SECTION 6

Evidence For Genotoxic Effects

(RFR AND ELF Genotoxicity)

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I. Introduction

Toxicity to the genome can lead to a change in cellular functions, cancer, and cell death. A large number of studies have been carried out to investigate the effects of electromagnetic field (EMF) exposure on DNA and chromosomal structures. The single-cell gel electrophoresis (comet assay) has been widely used to determine DNA damages: single and double strand breaks and cross-links. Studies have also been carried out to investigate chromosomal conformation and micronucleus formation in cells after exposure to EMF.

II. Radiofrequency radiation (RFR) and DNA damage (*28 total studies – 14 reported effects (50%) and 14 reported no significant effect (50%))*

II A. DNA studies that reported effects*:*

The following is a summary of the research data reported in the literature.

Aitken et al. [2005] exposed mice to 900-MHz RFR at a specific absorption rate (SAR) of 0.09 W/kg for 7 days at 12 h per day. DNA damage in caudal epididymal spermatozoa was assessed by quantitative PCR (QPCR) as well as alkaline and pulsed-field gel electrophoresis postexposure. Gel electrophoresis revealed no significant change in single- or double-DNA strand breakage in spermatozoa. However, QPCR revealed statistically significant damage to both the mitochondrial genome ($p < 0.05$) and the nuclear β-globin locus ($p < 0.01$).

Diem et al [2005] exposed human fibroblasts and rat granulosa cells to mobile phone signal (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; during 4, 16 and 24 h; intermittent 5 min on/10min off or continuous). RFR exposure induced DNA single- and double-strand breaks as measured by the comet assay. Effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect in the than continuous exposure.

Gandhi and Anita [2005] reported increases in DNA strand breaks and micronucleation in lymphocytes obtained from cell phone users.

Garaj-Vrhovac et al [1990] reported changes in DNA synthesis and structure in Chinese hamster cells after various durations of exposure to 7.7 GHz field at 30 mW/cm^2.

Lai and Singh [1995; 1996; 1997a; 2005] and Lai et al. [1997] reported increases in single and double strand DNA breaks in brain cells of rats exposed for 2 hrs to 2450-MHz field at 0.6-1.2 W/kg.

Lixia et al. [2006] reported an increase in DNA damage in human lens epithelial cells at 0 and 30 min after 2 hrs of exposure to 1.8 GHz field at 3 W/kg.

Markova et al. [2005] reported that GSM signals affected chromatin conformation and gama-H2AX foci that colocalized in distinct foci with DNA double strand breaks in human lymphocytes.

Narasimhan and Huh [1991] reported changes in lambdaphage DNA suggesting single strand breaks and strand separation.

Nikolova et al. [2005] reported a low and transient increase in DNA double strand break in mouse embryonic stem cells after acute exposure to 1.7- GHz field.

Paulraj and Behari [2006] reported an increased in single strand breaks in brain cells of rats after 35 days of exposure to 2.45 and 16.5 GHz fields at 1 and 2.01 W/kg.

Phillips et al. [1998] found increase and decrease in DNA strand breaks in cells exposure to various forms of cell phone radiation.

Sun et al. [2006] reported an increase in DNA single strand breaks in human lens epithelial cells after 2 hrs of exposure to 1.8 GHz field at 3 and 4 W/kg. The DNA damages caused by 4 W/kg field were irreversible.

Zhang et al. [2002] reported that 2450-MHz field at 5 mW/cm^2 did not induce DNA and chromosome damage in human blood cells after 2 hrs of exposure, but could increase DNA damage effect induced by mitomycin-C.

Zhang et al. [2006] reported that 1800-MHz field at 3.0 W/kg induced DNA damage in Chinese hamster lung cells after 24 hrs of exposure.

II B. DNA studies that reported no significant effect*:*

Chang et al. [2005] using the Ames assay found no significant change in mutation frequency in bacteria exposed for 48 hrs at 4W/kg to an 835-MHz CDMA signal.

Hook et al. [2004] showed that 24-hr exposure of Molt-4 cells to CDMA, FDMA, iDEN or TDMA modulated RF radiation did not significantly alter the level of DNA damage.

Lagroye et al. [2004a] reported no significant change in DNA strand breaks in brain cells of rats exposed for 2 hrs to 2450-MHz field at 1.2 W/kg.

Lagroye et al. [2004b] found no significant increases in DNA-DNA and DNA-protein cross-link in C3H10T(1/2) cells after a 2-hr exposure to CW 2450 MHz field at 1.9 W/kg.

Li et al. [2001] reported no significant change in DNA strand breaks in murine C3H10T(1/2) fibroblasts after 2 hrs of exposure to 847.74 and 835.02 MHz fields at 3-5 W/kg.

Maes et al. [1993, 1996, 1997, 2000, 2001, 2006] published a series of papers on in vitro genotoxic effects of radiofrequency radiation and interaction with chemicals. Their mostly found no significant effect.

Malyapa et al. [1997a,b, 1998] reported no significant change in DNA strand-breaks in cells exposed to 2450-Hz and various forms of cell phone radiation. Both in vitro and in vivo experiments were carried out.

McNamee et al. [2002a,b, 2003] found no significant increase in DNA breaks and micronucleus formation in human leukocytes exposed for 2 hrs to 1.9 GHz field at SAR up to 10 W/kg.

Sakuma et al. [2006] exposed human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs to mobile communication radiation for 2 and 24 hrs. No significant change in DNA strand breaks were observed up to 800 mW/kg.

Stronati et al. [2006] showed that 24 hrs of exposure to 935-MHz GSM basic signal at 1 or 2 W/Kg did not cause DNA strand breaks in human blood cells.

Tice et al. [2002] measured DNA single strand breaks in human leukocytes using the comet assay after exposure to various forms of cell phone signals. Cells were exposed at 37±1°C, for 3 or 24 h at average specific absorption rates (SARs) of 1.0-10.0 W/kg. Exposure for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes.

Vershaeve et al. [2006] long-term exposure (2 hrs/day, 5 days/week for 2 years) of rats to 900 MHz GSM signal at 0.3 and 0.9 W/kg did not significantly affect levels of DNA strand breaks in cells.

Vijayalaximi et al [2000] reported no significant increase in single strand breaks in human lymphocytes after 2 hrs of exposure to 2450-MHz field at 2 W/kg.

Zeni et al. [2005] reported that a 2-hr exposure to 900-MHz GSM signal at 0.3 and 1 W/kg did not significantly affect levels of DNA strand breaks in human leukocytes.

III. Micronucleus studies (29 Total studies: 16 reported effects (55%) and 13 reported no significant effect (45%))

III A. Micronucleus studies that reported effects:

Balode [1996] obtained blood samples from female Latvian Brown cows from a farm close to and in front of the Skrunda Radar and from cows in a control area. Micronuclei in peripheral erythrocytes were significantly higher in the exposed cows.

Busljeta et al. [2004] exposed male rats to 2.45 GHz RFR fields for 2 hours daily, 7 days a week, at 5-10 mW/cm^2 for up to 30 days. Erythrocyte count, haemoglobin and haematocrit were increased in peripheral blood on irradiation days 8 and 15. Anuclear cells and erythropoietic precursor cells were significantly decreased in the bone marrow on day 15, but micronucleated cells were increased.

D'Ambrosio et al. [2002] exposed human peripheral blood to 1.748 GHz continuous wave (CW) or phase-modulated wave (GMSK) for 15 min at a maximum specific absorption rate of ~5 W/kg. No changes were found in cell proliferation kinetics after exposure to either CW or GMSK fields. Micronucleus frequency result was not affected by CW exposure but a statistically significant increase in micronucleus was found following GMSK exposure.

Ferreira et al. [2006] found that rat offspring exposed to radiation from a cellular phone during their embryogenesis showed a significant increase in micronucleus frequency.

Fucic et al. [1992] reported increase in frequencies of micronuclei in the lymphocytes of humans exposed to microwaves.

Gandhi and Singh [2005] analyzed short term peripheral lymphocyte cultures for chromosomal aberrations and the buccal mucosal cells for micronuclei. They reported an increase in the number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes.

Garaj-Vrhovac et al [1992] exposed human whole-blood samples to continuous-wave 7.7 GHz radiation at power density of 0.5, 10 and 30 mW/cm^2 for 10, 30 and 60 min. In all experimental conditions, the frequencies of all types of chromosomal aberrations

(dicentric and ring chromosomes) and micronucleus were significantly higher than in the control samples.

Garaj-Vrhovac et al. [1999] investigated peripheral blood lymphocytes of 12 subjects occupationally exposed to microwave radiation. Results showed an increase in frequency of micronuclei as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls.

Haider et al. [1994] exposed plant cuttings bearing young flower buds for 30 h on both sides of a slewable curtain antenna (300/500 kW, 40-170 V/m) and 15 m (90 V/m) and 30 m (70 V/m) distant from a vertical cage antenna (100 kW) as well as at the neighbors living near the broadcasting station (200 m, 1-3 V/m). Laboratory controls were maintained for comparison. Higher micronucleus frequencies than in laboratory controls were found for all exposure sites in the immediate vicinity of the antennae,

Tice et al. [2002] measured micronucleus frequency in human leukocytes using the comet assay after exposure to various forms of cell phone signals. Cells were exposed at 37±1°C, for 3 or 24 h at average specific absorption rates (SARs) of 1.0-10.0 W/kg. Exposure for 3 h did not induce a significant increase in micronucleated lymphocytes. However, exposure to each of the signals for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase in the frequency of micronucleated lymphocytes. The magnitude of the response (approximately four fold) was independent of the technology, the presence or absence of voice modulation, and the frequency.

Trosic et al. [2001] investigated the effect of a 2450-MHz microwave irradiation on alveolar macrophage kinetics and formation of multinucleated giant cells after whole body irradiation of rats at 5-15 mW/cm^2. A group of experimental animals was divided in four subgroups that received 2, 8, 13 and 22 irradiation treatments of two hours each. The animals were killed on experimental days 1, 8, 16, and 30. Multinucleated cells were significantly increased in treated animals. The increase in number of nuclei per cell was time- and dose-dependent. Macrophages with two nucleoli were more common in animals treated twice or eight times. Polynucleation was frequently observed after 13 or 22 treatments.

Trosic et al. [2002] exposed adult male Wistar for 2 h a day, 7 days a week for up to 30 days to continuous 2450-MHz microwaves at a power density of 5-10mW/cm^2. Frequency of micronuclei in polychromatic erythrocytes showed a significant increase in the exposed animals after 2, 8 and 15 days of exposure compared to sham-exposed control.

Trosic et al. [2004] investigated micronucleus frequency in bone marrow red cells of rats exposed to a 2450-MHz continuous–wave microwaves for 2 h daily, 7 days a week, at a power density of 5-10 mW/cm^2 (whole body SAR 1.25 +/- 0.36 (SE) W/kg). The frequency of micronucleated polychromatic erythrocytes was significantly increased on experimental day 15.

Trosic et al. [2006] exposed rats 2 h/day, 7 days/week to 2450-MHz microwaves at a whole-body SAR of 1.25 +/- 0.36W/kg. Control animals were included in the study. Bone marrow micronucleus frequency was increased on experimental day 15, and polychromatic erythrocytes micronucleus frequency in the peripheral blood was increased on day 8.

Zotti-Martelli et al. [2000] exposed human peripheral blood lymphocytes in G(0) phase to electromagnetic fields at different frequencies (2.45 and 7.7 GHz) and power

densities (10, 20 and 30 mW/cm^2) for 15, 30 or 60min. The results showed for both radiation frequencies an induction of micronuclei as compared to control cultures at a power density of 30mW/cm^2 and after an exposure of 30 and 60 min.

Zotti-Martelli et al. [2005] exposed whole blood samples from nine different healthy donors for 60, 120 and 180 min to continuous-wave 1800-MHz microwaves at power densities of 5, 10 and 20 mW/cm^2. A statistically significant increase of micronucleus in lymphocytes was observed dependent on exposure time and power density. A considerable decrease in spontaneous and induced MN frequencies was measured in a second experiment.

III B. Micronucleus studies that reported no significant effects:

Bisht et al. [2002] exposed C3H 10T½ cells to 847.74 MHz CDMA (3.2 or 4.8 W/kg) or 835.62 MHz FDMA (3.2 or 5.1 W/kg) RFR for 3, 8, 16 or 24 h. No exposure condition was found to result in a significant increase relative to sham-exposed cells either in the percentage of binucleated cells with micronuclei or in the number of micronuclei per 100 binucleated cells.

Juutilainen et al. [2007] found no significant change in micronucleus frequency in erythrocytes of mice after long-term exposure to various mobile phone frequencies.

Koyama et al. [2004] exposed Chinese hamster ovary (CHO)-K1 cells to 2450-MHz microwaves for 2 h at average specific absorption rates (SARs) of 5, 10, 20, 50, 100, and 200 W/kg. Micronucleus frequency in cells exposed at SARs of 100 and 200 W/kg were significantly higher when compared with sham-exposed controls. They speculated that the effect observed was a thermal effect.

Port et al. [2003] reported that exposure of HL-60 cells to EMFs 25 times higher than the ICNIRP reference levels for occupational exposure did not induce any significant changes in apoptosis, micronucleation, abnormal morphologies and gene expression.

Scarfi et al [2006] exposed human peripheral blood lymphocytes to 900 MHz GSM signal at specific absorption rates of 0, 1, 5 and 10 W/kg peak values. No significant change in micronucleus frequency was observed.

Vijayalaximi et al. [1997a] exposed human blood to continuous-wave 2450- MHz microwaves, either continuously for a period of 90 min or intermittently for a total exposure period of 90 min (30 min on and 30 min off, repeated three times). The mean power density at the position of the cells was 5.0 mW/cm^2 and mean specific absorption rate was 12.46 W/kg. There were no significant differences between RFR-exposed and sham-exposed lymphocytes with respect to; (a) mitotic indices; (b) incidence of cells showing chromosome damage; (c) exchange aberrations; (d) acentric fragments; (e) binucleate lymphocytes, and (f) micronuclei.

Vijayalaximi et al. [1997b] exposed C3H/HeJ mice for 20 h/day, 7 days/week, over 18 months to continuous-wave 2450 MHz microwaves at a whole-body average specific absorption rate of 1.0 W/kg. At the end of the 18 months, peripheral blood and bone marrow smears were examined for the extent of genotoxicity as indicated by the presence of micronuclei in polychromatic erythrocytes. The results indicate that the incidence of micronuclei/1,000 polychromatic erythrocytes was not significantly different between groups exposed to RF radiation and sham-exposed groups.

Vijayalaximi et al. [1999] exposed CF-1 male mice to ultra-wideband electromagnetic radiation (UWBR) for 15 min at an estimated whole-body average specific absorption rate of 37 mW/kg. Peripheral blood and bone marrow smears were examined to determine the extent of genotoxicity, as assessed by the presence of micronuclei (MN) in polychromatic erythrocytes (PCE). There was no evidence for excess genotoxicity in peripheral blood or bone marrow cells of mice exposed to UWBR.

Vijayalaximi et al. [2001a] reported that there was no evidence for the induction of micronuclei in peripheral blood and bone marrow cells of rats exposed for 24h to 2450-MHz continuous-wave microwaves at a whole body average SAR of 12 W/kg.

Vijayalaximi et al. [2001b] reported that there is no evidence for the induction of chromosomal aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 835.62 MHz RF radiation at SARs of 4.4 or 5.0 W/kg.

Vijayalaximi et al. [2001c] reported no evidence for induction of chromosome aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 847.74 MHz RF radiation (CDMA) at SARs of 4.9 or 5.5 W/kg.

Vijayalaximi et al. [2003] exposed timed-pregnant Fischer 344 rats (from nineteenth day of gestation) and their nursing offspring (until weaning) to a far-field 1.6 GHz Iridium wireless communication signal for 2 h/day, 7 days/week at power density of 0.43 mW/cm^2 and whole-body average specific absorption rate of 0.036 to 0.077 W/kg (0.10 to 0.22 W/kg in the brain). This was followed by chronic, head-only exposures of male and female offspring to a near-field 1.6 GHz signal for 2 h/day, 5 days/week, over 2 years. Near-field exposures were conducted at an SAR of 0.16 or 1.6 W/kg in the brain. At the end of 2 years, all rats were necropsied. Bone marrow smears were examined for the extent of genotoxicity, assessed from the presence of micronuclei in polychromatic erythrocytes. There was no evidence for excess genotoxicity in rats that were chronically exposed to 1.6 GHz microwaves compared to sham-exposed and cage controls.

Zeni et al. [2003] investigated the induction of micronucleus in human peripheral blood lymphocytes after exposure to electromagnetic fields at various duration of exposure, specific absorption rate (SAR), and signal [continuous-wave (CW) or GSM (Global System of Mobile Communication)-modulated signal]. No statistically significant difference was detected in any case.

IV. Chromosome and genome effects (21 studies total: 13 reported effects (62%) and 8 reported no significant effect (38%))

IV A. Chromosome and genome studies that reported effects:

Belyaev et al. [I992] studied the effect of low intensity microwaves on the conformational state of the genome of X-irradiated E. coli cells by the method of viscosity anomalous time dependencies. A power density of 1 $microW/cm^2$ is sufficient to suppress radiation-induced repair of the genome conformational state.

Belyaev et al. [1996] studied the effect of millimeter waves on the genome conformational state of E. coli AB1157 by the method of anomalous viscosity time dependencies in the frequency range of 51.64-51.85 GHz. Results indicate an electron-conformational interactions.

Belyaev et al. [2005] investigated response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to microwaves from GSM mobile phone (915 MHz, specific absorption rate 37 mW/kg), and power frequency magnetic field (50 Hz, 15 microT peak value). Changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected.

Belyaev et al. [2006] investigated whether exposure of rat brain to microwaves of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression at a specific absorption rate (SAR) of 0.4 mW/g for 2 h. Data showed that GSM MWs at 915 MHz did not induce DNA double stranded breaks detectable by pulsed-field gel electrophoresis or changes in chromatin conformation, but affected expression of genes in rat brain cells.

Gadhia et al. [2003] reported a significant increase in dicentric chromosomes in blood cells among mobile users who were smoker–alcoholic as compared to nonsmoker–nonalcoholic; the same held true for controls of both types.

Garaj-Vrhovac et al. [1990] exposed V79 Chinese hamster cells to continuous-wave 7.7 GHz RFR at power density of 30 mW/cm^2 for 15, 30, and 60 min. Results suggest that the radiation causes changes in the synthesis as well as in the structure of DNA molecules.

Garaj-Vrhovac et al. [1991] exposed V79 Chinese hamster fibroblast cells to continuous wave 7.7 GHz radiation at power density of 0.5 mW/cm^2 for 15, 30 and 60 min. There was a significantly higher frequency of specific chromosome aberrations such as dicentric and ring chromosomes in irradiated cells.

Mashevich et al. [2003] found that human peripheral blood lymphocytes exposed to continuous 830-MHz electromagnetic fields (1.6-8.8 W/kg for 72 hr) showed a SAR-dependent chromosome aneuploidy, a major "somatic mutation" leading to genomic instability and thereby to cancer. The aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in the SAR dependent genetic toxicity. The effects were non-thermal.

Ono et al. (2004) exposed pregnant mice intermittently at a whole-body averaged specific absorption rate of 0.71 W/kg (10 seconds on, 50 seconds off which is 4.3 W/kg during the 10 seconds exposure) for 16 hours a day, from the embryonic age of 0 to 15 days. At 10 weeks of age, mutation frequencies at the lacZ gene in spleen, liver, brain, and testis were examined. Quality of mutation assessed by sequencing the nucleotides of mutant DNAs revealed no appreciable difference between exposed and non-exposed samples.

Sarimov et al. [2004] reported that exposure to microwaves of 895-915 MHz at 5.4 mW/kg resulted in statistically significant changes in condensation of chromatin in human lymphocytes. Effects are similar to stress response, differ at various frequencies, and vary among donors.

Sarkar et al. [1994] exposed mice to 2450-MHz microwaves at a power density of 1 mW/cm^2 for 2 h/day over a period of 120, 150 and 200 days. Rearrangement of DNA segments were observed in testis and brain of exposed animals.

Semin et al. [1995] exposed DNA samples at 18^oC at 10 different microwave frequencies (4- to 8 GHz, 25 ms pulses, 0.4 to 0.7 mW/cm^2 peak power, 1- to 6-Hz repetition rate, no heating). Irradiation at 3 or 4 Hz and 0.6 mW/cm^2 peak power clearly increased the accumulated damage to the DNA secondary structure ($P< .00001$). However, changing the pulse repetition rate to 1, 5, 6 Hz, as well as changing the peak power to 0.4 or 0.7 mW/cm^2 did not induce significant effect. Thus, the effect occurred only within narrow 'windows' of the peak intensities and modulation frequencies.

Sykes et al. [2001] exposed mice daily for 30 min to plane-wave fields of 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms for 1, 5 or 25 days. Three days after the last exposure, spleen sections were screened for DNA inversion events. There was no significant difference between the control and treated groups in the 1- and 5-day exposure groups, but there was a significant reduction in inversions below the spontaneous frequency in the 25-day exposure group. This observation suggests that exposure to RF radiation can lead to a perturbation in recombination frequency which may have implications for recombination repair of DNA.

IV. B. Chromosome and genome studies that reported no significant effects:

Antonopoulos et al. [1997] found no significant change in cell cycle progression and the frequencies of sister-chromatid exchanges in human lymphocytes exposed to electromagnetic fields of 380, 900 and 1800 MHz.

Ciaravino et al. [1991] reported that RFR did not affect changes in cell progression caused by adriamycin, and the RFR did not change the number of sister chromatid exchanges that were induced by the adriamycin.

Garson et al. [1991] analyzed lymphocytes from Telecom Australia radio-linemen who had all worked with RFR in the range 400 kHz-20 GHz with exposures at or below the Australian occupational limits. There was no significant increase in chromosomal damage in circulating lymphocytes.

Gos et al. [2000] exposed actively growing and resting cells of the yeast Saccharomyces cerevisiae to 900-MHz Global System for Mobile Communication (GSM) pulsed modulation format signals at specific absorption rates (SAR) of 0.13 and 1.3 W/kg. They reported no significant effect of the fields on forward mutation rates on the frequency of petite formation, on rates of intrachromosomal deletion formation, or on rates of intragenic recombination in the absence or presence of the genotoxic agent methyl methansulfonate.

Kerbacher et al (1990) reported that exposure to pulsed 2450-MHz microwaves for 2 h at an SAR of 33.8 W/kg did not significantly cause chromosome aberrations in CHO cells. The radiation also did not interact with Mitomycin C and Adriamycin.

Komatsubara et al. [2005] reported that exposure to 2.45-GHz microwaves for 2 h with up to 100 W/kg SAR CW and an average 100 W/kg PW (a maximum SAR of 900 W/kg) did not induce chromosomal aberrations in mouse m5S cells.

Meltz et al. [1990] reported no significant mutagenic effect of exposure to 2.45-GHz RFR (40 W/kg) alone and interaction with proflavin, a DNA-intercalating drug, in L5178Y mouse leukemic cells.

Roti-Roti et al. [2001] reported no significant effect of exposure to radiofrequency radiation in the cellular phone communication range (835.62 MHz frequency division multiple access, FDMA; 847.74 MHz code division multiple access, CDMA) on neoplastic transformation frequency using the in vitro C3H 10T(1/2) cell transformation assay system.

Takahashi et al. [2002] exposed mice to 1.5 GHz EMF in the head region at 2.0, 0.67, and 0 W/kg specific absorption rate for 90 min/day, 5 days/week, for 4 weeks. No mutagenic effect in mouse brain cells was detected.

V. Conclusions

From this literature survey, since only 50% of the studies reported effects, it is apparent that there is no consistent pattern that radiofrequency radiation exposure could induce genetic damages/changes in cells and organisms. However, one can conclude that under certain conditions of exposure, radiofrequency radiation is genotoxic. Data available are mainly applicable only to cell phone radiation exposure. Other than the study by Phillips et al [1998], there is no indication that RFR at levels that one can experience in the vicinity of base stations and RF-transmission towers could cause DNA damage.

During cell phone use, a relatively constant mass of tissue in the brain is exposed to the radiation at relatively high intensity (peak SAR of 4 - 8 W/kg). Several studies reported DNA damage at lower than 4 W/kg. This questions the wisdom of the IEEE Committee in using 4 W/kg as the threshold of effect for exposure-standard setting. Furthermore, since critical genetic mutations in one single cell are sufficient to lead to cancer and there are millions of cells in a gram of tissue, it is inconceivable that the base of SAR standard was changed from averaged over 1 gm of tissue to 10 gm. (The limit of localized tissue exposure has been changed from 1.6 W/kg averaged over 1 gm of tissue to 2 W/kg over 10 gm of tissue. Since distribution of radiofrequency energy is non-homogenous inside tissue, this change allows a higher peak level of exposure.) What actually needed is a better refinement of SAR calculation to identify ‘peak values’ of SAR inside the brain,

Aside from influences that are not directly related to experimentation [Huss et al., 2007], many factors could influence the outcome of an experiment in bioelectromagnetics research.

Any effect of EMF has to depend on the energy absorbed by a biological entity and on how the energy is delivered in space and time. Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different effects. In addition, in order to understand the biological consequence of EMF exposure, one must know whether the effect is cumulative, whether compensatory responses result, and when homeostasis will break down. The contributions of these physical factors are discussed in a talk presented in

Vienna, Austria in 1998. The paper is posted in many websites (e.g., http://www.wave-guide.org/library/lai.html).

Thus, differences in outcomes of the research on genotoxic effects of RFR could be explained by the many different exposure conditions used in the studies. An example is the study of Phillips et al. [1998] showing that different cell phone signals could cause different effects on DNA (i.e., an increase in strand breaks with exposure to one type of signal and a decrease with another). This is further complicated by the fact that some of the studies listed above used very poor exposure procedures with very limited documentation of exposure parameters, e.g., using a cell phone to expose cells and even animals. Data from these experiments are questionable.

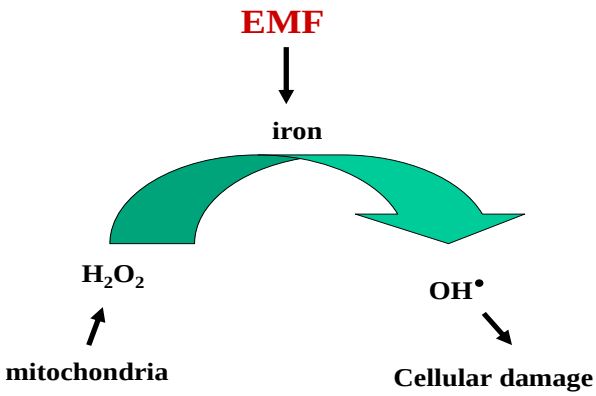
Another source of influence on an experimental outcome is the cell or organism studied. Many different biological systems were used in the genotoxicity studies. Different cell types [Hoyto et al., 2007] and organisms [Anderson et al., 2000; DiCarlo and Litovitz, 1999] may respond differently to EMF.

A few words have to be said on the 'comet assay', since it was used in most of the EMF studies to determine DNA damage. Different versions of the assay have been developed. These versions have different detection sensitivities and can be used to measure different aspects of DNA strand breaks. A comparison of data from experiments using different versions of the assay may be misleading. Another concern is that most of the 'comet assay' studies were carried out by experimenters who had no prior experience on the assay. My experience with the 'comet assay' is that it is a very sensitive assay and requires great care in performing. Thus, different detection sensitivities could result from different experimenters, even following the same procedures. One way to solve this experimental variation problem is for each researcher or laboratory to report their sensitivity of the 'comet assay', e.g., threshold of detecting strand breaks in human lymphocytes exposed to x-rays. This information is generally not available from the EMF-genotoxicity studies. However, in one incidence, an incredibly high sensitivity was even reported [Malyapa et al., 1998], suggesting the inexperience of the researchers on the assay.

A drawback in the interpretation and understanding of experimental data from bioelectromagnetic research is that there is no general acceptable mechanism on how EMF affects biological systems. The mechanism by which RFR causes genetic effect is unknown. Since the energy level is not sufficient to cause direct breakage of chemical bonds within molecules, the effects are probably indirect and secondary to other induced-chemical changes in the cell.

One possibility is via free radical formation inside cells. Free radicals kill cells by damaging macromolecules, such as DNA, protein and membrane. Several reports have indicated that electromagnetic fields (EMF) enhance free radical activity in cells [e.g., Lai and Singh, 1997a, b; 2004; Oral et al., 2006; Simko, 2007], particularly via the Fenton reaction [Lai and Singh, 2004]. The Fenton reaction is a catalytic process of iron

to convert hydrogen peroxides, a product of oxidative respiration in the mitochondria, into hydroxyl free radical, which is a very potent and toxic free radical.



THE FENTON REACTION

What is interesting that extremely-low frequency EMF has also been shown to cause DNA damage (see the list of papers on ELF EMF and DNA at the end of this chapter). Free radicals have also been implicated in this effect of ELF EMF. This further supports the view that EMF affects DNA via an indirect secondary process, since the energy content of ELF EMF is much lower than that of RFR.

Effects via the Fenton reaction predict how a cell would respond to EMF:

1. Cells that are metabolic active would be more susceptible to the effect because more hydrogen peroxide is generated by the mitochondria to fuel the reaction.

2. Cells that have high level of intracellular free iron would be more vulnerable. Cancer cells and cells undergoing abnormal proliferation have high concentration of free iron because they uptake more iron and have less efficient iron storage regulation. Thus, these cells could be selectively damaged by EMF, and EMF could potentially be used for the treatment of cancer and hyperplasia diseases. The effect could be further enhanced if one could shift anaerobic glycolysis of cancer cells to oxidative glycolysis. There is quite a large database of information on the effects of EMF (mostly in the ELF range) on cancer cells and tumors. The data tend to indicate that EMF could retard tumor growth and kill cancer cells.

3. Since the brain is exposed to rather high levels of EMF during cell phone use, the consequences of EMF-induced genetic damage in brain cells are of particular importance. Brain cells have high level of iron. Special molecular pumps are present on nerve cell nucleus membrane to pump iron into the nucleus. Iron atoms have been found to intercalate within DNA molecules. In addition, nerve cells have a low capability for DNA repair and DNA breaks could accumulate. Another concern is the presence of superparamagnetic iron-particles (magnetites) in body tissues,

particularly in the brain. These particles could enhance free radical activity in cells and cellular-damaging effects of EMF. These factors make nerve cells more vulnerable to EMF. Thus, the effect of EMF on DNA could conceivably be more significant on nerve cells than on other cell types of the body. Since nerve cells do not divide and are not likely to become cancerous, more likely consequences of DNA damage in nerve cells are changes in functions and cell death, which could either lead to or accelerate the development of neurodegenerative diseases. Double strand breaks, if not properly repaired, are known to lead to cell death. Cumulative DNA damage in nerve cells of the brain has been associated with neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases. However, another type of brain cells, the glial cells, can become cancerous, resulting from DNA damage. The question is whether the damaged cells would develop into tumors before they are killed by EMF due to over accumulation of genetic damages. The outcome depends on the interplay of these different physical and biological factors: an increase, decrease, or no significant change in cancer risk could result.

4. On the other hand, cells with high antioxidant potentials would be less susceptible to EMF. These include the amount of antioxidants and anti-oxidative enzymes in the cells. Furthermore, the effect of free radicals could depend on the nutritional status of an individual, e.g., availability of dietary antioxidants, consumption of alcohol, and amount of food consumption. Various life conditions, such as psychological stress and strenuous physical exercise, have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can also speculate that some individuals may be more susceptible to the effects of EMF exposure.

More research has to be carried out to prove the involvement of the free radicals in the biological effects of EMF. However, the Fenton reaction obviously can only explain some the genetic effects observed. For example, RF- and ELF EMF-induced DNA damages have been reported in normal lymphocytes, which contain a very low concentration of intracellular free iron.

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VI. References for Radiofrequency Radiation Studies

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APPENDIX 6-A
Abstracts on Effects of Extremely Low Frequency (ELF) EMF on DNA

27 (E)- effect reported; 14 (NE)- no significant effect reported

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

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

Cantoni O, **Sestili P**, **Fiorani M**, **Dacha M**. **Effect of 50 Hz sinusoidal electric and/or magnetic fields on the rate of repair of DNA single strand breaks in cultured mammalian cells exposed to three different carcinogens: methylmethane sulphonate, chromate and 254 nm U.V. radiation.** Biochem Mol Biol Int. 38(3):527-533, 1996. (NE)

Treatment of cultured mammalian cells with three different carcinogens, namely methylmethane sulphonate (MMS), chromate and 254 U.V. radiation, produces DNA single strand breaks (SSB) in cultured mammalian cells. The rate of removal of these lesions is not affected by exposure to 50 Hz electric (0.2 - 20 kV/m), magnetic (0.0002-0.2 mT), or combined electric and magnetic fields. These results indicate that, under the experimental conditions utilized in this study, 50 Hz electric, magnetic and electromagnetic fields (over a wide range of intensities) do not affect the machinery involved in the repair of DNA SSBs generated by different carcinogens in three different cultured mammalian cell lines, making it unlikely that field exposure enhances the ability of these carcinogens to induce transformation via inhibition of DNA repair.

Chahal R, **Craig DQ**, **Pinney RJ**. **Investigation of potential genotoxic effects of low frequency electromagnetic fields on Escherichia coli.** J Pharm Pharmacol. 45(1):30-33, 1993. (NE)

Exposure of growing cells of Escherichia coli strain AB1157 to a frequency of 1 Hz with field strengths of 1 or 3 kV m-1 did not affect spontaneous or ultraviolet light (UV)-induced mutation frequencies to rifampicin resistance. Neither did growth in the presence of charge alter the sensitivities of strains AB1157, TK702 umuC or TK501 umuC uvrB to UV. Similarly, although the resistance of strains TK702 umuC and TK501 umuC uvrB to UV was increased by the presence of plasmid pKM101, which carries DNA repair genes, pregrowth of plasmid-containing strains in electric fields did not increase UV resistance. Finally, growth in a low frequency field in the presence of sub-inhibitory concentrations of mitomycin C did not affect mitomycin C-induced mutation frequencies. It is concluded that low frequency electromagnetic fields do not increase spontaneous mutation, induce DNA repair or increase the mutagenic effects of UV or mitomycin C.

Chow K, **Tung WL** **Magnetic field exposure enhances DNA repair through the induction of DnaK/J synthesis.** FEBS Lett. 478(1-2):133-136, 2000. (E)

In contrast to the common impression that exposure to a magnetic field of low frequency causes mutations to organisms, we have demonstrated that a magnetic field can actually enhance the efficiency of DNA repair. Using Escherichia coli strain XL-1 Blue as the host and plasmid pUC8 that had been mutagenized by hydroxylamine as the vector for assessment, we found that bacterial transformants that had been exposed to a magnetic field of 50 Hz gave lower percentages of white colonies as compared to transformants that had not been exposed to the magnetic field. This result was indicative that the efficiency of DNA repair had been improved. The improvement was found to be mediated by the induced overproduction of heat shock proteins DnaK/J (Hsp70/40).

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

The effects of pulsed electric fields of low frequency (50 Hz) on DNA of human lymphocytes were investigated. The influence of additional external factors, such as hydrogen peroxide (H_2O_2) and gamma-irradiation, as well as the repair efficiency in these lymphocytes, was also evaluated. The comet assay, a very sensitive and rapid method for detecting DNA damage at the single cells level was the method used. A significant amount of damage was observed after exposure to the electric fields, compared to the controls. After 2 h incubation at 37 degrees C, a proportion of damage was repaired. H_2O_2 and gamma-irradiation increased the damage to lymphocytes exposed to pulsed electric fields according to the dose used, while the amount of the repair was proportional to the damage.

Fairbairn DW, **O'Neill KL** **The effect of electromagnetic field exposure on the formation of DNA single strand breaks in human cells.** Cell Mol Biol (Noisy-le-grand). 40(4):561-567, 1994. (NE)

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

Fiorani M, **Cantoni O**, **Sestili P**, **Conti R**, **Nicolini P**, **Vetrano F**, **Dacha M**. **Electric and/or magnetic field effects on DNA structure and function in cultured human cells.** Mutat Res. 282(1):25-29, 1992. (NE)

Exposure of cultured K562 cells to 50 Hz electric (0.2-20 kV/m), magnetic (0.002-2 G), or combined electric and magnetic fields for up to 24 h did not result in the production of detectable DNA lesions, as assayed by the filter elution technique. The rate of cell growth was also unaffected as well as the intracellular ATP and NAD+ levels. These results indicate that, under the experimental conditions utilized in this study, 50 Hz electric, magnetic and electromagnetic fields are not geno- and cyto-toxic in cultured mammalian cells.

Frazier ME, **Reese JA**, **Morris JE**, **Jostes RF**, **Miller DL** **Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis of DNA repair of induced, single-strand breaks.** Bioelectromagnetics. 11(3):229-234, 1990. (NE)

DNA damage was induced in isolated human peripheral lymphocytes by exposure at 5 Gy to 60Co radiation. Cells were permitted to repair the DNA damage while exposed to 60-Hz fields or while sham-exposed. Exposed cells were subjected to magnetic (B) or electric (E) fields, alone or in combination, throughout their allotted repair time. Repair was stopped at specific times, and the cells were immediately lysed and then analyzed for the presence of DNA single-strand breaks (SSB) by the alkaline-elution technique. Fifty to 75 percent of the induced SSB were repaired 20 min after exposure, and most of the remaining damage was repaired after 180 min. Cells were exposed to a 60-Hz ac B field of 1 mT; an E field of 1 or 20 V/m; or combined E and B fields of 0.2 V/m and 0.05 mT, 6 V/m and 0.6 mT, or 20 V/m and 1 mT. None of the exposures was observed to affect significantly the repair of DNA SSB.

Hong R, **Zhang Y**, **Liu Y**, **Weng EQ**. [Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 23(6):414-417, 2005. (E)

[Article in Chinese]

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

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

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

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

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

which could point to an age-related decrease of DNA repair efficiency of ELF-EMF induced DNA strand breaks.

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

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

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

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

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 (FeCl2, 10microg/ml), while the rest served as controls. A significant increase in the number of cells with DNA damage was found only after simultaneous exposure of lymphocytes to FeCl2 and 7mT 50Hz MF, compared to the control samples or those incubated with FeCl2 alone. However, when the cells were treated with melatonin and then exposed to iron ions and 50Hz MF, the number of damaged cells was significantly reduced, and the effect depended on the concentration of melatonin. The reduction reached about 50% at 0.5mM and about 100% at 1.0mM. Our results indicate that melatonin provides protection against DNA damage in rat lymphocytes exposed in vitro to iron ions and 50Hz MF (7mT). Therefore, it can be suggested that free radicals may be involved in 50Hz magnetic field and iron ions-induced DNA damage in rat blood lymphocytes. The future experimental studies, in vitro and in vivo, should provide an answer to the question concerning the role of melatonin in the free radical processes in the power frequency magnetic field.

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

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

after prolonged metabolic resonance using both single cell gel electrophoresis ('comet' assay) and 3'-OH DNA labeling using terminal deoxynucleotidyl transferase. These results provide insights into transmembrane signal processing and cell interactions with weak electric fields.

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

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

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

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

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

In previous research, we have found an increase in DNA single- and double-strand breaks in brain cells of rats after acute exposure (two hours) to a sinusoidal 60-Hz magnetic

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

Li SH, **Chow KC**. **Magnetic field exposure induces DNA degradation.** Biochem Biophys Res Commun. 280(5):1385-1388, 2001. (E)

In our earlier experiments, we discovered that magnetic field exposure could bring both stabilizing and destabilizing effects to the DNA of Escherichia coli, depending on our parameters of assessment, and both of these effects were associated with the induced synthesis of the heat shock proteins Hsp70/Hsp40 (DnaK/DnaJ). These contradicting results prompted us to explore in this study the effect of magnetic field exposure on the DNA stability in vivo when the heat shock response of the cell was suppressed. By using plasmid pUC18 in E. coli as the indicator, we found that without the protection of the heat shock response, magnetic field exposure indeed induced DNA degradation and this deleterious effect could be diminished by the presence of an antioxidant, Trolox C. In our in vitro test, we also showed that the magnetic field could potentiate the activity of oxidant radicals.

Lopucki M, **Schmerold I**, **Dadak A**, **Wiktor H**, **Niedermuller H**, **Kankofer M**. **Low dose magnetic fields do not cause oxidative DNA damage in human placental cotyledons in vitro.** Virchows Arch. 446(6):634-639, 2005. (NE)

The biological impact of low dose magnetic fields generated by electric appliances present in the human environment is still uncertain. In this study, human placentas served as a model tissue for the evaluation of the potential effect of oscillating low intensity magnetic fields on the concentration of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in cellular DNA. Cotyledons were dissected from placentas obtained immediately after physiological labours and exposed to magnetic fields (groups MF A, 2 mT, 50 Hz and MF B, 5 mT, 50 Hz) or sham exposed (group C) during an in vitro perfusion of 3 h. Cellular DNA was isolated, hydrolyzed and analyzed by HPLC. Native nucleosides were monitored at 254 nm and 8-OH-dG by electrochemical detection. Results were expressed as mumol 8-OH-dG/mol deoxyguanosine (dG). The concentrations of 8-OH-dG in group C, MF A and MF B were 28.45+/-15.27 micromol/mol dG, 62.80+/-31.91 mumol/mol dG, and 27.49+/-14.23 micromol/mol dG, respectively, demonstrating no significant difference between the groups. The results suggest that placental tissues possess a capacity to protect DNA against oxidative alterations by magnetic field of intensities previously shown to produce radical mediated DNA damage in rat brain cells in vivo and imbalances in electrolyte release of cotyledons under in vitro conditions.

Lourencini da Silva R, **Albano F**, **Lopes dos Santos LR**, **Tavares AD Jr**, **Felzenszwalb I**. **The effect of electromagnetic field exposure on the formation of DNA lesions.** Redox Rep. 5(5):299-301, 2000. (E)

In an attempt to determine whether electromagnetic field (EMF) exposure might lead to DNA damage, we exposed SnCl2-treated pBR322 plasmids to EMF and analysed the resulting conformational changes using agarose gel electrophoresis. An EMF-dependent potentiation of DNA scission (i.e. the appearance of relaxed plasmids) was observed. In confirmation of this, plasmids pre-exposed to EMF also were less capable of transforming Escherichia coli. The results indicate that EMF, in the presence of a transition metal, is capable of causing DNA damage. These observations support the idea that EMF, probably through secondary generation of reactive oxygen species, can be clastogenic and provide a possible explanation for the observed correlation between EMF exposure and the frequency of certain types of cancers in humans.

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

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

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

Several recent studies have reported that whole-body exposure of rodents to power frequency magnetic fields (MFs) can result in DNA single- and double-strand breaks in the brains of these animals. The current study was undertaken to investigate whether an acute 2h exposure of a 1 mT, 60 Hz MF could elicit DNA damage, and subsequently apoptosis, in the brains of immature (10-day-old) mice. DNA damage was quantitated at 0, 2, 4, and 24h after exposure using the alkaline comet assay. Apoptosis was quantitated in the external granule cell layer (EGCL) of the immature mouse cerebellum at 0 and 24h after exposure to MF by the TdT-mediated dUTP nick-end labeling (TUNEL) assay. Four

parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. While increased DNA damage was detected by tail ratio at 2h after MF exposure, no supporting evidence of increased DNA damage was detected by the other parameters. In addition, no similar differences were observed using these parameters at any of the other post-exposure times. No increase in apoptosis was observed in the EGCL of MF-exposed mice, when compared to sham mice. Taken together, these results do not support the hypothesis that acute MF exposure causes DNA damage in the cerebellums of immature mice.

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

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

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

We examined the effect of an extremely low-frequency magnetic field (ELFMF) at 5, 50 and 400 mT on DNA strand breaks in human glioma MO54 cells. A DNA damage analysis was performed using the method of alkaline comet assay. The cells were exposed to X-rays alone (5 Gy), ELFMF alone, or X-rays followed by ELFMF at 4 degrees C or on ice. No significant difference in the tail moment was observed between control and ELFMF exposures up to 400 mT. X-ray irradiation increased DNA strand breaks. When cells were exposed to X-rays followed by ELFMF at 50 and 400 mT, the tail moment increased significantly compared with that for X-rays alone. When the exposure of cells was performed at 37 degrees C, no significant change was observed between X-rays alone and X-rays plus 400 mT. We previously observed that exposure to

400 mT ELFMF for 2 h increased X-ray-induced mutations (Miyakoshi et al, Mutat. Res., 349: 109-114, 1996). Additionally, an increase in the mutation by exposure to the ELFMF was observed in cells during DNA-synthesizing phase (Miyakoshi et al., Int. J. Radiat. Biol., 71: 75-79, 1997). From these results, it appears that exposure to the high density ELFMF at more than 50 mT may potentiate X-ray-induced DNA strand breaks.

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

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

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

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45

genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

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

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

Robison JG, **Pendleton AR**, **Monson KO**, **Murray BK**, **O'Neill KL**. **Decreased DNA repair rates and protection from heat induced apoptosis mediated by electromagnetic field exposure.** Bioelectromagnetics. 23(2):106-112, 2002. (E)

In this study, we demonstrate that electromagnetic field (EMF) exposure results in protection from heat induced apoptosis in human cancer cell lines in a time dependent manner. Apoptosis protection was determined by growing HL-60, HL-60R, and Raji cell lines in a 0.15 mT 60 Hz sinusoidal EMF for time periods between 4 and 24 h. After induction of apoptosis, cells were analyzed by the neutral comet assay to determine the percentage of apoptotic cells. To discover the duration of this protection, cells were grown in the EMF for 24 h and then removed for 24 to 48 h before heat shock and neutral comet assays were performed. Our results demonstrate that EMF exposure offers significant protection from apoptosis ($P<.0001$ for HL-60 and HL-60R, $P<.005$ for Raji) after 12 h of exposure and that protection can last up to 48 h after removal from the EMF. In this study we further demonstrate the effect of the EMF on DNA repair rates. DNA repair data were gathered by exposing the same cell lines to the EMF for 24 h before damaging the exposed cells and non-exposed cells with H2O2. Cells were allowed to repair for time periods between 0 and 15 min before analysis using the alkaline comet assay. Results showed that EMF exposure significantly decreased DNA repair rates in HL-60 and HL-60R cell lines ($P<.001$ and $P<.01$ respectively), but not in the Raji cell line. Importantly, our apoptosis results show that a minimal time exposure to an EMF is

needed before observed effects. This may explain previous studies showing no change in apoptosis susceptibility and repair rates when treatments and EMF exposure were administered concurrently. More research is necessary, however, before data from this in vitro study can be applied to in vivo systems.

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

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

Schmitz C, **Keller E**, **Freuding T**, **Silny J**, **Korr H**. **50-Hz magnetic field exposure influences DNA repair and mitochondrial DNA synthesis of distinct cell types in brain and kidney of adult mice.** Acta Neuropathol (Berl). 107(3):257-264, 2004. (E)

Despite several recent investigations, the impact of whole-body magnetic field exposure on cell-type-specific alterations due to DNA damage and DNA repair remains unclear. In this pilot study adult mice were exposed to 50-Hz magnetic field (mean value 1.5 mT) for 8 weeks or left unexposed. Five minutes after ending exposure, the mice received [(3)H]thymidine and were killed 2 h later. Autoradiographs were prepared from paraffin sections of brains and kidneys for measuring unscheduled DNA synthesis and mitochondrial DNA synthesis, or in situ nick translation with DNA polymerase-I and [(3)H]dTTP. A significant ($P<0.05$) increase in both unscheduled DNA synthesis and in situ nick translation was only found for epithelial cells of the choroid plexus. Thus, these two independent methods indicate that nuclear DNA damage is produced by long-lasting and strong magnetic field exposure. The fact that only plexus epithelial cells were affected might point to possible effects of magnetic fields on iron transport across the blood-cerebrospinal fluid barrier, but the mechanisms are currently not understood. Mitochondrial DNA synthesis was exclusively increased in renal epithelial cells of distal

convoluted tubules and collecting ducts, i.e., cells with a very high content of mitochondria, possibly indicating increased metabolic activity of these cells.

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

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

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

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

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

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

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

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

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

In the present study, we used human peripheral blood leukocytes from 4 different donors, to investigate in vitro the possible genotoxic and/or co-genotoxic activity of extremely low frequency magnetic fields (ELF-MF) at 3 mT intensity. Two model mutagens were used to study the possible interaction between ELF-MF and xenobiotics: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 4-nitroquinoline N-oxide (4NQO). Primary DNA damage was evaluated by the alkaline single-cell microgel-electrophoresis ("comet") assay. Control cells (leukocytes not exposed to ELF-MF, nor treated with genotoxins) from the different blood donors showed a comparable level of basal DNA damage, whereas the contribution of individual susceptibility toward ELF-MF and the tested genotoxic compounds led to differences in the extent of DNA damage observed following exposure to the genotoxins, both in the presence and in the absence of an applied ELF-MF. A 3 mT ELF-MF alone was unable to cause direct primary DNA damage. In leukocytes exposed to ELF-MF and genotoxins, the extent of MNNG-induced DNA damage increased with exposure duration compared to sham-exposed cells. The

opposite was observed in cells treated with 4NQO. In this case the extent of 4NQO-induced DNA damage was somewhat reduced in leukocytes exposed to ELF-MF compared to sham-exposed cells. Moreover, in cells exposed to ELF-MF an increased concentration of GSH was always observed, compared to sham-exposed cells. Since following GSH conjugation the genotoxic pattern of MNNG and 4NQO is quite different, an influence of ELF-MF on the activity of the enzyme involved in the synthesis of GSH leading to different activation/deactivation of the model mutagens used was hypothesized to explain the different trends observed in MNNG and 4NQO genotoxic activity in the presence of an applied ELF-MF. The possibility that ELF-MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

Williams PA, **Ingebretsen RJ**, **Dawson RJ**. 14.6 mT ELF magnetic field exposure yields no DNA breaks in model system Salmonella, but provides evidence of heat stress protection. Bioelectromagnetics. 27(6):445-450, 2006. (NE)

In this study, we demonstrate that common extremely low frequency magnetic field (MF) exposure does not cause DNA breaks in this Salmonella test system. The data does, however, provide evidence that MF exposure induces protection from heat stress. Bacterial cultures were exposed to MF (14.6 mT 60 Hz field, cycled 5 min on, 10 min off for 4 h) and a temperature-matched control. Double- and single-stranded DNA breaks were assayed using a recombination event counter. After MF or control exposure they were grown on indicator plates from which recombination events can be quantified and the frequency of DNA strand breaks deduced. The effect of MF was also monitored using a recombination-deficient mutant (recA). The results showed no significant increase in recombination events and strand breaks due to MF. Evidence of heat stress protection was determined using a cell viability assay that compared the survival rates of MF exposed and control cells after the administration of a 10 min 53 degrees C heat stress. The control cells exhibited nine times more cell mortality than the MF exposed cells. This Salmonella system provides many mutants and genetic tools for further investigation of this phenomenon.

Winker R, **Ivancsits S**, **Pilger A**, **Adlkofer F**, **Rudiger HW**. **Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields.** Mutat Res. 585(1-2):43-49, 2005. (E)

Environmental exposure to extremely low-frequency electromagnetic fields (ELF-EMFs) has been implicated in the development of cancer in humans. An important basis for assessing a potential cancer risk due to ELF-EMF exposure is knowledge of biological effects on human cells at the chromosomal level. Therefore, we investigated in the present study the effect of intermittent ELF electromagnetic fields (50 Hz, sinusoidal, 5'field-on/10'field-off, 2-24 h, 1 mT) on the induction of micronuclei (MN) and chromosomal aberrations in cultured human fibroblasts. ELF-EMF radiation resulted in a time-dependent increase of micronuclei, which became significant after 10 h of intermittent exposure at a flux density of 1 mT. After approximately 15 h a constant level

of micronuclei of about three times the basal level was reached. In addition, chromosomal aberrations were increased up to 10-fold above basal levels. Our data strongly indicate a clastogenic potential of intermittent low-frequency electromagnetic fields, which may lead to considerable chromosomal damage in dividing cells.

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. (E)

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.

Yaguchi H, **Yoshida M**, **Ejima Y**, **Miyakoshi J**. **Effect of high-density extremely low frequency magnetic field on sister chromatid exchanges in mouse m5S cells.** Mutat Res. 440(2):189-194, 1999. (E)

The induction of sister chromatid exchanges (SCEs) was evaluated in the cultured mouse m5S cells after exposure to extremely low frequency magnetic field (ELFMF; 5, 50 and 400 mT). Exposure to 5 mT and 50 mT ELFMF led to a very small increase in the frequency of SCEs, but no significant difference was observed between exposed and unexposed control cells. The cells exposed to 400 mT ELFMF exhibited a significant elevation of the SCE frequencies. There was no significant difference between data from treatments with mitomycin-C (MMC) alone and from combined treatments of MMC plus ELFMF (400 mT) at any MMC concentrations from 4 to 40 nM. These results suggest that exposure to highest-density ELFMF of 400 mT may induce DNA damage, resulting

in an elevation of the SCE frequencies. We suppose that there may be a threshold for the elevation of the SCE frequencies, that is at least over the magnetic density of 50 mT.

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

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 asses the cancer risk. We examined the effects of extremely low frequency electro magnetic 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.

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

The present study was undertaken to verify a hypothesis that exposure of the cells to static or 50 Hz magnetic fields (MF) and simultaneous treatment with a known oxidant, ferrous chloride, may affect the oxidative deterioration of DNA molecules.The comet assay was chosen for the assessment of DNA damage. The experiments were performed on isolated rat lymphocytes incubated for 3h in Helmholtz coils at 7 mT static or 50 Hz MF. During MF exposure, part of the cell samples were incubated with 0.01 microM H(2)O(2) and another one with 10 microg/ml FeCl(2,) the rest serving as controls.Lymphocyte exposure to MF at 7 mT did not increase the number of cells with DNA damage in the comet assay. Incubation of lymphocytes with 10 microg/ml FeCl(2) did not produce a detectable damage of DNA either. However, when the FeCl(2)-incubated lymphocytes were simultaneously exposed to 7 mT MF, the number of damaged cells was significantly increased and reached about 20% for static MF and 15% for power frequency MF. In the control samples about 97% of the cells did not have any DNA damage.It is not possible at present to offer a reasonable explanation for the findings of this investigation - the high increase in the number of lymphocytes showing symptoms of DNA damage in the comet assay, following simultaneous exposure to the

combination of two non-cytotoxic factors -10 microg/ml FeCl(2) and 7 mT MF. In view of the obtained results we can only hypothesise that under the influence of simultaneous exposure to FeCl(2) and static or 50 Hz MF, the number of reactive oxygen species generated by iron cations may increase substantially. Further studies will be necessary to confirm this hypothesis and define the biological significance of the observed effect.

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

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