: The cells were exposed to an ELF magnetic field alone, to genotoxic agents (methyl methane sulfonate (MMS) and hydrogen peroxide (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) Koyama S, Nakahara T, Hirose H, Ding GR, Takashima Y, Isozumi Y, Miyakoshi J. ELF electromagnetic fields increase hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-induced mutations in pTN89 plasmids. Mutat Res. 560(1):27-32, 2004.**

We have examined the mutational effects of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in the presence and absence of an extremely low-frequency magnetic field (ELFMF), using pTN89 plasmids. Mutations were detected in the supF gene carried by these plasmids in Escherichia coli. The plasmids were either treated with H<sub>2</sub>O<sub>2</sub> (1microM) alone at 37 degrees C for 4h, or were exposed to an ELFMF (60Hz, 5millitesla (mT)) simultaneously with H<sub>2</sub>O<sub>2</sub> treatment. The mutation frequency was 2.28 x 10<sup>(-4)</sup> for H<sub>2</sub>O<sub>2</sub> treatment alone, and 5.81 x 10<sup>(-4)</sup> for ELFMF exposure with H<sub>2</sub>O<sub>2</sub> treatment. We did not observe any mutations using treatment with ELFMF exposure alone. This indicates that the ELFMF may potentiate H<sub>2</sub>O<sub>2</sub>-induced mutation. Sequence analysis of the supF mutant plasmids revealed that base substitutions, G: C-->A :T transitions and G:C-->T:A transversions were dominant in both treatment groups, and there was no difference in the mutation spectrum or the hotspots between the groups. Therefore, ELFMFs may interact and potentiate the damage induced by H<sub>2</sub>O<sub>2</sub>, resulting in an increase in the number of mutations.

**(E) Lai H, Singh NP. Melatonin and N-tert-butyl-alpha-phenylnitron 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-phenylnitron (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.

**(E) 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 N-tert-butyl-alpha-phenylnitron, 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) Lee BC, Johng HM, Lim JK, Jeong JH, Baik KY, Nam TJ, Lee JH, Kim J, Sohn UD, Yoon G, Shin S, Soh KS. Effects of extremely low frequency magnetic field on the antioxidant defense system in mouse brain: a chemiluminescence study. J Photochem Photobiol B. 73(1-2):43-48, 2004.**

Among the putative mechanisms, by which extremely low frequency (ELF) magnetic field (MF) may affect biological systems is that of increasing free radical life span in organisms. To test this hypothesis, we investigated whether ELF (60 Hz) MF can modulate antioxidant system in mouse brain by detecting chemiluminescence and measuring superoxide dismutase (SOD) activity in homogenates of the organ. Compared to sham exposed control group, lucigenin-initiated chemiluminescence in exposed group was not significantly increased. However, lucigenin-amplified t-butyl hydroperoxide (TBHP)-initiated brain homogenates chemiluminescence, was significantly increased in mouse exposed to 60 Hz, MF, 12 G for 3 h compared to sham exposed group. We also measured SOD activity, that plays a critical role of the antioxidant defensive system in brain. In the group exposed to 60 Hz, MF, 12 G for 3 h, brain SOD activity was significantly increased. These results suggest that 60 Hz, MF could deteriorate antioxidant defensive system by reactive oxygen species (ROS), other than superoxide radicals. Further studies are needed to identify the kind of ROS generated by the exposure to 60 Hz, MF and elucidate how MF can affect biological system in connection with oxidative stress.

**(E) 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.**

**PURPOSE:** The purpose of this study is to investigate the mechanism of cellular proliferation of electromagnetic field (EMF) on human intervertebral disc (IVD) cells. **MATERIALS AND METHODS:** Human IVD cells were cultured three-dimensionally in alginate beads. EMF was exposed to IVD cells with 650  $\Omega$ , 1.8 millitesla magnetic flux density, 60 Hz sinusoidal wave.

Cultures were divided into a control and EMF group. Cytotoxicity, DNA synthesis and proteoglycan synthesis were measured by MTT assay, [(3)H]-thymidine, and [(35)S]-sulfate incorporation. To detect phenotypical expression, reverse transcription-polymerase chain reactions (RT-PCR) were performed for aggrecan, collagen type I, and type II mRNA expression. To assess action mechanism of EMF, IVD cells were exposed to EMF with N(G)-Monomethyl-L-arginine (NMMA) and acetylsalicylic acid (ASA). RESULTS: There was no cytotoxicity in IVD cells with the EMF group in MTT assay. Cellular proliferation was observed in the EMF group ( $p < 0.05$ ). There was no difference in newly synthesized proteoglycan normalized by DNA synthesis between the EMF group and the control. Cultures with EMF showed no significant change in the expression of aggrecan, type I, and type II collagen mRNA compared to the control group. Cultures with NMMA (blocker of nitric oxide) or ASA (blocker of prostaglandin E2) exposed to EMF demonstrated decreased DNA synthesis compared to control cultures without NMMA or ASA ( $p < 0.05$ ). CONCLUSION: EMF stimulated DNA synthesis in human IVD cells while no significant effect on proteoglycan synthesis and chondrogenic phenotype expressions. DNA synthesis was partially mediated by nitric oxide and prostaglandin E2. EMF can be utilized to stimulate proliferation of IVD cells, which may provide efficient cell amplification in cell therapy to degenerative disc disease.

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

Extremely low frequency electromagnetic field (ELF-EMF) exposure is attracting increased attention as a possible disease-inducing factor. The in vivo effects of short-term and long-term ELF-EMF exposure on male *Drosophila melanogaster* were studied using transcriptomic analysis for preliminary screening and QRT-PCR for further verification. Transcriptomic analysis indicated that 439 genes were up-regulated and 874 genes were down-regulated following short-term exposures and that 514 genes were up-regulated and 1206 genes were down-regulated following long-term exposures (expression  $>2$ - or  $<0.5$ -fold, respectively). In addition, there are 238 up-regulated genes and 598 down-regulated genes in the intersection of short-term and long-term exposure (expression  $>2$ - or  $<0.5$ -fold). The DEGs (differentially expressed genes) in *D. melanogaster* following short-term exposures were involved in metabolic processes, cytoskeletal organization, mitotic spindle organization, cell death, protein modification and proteolysis. Long-term exposure led to changes in expression of genes involved in metabolic processes, response to stress, mitotic spindle organization, aging, cell death and cellular respiration. In the intersection of short-term and long-term exposure, a series of DEGs were related to apoptosis, aging, immunological stress and reproduction. To check the ELF-EMF effects on reproduction, some experiments on male reproduction ability were performed. Their results indicated that short-term ELF-EMF exposure may decrease the reproductive ability of males, but long-term exposures had no effect on reproductive ability. Down-regulation of *ark* gene in the exposed males suggests that the decrease in reproductive capacity may be induced by the effects of ELF-EMF exposure on spermatogenesis through the caspase pathway. QRT-PCR analysis confirmed that *jra*, *ark* and *decay* genes were down regulated in males exposed for 1 Generation (1G) and 72h, which suggests that apoptosis may be inhibited in vivo. ELF-EMF exposure may have accelerated cell senescence, as suggested by the down-regulation of both *cat* and *jra* genes and the up-regulation of *hsp22* gene. Up-regulation of *totA* and *hsp22* genes during exposure suggests that exposed flies might induce an in vivo immune response to counter the adverse effects encountered during ELF-EMF exposure. Down-regulation of *cat* genes suggests that the partial oxidative protection system might be restrained, especially during short-term exposures. This study demonstrates the bioeffects of ELF-EMF exposure and provides evidence for understanding the in vivo mechanisms of ELF-EMF exposure on male *D. melanogaster*.

**(E) Liu Y, Weng E, Zhang Y, Hong R. [Effects of extremely low frequency electromagnetic field and its combination with lead on the antioxidant system in mouse] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 20(4):263-265, 2002. [Article in Chinese]**

**OBJECTIVE:** To study the effects of extremely low frequency electromagnetic field(ELF EMF) and its combination with lead on the antioxidant system in mouse brain and liver tissues.

**METHOD:** Mice were exposed to a 50 Hz sinusoidal 0.2 mT or 6.0 mT EMF for 2 weeks. At the same time, some groups were exposed to lead(50 mg/kg). After the exposure, the antioxidant system and cell membrane fluidity in brain and liver were measured. **RESULTS:**

Malondialdehyde(MDA) content in brain and liver increased from the control levels of (1.33 +/- 0.12) and (3.95 +/- 0.21) nmol/mg pro to (1.35 +/- 0.09) and (6.15 +/- 0.28) nmol/mg pro respectively following 0.2 mT exposure, and to (3.98 +/- 0.10) and (6.50 +/- 0.79) nmol/mg pro respectively following 6.0 mT exposure. Total antioxidant capability(T-AOC) in brain and liver decreased from the control levels of (4.39 +/- 0.48) and (2.45 +/- 0.21) U/mg pro to (3.99 +/- 0.39) and (1.92 +/- 0.32) U/mg pro respectively following 0.2 mT, and to (3.12 +/- 0.37) and (1.57 +/- 0.14) U/mg pro respectively following 6.0 mT. GSH content decreased only in liver tissue from the control level of (194.60 +/- 20.93) mg/g pro to (189.24 +/- 5.61) mg/g pro(0.2 mT) and (153.04 +/- 1.18) mg/g pro(6.0 mT). Cellular membrane fluidity decreased from the control levels of (1.396 +/- 0.040) and (2.899 +/- 0.552) to (1.224 +/- 0.190) and (1.894 +/- 0.0761) (0.2 mT), (1.159 +/- 0.179) and (1.516 +/- 0.204)(6.0 mT) respectively. Compared with single EMF exposure(6.0 mT), EMF combined with lead exposure induced remarkable increase in MDA, GSH content and T-AOC and decrease in cell membrane fluidity both in the brain and liver, and increase in SOD activity only in liver. **CONCLUSION:** ELF EMF might alter the metabolism of free radicals, decrease anti-oxidant capability and enhance lipid peroxidation. The combination of EMF with lead showed synergic effects on lipid peroxidation.

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

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

**(E) \*Manikonda PK, Rajendra P, Devendranath D, Gunasekaran B, Channakeshava, Aradhya SR, Sashidhar RB, Subramanyam C. Extremely low frequency magnetic fields induce oxidative stress in rat brain. Gen Physiol Biophys. 2013 Dec 13. [Epub ahead of print]**

The present investigation was conducted to understand the influence of long-term exposure of rats to extremely low frequency magnetic fields (ELF-MF), focusing on oxidative stress (OS) on different regions of rat's brain. Male Wistar rats (21-day-old) were exposed to ELF-MF (50 Hz; 50 and 100  $\mu$ T) for 90 days continuously; hippocampal, cerebellar and cortical regions from rats were analyzed for (i) reactive oxygen species (ROS), (ii) metabolites indicative of OS and (iii) antioxidant enzymes. In comparison to control group rats, the rats that were continuously exposed to ELF-MF caused OS and altered glutathione (GSH/GSSG) levels in dose-dependent manner in all the regions of the brain. Accumulation of ROS, lipid peroxidation end products and activity of superoxide dismutase in different regions was in the descending order of cerebellum < hippocampus < cortex. Decrement in GSH/GSSG levels and increment in glutathione peroxidase activity were in the descending order of hippocampus < cerebellum < cortex. The continuous exposure to ELF-MF caused OS in all the examined regions of brain more significantly at 100  $\mu$ T than at 50  $\mu$ T. Varied influences observed in different regions of the brain, as documented in this study, may contribute to altered metabolic patterns in its related regions of the central nervous system, leading to aberrant neuronal functions.

**(E) Mannerling AC, Simkó M, Mild KH, Mattsson MO. Effects of 50-Hz magnetic field exposure on superoxide radical anion formation and HSP70 induction in human K562 cells. Radiat Environ Biophys. 49(4):731-741, 2010.**

Epidemiological studies suggest a correlation between exposure to low-level extremely low-frequency (ELF) magnetic fields (MF) and certain cancers and neurodegenerative diseases. Experimental studies have not provided any mechanism for such effects, although at flux density levels significantly higher than the ones encountered in epidemiological studies, radical homeostasis and levels of stress response proteins can be affected. Here, we report on the influence of MF exposure (50-Hz sine wave; 1 h; 0.025-0.10 mT; vertical or horizontal MF exposure direction) on different cellular parameters (proliferation, cell cycle distribution, superoxide radical anion, and HSP70 protein levels) in the human leukaemia cell line K562. The positive control heat treatment (42 degrees C, 1 h) did not affect either cell proliferation or superoxide radical anion production but caused accumulation of cells in the G2 phase and increased the stress protein HSP70. MF exposure (0.10 mT, 1 h) did not affect either cell cycle kinetics or proliferation. Both vertical and horizontal MF exposures for 1 h caused significantly and transiently increased HSP70 levels (>twofold), at several flux densities, compared to sham controls and also compared to heat treatment. This exposure also increased (30-40%) the levels of the superoxide radical anion, comparable to the positive control PMA. Addition of free radical scavengers (melatonin or 1,10-phenanthroline) inhibited the MF-induced increase in HSP70. In conclusion, an early response to ELF MF in K562 cells seems to be an increased amount of oxygen radicals, leading to HSP70 induction. Furthermore, the results suggest that there is a flux density threshold where 50-Hz MF exerts its effects on K562 cells, at or below 0.025 mT, and also that it is the MF, and not the induced electric field, which is the active parameter.

**(NE) Markkanen A, Naarala J, Juutilainen J. A Study on the effects of 50 Hz magnetic fields on UV-induced radical reactions in murine fibroblasts. J Radiat Res (Tokyo). 51(5):609-613, 2010.**

The aim of this study was to test the hypothesis that the "radical pair mechanism" (magnetic field effect on recombination rate of radical pairs) explains our previous findings indicating that 50 Hz magnetic fields (MF) of about 100  $\mu$ T modify biological responses to ultraviolet (UV) radiation. In the present study, the effects of 50 Hz MF on cellular oxidative processes induced by UV radiation were investigated. Murine L929 fibroblast cells were exposed to 50 Hz MF of 100 or 300  $\mu$ T during a 1-h UV exposure or for 24 h before it. The decay kinetics of oxidative reactions were analysed by measuring ultraweak chemiluminescence (photon emissions) of the exposed cells by scintillation counter in the out-of-coincidence mode. No significant MF effects were found. The results do not support the hypothesis that 100-300  $\mu$ T MF modify biological responses to UV radiation by causing an overall change in oxidative reactions at cellular level.

**(E) \*Martínez-Sámamo J, Torres-Durán PV, Juárez-Oropeza MA, Elías-Viñas D, Verdugo-Díaz L. Effects of acute electromagnetic field exposure and movement restraint on antioxidant system in liver, heart, kidney and plasma of Wistar rats: a preliminary report. Int J Radiat Biol. 86(12):1088-1094, 2010.**

PURPOSE: The aim of the present study was to evaluate the early effects of acute (2 h) exposure to extremely low frequency electromagnetic fields (ELF-EMF), as well as movement restraint (MR) and the combination of both on the antioxidant systems in the plasma, liver, kidney, and heart of rats. MATERIALS AND METHODS: Twenty-four adult male Wistar rats were divided in two groups, restrained and unrestrained. The restrained animals were confined into an acrylic tube for 120 min. Half of the animals of each group were exposed to ELF-EMF (60 Hz, 2.4 mT) during the period of restriction. Immediately after treatment, reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and thiobarbituric acid reactive substances (TBARS) were measured in tissues. RESULTS: GSH concentration was significantly lower in the heart of all experimental animals when compared to the control group; furthermore, the decrease was higher in the liver of restrained animals. SOD activity was lower in the plasma of restrained and EMF exposed animals compared to unrestrained rats. There were no significant differences in CAT activity and TBARS levels among all the experimental groups vs. the control group. CONCLUSION: Two hours of 60 Hz EMF exposure might immediately alter the metabolism of free radicals, decreasing SOD activity in plasma and GSH content in heart and kidney, but does not induce immediate lipid peroxidation. Oxidative stress induced by movement restraint was stronger than that produced by EMF.

**(E) Martínez-Sámamo J, Torres-Durán PV, Juárez-Oropeza MA, Verdugo-Díaz L. Effect of acute extremely low frequency electromagnetic field exposure on the antioxidant status and lipid levels in rat brain. Arch Med Res. 43(3):183-189, 2012.**

BACKGROUND AND AIMS: It is generally accepted that electromagnetic fields (EMF) can exert biological effects; however, the mechanisms by which EMF elicits responses are still unknown. The present study was designed to assess the immediate effects of acute EMF exposure, movement restriction, and the combination of both on the antioxidant systems and lipid content in the whole brain of rat. METHODS: Thirty two male Wistar rats were arranged in four groups: control, EMF exposed, movement restrained (MR), and EMF + MR for 2 h. Rats were then sacrificed and their brains analyzed for superoxide dismutase and catalase activities, reduced glutathione, nitric oxide, total cholesterol, and triacylglycerol levels, as well as plasma

corticosterone concentrations. RESULTS: Acute exposure to EMF induces reduction in catalase and superoxide dismutase activities, whereas the combination of EMF + MR also decreases both reduced glutathione and nitric oxide levels. Our results show that the acute exposure to EMF does not induce elevation of stress-hormone corticosterone but impairs the antioxidant status in rat brain. CONCLUSIONS: Plasma corticosterone concentration and antioxidant data indicate that the acute exposure to EMF appears to be a mild stressor that leads to some adaptive responses due to the activation of systems controlling the brain oxidative balance.

**(E) Martino CF. Static magnetic field sensitivity of endothelial cells. Bioelectromagnetics. 32(6):506-508, 2011.**

In this manuscript, data demonstrating the magnetic sensitivity of human umbilical vein endothelial cells (HUVECs) is presented. The effects of low level fields (LLF; 0.2-1  $\mu$ T), 30 and 120  $\mu$ T magnetic fields on the proliferation of endothelial cells were investigated. Primary HUVECs were cultured and exposed to the distinct magnetic conditions in the same incubator. Although cell numbers were slightly affected between 30 and 120  $\mu$ T magnetic fields, reducing the magnetic field to low levels clearly inhibited proliferation. The rationale of introducing LLF is to elucidate a possible mechanism of interaction. Small differences of 30  $\mu$ T reduce endothelial cell numbers significantly. The addition of free radical scavenger superoxide dismutase suppressed the enhanced proliferation caused by 120  $\mu$ T static magnetic fields. It is proposed that the static magnetic field interacts with endothelial cells via a free radical mechanism.

**(E) \*Martino CF, Castello PR. Modulation of hydrogen peroxide production in cellular systems by low level magnetic fields. PLoS One. 2011;6(8):e22753.**

Increased generation of reactive oxygen species (ROS) and an altered redox status have long been observed in cancer cells, suggesting that ROS might be involved in the development of these cells. However, recent studies suggest that inducing an excess of ROS in cancer cells can be exploited for therapeutic benefits. Cancer cells in advanced stage tumors frequently exhibit multiple genetic alterations and high oxidative stress, suggesting that it might be possible to preferentially modulate the development of these cells by controlling their ROS production. Low levels of ROS are also important for the development and survival of normal cells. In this manuscript, we present data on the influence of the suppression of the Earth's magnetic field (low level magnetic fields or LLF) which magnitudes range from 0.2  $\mu$ T to 2  $\mu$ T on the modulation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in human fibrosarcoma cancer cell line HT1080, pancreatic AsPC-1 cancer cell line, and bovine pulmonary artery endothelial cells (PAEC) exposed to geomagnetic field (control; 45  $\mu$ T-60  $\mu$ T). Reduction of the Earth's magnetic field suppressed H<sub>2</sub>O<sub>2</sub> production in cancer cells and PAEC. The addition of catalase and superoxide dismutase (SOD) mimetic MnTBAP inhibited the magnetic field effect. Modulating ROS production by magnetic fields may open new venues of biomedical research and therapeutic strategies.

**(E) \*Morabito C, Guarnieri S, Fanò G, Mariggio MA. Effects of acute and chronic low frequency electromagnetic field exposure on PC12 cells during neuronal differentiation. Cell Physiol Biochem. 26(6):947-958, 2010.**

BACKGROUND/AIMS: The purpose of this study was to provide information about the in vitro neuritogenesis during cell exposure to extremely low frequency electromagnetic fields (ELF-EMFs) of different intensities and durations using pheochromocytoma-derived cell line (PC12 cells) as neuronal model. METHODS: Proliferative rates and neuritogenesis were tested by colorimetric assay and morphological analysis, respectively; reactive oxygen species (ROS)

levels and intracellular Ca(2+) variations monitored using single cell videomicroscopy. RESULTS: The long-lasting ELF-EMF exposure (0.1-1.0 mT) did not appear to significantly affect the biological response (proliferation and neuritogenesis). However, during the acute ELF-EMF exposure (30 min), in undifferentiated PC12 cells, there were increased ROS levels and decreased catalase activity, that, conversely, resulted increased after chronic exposure (7 days) at 1.0 mT. Acute exposure (0.1-1.0 mT) affected the spontaneous intracellular Ca(2+) variations in undifferentiated cells, in which basal intracellular Ca(2+) resulted increased after chronic exposure. In addition acute exposure affected cell response to a depolarizing agent, while basal membrane potential was not changed. CONCLUSION: Even if further studies remain necessary to identify the ROS/intracellular Ca(2+)cross-talking pathway activated by ELF-EMF exposure, we support the hypothesis that ROS and Ca(2+) could be the cellular "primum movens" of the ELF-EMF induced effects on biological systems

**(E) \*Morabito C, Rovetta F, Bizzarri M, Mazzoleni G, Fanò G, Mariggìò MA. Modulation of redox status and calcium handling by extremely low frequency electromagnetic fields in C2C12 muscle cells: A real-time, single-cell approach. Free Radic Biol Med. 48(4):579-589, 2010.**

The biological effects of electric and magnetic fields, which are ubiquitous in modern society, remain poorly understood. Here, we applied a single-cell approach to study the effects of short-term exposure to extremely low frequency electromagnetic fields (ELF-EMFs) on muscle cell differentiation and function using C2C12 cells as an in vitro model of the skeletal muscle phenotype. Our focus was on markers of oxidative stress and calcium (Ca(2+)) handling, two interrelated cellular processes previously shown to be affected by such radiation in other cell models. Collectively, our data reveal that ELF-EMFs (1) induced reactive oxygen species production in myoblasts and myotubes with a concomitant decrease in mitochondrial membrane potential; (2) activated the cellular detoxification system, increasing catalase and glutathione peroxidase activities; and (3) altered intracellular Ca(2+)homeostasis, increasing the spontaneous activity of myotubes and enhancing cellular reactivity to a depolarizing agent (KCl) or an agonist (caffeine) of intracellular store Ca(2+)channels. In conclusion, our data support a possible link between exposure to ELF-EMFs and modification of the cellular redox state, which could, in turn, increase the level of intracellular Ca(2+)and thus modulate the metabolic activity of C2C12 cells.

**(E) Morimoto S, Takahashi T, Shimizu K, Kanda T, Okaishi K, Okuro M, Murai H, Nishimura Y, Nomura K, Tsuchiya H, Ohashi I, Matsumoto M. Electromagnetic fields inhibit endothelin-1 production stimulated by thrombin in endothelial cells. J Int Med Res. 33(5):545-554, 2005.**

Electromagnetic field (EMF) radiation has been found to induce arteriolar dilatation, but the mechanism of action remains largely unknown. This study investigated the effect of EMF radiation on the production of endothelin-1 (ET-1), a potent vasoconstrictor, by cultured endothelial cells. EMF radiation reduced ET-1 basal levels in human umbilical vein and microvascular endothelial cells, but failed to reduce ET-1 basal levels in bovine and human aortic endothelial cells. EMF radiation significantly inhibited thrombin-stimulated ET-1 production in all four endothelial cell types in a dose-dependent manner. EMF radiation significantly inhibited thrombin-induced endothelin-1 mRNA expression in all four cell types. The inhibitory effect of EMF radiation on ET-1 production was abolished by the nitric oxide synthase inhibitor NG-monomethyl-L-arginine (10(-3) mol/l). These results demonstrate that EMF radiation modulates ET-1 production in cultured vascular endothelial cells and the inhibitory effect of EMF radiation is, at least partly, mediated through a nitric oxide-related pathway.

**(E) Osera C, Fassina L, Amadio M, Venturini L, Buoso E, Magenes G, Govoni S, Ricevuti G, Pascale A. Cytoprotective Response Induced by Electromagnetic Stimulation on SH-SY5Y Human Neuroblastoma Cell Line. Tissue Eng Part A. 17(19-20)3573-3582, 2011.**

It is well known that physiological functions and pathological conditions of cells and tissues can be influenced not only by chemical molecules, but also by physical stimuli such as electromagnetic waves. In particular, epidemiological studies suggest possible associations between exposure to electromagnetic fields and an increased risk of tumors and neurodegenerative disorders, such as Alzheimer's disease. However, depending on the dose and on the length of treatment, the electromagnetic stimuli can be harmful or induce a cytoprotective cellular response, suggesting a possible application in medical therapy. In this study, under a tissue engineering viewpoint, we investigated the effects of an electromagnetic wave (magnetic field intensity, 2 mT; frequency, 75 Hz) on a neuronal cellular model characterized by the overexpression of the amyloid precursor protein (APP). After a prolonged electromagnetic treatment, lower mitochondrial activity and proliferation rate, resulting in a higher cellular quiescence, were observed. Focusing on the stress and oxidative pathways, we detected an overall increase of two fundamental proteins, the chaperone heat shock protein HSP70 and the free radical scavenger superoxide dismutase-1 enzyme (SOD-1). Interestingly, we found that the electromagnetic stimulation promotes the nonamyloidogenic processing of APP through an increased expression of the  $\alpha$ -secretase ADAM10 and an enhanced release of the soluble neurotrophic factor sAPP $\alpha$  (a product of the ADAM10-mediated cleavage of APP). In conclusion, these findings suggest that the electromagnetic stimulus, if properly administered in terms of dose and timing, is able to induce a cytoprotective response in the cell. Moreover, these results suggest a possible use of this particular physical stimulation to improve the functional capability of the cells to face noxae.

**\*Pal A, Singh A, Nag TC, Chattopadhyay P, Mathur R, Jain S. Iron oxide nanoparticles and magnetic field exposure promote functional recovery by attenuating free radical-induced damage in rats with spinal cord transection. Int J Nanomedicine. 8:2259-2272, 2013.**

BACKGROUND: Iron oxide nanoparticles (IONPs) can attenuate oxidative stress in a neutral pH environment in vitro. In combination with an external electromagnetic field, they can also facilitate axon regeneration. The present study demonstrates the in vivo potential of IONPs to recover functional deficits in rats with complete spinal cord injury. METHODS: The spinal cord was completely transected at the T11 vertebra in male albino Wistar rats. Iron oxide nanoparticle solution (25  $\mu$ g/mL) embedded in 3% agarose gel was implanted at the site of transection, which was subsequently exposed to an electromagnetic field (50 Hz, 17.96  $\mu$ T for two hours daily for five weeks). RESULTS: Locomotor and sensorimotor assessment as well as histological analysis demonstrated significant functional recovery and a reduction in lesion volume in rats with IONP implantation and exposure to an electromagnetic field. No collagenous scar was observed and IONPs were localized intracellularly in the immediate vicinity of the lesion. Further, in vitro experiments to explore the cytotoxic effects of IONPs showed no effect on cell survival. However, a significant decrease in H<sub>2</sub>O<sub>2</sub>-mediated oxidative stress was evident in the medium containing IONPs, indicating their free radical scavenging properties. CONCLUSION: These novel findings indicate a therapeutic role for IONPs in spinal cord injury and other neurodegenerative disorders mediated by reactive oxygen species.

**(E) Politański P, Rajkowska E, Pawlaczyk-Łuszczynska M, Dudarewicz A, Wiktorek-Smagur A, Sliwińska-Kowalska M, Zmysłony M. Static magnetic field affects oxidative stress in mouse cochlea. *Int J Occup Med Environ Health*. 23(4):377-384, 2010.**

OBJECTIVE: It has been shown that oxidative stress plays an important role in development of noise induced hearing loss. Since static magnetic fields (SMF) exposure may alter dynamics of oxidative processes in the tissue, the aim of the study was to assess the influence of SMF on noise-induced alteration in the cochlear level of reactive oxygen species (ROS) and hearing thresholds. MATERIALS AND METHODS: Auditory brainstem response (ABR), lipid peroxidation (LPO) levels, super-oxide dismutase (SOD) activity and catalase activity were assessed in the cochlea prior to, and at five time-points over two weeks following exposure of C57BL/6 mice to 8h, 119 dB SPL, 4 kHz octave band noise. RESULTS: The ABR indicated no permanent functional damage due to noise exposure either for the 4 kHz and 8 kHz SMF-exposed group or for animals not exposed to SMF. However, significant differences in LPO level, catalase and SOD activity between animals exposed to noise and SMF and those exposed to noise only were observed. CONCLUSIONS: The results suggest that SMF causes an increase in ROS level in the cochlea after noise exposure and, at the same time, it speeds up activation of antioxidative enzymes.

**(E) \*Poniedziałek B, Rzymiski P, Karczewski J, Jaroszyk F, Wiktorowicz K. Reactive oxygen species (ROS) production in human peripheral blood neutrophils exposed in vitro to static magnetic field. *Electromagn Biol Med*. 2013 Apr 30. [Epub ahead of print]**

The aim of this study was to determine the effect of gradient static magnetic field (SMF) on reactive oxygen species (ROS) production in human neutrophils in peripheral blood in vitro. Blood samples collected from healthy individuals were incubated in an inhomogeneous SMF (in a south or north pole of the field) for 15, 30 or 45 minutes. The maximum value of induction ( $B_{max}$ ) amounted to  $\approx 60$  mT. To determine the strength of the ROS production, dihydrorhodamine (123DHR) as fluorophore and phorbol 12-myristate 13-acetate (PMA) as respiratory burst stimulator were used. 123DHR oxidation by ROS was measured by flow cytometry. The exposure of blood samples to SMF induced statistically significant changes in ROS production in unstimulated and PMA-stimulated neutrophils. The observed effects were highly correlated with the exposure time and depended on the orientation of the field. Although intracellular mechanisms underlying such interactions are not thoroughly understood, it could be presumed that SMF affects ROS metabolic oscillations and their formation and inactivation. This study emphasizes the importance of proper adjustment of exposure time to SMF for any potential therapeutic applications.

**(E) \*Poniedziałek B, Rzymiski P, Nawrocka-Bogusz H, Jaroszyk F, Wiktorowicz K. The effect of electromagnetic field on reactive oxygen species production in human neutrophils in vitro. *Electromagn Biol Med*. 2012 Nov 8. [Epub ahead of print]**

The present study was undertaken in order to determine the effect of low frequency electromagnetic field (EMF) on reactive oxygen species (ROS) production in human neutrophils in peripheral blood in vitro. We investigated how differently generated EMF and several levels of magnetic induction affect ROS production. To evaluate the level of ROS production, two fluorescent dyes were used: 2'7'-dichlorofluorescein-diacetate and dihydrorhodamine. Phorbol 12-myristate 13-acetate (PMA), known as strong stimulator of the respiratory burst, was also used. Alternating magnetic field was generated by means of Viofor JPS apparatus. Three different levels of magnetic induction have been analyzed (10, 40 and 60  $\mu$ T). Fluorescence of

dichlorofluorescein and 123 rhodamine was measured by flow cytometry. The experiments demonstrated that only EMF tuned to the calcium ion cyclotron resonance frequency was able to affect ROS production in neutrophils. Statistical analysis showed that this effect depended on magnetic induction value of applied EMF. Incubation in EMF inhibited cell activity slightly in unstimulated neutrophils, whereas the activity of PMA-stimulated neutrophils has increased after incubation in EMF.

**(E) Potenza L, Martinelli C, Polidori E, Zeppa S, Calcabrini C, Stocchi L, Sestili P, Stocchi V. Effects of a 300 mT static magnetic field on human umbilical vein endothelial cells. *Bioelectromagnetics*. 31(8):630-639, 2010.**

This study describes the effects of a static magnetic field (SMF) on cell growth and DNA integrity of human umbilical vein endothelial cells (HUVECs). Fast halo assay was used to investigate nuclear damage; quantitative polymerase chain reaction (QPCR), standard PCR, and real-time PCR were used to evaluate mitochondrial DNA integrity, content, and gene expression. HUVECs were continually exposed to a 300 mT SMF for 4, 24, 48, and 72 h. Compared to control samples (unexposed cultures) the SMF-exposed cells did not show a statistically significant change in their viability. Conversely, the static field was shown to be significant after 4 h of exposure, inducing damage on both the nuclear and mitochondrial levels, reducing mitochondrial content and increasing reactive oxygen species. Twenty-four hours of exposure increased mitochondrial DNA content as well as expression of one of the main genes related to mitochondrial biogenesis. No significant differences between exposed and sham cultures were found after 48 and 72 h of exposure. The results suggest that a 300 mT SMF does not cause permanent DNA damage in HUVECs and stimulates a transient mitochondrial biogenesis.

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

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

**(E) Raggi F, Vallesi G, Rufini S, Gizzi S, Ercolani E, Rossi R. ELF magnetic therapy and oxidative balance. *Electromagn Biol Med*. 27(4):325-339, 2008.**

Knowledge about the relationship between exposure to extremely low-frequency (ELF) EMF and formation (or neutralization) of free radicals in the living cells is limited. Studies performed on

animals and plants have shown conflicting effects on the relation between EMF and oxidative stress. Very few experiments have been performed on humans. The present study reports on the effects of an ELF magnetic therapy device (Seqex) on oxidative scale in humans. This device supplies complex magnetic signals with specific choices of frequency, intensity, and shape that are based on Liboff's ion cyclotron resonance hypothesis. Thirty-two healthy volunteers were treated using the Seqex cycle. A quantitative determination of oxidative stress was obtained at three time points by measuring Malondialdehyde (MDA) concentrations in peripheral blood before and after the cycle and one month following completion of the cycle. A highly significant reduction in mean MDA (53.8%,  $p = 0.0002$ ) was found at the end of the treatment. One month later the mean MDA had again risen, but there was still a significant overall reduction of 15.6% ( $p = 0.010$ ) compared to original values.

**(E) \*Rajabbeigi E, Ghanati F, Abdolmaleki P, Payez A. Antioxidant capacity of parsley cells (*Petroselinum crispum* L.) in relation to iron-induced ferritin levels and static magnetic field. *Electromagn Biol Med.* 2013 Jan 16. [Epub ahead of print]**

This study was aimed to evaluate antioxidant response of parsley cells to 21 ppm iron and static magnetic field (SMF; 30 mT). The activity of catalase (CAT) and ascorbate peroxidase (APX) and the contents of malonyldialdehyde, iron and ferritin were measured at 6 and 12 h after treatments. Exposure to SMF increased the activity of CAT in treated cells, while combination of iron and SMF treatments as well as iron supply alone decreased CAT activity, compared to that of control cells. Combination of SMF with iron treatment reduced iron content of the cells and ameliorated mal effect of iron on CAT activity. All treatments reduced APX activity; however, the content of total ascorbate increased in response to iron and SMF+iron. The results showed that among the components of antioxidant system of parsley cells, enhanced activity of CAT in SMF-treated cells and increase of ascorbate in SMF+Fe-treated ones were responsible for the maintenance of membranes integrity. Ferritin contents of SMF- and SMF+Fe-treated cells also decreased significantly 12 h after treatments, compared to those of the control cells. These results cast doubt on the proposed functions of ferritin as a putative reactive oxygen species detoxifying molecule.

**(E) \*Rauš Balind S, Selaković V, Radenović L, Prolić Z, Janać B. Extremely Low Frequency Magnetic Field (50 Hz, 0.5 mT) Reduces Oxidative Stress in the Brain of Gerbils Submitted to Global Cerebral Ischemia. *PLoS One.* 2014 Feb 19;9(2):e88921. doi: 10.1371/journal.pone.0088921. eCollection 2014.**

Magnetic field as ecological factor has influence on all living beings. The aim of this study was to determine if extremely low frequency magnetic field (ELF-MF, 50 Hz, 0.5 mT) affects oxidative stress in the brain of gerbils submitted to 10-min global cerebral ischemia. After occlusion of both carotid arteries, 3-month-old gerbils were continuously exposed to ELF-MF for 7 days. Nitric oxide and superoxide anion production, superoxide dismutase activity and index of lipid peroxidation were examined in the forebrain cortex, striatum and hippocampus on the 7(th) (immediate effect of ELF-MF) and 14(th) day after reperfusion (delayed effect of ELF-MF). Ischemia per se increased oxidative stress in the brain on the 7(th) and 14(th) day after reperfusion. ELF-MF also increased oxidative stress, but to a greater extent than ischemia, only immediately after cessation of exposure. Ischemic gerbils exposed to ELF-MF had increased oxidative stress parameters on the 7(th) day after reperfusion, but to a lesser extent than ischemic or ELF-MF-exposed animals. On the 14(th) day after reperfusion, oxidative stress parameters in the brain of these gerbils were mostly at the control levels. Applied ELF-MF decreases oxidative stress induced by global cerebral ischemia and thereby reduces possible negative consequences

which free radical species could have in the brain. The results presented here indicate a beneficial effect of ELF-MF (50 Hz, 0.5 mT) in the model of global cerebral ischemia.

**(E) Regoli F, Gorbi S, Machella N, Tedesco S, Benedetti M, Bocchetti R, Notti A, Fattorini D, Piva F, Principato G. Pro-oxidant effects of extremely low frequency electromagnetic fields in the land snail *Helix aspersa*. *Free Radic Biol Med.* 39(12):1620-1628, 2005.**

Pro-oxidant effects of extremely low frequency (ELF) 50-Hz magnetic fields were investigated in the land snail *Helix aspersa* exposed both in short-term laboratory treatments and under field conditions by maintaining the organisms in the proximity of a power line for up to 2 months. Oxidative perturbations were investigated as individual antioxidants (catalase, glutathione reductase, glutathione S-transferases, and total glutathione) and total scavenging capacity toward peroxy radicals and hydroxyl radicals. Accumulation of lipid peroxidation products, destabilization of lysosomal membranes, and loss of DNA integrity were also evaluated as markers of cell damage. The overall results indicated an oxidative challenge caused by ELF magnetic fields with particularly prompt and sensitive responses for catalase, glutathione reductase, and the overall capability to neutralize peroxy radicals. Cell injuries occurred to different extents according to duration and intensity of electromagnetic exposure and confirmed complex cause-effect relationships between pro-oxidant factors, efficiency of antioxidant defenses, and the onset of oxidative toxicity. This study highlights the importance of a multimarker approach for detecting a wide panel of biological responses, the necessity of investigating the long-term effects of early oxidative responses, and the role of ELF in enhancing susceptibility to other forms of pathologies or diseases.

**(E) Roy S, Noda Y, Eckert V, Traber MG, Mori A, Liburdy R, Packer L. The phorbol 12-myristate 13-acetate (PMA)-induced oxidative burst in rat peritoneal neutrophils is increased by a 0.1 mT (60 Hz) magnetic field. *FEBS Lett.* 376(3):164-166, 1995.**

Magnetic fields (MF) may affect biological systems by increasing free radical concentrations. To test this, we have investigated whether low frequency (60 Hz) low intensity (0.1 mT) MF can modulate the phorbol 12-myristate 13-acetate (PMA) induced respiratory burst in primed rat peritoneal neutrophils, followed in real time using the dye 2',7'-dichlorofluorescein (DCFH), which reacts with free radical-derived oxidants such as H<sub>2</sub>O<sub>2</sub> (which is formed from the dismutation of superoxide) to become 2',7'-dichlorofluorecein (DCF), a highly fluorescent compound. In the presence of the MF, a 12.4% increase in the fluorescence signal was observed in PMA-stimulated neutrophils (n = 5, P < 0.02, 18 pairs of measurements). We believe this represents the first experimental observation of MF influencing events involving free radical species generated during signal transduction in living cells.

**(E) \*Sadeghipour R, Ahmadian S, Bolouri B, Pazhang Y, Shafieezadeh M. Effects of extremely low-frequency pulsed electromagnetic fields on morphological and biochemical properties of human breast carcinoma cells (T47D). *Electromagn Biol Med.* 31(4):425-435, 2012.**

This study was carried out to investigate the effects of 100 and 217 Hz extremely low-frequency pulsed electromagnetic fields (ELF-PEMF) on cell proliferation, actin reorganization, and ROS generation in a human breast carcinoma cells (T47D). Cells were exposed for 24-72 h, at 100 and 217 Hz, 0.1 mT. The treatment induced a time dependent decrease in cell growth after 72 h and revealed an increase in fluorescence intensity in cytoplasm and actin aggregations around the nucleus as detected by fluorescence microscopy. The amount of actin in T47D cells increased

after 48 h exposure to 100 Hz and 24 h to 217 Hz while no changes in nuclear morphology were detected. Exposing the cells to 217 Hz for 72 h caused a dramatic increase of intracellular ROS generation while with exposure to 100 Hz it remained nearly unchanged. These results suggest that exposure to ELF-PEMF (100, 217 Hz, 0.1 mT) are able inducing an increase of actin level, its migration toward nucleus but despite of these changes and dramatic increase in ROS generation the symptoms of apoptosis were not observed. Our results support the hypothesis that cell response to EMF may only be observed at certain window effects; such as frequency and intensity of EMF parameters.

**(E) Sahebamei H, Abdolmaleki P, Ghanati F. Effects of magnetic field on the antioxidant enzyme activities of suspension-cultured tobacco cells. Bioelectromagnetics. 28(1):42-47, 2007.**

Effects of magnetic fields (MFs) on the activities of antioxidant enzymes of suspension-cultured tobacco cells were investigated. Compared with the control cells, exposure of the cells to static MF with the magnitudes of 10 and 30 mT for 5 days, 5 h each day, increased the activity of superoxide dismutase (SOD). In contrast, the activity of the catalase (CAT) and ascorbate peroxidase (APX) was decreased by MF, compared with those of the control cells. Level of lipid peroxidation was also increased by MF. It suggests that MF could deteriorate antioxidant defense system of plant cells.

**(E) \*Selaković V, Rauš Balind S, Radenović L, Prolić Z, Janać B. Age-Dependent Effects of ELF-MF on Oxidative Stress in the Brain of Mongolian Gerbils. Cell Biochem Biophys. 2013 Jan 6. [Epub ahead of print]**

The aim of study was to investigate the effects of extremely low frequency magnetic field (ELF-MF; 50 Hz; 0.1, 0.25 and 0.5 mT) on oxidative stress in the brain of 3- (adult) and 10-month-old (middle-aged) gerbils. Nitric oxide (NO) level, superoxide (O<sub>2</sub><sup>-</sup>) production, superoxide dismutase (SOD) activity, and index of lipid peroxidation (ILP) were measured in the forebrain cortex, striatum, hippocampus, and cerebellum immediately and 3 days after cessation of 7-day exposure. In all gerbils, ELF-MF significantly increased oxidative stress in all tested brain regions. This effect was correlated with the value of magnetic induction and was higher in middle-aged gerbils. Three days after cessation of exposure, the values of examined parameters were closer to control levels. In adult gerbils, the effect of ELF-MF of 0.1 mT on NO level, O<sub>2</sub><sup>-</sup> production and SOD activity was almost fully disappeared, and ILP was at the control level regardless of the value of magnetic induction. In middle-aged gerbils, the effect of ELF-MF was still present but to a lesser degree than those observed immediately after cessation of exposure. These findings pointed out the ability of ELF-MF to induce age- and magnetic induction-dependent modification of oxidative stress in the brain.

**Seyhan N, Güler G. Review of in vivo static and ELF electric fields studies performed at Gazi Biophysics Department. Electromagn Biol Med. 25(4):307-323, 2006.**

In vivo effects of Static Electric and ELF Magnetic and Electric fields have been carried out for more than 20 years in the Bioelectromagnetic Laboratory at the Biophysics Department of the Medical Faculty of Gazi University. In this article, the results of in vivo ELF Electric field studies are presented as a review. Static and 50 Hz ELF (Extremely Low Frequency) Electric (E) fields effects on free radical synthesis, antioxidant enzyme level, and collagen synthesis were analyzed on tissues of guinea pigs, such as brain, liver, lung, kidney, spleen, testis, and plasma. Animals were exposed to static and ELF electric fields with intensities ranging from 0.3 kV/m to 1.9 kV/m

in vertical and horizontal directions. Exposure periods were 1, 3, 5, 7, and 10 days. Electric fields were generated from a specially designed parallel plate capacitor system. The results indicate that the effects of electric fields on the tissues studied depend significantly on the type and magnitude of electric field and exposure period.

**(E) Sharifian A, Gharavi M, Pasalar P, Aminian O. Effect of extremely low frequency magnetic field on antioxidant activity in plasma and red blood cells in spot welders. Int Arch Occup Environ Health. 82(2):259-266, 2009.**

**OBJECTIVE:** The purpose of this study was to determine a possible relation between exposure to extremely low frequency magnetic field (ELF-MF) and the human antioxidant activity.

**METHODS:** The total serum antioxidant status (TAS), red blood cells (RBCs) glutathione peroxidase (GPX) and superoxide dismutase (SOD) were measured in 46 spot welders who were occupationally exposed to ELF-MF (magnetic field strength = 8.8-84 microTesla (microT), frequency = 50 Hertz (Hz) and electric field strength = 20-133 V/m). The results were compared with a nonexposed ELF-MF control group. The correlation between magnetic field strength and antioxidant activity in RBCs and plasma was then assessed. **RESULTS:** No significant differences in TAS levels were observed (P value = 0.065). However, in RBCs of exposed group, a significant decrease in SOD and GPX activities was observed (P value = 0.001 and 0.003, respectively). This decrease was measured as 22 and 12.3%, respectively. Furthermore, a significant negative correlation between SOD/GPX activities and magnetic field intensity was observed (coefficients of SOD: -0.625, significance: 0.0001 and coefficients of GPX: -0.348, significance: 0.018). **CONCLUSION:** The results of this study indicate that ELF-MF could influence the RBC antioxidant activity and might act as an oxidative stressor. Intracellular antioxidant enzymes such as SOD and GPX were found to be the most important markers involving in this process. The influence of magnetic field on the antioxidant activity of RBCs might occur even at the recommended levels of exposure.

**(E) Simkó M, Droste S, Kriehuber R, Weiss DG. Stimulation of phagocytosis and free radical production in murine macrophages by 50 Hz electromagnetic fields. Eur J Cell Biol. 80(8):562-566, 2001.**

Effects of 50 Hz electromagnetic fields on phagocytosis and free radical production were examined in mouse bone marrow-derived macrophages. Macrophages were in vitro exposed to electromagnetic fields using different magnetic field densities (0.5-1.5 mT). Short-time exposure (45 min) to electromagnetic fields resulted in significantly increased phagocytic uptake (36.3% +/- 15.1%) as quantified by measuring the internalization rate of latex beads. Stimulation with 1 nM 12-O-tetradecanoylphorbol-13-acetate (TPA) showed the same increased phagocytic activity as 1 mT electromagnetic fields. However, co-exposure to electromagnetic fields and TPA showed no further increase of bead uptake, and therefore we concluded that because of the absence of additive effects, the electromagnetic fields-induced stimulation of mouse bone marrow-derived macrophages does not involve the protein kinase C signal transduction pathway. Furthermore, a significant increased superoxide production after exposure to electromagnetic fields was detected.

(review) **Simkó M. Cell type specific redox status is responsible for diverse electromagnetic field effects. Curr Med Chem. 14(10):1141-1152, 2007.**

Epidemiologic and experimental research on the potential carcinogenic effects of extremely low frequency electromagnetic fields (ELF-EMF) has been performed for a long time. Epidemiologic studies regarding ELF-EMF-exposure have focused primarily on leukaemia development due to

residential sources in children and adults, and from occupational exposure in adults, but also on other kinds of cancer. Genotoxic investigations of EMF have shown contradictory results, a biological mechanism is still lacking that can explain the link between cancer development and ELF-EMF-exposure. Recent laboratory research has attempted to show general biological effects, and such that could be related to cancer development and/or promotion. Metabolic processes which generate oxidants and antioxidants can be influenced by environmental factors, such as ELF-EMF. Increased ELF-EMF exposure can modify the activity of the organism by reactive oxygen species leading to oxidative stress. It is well established that free radicals can interact with DNA resulting in single strand breaks. DNA damage could become a site of mutation, a key step to carcinogenesis. Furthermore, different cell types react differently to the same stimulus, because of their cell type specific redox status. The modulation of cellular redox balance by the enhancement of oxidative intermediates, or the inhibition or reduction of antioxidants, is discussed in this review. An additional aspect of free radicals is their function to influence other illnesses such as Parkinson's and Alzheimer's diseases. On the other hand, modulation of antioxidants by ELF-EMF can lower the intracellular defence activity promoting the development of DNA damage. It has also been demonstrated that low levels of reactive oxygen species trigger intracellular signals that involve the transcription of genes and leading to responses including cell proliferation and apoptosis. In this review, a general overview is given about oxidative stress, as well as experimental studies are reviewed as they are related to changes in oxidant and antioxidant content after ELF-EMF exposure inducing different biological effects. Finally, we conclude from our review that modulations on the oxidant and antioxidant level through ELF-EMF exposure can play a causal role in cancer development.

**(Review) Simkó M, Mattsson MO. Extremely low frequency electromagnetic fields as effectors of cellular responses in vitro: possible immune cell activation. J Cell Biochem. 93(1):83-92, 2004.**

There is presently an intense discussion if electromagnetic field (EMF) exposure has consequences for human health. This include exposure to structures and appliances that emit in the extremely low frequency (ELF) range of the electromagnetic spectrum, as well as emission coming from communication devices using the radiofrequency part of the spectrum. Biological effects of such exposures have been noted frequently, although the implication for specific health effects is not that clear. The basic interaction mechanism(s) between such fields and living matter is unknown. Numerous hypotheses have been suggested, although none is convincingly supported by experimental data. Various cellular components, processes, and systems can be affected by EMF exposure. Since it is unlikely that EMF can induce DNA damage directly, most studies have examined EMF effects on the cell membrane level, general and specific gene expression, and signal transduction pathways. In addition, a large number of studies have been performed regarding cell proliferation, cell cycle regulation, cell differentiation, metabolism, and various physiological characteristics of cells. Although 50/60 Hz EMF do not directly lead to genotoxic effects, it is possible that certain cellular processes altered by exposure to EMF indirectly affect the structure of DNA causing strand breaks and other chromosomal aberrations. The aim of this article is to present a hypothesis of a possible initial cellular event affected by exposure to ELF EMF, an event which is compatible with the multitude of effects observed after exposure. Based on an extensive literature review, we suggest that ELF EMF exposure is able to perform such activation by means of increasing levels of free radicals. Such a general activation is compatible with the diverse nature of observed effects. Free radicals are intermediates in natural processes like mitochondrial metabolism and are also a key feature of phagocytosis. Free radical release is inducible by ionizing radiation or phorbol ester treatment, both leading to genomic instability. EMF might be a stimulus to induce an "activated state" of the cell such as phagocytosis, which then enhances the release of free radicals, in turn leading to genotoxic events. We envisage that

EMF exposure can cause both acute and chronic effects that are mediated by increased free radical levels: (1) Direct activation of, for example macrophages (or other cells) by short-term exposure to EMF leads to phagocytosis (or other cell specific responses) and consequently, free radical production. This pathway may be utilized to positively influence certain aspects of the immune response, and could be useful for specific therapeutic applications. (2) EMF-induced macrophage (cell) activation includes direct stimulation of free radical production. (3) An increase in the lifetime of free radicals by EMF leads to persistently elevated free radical concentrations. In general, reactions in which radicals are involved become more frequent, increasing the possibility of DNA damage. (4) Long-term EMF exposure leads to a chronically increased level of free radicals, subsequently causing an inhibition of the effects of the pineal gland hormone melatonin. Taken together, these EMF induced reactions could lead to a higher incidence of DNA damage and therefore, to an increased risk of tumour development. While the effects on melatonin and the extension of the lifetime of radicals can explain the link between EMF exposure and the incidence of for example leukaemia, the two additional mechanisms described here specifically for mouse macrophages, can explain the possible correlation between immune cell system stimulation and EMF exposure.

**(E) Sullivan K, Balin AK, Allen RG. Effects of static magnetic fields on the growth of various types of human cells. Bioelectromagnetics. 32(2):140-147, 2011.**

The effects of a static magnetic field (SMF) on the proliferation of various types of human cells were determined. All cultures were maintained at 37 °C throughout the experiment. SMF was generated by placing two magnets oppositely oriented on either side of a T25 flask. The flux density in the flask ranged from 35 to 120 mT. Growth curves were constructed by plotting cell number at 18 h and 4, 7, 11, and 14 days after seeding, with the 18-h point being a measure of attachment efficiency. Exposure to SMF significantly decreased initial attachment of fibroblasts and decreased subsequent growth compared to sham-exposed control. Significant effects were observed in both fetal lung (WI-38) and adult skin fibroblasts, but they were generally larger in the fetal lung fibroblast line. SMF did not affect attachment of human melanoma cells, but inhibited their growth by 20% on day 7. SMF produced no effects in a human adult stem cell line. Oxidant production increased 37% in WI-38 cells exposed to SMF (230-250 mT) during the first 18 h after seeding, when cell attachment occurs. Conversely, no elevation in oxidant levels was observed after a prolonged 5-day exposure. These results indicate that exposure to SMF has significant biological effects in some, but not all types of human cells.

**(NE) Suri A, deBoer J, Kusser W, Glickman BW. A 3 milliTesla 60 Hz magnetic field is neither mutagenic nor co-mutagenic in the presence of menadione and MNU in a transgenic rat cell line. Mutat Res. 372(1):23-31, 1996.**

The mechanisms by which an electromagnetic field (EMF) influences biological material are poorly understood. One potentially important model suggests that a magnetic field can stabilize free radicals in such a way as to permit their dispersment rather than their return to the ground state (Okazaki et al., 1988; Scaiano, 1995). We have tested this hypothesis by examining mutagenesis in the E. coli lacI gene target carried in the Big Blue rat embryo fibroblast cell line, R2 lambda LIZ. Mutant frequencies were determined in cells exposed to a magnetic field, cells pretreated with the mutagens N-methylnitrosourea (MNU) or 2-methyl-1,4-naphthoquinone (menadione), prior to being held in a 60 Hz 3 milliTesla (mT) magnetic field and cells concurrently exposed to the mutagens and the magnetic field. Menadione was selected because its mutagenic mechanism involves the formation of free radicals, while MNU is an alkylating agent not thought to act through radical formation. According to the radical stabilization hypothesis the

application of a magnetic field to menadione treated cells would accentuate the mutagenic effects. Our results failed to indicate that the magnetic field affects mutagenesis by the oxygen-radical mediated mutagen, menadione.

**(E) \*Tasset I, Medina FJ, Jimena I, Agüera E, Gascón F, Feijóo M, Sánchez-López F, Luque E, Peña J, Drucker-Colín R, Túnez I. Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: effects on neurotrophic factors and neuronal density. *Neuroscience*. 209:54-63, 2012.**

There is evidence to suggest that the neuroprotective effect of exposure of extremely low-frequency electromagnetic fields (ELF-EMF) may be due, at least in part, to the effect of these fields on neurotrophic factors levels and cell survival, leading to an improvement in behavior. This study was undertaken to investigate the neuroprotective effects of ELFEF in a rat model of 3-nitropropionic acid (3NP)-induced Huntington's disease. Behavior patterns were evaluated, and changes in neurotrophic factor, cell damage, and oxidative stress biomarker levels were monitored in Wistar rats. Rats were given 3NP over four consecutive days (20 mg/kg body weight), whereas ELFEF (60 Hz and 0.7 mT) was applied over 21 days, starting after the last injection of 3NP. Rats treated with 3NP exhibited significantly different behavior in the open field test (OFT) and the forced swim test (FST), and displayed significant differences in neurotrophic factor levels and oxidative stress biomarkers levels, together with a neuronal damage and diminished neuronal density, with respect neuronal controls. ELFEF improved neurological scores, enhanced neurotrophic factor levels, and reduced both oxidative damage and neuronal loss in 3NP-treated rats. ELFEF alleviates 3NP-induced brain injury and prevents loss of neurons in rat striatum, thus showing considerable potential as a therapeutic tool.

**Tasset I, Pérez-Herrera A, Medina FJ, Arias-Carrión O, Drucker-Colín R, Túnez I. Extremely low-frequency electromagnetic fields activate the antioxidant pathway Nrf2 in a Huntington's disease-like rat model. *Brain Stimul*. 2012 Apr 15. [Epub ahead of print]**

Transcranial magnetic stimulation (TMS) is a non-invasive technique used recently to treat different neuropsychiatric and neurodegenerative disorders. Despite its proven value, the mechanisms through which TMS exerts its beneficial action on neuronal function remain unclear. Recent studies have shown that its beneficial effects may be at least partly due to a neuroprotective effect on oxidative and cell damage. This study shows that TMS can modulate the Nrf2 transcription factor in a Huntington's disease-like rat model induced by 3-nitropropionic acid (3-NP). Western blot analysis demonstrated that 3-NP caused a reduction in Nrf2 in both cytoplasm and nucleus, while TMS applied to 3-NP-treated rats triggered an increase in cytoplasm and nucleus Nrf2 levels. It was therefore concluded that TMS modulates Nrf2 expression and translocation and that these mechanisms may partly explain the neuroprotective effect of TMS, as well as its antioxidant and cell protection capacity

**Túnez I, Drucker-Colín R, Jimena I, Medina FJ, Muñoz Mdel C, Peña J, Montilla P. Transcranial magnetic stimulation attenuates cell loss and oxidative damage in the striatum induced in the 3-nitropropionic model of Huntington's disease. *J Neurochem*. 97(3):619-630, 2006.**

An investigation was conducted on the effect of transcranial magnetic field stimulation (TMS) on the free radical production and neuronal cell loss produced by 3-nitropropionic acid in rats. The effects of 3-nitropropionic acid were evaluated by examining the following changes in: the quantity of hydroperoxides and total radical-trapping antioxidant potential (TRAP), lipid

peroxidation products, protein carbonyl groups, reduced glutathione (GSH) content, glutathione peroxidase (GSH-Px), catalase and succinate dehydrogenase (SDH) activities; total nitrite and cell death [morphological changes, quantification of neuronal loss and lactate dehydrogenase (LDH) levels]. Our results reveal that 3-nitropropionic acid induces oxidative and nitrosative stress in the striatum, prompts cell loss and also shows that TMS prevents the harmful effects induced by the acid. In conclusion, the results show the ability of TMS to modify neuronal response to 3-nitropropionic acid.

**(NE) Türközer Z, Güler G, Seyhan N. Effects of exposure to 50 Hz electric field at different strengths on oxidative stress and antioxidant enzyme activities in the brain tissue of guinea pigs. Int J Radiat Biol. 84(7):581-590, 2008.**

**PURPOSE:** The aim of this study was to evaluate the possible effects of varied exposure to 50 Hz extremely low frequency (ELF) electric field (EF) on the lipid peroxidation levels and antioxidant enzyme activities in the brain homogenates of guinea pigs. Subjects were exposed to 2 kV/m, 2.5 kV/m, 3 kV/m, 3.5 kV/m, 4 kV/m, 4.5 kV/m and 5 kV/m electric fields for three days, 8 h a day in both vertical and horizontal directions. **MATERIALS AND METHODS:** Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were measured in order to identify possible alterations in lipid peroxidation levels and antioxidant status due to electric field exposure. Xanthine oxidase (XO), myeloperoxidase (MPO) and adenosine deaminase (ADA) activities were also evaluated in the same samples. **RESULTS:** Although the study showed several positive but non-significant findings ( $p > 0.05$ ), we did not find significant differences among all of the exposed groups and sham groups in lipid peroxidation levels and enzyme activities ( $p > 0.05$ ) at all strengths and in both directions. Furthermore, the result was the same when the comparison was made between the groups in vertical directions and horizontal directions ( $p > 0.05$ ). **CONCLUSION:** The present study observed effects of 50 Hz EF exposure on lipid peroxidation levels and antioxidant defense mechanisms but these were not statistically significant at the 95% confidence level. Further research on the effects ELF-EF exposure on lipid peroxidation levels and antioxidant defence mechanisms are warranted.

**(E) \*Tuschl H, Neubauer G, Schmid G, Weber E, Winker N. Occupational exposure to static, ELF, VF and VLF magnetic fields and immune parameters. Int J Occup Med Environ Health. 13(1):39-50, 2000.**

Despite the important role of the immune system in defending the body against infections and cancer, very few investigations have been undertaken to study possible effects of electromagnetic fields on human immunity. The aim of the present study was to examine the effect of occupational exposure on hospital personnel operating magnetic resonance tomographs and on industrial workers at induction heaters. In both categories of workplaces, magnetic flux densities exceeding Austrian exposure standards have been registered. Because of the complexity and high redundancy of the immune system, an extensive range of assay systems was applied: relative and absolute numbers of lymphocytic subsets were counted, the proliferative activity of T and B cells determined, the production of interleukin 2, interferon gamma and tumour necrosis factor alpha analysed, serum immunoglobulins evaluated, as well as non-specific immunity of monocytes and granulocytes measured by their oxidative burst. The number of natural killer cells and oxidative burst in monocytes showed statistically significant differences in workers at induction heaters and controls. The observed effect on oxidative burst was counteracted by a higher number of active cells in the exposed group, indicating normal non-specific immunity. The high number of natural

killer cells, observed in some of the study subjects working at induction heaters, was reconfirmed in another investigation and deserves a further follow-up.

**(E) Vañner LM, Podoplelov AV, Leshina TV, Sagdeev RZ, Molin IuN. [Effect of a magnetic field on the rate of H<sub>2</sub>O<sub>2</sub> breakdown by catalase and by an Fe<sup>3+</sup>--EDTA complex] Biofizika. 23(2):234-241, 1978.[Article in Russian]**

The acceleration of H<sub>2</sub>O<sub>2</sub> decomposition induced by catalase and the dimer complex [Fe<sup>3+</sup>(EDTA)]<sub>2</sub> has been observed in a constant magnetic field. The effect increases with the field increasing up to 8000 Oe, reaching 20 +/- 5% and 24 +/- 5% for catalase and [Fe<sup>3+</sup>(EDTA)]<sub>2</sub> respectively. The results are discussed within the hypothesis of a one-electron reaction mechanism using the models developed specially to explain the magnetic effects in radical reactions. It is supposed that the stage which is affected by the magnetic field is the electron transfer coupled with Fe<sup>3+</sup>+O<sub>2</sub>/2 paramagnetic species. The interpretation proposed does not exclude an alternative possibility of the magnetic effects during the electron transfer to two iron atoms by a two-electron (synchronous) mechanism.

**(NE) \*Vannoni D, Albanese A, Battisti E, Aceto E, Giglioni S, Corallo C, Carta S, Ferrata P, Fioravanti A, Giordano N. In vitro exposure of human osteoarthritic chondrocytes to ELF fields and new therapeutic application of musically modulated electromagnetic fields: biological evidence. J Biol Regul Homeost Agents. 26(1):39-49, 2012.**

Osteoarthritis (OA) is the most frequently occurring rheumatic disease, caused by metabolic changes in chondrocytes, the cells that maintain cartilage. Treatment with electromagnetic fields (MF) produces benefits in patients affected by this pathology. Isolated human osteoarthritic (OA) chondrocytes were cultured in vitro under standard conditions or stimulated with IL-1beta or IGF-1, to mimic the imbalance between chondroformation and chondroresorption processes observed in OA cartilage in vivo. The cells were exposed for a specific time to extremely low frequency (ELF; 100-Hz) electromagnetic fields and to the Therapeutic Application of Musically Modulated Electromagnetic Fields (TAMMEF), which are characterized by variable frequencies, intensities, and waveforms. Using flow cytometry, we tested the effects of the different types of exposure on chondrocyte metabolism. The exposure of the cells to both systems enhances cell proliferation, does not generate reactive oxygen species, does not cause glutathione depletion or changes in mitochondrial transmembrane potential and does not induce apoptosis. This study presents scientific support to the fact that MF could influence OA chondrocytes from different points of view (viability, ROS production and apoptosis). We can conclude that both ELF and TAMMEF systems could be recommended for OA therapy and represent a valid non-pharmacological approach to the treatment of this pathology.

**(E) \*Vignola MB, Dávila S, Cremonuzzi D, Simes JC, Palma JA, Campana VR. Evaluation of inflammatory biomarkers associated with oxidative stress and histological assessment of magnetic therapy on experimental myopathy in rats. Electromagn Biol Med. 31(4):320-332, 2012.**

The effect of pulsed electromagnetic field (PEMF) therapy, also called magnetic therapy, upon inflammatory biomarkers associated with oxidative stress plasma fibrinogen, nitric oxide (NO), L-citrulline, carbonyl groups, and superoxide dismutase (SOD) was evaluated through histological assessment, in rats with experimental myopathy. The groups studied were: (A) control (intact rats that received PEMF sham exposures); (B) rats with myopathy and sacrificed 24 h later; (C) rats with myopathy; (D) rats with myopathy and treated with PEMF; and (E) intact

rats treated with PEMF. Groups A, C, D, and E were sacrificed 8 days later. Myopathy was induced by injecting 50 µl of 1% carrageenan λ (type IV) once sub-plantar. Treatment was carried out with PEMF emitting equipment with two flat solenoid disks for 8 consecutive days in groups D and E, at 20 mT and 50 Hz for 30 min/day/rat. The biomarkers were determined by spectrophotometry. The muscles (5/8) were stained with Hematoxylin-Eosin and examined by optic microscopy. Quantitative variables were statistically analyzed by the Fisher test, and categorical applying Pearson's Chi Squared test at  $p < 0.05$  for all cases. In Groups B and C, the biomarkers were significantly increased compared to A, D, and E groups: fibrinogen ( $p < 0.001$ ); NO, L-citrulline and carbonyl groups ( $p < 0.05$ ); SOD ( $p < 0.01$ ) as well as the percentage of area with inflammatory infiltration ( $p < 0.001$ ). PEMF caused decreased levels of fibrinogen, L-citrulline, NO, SOD, and carbonyl groups and significant muscle recovery in rats with experimental myopathies.

(Review) **Vojtíšek M, Knotková J, Kasparová L, Svandová E, Markvartová V, Tůma J, Vozeh F, Patková J. Metal, EMF, and brain energy metabolism. Electromagn Biol Med. 28(2):188-193, 2009.**

Some implications of cooperative potential of metal ions and electromagnetic fields' radiation (EMF) in carcinogenic processes are discussed. It is known that these factors, chemical and physical individually have connections with processes of oxidative stress. Special attention was paid to possible manifestation within the brain. Therefore, the entry of a few potentially neurotoxic metals into the brain is discussed.

**(E) Wartenberg M, Wirtz N, Grob A, Niedermeier W, Hescheler J, Peters SC, Sauer H. Direct current electrical fields induce apoptosis in oral mucosa cancer cells by NADPH oxidase-derived reactive oxygen species. Bioelectromagnetics. 29(1):47-54, 2008.**

The presence of more than one dental alloy in the oral cavity often causes pathological galvanic currents and voltage resulting in superficial erosions of the oral mucosa and eventually in the emergence of oral cancer. In the present study the mechanisms of apoptosis of oral mucosa cancer cells in response to electromagnetic fields was investigated. Direct current (DC) electrical fields with field strengths between 2 and 16 V/m, applied for 24 h to UM-SCC-14-C oral mucosa cancer cells, dose-dependently resulted in decreased cell proliferation as evaluated by Ki-67 immunohistochemistry and upregulation of the cyclin-dependent kinase (CDK) inhibitors p21(cip1/waf1) and p27(kip1), which are associated with cell cycle arrest. Electrical field treatment (4 V/m, 24 h) increased apoptosis as evaluated by immunohistochemical analysis of cleaved caspase-3 and poly-(ADP-ribose)-polymerase-1 (PARP-1). Furthermore, robust reactive oxygen species (ROS) generation, increased expression of NADPH oxidase subunits as well as Hsp70 was observed. Electrical field treatment (4 V/m, 24 h) resulted in increased expression of Cu/Zn superoxide dismutase and decreased intracellular concentration of reduced glutathione (GSH), whereas the expression of catalase remained unchanged. Pre-treatment with the free radical scavenger N-acetyl cysteine (NAC) and the superoxide dismutase mimetic EUK-8 abolished caspase-3 and PARP-1 induction, suggesting that apoptosis in oral mucosa cancer cells is initiated by ROS generation in response to DC electrical field treatment.

**(E) 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 ELF-EMF on cell proliferation and DNA damage were prevented by pretreatment of cells with an antioxidant like alpha-tocopherol, suggesting that redox reactions were involved. Accordingly, Rat-1 fibroblasts that had been exposed to ELF-EMF for 3 or 24 h exhibited a significant increase in dichlorofluorescein-detectable reactive oxygen species, which was blunted by alpha-tocopherol pretreatment. Cells exposed to ELF-EMF and examined as early as 6 h after treatment initiation also exhibited modifications of NF kappa B-related proteins (p65-p50 and I kappa B alpha), which were suggestive of increased formation of p65-p50 or p65-p65 active forms, a process usually attributed to redox reactions. These results suggest that ELF-EMF influence proliferation and DNA damage in both normal and tumor cells through the action of free radical species. This information may be of value for appraising the pathophysiologic consequences of an exposure to ELF-EMF.

**(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.**

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

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

Extremely low frequency (ELF) electromagnetic field (EMF) is thought to prolong the life of free radicals and can act as a promoter or co-promoter of cancer. 8-hydroxy-2'-deoxyguanosine (8OHdG) is one of the predominant forms of radical-induced lesions to DNA and is a potential tool to assess the cancer risk. We examined the effects of extremely low frequency electromagnetic field (ELF-EMF) (50 Hz, 0.97 mT) on 8OHdG levels in DNA and thiobarbituric acid reactive substances (TBARS) in plasma. To examine the possible time-dependent changes

resulting from magnetic field, 8OHdG and TBARS were quantitated at 50 and 100 days. Our results showed that the exposure to ELF-EMF induced oxidative DNA damage and lipid peroxidation (LPO). The 8OHdG levels of exposed group (4.39±/0.88 and 5.29±/1.16 8OHdG/dG.10(5), respectively) were significantly higher than sham group at 50 and 100 days (3.02±/0.63 and 3.46±/0.38 8OHdG/dG.10(5)) (p<0.001, p<0.001). The higher TBARS levels were also detected in the exposure group both on 50 and 100 days (p<0.001, p<0.001). In addition, the extent of DNA damage and LPO would depend on the exposure time (p<0.05 and p<0.05). Our data may have important implications for the long-term exposure to ELF-EMF which may cause oxidative DNA damage.

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

Abstract Purpose: Genotoxic effects have been considered the gold standard to determine if an environmental factor is a carcinogen, but the currently available data for extremely low frequency time-varying magnetic fields (ELF-MFs) remain controversial. As an environmental stimulus, the effect of ELF-MF on cellular DNA may be subtle. Therefore, a more sensitive method and systematic research strategy are warranted to evaluate genotoxicity. Materials and methods: We investigated the effect of ELF-MFs in combination with ionizing radiation (IR) or H<sub>2</sub>O<sub>2</sub> on the DNA damage response of expression of phosphorylated H2AX ( $\gamma$ -H2AX) and production of  $\gamma$ -H2AX foci in non-tumorigenic human cell systems consisting of human lung fibroblast WI38 cells and human lung epithelial L132 cells. Results: Exposure to a 60-Hz, 2 mT ELF-MFs for 6 h produced increased  $\gamma$ -H2AX expression, as well as  $\gamma$ -H2AX foci production, a common DNA double-strand break (DSB) marker. However, exposure to a 1 mT ELF-MFs did not have the same effect. Moreover, 2 mT ELF-MFs exposure potentiated the expression of  $\gamma$ -H2AX and  $\gamma$ -H2AX foci production when combined with IR, but not when combined with H<sub>2</sub>O<sub>2</sub>. Conclusions: ELF-MFs could affect the DNA damage response and, in combination with different stimuli, provide different effects on  $\gamma$ -H2AX.

**(NE) Yoshikawa T, Tanigawa M, Tanigawa T, Imai A, Hongo H, Kondo M. Enhancement of nitric oxide generation by low frequency electromagnetic field. *Pathophysiology.* 7(2):131-135, 2000.**

Oxidative stress is implicated in the intracellular signal transduction pathways for nitric oxide synthase (NOS) induction. The electromagnetic field (EMF) is believed to increase the free radical lifespan [S. Roy, Y. Noda, V. Eckert, M.G. Traber, A. Mori, R. Liburdy, L. Packer, The phorbol 12-myristate 13-acetate (PMA)-induced oxidative burst in rat peritoneal neutrophils is increased by a 0.1 mT (60 Hz) magnetic field, *FEBS Lett.* 376 (1995) 164-6; F.S. Prato, M. Kavaliers, J.J. Carson, Behavioural evidence that magnetic field effects in the land snail, *Cepaea nemoralis*, might not depend on magnetite or induced electric currents, *Bioelectromagnetics* 17 (1996) 123-30; A.L. Hulbert, J. Metcalfe, R. Hesketh, Biological response to electromagnetic fields, *FASEB* 12 (1998) 395-420]. We tested the effects of EMF on endotoxin induced nitric oxide (NO) generation in vivo. Male BALB/C mice were injected with lipopolysaccharide (LPS) intraperitoneously (i.p.), followed by the exposure to EMF (0.1 mT, 60 Hz). Five hours and 30 min after the LPS administration, mice were administered with a NO spin trap, ferrous N-methyl-D-glucaminedithiocarbamate (MGD-Fe). Thirty minutes later, mice were sacrificed, and their livers were removed. The results were compared to three control groups: group A (LPS (-) EMF(-)); group B (LPS(-) EMF(+)); group C (LPS(+) EMF(-)). The ESR spectra of obtained livers were examined at room temperature. Three-line spectra of NO adducts were observed in the livers of

all groups. In groups A and B very weak signals were observed, but in groups C and D strong spectra were observed. The signal intensity of the NO adducts in Group D was also significantly stronger than that in Group C. EMF itself did not induce NO generation, however, it enhanced LPS induced NO generation in vivo.

**(E) Zhao G, Chen S, Wang L, Zhao Y, Wang J, Wang X, Zhang W, Wu R, Wu L, Wu Y, Xu A. Cellular ATP content was decreased by a homogeneous 8.5 T static magnetic field exposure: role of reactive oxygen species. *Bioelectromagnetics*. 32(2):94-101, 2011.**

The literature on the impact of strong static magnetic fields (SMF) on human health is vast and contradictory. The present study focused on the cellular effects of strong homogeneous SMF in human-hamster hybrid (A(L)) cells, mitochondria-deficient ( $\rho(0)$  A(L)) cells, and double-strand break (DSB) repair-deficient (XRS-5) cells. Adenosine triphosphate (ATP) content was significantly decreased in A(L) cells exposed to 8.5 Tesla (T) but not 1 or 4 T SMF for either 3 or 5 h. In addition, ATP content significantly decreased in the two deficient cell lines exposed to 8.5 T SMF for 3 h. With further incubation of 12 or 24 h without SMF exposure, ATP content could retrieve to the control level in the A(L) cells but not  $\rho(0)$  A(L) and XRS-5 cells. Under a fluorescence reader, the levels of reactive oxygen species (ROS) in the three cell lines were significantly increased by exposure to 8.5 T SMF for 3 h. Concurrent treatment with ROS inhibitor, DMSO, dramatically suppressed the ATP content in exposed A(L) cells. However, the CD59 mutation frequency and the cell cycle distribution were not significantly affected by exposure to 8.5 T SMF for 3 h. Our results indicated that the cellular ATP content was reduced by 8.5 T SMF for 3 h exposure, which was partially mediated by mitochondria and the DNA DSB repair process. Moreover, ROS were involved in the process of the cellular perturbations from the SMF.

**(E) Zwirska-Korczala K, Adamczyk-Sowa M, Polaniak R, Sowa P, Birkner E, Drzazga Z, Brzozowski T, Konturek SJ. Influence of extremely-low-frequency magnetic field on antioxidative melatonin properties in AT478 murine squamous cell carcinoma culture. *Biol Trace Elem Res*. 102(1-3):227-243, 2004.**

Effects of melatonin, extremely-low-frequency magnetic field (ELF-MF), and their combination on AT478 murine squamous cell carcinoma line were studied. Manganese superoxide dismutase (MnSOD), copper-zinc superoxide dismutase (Cu/ZnSOD), and glutathione peroxidase (GSH-Px) were used as markers of cells antioxidative status, and malondialdehyde (MDA) level was used as a marker of lipid peroxidation. After melatonin treatment, antioxidative enzyme activities were increased and MDA level was decreased. Application of ELF-MF on treated cells caused an increase of both superoxide dismutases activity and MDA level, but influence of ELF-MF on GSH-Px activity was negligible. All enzyme activity in culture medium containing melatonin ( $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  M) after exposure to ELF-MF were significantly diminished compared to cells treated only with melatonin. Also MDA levels after combined treatment with melatonin and ELF-MF were significantly decreased. Observed changes were statistically significant ( $p < 0.05$ ). These results strongly suggest that ELF-MF attenuates antioxidative actions of melatonin on cellular level.