

# Studies that show **WiFi** and Devices Health Effects

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### Effects On Brain and Neurons

**Lai, H, Carino, MA, Singh, NP, Naltrexone blocks RFR-induced DNA double strand breaks in rat brain cells. *Wireless Networks* 3:471-476, 1997.**

Previous research in our laboratory has shown that various effects of radiofrequency electromagnetic radiation (RFR) exposure on the nervous system are mediated by endogenous opioids in the brain. We have also found that acute exposure to RFR induced DNA strand breaks in brain cells of the rat. The present experiment was carried out to investigate whether endogenous opioids are also involved in RFR-induced DNA strand breaks. Rats were treated with the opioid antagonist naltrexone (1 mg/kg, IP) immediately before and after exposure to **2450-MHz pulsed** (2  $\mu$ s pulses, 500 pps) RFR at a power density of 2 mW/cm<sup>2</sup> (average whole body specific absorption rate of 1.2 W/kg) for 2 hours. DNA double strand breaks were assayed in brain cells at 4 hours after exposure using a microgel electrophoresis assay. Results showed that the RFR exposure significantly increased DNA double strand breaks in brain cells of the rat, and the effect was partially blocked by treatment with naltrexone. Thus, these data indicate that endogenous opioids play a mediating role in RFR-induced DNA strand breaks in brain cells of the rat.

**Kesari KK, Behari J, Kumar S. Mutagenic response of 2.45 GHz radiation exposure on rat brain. *Int J Radiat Biol.* 86(4):334-343, 2010.**

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

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**Lai, H, Singh, NP, Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. Bioelectromagnetics 18(6):446-454, 1997.**

Effects of in vivo microwave exposure on DNA strand breaks, a form of DNA damage, were investigated in rat brain cells. In previous research, we have found that acute (2 hours) exposure to pulsed (2 microseconds pulses, 500 pps) **2450-MHz radiofrequency electromagnetic radiation (RFR)** (power density 2 mW/cm<sup>2</sup>, average whole body specific absorption rate 1.2 W/kg) caused an increase in DNA single- and double-strand breaks in brain cells of the rat when assayed 4 hours post exposure using a microgel electrophoresis assay. In the present study, we found that treatment of rats immediately before and after RFR exposure with either melatonin (1 mg/kg/injection, SC) or the spin-trap compound N-tert-butyl-alpha-phenylnitron (PBN) (100 mg/kg/injection, i.p.) blocks this effects of RFR. Since both melatonin and PBN are efficient free radical scavengers it is hypothesized that free radicals are involved in RFR-induced DNA damage in the brain cells of rats. Since cumulated DNA strand breaks in brain cells can lead to neurodegenerative diseases and cancer and an excess of free radicals in cells has been suggested to be the cause of various human diseases, data from this study could have important implications for the health effects of RFR exposure.

**Lai H, Singh NP, Interaction of Microwaves and a Temporally Incoherent Magnetic Field on Single and Double DNA Strand Breaks in Rat Brain Cells. Electromag Biol Med 24:23-29, 2005.**

The effect of a temporally incoherent magnetic field ('noise') on microwave-induced DNA single and double strand breaks in rat brain cells was investigated. Four treatment groups of rats were studied: microwave-exposure (continuous-wave **2450-MHz microwaves**, power density 1 mW/cm<sup>2</sup>, average whole body specific absorption rate of 0.6 W/kg), 'noise'-exposure (45 mG), 'microwave + noise'-exposure, and sham-exposure. Animals were exposed to these conditions for 2 hrs. DNA single and double strand breaks in brain cells of these animals were assayed 4 hrs later using a microgel electrophoresis assay. Results show that brain cells of microwave-exposed rats had significantly higher levels of DNA single and double strand breaks when compared with sham-exposed animals. Exposure to 'noise' alone did not significantly affect the levels (i.e., they were similar to those of the sham-exposed rats). However, simultaneous 'noise' exposure blocked microwave-induced increases in DNA strand breaks. These data indicate that simultaneous exposure to a temporally incoherent magnetic field could block microwave-induced DNA damage in brain cells of the rat.

**Papageorgiou CC, Hountala CD, Maganioti AE, Kyprianou MA, Rabavilas AD, Papadimitriou GN, Capsalis CN. Effects of wi-fi signals on the p300 component of event-related potentials during an auditory hayling task. J Integr Neurosci. 10(2):189-202, 2011.**

The P300 component of event-related potentials (ERPs) is believed to index attention and working memory (WM) operation of the brain. The present study focused on the possible gender-related **effects of Wi-Fi** (Wireless Fidelity) electromagnetic fields (EMF)

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on these processes. Fifteen male and fifteen female subjects, matched for age and education level, were investigated while performing a modified version of the Hayling Sentence Completion test adjusted to induce WM. ERPs were recorded at 30 scalp electrodes, both without and with the exposure to a Wi-Fi signal. P300 amplitude values at 18 electrodes were found to be significantly lower in the response inhibition condition than in the response initiation and baseline conditions. Independent of the above effect, within the response inhibition condition there was also a significant gender X radiation interaction effect manifested at 15 leads by decreased P300 amplitudes of males in comparison to female subjects only at the presence of EMF. In conclusion, the present findings suggest that Wi-Fi exposure may exert gender-related alterations on neural activity associated with the amount of attentional resources engaged during a linguistic test adjusted to induce WM.

**Ghazizadeh V, Nazıroğlu M. Electromagnetic radiation (Wi-Fi) and epilepsy induce calcium entry and apoptosis through activation of TRPV1 channel in hippocampus and dorsal root ganglion of rats. *Metab Brain Dis.* 29(3):787-799, 2014.**

Incidence rates of epilepsy and use of Wi-Fi worldwide have been increasing. TRPV1 is a  $\text{Ca}^{2+}$  permeable and non-selective channel, gated by noxious heat, oxidative stress and capsaicin (CAP). The hyperthermia and oxidant effects of Wi-Fi may induce apoptosis and  $\text{Ca}^{2+}$  entry through activation of TRPV1 channel in epilepsy. Therefore, we tested the **effects of Wi-Fi (2.45 GHz)** exposure on  $\text{Ca}^{2+}$  influx, oxidative stress and apoptosis through TRPV1 channel in the murine dorsal root ganglion (DRG) and hippocampus of pentylentetrazol (PTZ)-induced epileptic rats. Rats in the present study were divided into two groups as controls and PTZ. The PTZ groups were divided into two subgroups namely PTZ + Wi-Fi and PTZ + Wi-Fi + capsazepine (CPZ). The hippocampal and DRG neurons were freshly isolated from the rats. The DRG and hippocampus in PTZ + Wi-Fi and PTZ + Wi-Fi + CPZ groups were exposed to Wi-Fi for 1 hour before CAP stimulation. The cytosolic free  $\text{Ca}^{2+}$ , reactive oxygen species production, apoptosis, mitochondrial membrane depolarization, caspase-3 and -9 values in hippocampus were higher in the PTZ group than in the control although cell viability values decreased. The Wi-Fi exposure induced additional effects on the cytosolic  $\text{Ca}^{2+}$  increase. However, pretreatment of the neurons with CPZ, results in a protection against epilepsy-induced  $\text{Ca}^{2+}$  influx, apoptosis and oxidative damages. In results of whole cell patch-clamp experiments, treatment of DRG with  $\text{Ca}^{2+}$  channel antagonists [thapsigargin, verapamil + diltiazem, 2-APB, MK-801] indicated that Wi-Fi exposure induced  $\text{Ca}^{2+}$  influx via the TRPV1 channels. In conclusion, epilepsy and Wi-Fi in our experimental model is involved in  $\text{Ca}^{2+}$  influx and oxidative stress-induced hippocampal and DRG death through activation of TRPV1 channels, and negative modulation of this channel activity by CPZ pretreatment may account for the neuroprotective activity against oxidative stress.

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

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

**Lai H, Carino MA, Horita A, Guy AW, Intraseptal microinjection of beta-funaltrexamine blocked a microwave-induced decrease of hippocampal cholinergic activity in the rat. Pharmacol Biochem Behav 53(3):613-616, 1994.**

Acute (45 min) exposure to pulsed (2 microseconds pulse width, 500 pulses per second) **2450-MHz microwaves** at a power density of 1 mW/cm<sup>2</sup> (whole body specific absorption rate 0.6 W/kg) microwaves caused a decrease in cholinergic activity in the hippocampus of the rat as measured by the sodium-dependent high-affinity choline uptake. Microinjection of beta-funaltrexamine (1 microgram) into the septum before microwave exposure blocked this effect. These data indicate that mu-opioid receptors in the septum mediate a microwave-induced decrease in cholinergic activity in the hippocampus and support our hypothesis that microwaves at a whole body SAR of 0.6 W/kg can activate endogenous opioids in the brain.

**Lai H, Carino MA, Horita A, Guy AW, Single vs. repeated microwave exposure: effects on benzodiazepine receptors in the brain of the rat. Bioelectromagnetics 13(1):57-66, 1992.**

We studied the effects of single (45 min) and repeated (ten daily 45-min sessions) microwave exposures (**2450-MHz**, 1 mW/cm<sup>2</sup>, average whole-body SAR of 0.6 W/kg, pulsed at 500 pps with pulse width of 2 microseconds) on the concentration and affinity of benzodiazepine receptors in the cerebral cortex, hippocampus, and cerebellum of the rat. We used a receptor-binding assay with 3H-flunitrazepam as ligand. Immediately after a single exposure, an increase in the concentration of receptor was observed in the cerebral cortex, but no significant effect was observed in the hippocampus or cerebellum. No significant change in binding affinity of the receptors was observed in any of the brain-regions studied. In rats subjected to repeated exposures, no significant change in receptor concentration was found in the cerebral cortex immediately after the last exposure, which may indicate an adaptation to repeated exposures. Our data also

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show that handling and exposure procedures in our experiments did not significantly affect benzodiazepine receptors in the brain. Because benzodiazepine receptors in the brain are responsive to anxiety and stress, our data support the hypothesis that low-intensity microwave irradiation can be a source of stress.

**Lai H, Carino MA, Horita A, Guy AW, Opioid receptor subtypes that mediate a microwave-induced decrease in central cholinergic activity in the rat. *Bioelectromagnetics* 13(3):237-246, 1992.**

We performed experiments to investigate subtypes of opioid receptors in the brain involved in the effect of acute (45 min) pulsed microwave exposure (**2,450-MHz**, 2-microseconds pulses, 500 pps, average power density 1 mW/cm<sup>2</sup>, peak-power density, 1 W/cm<sup>2</sup>, average whole body SAR 0.6 W/kg) on cholinergic activity in the rat brain. Rats were pretreated by microinjection of specific antagonists of mu, delta, and kappa opioid-receptors into the lateral cerebroventricle before exposure to microwaves. The data showed that all three subtypes of opioid receptors are involved in the microwave-induced decrease in cholinergic activity in the hippocampus. However, the microwave-induced decrease in cholinergic activity in the frontal cortex was not significantly affected by any of the drug treatments, confirming our previous conclusion that the effect of microwaves on the frontal cortex is not mediated by endogenous opioids.

**Yang XS, He GL, Hao YT, Xiao Y, Chen CH, Zhang GB, Yu ZP. Exposure to 2.45 GHz electromagnetic fields elicits an HSP-related stress response in rat hippocampus. *Brain Res Bull.* 88(4):371-378, 2012.**

The issue of possible neurobiological effects of the electromagnetic field (EMF) exposure is highly controversial. To determine whether electromagnetic field exposure could act as an environmental stimulus capable of producing stress responses, we employed the hippocampus, a sensitive target of electromagnetic radiation, to assess the changes in its stress-related gene and protein expression after EMF exposure. Adult male Sprague-Dawley rats with body restrained were exposed to a **2.45 GHz EMF** at a specific absorption rate (SAR) of 6 W/kg or sham conditions. cDNA microarray was performed to examine the changes of gene expression involved in the biological effects of electromagnetic radiation. Of 2048 candidate genes, 23 upregulated and 18 downregulated genes were identified. Of these differential expression genes, two heat shock proteins (HSP), HSP27 and HSP70, are notable because expression levels of both proteins are increased in the rat hippocampus. Result from immunocytochemistry revealed that EMF caused intensive staining for HSP27 and HSP70 in the hippocampus, especially in the pyramidal neurons of cornu ammonis 3 (CA3) and granular cells of dentate gyrus (DG). The gene and protein expression profiles of HSP27 and HSP70 were further confirmed by reverse transcription polymerase chain reaction (RT-PCR) and Western blot. Our data provide direct evidence that exposure to electromagnetic fields elicits a stress response in the rat hippocampus.

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**Lai H, Carino MA, Wen YF, Horita A, Guy AW, Naltrexone pretreatment blocks microwave-induced changes in central cholinergic receptors. *Bioelectromagnetics* 12(1):27-33, 1991.**

Repeated exposure of rats to pulsed, circularly polarized microwaves (2,450-MHz, 2-microseconds pulses at 500 pps, power density 1 mW/cm<sup>2</sup>, at an averaged, whole-body SAR of 0.6 W/kg) induced biphasic changes in the concentration of muscarinic cholinergic receptors in the central nervous system. An increase in receptor concentration occurred in the hippocampus of rats subjected to ten 45-min sessions of microwave exposure, whereas a decrease in concentration was observed in the frontal cortex and hippocampus of rats exposed to ten 20-min sessions. These findings, which confirm earlier work in the authors' laboratory, were extended to include pretreatment of rats with the narcotic antagonist naltrexone (1 mg/kg, IP) before each session of exposure. The drug treatment blocked the microwave-induced changes in cholinergic receptors in the brain. These data further support the authors' hypothesis that endogenous opioids play a role in the effects of microwaves on central cholinergic systems.

**Lai H, Horita A, Guy AW, Microwave irradiation affects radial-arm maze performance in the rat. *Bioelectromagnetics* 15(2):95-104, 1994.**

After 45 min of exposure to pulsed 2450 MHz microwaves (2 microseconds pulses, 500 pps, 1 mW/cm<sup>2</sup>, average whole body SAR 0.6 W/kg), rats showed retarded learning while performing in the radial-arm maze to obtain food rewards, indicating a deficit in spatial "working memory" function. This behavioral deficit was reversed by pretreatment before exposure with the cholinergic agonist physostigmine or the opiate antagonist naltrexone, whereas pretreatment with the peripheral opiate antagonist naloxone methiodide showed no reversal of effect. These data indicate that both cholinergic and endogenous opioid neurotransmitter systems in the brain are involved in the microwave-induced spatial memory deficit.

**Lai H. Interaction of microwaves and a temporally incoherent magnetic field on spatial learning in the rat. *Physiol Behav.* 82(5):785-789, 2004.**

The effect of a temporally incoherent magnetic field ('noise') on microwave-induced spatial learning deficit in the rat was investigated. Rats were trained in six sessions to locate a submerged platform in a circular water maze. Four treatment groups of rats were studied: microwave-exposure (2450-MHz continuous-wave microwaves, power density 2 mW/cm<sup>2</sup>, average whole-body specific absorption rate 1.2 W/kg), 'noise' exposure (60 mG), 'microwave+noise' exposure, and sham exposure. Animals were exposed to these conditions for 1 h immediately before each training session. One hour after the last training session, animals were tested in a 2-min probe trial in the maze during which the platform was removed. The time spent during the 2 min in the quadrant of the maze in which the platform had been located was scored. Results show that microwave-exposed rats had significant deficit in learning to locate the submerged platform when compared with the performance of the sham-exposed animals. Exposure to 'noise' alone did not significantly affect the performance of the animals (i.e., it was similar to that of the sham-exposed rats). However, simultaneous exposure to 'noise'

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significantly attenuated the microwave-induced spatial learning deficit (i.e. 'microwave+noise'-exposed rats learned significantly better than the microwave-exposed rats). During the probe trial, microwave-exposed animals spent significantly less time in the quadrant where the platform was located. However, response of the 'microwave+noise'-exposed animals was similar to that of the sham-exposed animals during the probe trial. Thus, simultaneous exposure to a temporally incoherent magnetic field blocks microwave-induced spatial learning and memory deficits in the rat.

### **Kesari KK, Kumar S, Behari J. Pathophysiology of microwave radiation: effect on rat brain. *Appl Biochem Biotechnol.* 166(2):379-388, 2012.**

The study aims to investigate the effect of **2.45 GHz microwave radiation** on Wistar rats. Rats of 35 days old with  $130 \pm 10$  g body weight were selected for this study. Animals were divided into two groups: sham exposed and experimental (six animals each). Animals were exposed for 2 h a day for 45 days at 2.45 GHz frequency (power density, 0.21 mW/cm<sup>2</sup>). The whole body specific absorption rate was estimated to be 0.14 W/kg. Exposure took place in a ventilated plexiglas cage and kept in an anechoic chamber under a horn antenna. After completion of the exposure period, rats were killed, and pineal gland and whole brain tissues were isolated for the estimation of melatonin, creatine kinase, caspase 3, and calcium ion concentration. Experiments were performed in a blind manner and repeated. A significant decrease ( $P < 0.05$ ) was recorded in the level of pineal melatonin of exposed group as compared with sham exposed. A significant increase ( $P < 0.05$ ) in creatine kinase, caspase 3, and calcium ion concentration was observed in whole brain of exposed group of animals as compared to sham exposed. One-way analysis of variance method was adopted for statistical analysis. The study concludes that a reduction in melatonin or an increase in caspase-3, creatine kinase, and calcium ion may cause significant damage in brain due to chronic exposure of these radiations. These biomarkers clearly indicate possible health implications of such exposures.

### **Hao Y, Yang X, Chen C, Yuan-Wang, Wang X, Li M, Yu Z. STAT3 signalling pathway is involved in the activation of microglia induced by 2.45 GHz electromagnetic fields. *Int J Radiat Biol.* 86(1):27-36, 2010.**

**PURPOSE:** Microglia activation plays a pivotal role in the initiation and progression of central nervous system (CNS) insult. The aim of the present work was to investigate the activation of microglia and involvement of signal transducer and activator of transcription 3 (STAT3) in microglia activation after 2.45 GHz electromagnetic fields (EMF) exposure. **MATERIALS AND METHODS:** In this study, murine N9 microglial cells were exposed to **2.45 GHz EMF**, the protein expressions of STAT3, Janus Tyrosine kinase 1 and 2 (JAK1 and JAK2), phosphor-(Try705)STAT3 and DNA binding activity of STAT3 were examined by Western blot analysis and electrophoresis mobility shift assay (EMSA). Levels of the nitric oxide (NO) derivative nitrite were determined in the culture medium by the Griess reaction. The mRNA expression of tumour necrosis factor alpha (TNF-alpha) and inducible nitric oxide synthase (iNOS) were detected by reverse transcription and polymerase chain reaction (RT-PCR). **RESULTS:** A significant increase of STAT3 DNA-



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binding ability was noted after exposure. Consistent with this, EMF rapidly induced phosphorylation of STAT3 and activated JAK1 and JAK2. In addition, EMF exposure increased transcription levels of the inflammation-associated genes, iNOS and TNF-alpha, which are reported to contain STAT-binding elements in their promoter region. P6, a JAK inhibitor, reduced induction of iNOS and TNF-alpha, nuclear factor binding activity, and activation of STAT3 in EMF-stimulated microglia. CONCLUSION: These results provide evidence that EMF exposure can initiate the activation of microglia cells and STAT3 signalling involves in EMF-induced microglial activation.

**Zhang Y, She F, Li L, Chen C, Xu S, Luo X, Li M, He M, Yu Z. p25/CDK5 is partially involved in neuronal injury induced by radiofrequency electromagnetic field exposure. Int J Radiat Biol. 2013 Jul 29. [Epub ahead of print]**

Purpose: Several studies suggest that radiofrequency electromagnetic field (RF-EMF) exposure can induce neuronal injury. The aim of the present work was to investigate whether the cyclin-dependent kinase 5 (CDK5) pathway is involved in neuronal injury induced by RF-EMF exposure. Materials and methods: Newborn Sprague-Dawley rats' primary cultured cortical neurons were exposed to pulsed 2.45 GHz RF-EMF for 10 min. The cellular viability was assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The apoptosis was assessed by Hoechst 33342 and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling co-staining. The protein expressions of CDK5, p35, p25, and phosphorylated tau at Ser<sup>404</sup> were examined by Western blot analysis. The CDK5 activity was detected using a histone-H1 kinase assay. Results: The cellular viability of neurons was significantly decreased ( $p < 0.01$ , Partial Eta Squared [ $\eta_p^2$ ]: 0.554), and the percentage of apoptotic nuclei ( $p < 0.01$ ,  $\eta_p^2 = 0.689$ ), activity of CDK5 ( $p < 0.05$ ,  $\eta_p^2 = 0.589$ ), ratio of p25 and p35 ( $p < 0.05$ ,  $\eta_p^2 = 0.670$ ), levels of tau phosphorylation at Ser<sup>404</sup> ( $p < 0.01$ ,  $\eta_p^2 = 0.896$ ) were significantly increased after RF-EMF exposure. No significant change was detected in CDK5 expression after RF-EMF exposure. Pretreatment with Roscovitine (a CDK5 inhibitor) significantly blocked the RF-EMF-induced decrease of cellular viability ( $p < 0.05$ ,  $\eta_p^2 = 0.398$ ) and tau hyperphosphorylation at Ser<sup>404</sup> ( $p < 0.01$ ,  $\eta_p^2 = 0.917$ ), but did not significantly block the RF-EMF-induced apoptosis ( $p > 0.05$ ,  $\eta_p^2 = 0.130$ ). Conclusions: These results suggest that abnormal activity of p25/CDK5 is partially involved in primary cultured cortical neuron injury induced by RF-EMF exposure.

**Eser O, Songur A, Aktas C, Karavelioglu E, Caglar V, Aylak F, Ozguner F, Kanter M. The effect of electromagnetic radiation on the rat brain: an experimental study. Turk Neurosurg. 23(6):707-715, 2013.**

AIM: The aim of this study is to determine the structural changes of electromagnetic waves in the frontal cortex, brain stem and cerebellum. MATERIAL and METHODS: 24 Wistar Albino adult male rats were randomly divided into four groups: group I consisted of control rats, and groups II-IV comprised electromagnetically irradiated (EMR) with 900, 1800 and **2450 MHz**. The heads of the rats were exposed to 900, 1800 and 2450 MHz microwaves irradiation for 1h per day for 2 months. RESULTS: While the histopathological changes in the frontal cortex and brain stem were normal in the control group, there were severe degenerative changes,

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shrunk cytoplasm and extensively dark pyknotic nuclei in the EMR groups. Biochemical analysis demonstrated that the Total Antioxidative Capacity level was significantly decreased in the EMR groups and also Total Oxidative Capacity and Oxidative Stress Index levels were significantly increased in the frontal cortex, brain stem and cerebellum. IL-1 $\beta$  level was significantly increased in the EMR groups in the brain stem. CONCLUSION: EMR causes to structural changes in the frontal cortex, brain stem and cerebellum and impair the oxidative stress and inflammatory cytokine system. This deterioration can cause to disease including loss of these areas function and cancer development.

### **Wang, BM, Lai, H, Acute exposure to pulsed 2450-MHz microwaves affects water maze learning in the rat. *Bioelectromagnetics* 21:52-56, 2000.**

Rats were trained in six sessions to locate a submerged platform in a circular water maze. They were exposed to pulsed **2450-MHz** microwaves (pulse width 2  $\mu$ s, 500 pulses/s, power density 2 mW/cm<sup>2</sup>, average whole body specific absorption rate 1.2 W/kg) for 1 hr in a circular waveguide system immediately before each training session. One hour after the last training session, they were tested in a probe trial during which the platform was removed and the time spent in the quadrant of the maze in which the platform had been located during the 1-min trial was scored. Three groups of animals: microwave-exposed, sham-exposed, and cage control were studied. Data show that microwave-exposed rats were slower than sham-exposed and cage control rats in learning to locate the platform. However, there was no significant difference in swim speed among the three groups of animals, indicating that the difference in learning was not due to a change in motor functions or motivation. During the probe trial, microwave-exposed animals spent significantly less time in the quadrant that had contained the platform, and their swim patterns were different from those of the sham-exposed and cage control animals. The latter observation indicates that microwave-exposed rats used a different strategy in learning the location of the platform. These results show that acute exposure to pulsed microwaves caused a deficit in spatial "reference" memory in the rat.

### **Neubauer C, Phelan AM, Kues H, Lange DG, Microwave irradiation of rats at 2.45 GHz activates pinocytotic-like uptake of tracer by capillary endothelial cells of cerebral cortex. *Bioelectromagnetics* 11(4):261-268, 1990.**

Far-field exposures of male albino rats to 2.45-GHz microwaves (10-microseconds pulses, 100 pps) at a low average power density (10 mW/cm<sup>2</sup>; SAR approximately 2 W/kg) and short durations (30-120 min) resulted in increased uptakes of tracer through the blood-brain barrier (BBB). The uptake of systemically administered rhodamine-ferritin complex by capillary endothelial cells (CECs) of the cerebral cortex was dependent on power density and on duration of exposure. At 5 mW/cm<sup>2</sup>, for example, a 15-min exposure had no effect. Near-complete blockade of uptake resulted when rats were treated before exposure to microwaves with a single dose of colchicine, which inhibits microtubular function. A pinocytotic-like mechanism is presumed responsible for the microwave-induced increase in BBB permeability.

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**Nazıroğlu M, Çelik Ö, Özgül C, Çiğ B, Doğan S, Bal R, Gümral N, Rodríguez AB, Pariente JA. Melatonin modulates wireless (2.45 GHz)-induced oxidative injury through TRPM2 and voltage gated Ca(2+) channels in brain and dorsal root ganglion in rat. *Physiol Behav.* 105(3):683-692, 2012.**

We aimed to investigate the protective effects of melatonin and **2.45 GHz electromagnetic radiation (EMR)** on brain and dorsal root ganglion (DRG) neuron antioxidant redox system, Ca(2+) influx, cell viability and electroencephalography (EEG) records in the rat. Thirty two rats were equally divided into four different groups namely group A1: Cage control, group A2: Sham control, group B: 2.45 GHz EMR, group C: 2.45 GHz EMR+melatonin. Groups B and C were exposed to 2.45 GHz EMR during 60 min/day for 30 days. End of the experiments, EEG records and the brain cortex and DRG samples were taken. Lipid peroxidation (LP), cell viability and cytosolic Ca(2+) values in DRG neurons were higher in group B than in groups A1 and A2 although their concentrations were increased by melatonin, 2-aminoethyldiphenyl borinate (2-APB), diltiazem and verapamil supplementation. Spike numbers of EEG records in group C were lower than in group B. Brain cortex vitamin E concentration was higher in group C than in group B. In conclusion, Melatonin supplementation in DRG neurons and brain seems to have protective effects on the 2.45 GHz-induced increase Ca(2+) influx, EEG records and cell viability of the hormone through TRPM2 and voltage gated Ca(2+) channels.

**Testylier G, Tonduli L, Malabiau R, Debouzy JC. Effects of exposure to low level radiofrequency fields on acetylcholine release in hippocampus of freely moving rats. *Bioelectromagnetics* 23:249-255, 2002.**

Some central cholinergic effects have been reported in animals after acute exposure to radiofrequency electromagnetic field at low intensity. We studied acetylcholine (ACh) release in the brain of freely moving rats exposed for 1 h during the day to a **2.45 GHz** continuous wave radiofrequency field (RF) (2 or 4 mW/cm<sup>2</sup>) or exposed for 1 or 14 h during the night to a 800 MHz field modulated at 32 Hz (AM 200 mW/cm<sup>2</sup>). Measurements were performed by microdialysis using a membrane implanted through the upper CA1 region of the hippocampus. After irradiation with the 2.45 GHz RF, rats exposed at 2 mW/cm<sup>2</sup> did not show a significant modification of ACh release, whereas those exposed at 4 mW/cm<sup>2</sup> showed a significant 40% decrease in mean ACh release from hippocampus. This decrease was maximal at 5 h post exposure. Exposure to the 800 MHz RF for 1 h did not cause any significant effect, but exposure for 14 hrs induced a significant 43% decrease in ACh release during the period 11 p.m.-4 a.m. compared to control rats. In the control group we observed an increase of ACh release at the beginning of the night, which was linked to the waking period of rats. This normal increase was disturbed in rats exposed overnight to the 800 MHz RF. This work indicates that neurochemical modification of the hippocampal cholinergic system can be observed during and after an exposure to low intensity RF.

**Lu Y, Xu S, He M, Chen C, Zhang L, Liu C, Chu F, Yu Z, Zhou Z, Zhong M. Glucose administration attenuates spatial memory deficits induced by chronic low-power-density microwave exposure. *Physiol Behav.* 106(5):631-637, 2012.**

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Extensive evidence indicates that glucose administration attenuates memory deficits in rodents and humans, and cognitive impairment has been associated with reduced glucose metabolism and uptake in certain brain regions including the hippocampus. In the present study, we investigated whether glucose treatment attenuated memory deficits caused by chronic low-power-density microwave (MW) exposure, and the effect of MW exposure on hippocampal glucose uptake. **We exposed Wistar rats to 2.45 GHz pulsed MW irradiation** at a power density of 1 mW/cm<sup>2</sup> for 3 h/day, for up to 30 days. MW exposure induced spatial learning and memory impairments in rats. Hippocampal glucose uptake was also reduced by MW exposure in the absence or presence of insulin, but the levels of blood glucose and insulin were not affected. However, these spatial memory deficits were reversed by systemic glucose treatment. Our results indicate that glucose administration attenuates the spatial memory deficits induced by chronic low-power-density MW exposure, and reduced hippocampal glucose uptake may be associated with cognitive impairment caused by MW exposure.

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

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

**Paulraj R, Behari J. Protein Kinase C Activity in developing rat brain cells exposed to 2.45 GHz radiation. Electromag Biol Med 25(1) 61-70, 2006.**

There is growing concern by the public regarding the potential human health hazard due to exposure to microwave frequencies. **2.45 GHz radiation** widespread use in industry, research, and medicine, and leakage into the environment is possible. In order to quantitate this, experiments were performed on developing rat brain. Male Wistar 35-day-old rats ( $n = 6$ ) were used for this study. Animals were exposed to 2.45 GHz radiation for 2 h/day for a period of 35 days at a power density of 0.344 mW/cm<sup>2</sup> (SAR 0.11 W/kg). The control group was sham irradiated. After 35 days these rats were sacrificed and whole brain tissue was isolated for protein kinase C (PKC) assay. For morphological study the forebrain was isolated from the whole brain and PKC activity was measured using P<sup>32</sup> labeled ATP. Our study reveals a statistically significant ( $p < 0.05$ ) decrease in PKC activity in hippocampus as compared to the remaining portion of the whole brain and the control group. A similar experiment conducted on hippocampus and the whole brain gave a similar result. Electron microscopic study shows an increase in the glial cell population in

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the exposed group as compared to the control group. This present study is indicative of a significant change after exposure to the above-mentioned field intensity. This suggests that chronic exposures may affect brain growth and development.

**Kubinyi G, Thuroczy G, Bakos J, Boloni E, Sinay H, Szabo LD, Effect of continuous-wave and amplitude-modulated 2.45 GHz microwave radiation on the liver and brain aminoacyl-transfer RNA synthetases of in utero exposed mice. *Bioelectromagnetics* 17(6):497-503, 1996.**

Investigations have been carried out concerning the effects of **microwave (MW)** exposure on the aminoacyl-transfer ribonucleic acid (tRNA) synthetase of the progeny of females that were exposed during their entire period of gestation (19 days). The changes caused by **continuous-wave (CW)** and amplitude-modulated (AM) MW radiation have been compared. CFLP mice were exposed to MW radiation for 100 min each day in an anechoic room. The MW frequency was **2.45 GHz**, and the amplitude modulation had a 50 Hz rectangular waveform (on/off ratio, 50/50%). The average power density exposure was 3 mW/cm<sup>2</sup>, and the whole body specific absorption rate (SAR) was 4.23 +/- 0.63 W/kg. The weight and mortality of the progeny were followed until postnatal day 24. Aminoacyl-tRNA synthetase enzymes and tRNA from the brains and livers of the offspring (461 exposed, 487 control) were isolated. The aminoacyl-tRNA synthetase activities were determined. The postnatal increase of body weight and organ weight was not influenced by the prenatal MW radiation. The activity of enzyme isolated from the brain showed a significant decrease after CW MW exposure, but the changes were not significant after 50 Hz AM MW exposure. The activity of the enzyme isolated from liver increased under CW and 50 Hz modulated MW.

**Jorge-Mora T, Folgueiras MA, Leiro-Vidal JM, Jorge-Barreiro FJ, Ares-Pena FJ, López-Martin E. Exposure to 2.45 GHz Microwave Radiation Provokes Cerebral Changes in Induction of Hsp-90  $\alpha/\beta$  Heat Shock Protein in Rat. *Prog Electromagn Res*, 100:351-379, 2010.**

Physical agents such as non-ionizing continuous-wave **2.45 GHz radiation** may cause damage that alters cellular homeostasis and may trigger activation of the genes that encode heat shock proteins (HSP). We used Enzyme-Linked ImmunoSorbent Assay (ELISA) and immunohistochemistry to analyze the changes in levels of HSP-90 and its distribution in the brain of Sprague-Dawley rats, ninety minutes and twenty-four hours after acute (30min) continuous exposure to 2.45 GHz radiation in a the Gigahertz Transverse Electromagnetic (GTEM cell). In addition, we studied further indicators of neuronal insult: dark neurons, chromatin condensation and nucleus fragmentation, which were observed under optical conventional or fluorescence microscopy after DAPI staining. The cellular distribution of protein HSP-90 in the brain increased with each corresponding SAR ( $0.034 \pm 3 \cdot 10^{-3}$ ,  $0.069 \pm 5 \cdot 10^{-3}$ ,  $0.27 \pm 21 \cdot 10^{-3}$  W/kg), in hypothalamic nuclei, limbic cortex and somatosensorial cortex after exposure to the radiation. At twenty-four hours post-irradiation, levels of HSP-90 protein remained high in all hypothalamic nuclei for all SARs, and in the parietal cortex, except the limbic system, HSP-90 levels were lower than in non-irradiated rats, almost half the levels in rats

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exposed to the highest power radiation. Non-apoptotic cellular nuclei and a some dark neurons were found ninety minutes and twenty-four hours after maximal SAR exposure. The results suggest that acute exposure to electromagnetic fields triggered an imbalance in anatomical HSP- 90 levels but the anti-apoptotic mechanism is probably sufficient to compensate the non-ionizing stimulus. Further studies are required to determine the regional effects of chronic electromagnetic pollution on heat shock proteins and their involvement in neurological processes and neuronal damage.

**Gürler HS, Bilgici B, Akar AK, Tomak L, Bedir A. Increased DNA oxidation (8-OHdG) and protein oxidation (AOPP) by Low level electromagnetic field (2.45 GHz) in rat brain and protective effect of garlic. Int J Radiat Biol. 2014 May 21:1-15. [Epub ahead of print]**

Purpose: To investigate the oxidative damage and protective effect of garlic on rats exposed to low level of electromagnetic fields (EMF) at **2.45 GHz Microwave** radiation (MWR). Methods: Thirty six Wistar rats were divided into three groups. Group I was the control group and not exposed to EMF. Group II and III were exposed to low level EMF ( $3.68 \pm 0.36$  V/m) at 2.45 GHz MWR for 1 hour/day for 30 consecutive days. Daily 500 mg/kg garlic was given to Group III during the study period. At the end of the study, thiobarbituric acid reactive substances (TBARS), advanced oxidation protein products (AOPP) and 8-hydroxydeoxyguanosine (8-OHdG) levels were investigated in brain tissue and blood samples. Results: Exposure to low level of EMF increased 8-OHdG level in both plasma and brain tissue whereas it increased AOPP level only in plasma. Garlic prevented the increase of 8-OHdG level in brain tissue and plasma AOPP levels. Conclusions: It may be concluded that low level EMF at 2.45 GHz MWR increases the DNA damage in both brain tissues and plasma of the rats whereas it increases protein oxidation only in plasma. It may also be argued that the use of garlic decreases these effects.

**Fukui Y, Hoshino K, Inouye M, Kameyama Y, Effects of hyperthermia induced by microwave irradiation on brain development in mice. J Radiat Res (Tokyo) 33(1):1-10, 1992.**

Pregnant mice were exposed to **2.45 GHz of microwave** radiation for 15 or 20 min on day 13 of gestation. The highest maternal core temperature during the exposure did not exceed 42.5 degrees C. Pregnant females also were immersed in hot water at 42 degrees C for 15 min to compare thermal effects on brain development. Animals were killed 9 hours after treatment, and the pyknotic cells in the ventricular zone of telencephalon were counted. The respective incidences of these cells in the groups exposed to microwaves for 15 and 20 min were 1.83% and 3.06%. Microwave radiation for 20 min had an effect that was comparable to that of immersion in 42 degrees C hot water for 15 min. In addition, some animals were examined on day 18 of gestation, and some of their offspring were examined at 6 weeks of age in an examination of long-term effects. Brain weight for the group exposed to microwaves for 20 min was significantly lower than for the control group, and the numerical density of the neurons in the cerebrum was higher. We concluded that microwave radiation at the dose tested mainly has a thermal effect.

**Nazıroğlu M, Çelik Ö, Özgül C, Çiğ B, Doğan S, Bal R, Gümral N, Rodríguez AB, Pariente JA. Melatonin modulates wireless (2.45 GHz)-induced oxidative injury through TRPM2**

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**and voltage gated Ca(2+) channels in brain and dorsal root ganglion in rat. *Physiol Behav.* 105(3):683-692, 2012.**

We aimed to investigate the protective effects of melatonin and 2.45 GHz electromagnetic radiation (EMR) on brain and dorsal root ganglion (DRG) neuron antioxidant redox system, Ca(2+) influx, cell viability and electroencephalography (EEG) records in the rat. Thirty two rats were equally divided into four different groups namely group A1: Cage control, group A2: Sham control, group B: 2.45 GHz EMR, group C: 2.45 GHz EMR+melatonin. Groups B and C were exposed to 2.45 GHz EMR during 60 min/day for 30 days. End of the experiments, EEG records and the brain cortex and DRG samples were taken. Lipid peroxidation (LP), cell viability and cytosolic Ca(2+) values in DRG neurons were higher in group B than in groups A1 and A2 although their concentrations were increased by melatonin, 2-aminoethyldiphenyl borinate (2-APB), diltiazem and verapamil supplementation. Spike numbers of EEG records in group C were lower than in group B. Brain cortex vitamin E concentration was higher in group C than in group B. In conclusion, Melatonin supplementation in DRG neurons and brain seems to have protective effects on the 2.45 GHz-induced increase Ca(2+) influx, EEG records and cell viability of the hormone through TRPM2 and voltage gated Ca(2+) channels.

**Field AS, Ginsburg K, Lin JC The effect of pulsed microwaves on passive electrical properties and interspike intervals of snail neurons. *Bioelectromagnetics* 14(6):503-520, 1993.**

The effects of pulsed microwaves (2.45 GHz, 10 microseconds, 100 pps, SAR: 81.5 kW/kg peak, 81.5 W/kg average) on membrane input resistance and action potential (AP) interval statistics were studied in spontaneously active ganglion neurons of land snails (*Helix aspersa*), at strictly constant temperature (20.8 +/- .07 degrees C worst case). Statistical comparison with sham-irradiated neurons revealed a significant increase in the mean input resistance of neurons exposed to pulsed microwaves (P < or = .05). Pulsed microwaves had no visible effect on mean AP firing rate; this observation was confirmed by analysis of interspike intervals (ISIs). Using an integrator model for spontaneously active neurons, we found the net input current to be more variable in neurons exposed to pulsed microwaves. The mean input current was not affected. The standard deviation of ISIs and the autocorrelation of the input current were marginally affected, but these changes were not consistent across neurons. Although the observed effects were less obvious than those reported in other studies, they represent evidence of a direct interaction between neurons and pulsed microwaves, in the absence of macroscopic temperature changes. The data do not suggest a single, specific mechanism for such interaction.

**Ding G, Xie X, Zhang L et al. Changes of nitric oxide synthase in hippocampus and cerebellum of the rat following exposure to electromagnetic pulse. *Chin J Phys Med* 20:81-83, 1998.**

Objective: In order to explore the role of nitric oxide in the obstruction of learning and memory of the rat caused by exposing to electromagnetic pulses (EMP), the distribution of nitric oxide synthase (NOS) expression was studied in hippocampus and cerebellum of

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the rat following exposure to EMP. Methods: SP immunohistochemical staining was employed to detect the distribution of NOS expression. Results: The number of NOS positive neurons and the intensity of positive staining in hippocampus were decreased at 1.5 and 24 h after exposure to EMP. At 48 h, the number of NOS positive neurons reversed to control level but the intensity of positive staining was still low. the expression of NOS in cerebellum had no obvious changes. Conclusion: Decrease of NOS expression in hippocampus relates to the obstruction of learning and memory of the rat after exposure to EMP.

### **Lange DG, Sedmak J, Japanese encephalitis virus (JEV): potentiation of lethality in mice by microwave radiation. Bioelectromagnetics 12(6):335-348, 1991.**

The expression of Japanese Encephalitis Virus (JEV) lethality in mice requires entry of the virus into the central nervous system. This entry is presumably through the capillary endothelial cells (CEC), because entry between CECs is inhibited by bands of circumferential tight-junctions. A viremic stage occurs during the first 4 to 5 days after JEV administration in mice, and both microwave **radiation (2.45-GHz, continuous wave, 10-min exposure)** and hypercarbia were employed to increase CEC permeability to JEV. Exposure to microwaves at power densities of 10-50 mW/cm<sup>2</sup> resulted in a dose-dependent increase in JEV-induced lethality. Mice did not become tolerant or sensitized to microwave potentiation of JEV-induced mortality because 4 daily exposures at 10 or 50 mW/cm<sup>2</sup> (SARS, approximately 24-98 W/kg) did not alter the lethality pattern to subsequent microwave radiation of JEV-exposed animals. Similarly, hypercarbia (5, 10, and 20% CO<sub>2</sub>) was observed to produce a dose-dependent increase in JEV-induced lethality. Both microwave radiation and hypercarbia are thought to promote pinocytosis in CNS capillary endothelial cells. This may be one mechanism by which they enhance JEV-induced lethality in adult Swiss-Cox mice.

### **Inaba R, Shishido K, Okada A, Moroji T. Effects of whole body microwave exposure on the rat brain contents of biogenic amines. Eur J Appl Physiol Occup Physiol. 65(2):124-128, 1992.**

The effects of whole body microwave exposure on the central nervous system (CNS) of the rat were investigated. Rats weighing from 250 to 320 g were exposed for 1 h to whole body **microwave with a frequency of 2450 MHz** at power densities of 5 and 10 mW.cm<sup>-2</sup> at an ambient temperature of 21-23 degrees C. The rectal temperatures of the rats were measured just before and after microwave exposure and mono-amines and their metabolites in various discrete brain regions were determined after microwave exposure. Microwave exposure at power densities of 5 and 10 mW.cm<sup>-2</sup> increased the mean rectal temperature by 2.3 degrees C and 3.4 degrees C, respectively. The noradrenaline content in the hypothalamus was significantly reduced after microwave exposure at a power density of 10 mW.cm<sup>-2</sup>. There were no differences in the dopamine (DA) content of any region of the brain between microwave exposed rats and control rats. The dihydroxyphenyl acetic acid (DOPAC) content, the main metabolite of DA, was significantly increased in the pons plus medulla oblongata only at a power density of 10 mW.cm<sup>-2</sup>. The DA turnover rates, the DOPAC:DA ratio, in the striatum and cerebral



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cortex were significantly increased only at a power density of 10 mW.cm<sup>-2</sup>. The serotonin (5-hydroxytryptamine, 5-HT) content in all regions of the brain of microwave exposed rats was not different from that of the control rats. The 5-hydroxyindoleacetic acid (5-HIAA) content in the cerebral cortex of microwave exposed rats was significantly increased at power densities of 5 and 10 mW.cm<sup>-2</sup>.

**Naziroğlu M, Gümral N. Modulator effects of L-carnitine and selenium on wireless devices (2.45 GHz)-induced oxidative stress and electroencephalography records in brain of rat. *Int J Radiat Biol.* 85(8):680-689, 2009.**

PURPOSE: Electromagnetic radiation (EMR) from wireless devices may affect biological systems by increasing free radicals. The present study was designed to determine the effects of **2.45 GHz EMR** on the brain antioxidant redox system and electroencephalography (EEG) records in rat. The possible protective effects of selenium and L-carnitine were also tested and compared to untreated controls. MATERIALS AND METHODS: Thirty rats were equally divided into five different groups, namely Group A(1): Cage control, Group A(2): Sham control, group B: 2.45 GHz EMR, group C: 2.45 GHz EMR + selenium, group D: 2.45 GHz EMR + L-carnitine. Groups B, C and D were exposed to 2.45 GHz EMR during 60 min/day for 28 days. End of the experiments, EEG records and the brain cortex samples were taken. RESULTS: The cortex brain vitamin A ( $p < 0.05$ ), vitamin C ( $p < 0.01$ ) and vitamin E ( $p < 0.05$ ) concentrations values were lower in group B than in group A1 and A2 although their concentrations were increased by selenium and L-carnitine supplementation. Lipid peroxidation, levels were lower in group C ( $p < 0.05$ ) and D ( $p < 0.01$ ) than in group B where as reduced glutathione levels were higher in group C ( $p < 0.05$ ) than in group A1, A2 and B. However, B-carotene levels did not change in the five groups. CONCLUSIONS: L-carnitine and selenium seem to have protective effects on the 2.45 GHz-induced decrease of the vitamins by supporting antioxidant redox system. L-carnitine on the vitamin concentrations seems to more protective affect than in selenium.

**De Roos AJ, Teschke K, Savitz DA, Poole C, Grufferman S, Pollock BH, Olshan AF. Parental occupational exposures to electromagnetic fields and radiation and the incidence of neuroblastoma in offspring. *Epidemiology* 12(5):508-517, 2001.**

We examined parental occupational exposures to electromagnetic fields and radiation and the incidence of neuroblastoma in offspring. Cases were 538 children diagnosed with neuroblastoma between 1992 and 1994 in the United States or Canada. Age-matched controls were selected by random-digit dialing. Occupational exposures to electrical equipment and radiation sources were classified by an industrial hygienist, and average exposures to extremely low frequency magnetic fields were estimated using a job exposure matrix. Maternal exposure to a broad grouping of sources that produce radiofrequency radiation was associated with an increased incidence of neuroblastoma (odds ratio = 2.8; 95% confidence interval = 0.9-8.7). Paternal exposure to battery-powered forklifts was positively associated with neuroblastoma (odds ratio = 1.6; 95% confidence interval = 0.8-3.2), as were some types of equipment that emit radiofrequency radiation (odds ratios congruent with 2.0); however, the broad groupings

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of sources that produce ELF fields, radiofrequency radiation, or ionizing radiation were not associated with neuroblastoma. Paternal average extremely low frequency magnetic field exposure >0.4 microTesla was weakly associated with neuroblastoma (odds ratio = 1.6; 95% confidence interval = 0.9-2.8), whereas maternal exposure was not. Overall, there was scant supportive evidence of strong associations between parental exposures in electromagnetic spectrum and neuroblastoma in offspring.

## Effects on Eyes

**Liu X, Shen H, Shi Y, Chen J, Chen Y, Ji A. The microarray study on the stress gene transcription profile in human retina pigment epithelial cells exposed to microwave radiation. Zhonghua Yu Fang Yi Xue Za Zhi 36(5):291-294, 2002.**

OBJECTIVE: To study the difference in stress and apoptosis related genes transcription between hTERT-RPE1 cells exposed to simulated microwave radiation and the cells with heat water bath, and the effects of microwave on gene transcription in cultured human retina pigment epithelial cells. METHODS: cDNA microarray technique was used to detect the mRNA isolated from hTERT-RPE1 cells exposed to **2 450 MHz** simulated microwave radiation and with heat water bath, respectively. RESULTS: Among the 97 related aim genes, there were seven genes up-regulating its transcription, i.e., M31166 (2.52fold), L24123 (2.66fold), AF039704 (2.22fold), U67156 (2.07fold), AF040958 (2.13fold), NM-001423 (2.63fold) and NM-005346 (3.68fold). But, no notably down-regulating gene in transcription was detected. CONCLUSIONS: Microwave could induce up-regulating in multiple stress and apoptosis related genes transcription in cultured human retina pigment epithelial cells, hTERT-RPE1 cells. Microwave radiation has unique effect itself in addition to its heat effect.

**Akar A, Karayığit MO, Bolat D, Gültiken ME, Yarım M, Castellani G. Effects of low level electromagnetic field exposure at 2.45 GHz on rat cornea. Int J Radiat Biol. 2012 Dec 3. [Epub ahead of print]**

Abstract Purpose: To investigate the effects of low level Electromagnetic Field (low level-EMF) exposure, as frequently encountered in daily life, on the yesmal rat cornea using histological and stereological method. Methods: Twenty-two adult male Wistar rats were randomly divided into two groups: study group (n=11) and control group (n=11). Rats in the study group were exposed to **2.45 GHz Microwave (MW) radiation** (11.96±0.89V/m), 0.25 W/kg specific absorption rate (SAR) for 2 hours each day for 21 days. The corneal thickness and the anterior epithelium corneal thickness were measured using two different methods. Results: Using the histological method, the mean corneal thicknesses in the control and study group were 278.9±54.5 µm, and 272.4±85.6 µm, respectively. There was no statistically significant difference between the groups (p>0.05). The anterior corneal epithelium thickness was 28.1±4.9 µm in the control group and 31.7±5.5 µm in the study group. There were statistically differences between the groups with regard to the thickness of anterior epithelium (p<0.05). In the measurement made by the stereological method, the percentage of the cornea occupied by anterior corneal

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epithelium was 15.94% in the control group and 17.9% in the study group. Despite the fact that there was a relation between increased anterior epithelial area (AEA) and radiation exposure, no statistically significant relationship in area fraction of each compartment was found between the control and study groups. Conclusions: Results of this preliminary study show that exposure to MW radiation might cause alterations in the rat cornea.

**Tök L, Nazıroğlu M, Doğan S, Kahya MC, Tök O. Effects of melatonin on Wi-Fi-induced oxidative stress in lens of rats. Indian J Ophthalmol. 62(1):12-15, 2014. doi: 10.4103/0301-4738.126166.**

Introduction: Melatonin has been considered a potent antioxidant that detoxifies a variety of reactive oxygen species in many pathophysiological states of eye. The present study was designed to determine the effects of Wi-Fi exposure on the lens oxidant, antioxidant redox systems, as well as the possible protective effects of melatonin on the lens injury induced by electromagnetic radiation (EMR). Materials and Methods: Thirty-two rats were used in the current study and they were randomly divided into four equal groups as follows: First and second groups were cage-control and sham-control rats. Rats in third group were exposed to **Wi-Fi (2.45 GHz)** for duration of 60 min/day for 30 days. As in the third group, the fourth group was treated with melatonin. The one-hour exposure to irradiation in second, third and fourth took place at noon each day. Results: Lipid peroxidation levels in the lens were slightly higher in third (Wi-Fi) group than in cage and sham control groups although their concentrations were significantly ( $P < 0.05$ ) decreased by melatonin supplementation. Glutathione peroxidase (GSH-Px) activity was significantly ( $P < 0.05$ ) lower in Wi-Fi group than in cage and sham control groups although GSH-Px ( $P < 0.01$ ) and reduced glutathione ( $P < 0.05$ ) values were significantly higher in Wi-Fi + melatonin group than in Wi-Fi group. Conclusions: There are poor oxidative toxic effects of one hour of Wi-Fi exposure on the lens in the animals. However, melatonin supplementation in the lens seems to have protective effects on the oxidant system by modulation of GSH-Px activity.

**Pologea-Moraru R, Kovacs E, Iliescu KR, Calota V, Sajin G. The effects of low level microwaves on the fluidity of photoreceptor cell membrane. Bioelectrochemistry 56(1-2):223-225, 2002.**

Due to the extensive use of electromagnetic fields in everyday life, more information is required for the detection of mechanisms of interaction and the possible side effects of electromagnetic radiation on the structure and function of the organism. In this paper, we study the effects of low-power microwaves (2.45 GHz) on the membrane fluidity of rod photoreceptor cells. The retina is expected to be very sensitive to microwave irradiation due to the polar character of the photoreceptor cells [Biochim. Biophys. Acta 1273 (1995) 217] as well as to its high water content [Stud. Biophys. 81 (1981) 39].

**Yang R, Chen J, Deng Z, Liu X, [Effect of vitamin E on morphological variation of retinal ganglion cells after microwave radiation]. Wei Sheng Yan Jiu 30(1):31-33, 2001. [Article in Chinese]**

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To determine the morphological variation in the primary cultured pig retinal ganglion cells induced by microwave and the protection of VE can supply some experiment foundation for study of effect of microwave and its protection. Retinal ganglion cells of pig were cultured in vitro and added VE of different concentration, Each group was taken after 30 mW/cm<sup>2</sup> microwave intensity radiated for 1 h in shielded room by **2450 MHz** continuous wave physiotherapy machine. Immediately after radiation, the morphological variation of cells was observed by optics microscope and transmission electronic microscope. The result showed that a portion of cells congregated, with their axon disappeared after radiation. Mitochondria and endoplasmic reticulum are detected swelling by transmission electronic microscope. The results showed that A poposis cells can be observed. Cells of VE added groups had not obvious changes with optics microscope, but could be found that mitochondria swelling lightly and integrate mitochondria cristae by transmission electronic microscope. The results showed that microwave induced the morphological damage in primary cultured retinal ganglion cells, VE could reduced the damage of retina ganglion cells by microwave in some extent.

**Behrens T, Lyngé E, Cree I, Sabroe S, Lutz JM, Afonso N, Eriksson M, Guénel P, Merletti F, Morales-Suarez-Varela M, Stengrevics A, Févotte J, Llopis-González A, Gorini G, Sharkova G, Hardell L, Ahrens W. Occupational exposure to electromagnetic fields and sex-differential risk of uveal melanoma. *Occup Environ Med.*67(11):751-759, 2010**

Objectives The association between occupational exposure to electromagnetic fields (EMF) and the risk of uveal melanoma was investigated in a case-control study in nine European countries. Methods Incident cases of uveal melanoma and population as well as hospital controls were included and frequency matched by country, 5-year birth cohort and sex. Subjects were asked whether they had worked close to high-voltage electrical transmission installations, computer screens and various electrical machines, or in complex electrical environments. Measurements of two Scandinavian job-exposure matrices were applied to estimate lifelong cumulative EMF exposure. Unconditional logistic regression analyses, stratified by sex and eye colour were calculated, adjusting for several potential confounders. Results 293 patients with uveal melanoma and 3198 control subjects were interviewed. Women exposed to electrical transmission installations showed elevated risks (OR 5.81, 95% CI 1.72 to 19.66). Positive associations with exposure to control rooms were seen among men and women, but most risk increases were restricted to subjects with dark iris colour. Application of published EMF measurements revealed stronger risk increases among women compared to men. Again, elevated risks were restricted to subjects with dark eye colour. Conclusion Although based on a low prevalence of exposure to potential occupational sources of EMF, our data indicate that exposed dark-eyed women may be at particular risk for uveal melanoma.

**Yao K, Wang KJ, Sun ZH, Tan J, Xu W, Zhu LJ, Lu de Q. Low power microwave radiation inhibits the proliferation of rabbit lens epithelial cells by upregulating P27Kip1 expression. *Mol Vis.* 10:138-143, 2004.**

PURPOSE: The goal of this study was to examine the effects of low power microwave

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radiation (<10 mW/cm<sup>2</sup>) on the proliferation of cultured rabbit lens epithelial cells (RLEC). METHODS: Cultured RLEC were exposed to continuous microwave radiation at a frequency of **2,450 MHz** and power densities of 0.10, 0.25, 0.50, 1.00, and 2.00 mW/cm<sup>2</sup> for 8 h. Cell morphologic changes were observed under a phase-contrast microscope. Cell viability was measured using the MTT assay and cell cycle analysis was measured using flow cytometry. After exposure to 2.00 mW/cm<sup>2</sup> microwave radiation for 4, 6, and 8 h, the expression of cell cycle-regulatory proteins, P21WAF1 and P27Kip1, was examined using western blot analysis. Finally, the levels of P21WAF1 and P27Kip1 mRNA were analyzed by reverse transcription-polymerase chain reaction (RT-PCR). RESULTS: After 8 h of radiation treatment, cells treated with 0.50, 1.00, and 2.00 mW/cm<sup>2</sup> microwave radiation exhibited decreased cell viability, increased cell condensation and an inhibition of DNA synthesis. RLEC showed significant G0/G1 arrest. No obvious changes could be detected in the 0.10 and 0.25 mW/cm<sup>2</sup> microwave treatment groups. Protein expression of P27Kip1 was markedly increased after microwave radiation. However, the mRNA levels were unchanged. On the other hand, there were no detectable differences in P21WAF1 protein expression and mRNA levels between microwave treatment and control groups. CONCLUSIONS: This study suggests that low power microwave radiation higher than 0.50 mW/cm<sup>2</sup> can inhibit lens epithelial cell proliferation, and increase the expression of P27Kip1. These effects may account for the decline of lens epithelial proliferation after exposure to microwave radiation.

**Kues HA, Monahan JC, D'Anna SA, McLeod DS, Luty GA, Koslov S, Increased sensitivity of the non-human primate eye to microwave radiation following ophthalmic drug pretreatment. Bioelectromagnetics 13(5):379-393, 1992.**

Previous studies in our laboratory have established that pulsed microwaves at 2.45 GHz and 10 mW/cm<sup>2</sup> are associated with production of corneal endothelial lesions and with disruption of the blood-aqueous barrier in the non-human primate eye. In the study reported here we examined ocular damage in monkeys (*M. mulatta* and *M. fascicularis*) following topical treatment with one of two ophthalmic drugs (timolol maleate and pilocarpine) that preceded exposure to pulsed microwaves. Anesthetized monkeys were sham exposed or exposed to pulsed, 2.45 GHz microwaves (10 microseconds, 100 pps) at average power densities of 0.2, 1, 5, 10, or 15 mW/cm<sup>2</sup> 4 h a day for 3 consecutive days (respective SARs were 0.052, 0.26, 1.3, 2.6, and 3.9 W/kg). Immediately before microwave exposure, one or both eyes were treated topically with one drop of 0.5% timolol maleate or of 2% pilocarpine. Following administration of a drug, we observed a significant reduction in the power-density threshold (from 10 to 1 mW/cm<sup>2</sup>) for induction of corneal endothelial lesions and for increased vascular permeability of the iris. Diagnostic procedures (in vivo specular microscopy and fluorescein iris angiography) were performed following each exposure protocol. In addition, increased vascular permeability was confirmed with horseradish peroxidase tracer techniques. Although we did not measure intraocular temperatures in experimental animals, the results suggest that a mechanism other than significant heating of the eye is involved. Our data indicate that pulsed microwaves at an average SAR of 0.26 W/kg, if administered after pretreatment with ophthalmic drugs, can produce significant ocular effects in the

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anesthetized primate.

**Kojima M, Hata I, Wake K, Watanabe S, Yamanaka Y, Kamimura Y, Taki M, Sasaki K. Influence of anesthesia on ocular effects and temperature in rabbit eyes exposed to microwaves. *Bioelectromagnetics* 25(3):228-233, 2004.**

To investigate the effect of systemic anesthesia on ocular effects and temperature in rabbit eyes exposed to microwaves, one eye each of 43 male pigmented rabbits (Dutch, 1.8-2.2 kg) was **exposed at 2.45 GHz** for 60-20 min (300 mW/cm<sup>2</sup>; 108 W/kg), either under anesthesia (ketamine hydrochloride (5 mg/kg) + xylazine (0.23 mg/kg)) or without anesthesia. Changes in the anterior segment were evaluated by image analysis utilizing a Scheimpflug camera, specular microscopy, and a laser flare cell meter. Temperatures within the eye were measured during microwave exposure by a Fluoroptic thermometer. The exposed eyes showed miosis, conjunctival congestion, corneal edema, and an increase in the light scattering of the anterior shallow cortex in the pupillary area of the lens. The group under systemic anesthesia showed much stronger symptoms than those treated without anesthesia. All of the anterior ocular changes disappeared within a week. The highest temperature during exposure was in the vitreous, followed by the anterior chamber, and the retrobulbar cavity of the orbit. The ocular temperatures of the rabbits under systemic anesthesia were 2-9 degrees C higher than those without anesthesia. Body temperature showed an increase of 1 degrees C during the exposure. Acute high intensity microwave exposure temporarily induced anterior segments inflammation and lens changes. The more pronounced ocular effects in the anesthetized rabbits were associated with the significantly higher ocular temperatures in the anesthetized animals. The influence of systemic anesthesia on ocular changes should be considered.

**Yu Y, Yao K. Non-thermal cellular effects of lowpower microwave radiation on the lens and lens epithelial cells. *J Int Med Res.* 38(3):729-736, 2010.**

Because of the increased use of modern radiofrequency devices, public concern about the possible health effects of exposure to microwave radiation has arisen in many countries. It is well established that high-power microwave radiation can induce cataracts via its thermal effects. It remains unclear whether low-power microwave radiation, especially at levels below the current exposure limits, is cataractogenic. This review summarizes studies on the biological effects of low-power microwave radiation on lens and lens epithelial cells (LECs). It has been reported that exposure affects lens transparency, alters cell proliferation and apoptosis, inhibits gap junctional intercellular communication, and induces genetic instability and stress responses in LECs. These results raise the question of whether the ambient microwave environment can induce non-thermal effects in the lens and whether such effects have potential health consequences. Further in vivo studies on the effects on the lens of exposure to low-power microwave radiation are needed.

**Balci M, Namuslu M, Devrim E, Durak I. Effects of computer monitor-emitted radiation on oxidant/antioxidant balance in cornea and lens from rats. *Mol Vis.* 15:2521-2525, 2009.**

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**PURPOSE:** This study aims to investigate the possible effects of computer monitor-emitted radiation on the oxidant/antioxidant balance in corneal and lens tissues and to observe any protective effects of vitamin C (vit C). **METHODS:** Four groups (PC monitor, PC monitor plus vitamin C, vitamin C, and control) each consisting of ten Wistar rats were studied. The study lasted for three weeks. Vitamin C was administered in oral doses of 250 mg/kg/day. The computer and computer plus vitamin C groups were exposed to computer monitors while the other groups were not. Malondialdehyde (MDA) levels and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activities were measured in corneal and lens tissues of the rats. **RESULTS:** In corneal tissue, MDA levels and CAT activity were found to increase in the computer group compared with the control group. In the computer plus vitamin C group, MDA level, SOD, and GSH-Px activities were higher and CAT activity lower than those in the computer and control groups. Regarding lens tissue, in the computer group, MDA levels and GSH-Px activity were found to increase, as compared to the control and computer plus vitamin C groups, and SOD activity was higher than that of the control group. In the computer plus vitamin C group, SOD activity was found to be higher and CAT activity to be lower than those in the control group. **CONCLUSION:** The results of this study suggest that computer-monitor radiation leads to oxidative stress in the corneal and lens tissues, and that vitamin C may prevent oxidative effects in the lens.

**Lu L, Xu H, Wang X, Guo G. Increased nitric oxide synthase activity is essential for electromagnetic-pulse-induced blood-retinal barrier breakdown in vivo. Brain Res. 1264:104-110, 2009.**

**PURPOSE:** To examine whether electromagnetic pulses (EMPs) affected the permeability of the blood-retinal barrier (BRB), gene expression of occludin and activity of nitric oxide synthase (NOS). **METHODS:** Sprague-Dawley (SD) rats were used and randomized into EMP and control groups. Retinas were removed immediately, and 2 h or 24 h after EMP radiation. BRB permeability was analyzed by transmission electron microscopy and Evans Blue staining. Retinal NOS activity and concentrations of nitrite and nitrate were measured. Occludin mRNA and protein levels were detected by RT-PCR and Western blotting. **RESULTS:** Exposure of SD rats to EMP resulted in increased BRB permeability, with the greatest decrease in occludin at 24 h. Moreover, this permeability defect was also correlated with significant increases in the formation of NO and induction of NOS activity in SD rats. Furthermore, we found that treatment with NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME) blocked BRB breakdown and prevented the increase in NO formation and induction of NOS activity, as well as the decrease in occluding expression. **CONCLUSION:** Taken together, these results support the view that NOS-dependent NO production is an important factor that contributes to EMP-induced BRB dysfunction, and suggests that NOS induction may play an important role in BRB breakdown.

**Wang KJ, Yao K, Lu DQ. [Effects of different dose microwave radiation on protein components of cultured rabbit lens] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 25(4):208-210, 2007. [Article in Chinese]**

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**OBJECTIVE:** To study the effects of different dose microwave radiation on protein components of cultured rabbit lens, and analyze the mechanisms of lens injury caused by microwave radiation. **METHODS:** Cultured rabbit lens were exposed to microwave radiation with frequency of **2450 MHz** and power density of 0.25, 0.50, 1.00, 2.00, 5.00 mW/cm(2) for 8 hours in vitro. The transparency of lens was observed. Changes of protein concentration were detected after different lens protein components were extracted, including water-soluble protein (WSP), urea soluble protein (USP), alkali soluble protein (ASP) and sonicated protein (SP). The influence of microwave radiation on WSP was analyzed using SDS-PAGE electrophoresis and coomassie-blue staining. **RESULTS:** Transparency of lens decreased after radiation. There was obvious opacification of lens cortex after 5.00 mW/cm(2) microwave radiation for 8 hours. After 1.00, 2.00 and 5.00 mW/cm(2) radiation, the percentage of WSP decreased while USP increased obviously. There was no change of ASP. The percentage of SP decreased when the power of microwave was 5.00 mW/cm(2). The low molecular weight protein of WSP decreased while high molecular weight protein increased after microwave radiation. **CONCLUSION:** Microwave radiation higher than 1.00 mW/cm(2) can affect the proportion of WSP and USP in cultured rabbit lens, and cause changes of lens transparency and refractive power, which leads to lens opacity.

## Effect on Fertility and Reproduction

**Atasoy HI, Gunal MY, Atasoy P, Elgun S, Bugdayci G. Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices. J Pediatr Urol. 9(2):223-229, 2013.**

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



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**Dasdag S, Taş M, Akdag MZ, Yegin K. Effect of long-term exposure of 2.4 GHz radiofrequency radiation emitted from Wi-Fi equipment on testes functions. Electromagn Biol Med. 2014 Jan 24. [Epub ahead of print]**

The aim of this study was to investigate long-term effects of radiofrequency radiation (RFR) emitted from a Wireless Fidelity (Wi-Fi) system on testes. The study was carried out on 16 Wistar Albino adult male rats by dividing them into two groups such as sham (n: 8) and exposure (n: 8). Rats in the exposure group were exposed to 2.4 GHz RFR radiation for 24 h/d during 12 months (1 year). The same procedure was applied to the rats in the sham control group except the Wi-Fi system was turned off. Immediately after the last exposure, rats were sacrificed and reproductive organs were removed. Motility (%), concentration ( $\times 10^6/\text{mL}$ ), tail defects (%), head defects (%) and total morphologic defects (%) of sperms and weight of testes (g), left epididymis (g), prostate (g), seminal vesicles (g) were determined. Seminiferous tubules diameter ( $\mu\text{m}$ ) and tunica albuginea thickness ( $\mu\text{m}$ ) were also measured. However, the results were evaluated by using Johnsen's score. Head defects increased in the exposure group ( $p < 0.05$ ) while weight of the epididymis and seminal vesicles, seminiferous tubules diameter and tunica albuginea thickness were decreased in the exposure group ( $p < 0.01$ ,  $p < 0.001$ ,  $p < 0.0001$ ). However, other alterations of other parameters were not found significant ( $p > 0.05$ ). In conclusion, we observed that long-term exposure of 2.4 GHz RF emitted from Wi-Fi (2420  $\mu\text{W}/\text{kg}$ , 1 g average) affects some of the reproductive parameters of male rats. We suggest Wi-Fi users to avoid long-term exposure of RF emissions from Wi-Fi equipment.

**Avendaño C, Mata A, Sanchez Sarmiento CA, Doncel GF. Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. Fertil Steril. 97(1):39-45, 2012.**

**OBJECTIVE:** To evaluate the effects of laptop computers connected to local area networks wirelessly (Wi-Fi) on human spermatozoa. **DESIGN:** Prospective in vitro study. **SETTING:** Center for reproductive medicine. **PATIENT(S):** Semen samples from 29 healthy donors. **INTERVENTION(S):** Motile sperm were selected by swim up. Each sperm suspension was divided into two aliquots. One sperm aliquot (experimental) from each patient was exposed to an internet-connected laptop by Wi-Fi for 4 hours, whereas the second aliquot (unexposed) was used as control, incubated under identical conditions without being exposed to the laptop. **MAIN OUTCOME MEASURE(S):** Evaluation of sperm motility, viability, and DNA fragmentation. **RESULT(S):** Donor sperm samples, mostly normozoospermic, exposed ex vivo during 4 hours to a wireless internet-connected laptop showed a significant decrease in progressive sperm motility and an increase in sperm DNA fragmentation. Levels of dead sperm showed no significant differences between the two groups. **CONCLUSION(S):** To our knowledge, this is the first study to evaluate the direct impact of laptop use on human spermatozoa. Ex vivo exposure of human spermatozoa to a wireless internet-connected laptop decreased motility and induced DNA fragmentation by a nonthermal effect. We speculate that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility. Further in vitro and in vivo studies are needed to prove this contention.

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**Nazıroğlu M, Yüksel M, Köse SA, Özkaya MO. Recent reports of Wi-Fi and mobile phone-induced radiation on oxidative stress and reproductive signaling pathways in females and males. J Membr Biol. 246(12):869-875, 2013.**

Environmental exposure to electromagnetic radiation (EMR) has been increasing with the increasing demand for communication devices. The aim of the study was to discuss the mechanisms and risk factors of EMR changes on reproductive functions and membrane oxidative biology in females and males. It was reported that even chronic exposure to EMR did not increase the risk of reproductive functions such as increased levels of neoantigens abort. However, the results of some studies indicate that EMR induced endometriosis and inflammation and decreased the number of follicles in the ovarium or uterus of rats. In studies with male rats, exposure caused degeneration in the seminiferous tubules, reduction in the number of Leydig cells and testosterone production as well as increases in luteinizing hormone levels and apoptotic cells. In some cases of male and female infertility, increased levels of oxidative stress and lipid peroxidation and decreased values of antioxidants such as melatonin, vitamin E and glutathione peroxidase were reported in animals exposed to EMR. In conclusion, the results of current studies indicate that oxidative stress from exposure to Wi-Fi and mobile phone-induced EMR is a significant mechanism affecting female and male reproductive systems. However, there is no evidence to this date to support an increased risk of female and male infertility related to EMR exposure.

**Saygin M, Caliskan S, Karahan N, Koyu A, Gumral N, Uguz A. Testicular apoptosis and histopathological changes induced by a 2.45 GHz electromagnetic field. Toxicol Ind Health. 27(5):455-463, 2011.**

There is a growing public concern about the potential human health hazard caused by exposure to electromagnetic radiation (EMR). The objective of this study is to investigate the effects of **2450 mhz** electromagnetic field on apoptosis and histopathological changes on rat testis tissue. Twelve-week-old male Wistar Albino rats were used in this study. Eighteen rats equally divided into three different groups which were named group I, II and III. Cage control (group I), sham control (group II) and 2.45 GHz EMR (group III) groups were formed. Group III were exposed to 2.45 GHz EMR, at 3.21 W/kg specific absorption rate for 60 minutes/ day for 28 days. There was no difference among the groups for the diameter of the seminiferous tubules, pyknotic, karyoleptic and karyotic cells. However, the number of Leydig cells of testis tissue of the rats in group III was significantly reduced comparing with the group I ( $p < 0.05$ ). Estimation of spermatogenesis using the Johnsen testicular biopsy score revealed that the difference between groups is statistically significant. The level of TNF- $\alpha$ , Caspase-3 and Bcl-2 were compared, and no significant difference was found between the groups. When Bax apoptosis genes and Caspase-8 apoptosis enzyme were compared, there were significant differences between the groups ( $p < 0.05$ ). Electromagnetic field affects spermatogenesis and causes to apoptosis due to the heat and other stress-related events in testis tissue.

**Wang H, Peng R, Zhou H, Wang S, Gao Y, Wang L, Yong Z, Zuo H, Zhao L, Dong J, Xu X, Su Z. Impairment of long-term potentiation induction is essential for the disruption of**

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### **spatial memory after microwave exposure. Int J Radiat Biol. 2013 Jul 24. [Epub ahead of print]**

Purpose: To assess the impact of microwave exposure on learning and memory and to explore the underlying mechanisms. Materials and methods: 100 Wistar rats were exposed to a 2.856 GHz pulsed microwave field at average power densities of 0 mW/cm<sup>2</sup>, 5 mW/cm<sup>2</sup>, 10 mW/cm<sup>2</sup> and 50 mW/cm<sup>2</sup> for 6 min. The spatial memory was assessed by the Morris Water Maze (MWM) task. An in vivo study was conducted soon after microwave exposure to evaluate the changes of population spike (PS) amplitudes of long-term potentiation (LTP) in the medial perforant path (MPP)-dentate gyrus (DG) pathway. The structure of the hippocampus was observed by the light microscopy and the transmission electron microscopy (TEM) at 7 d after microwave exposure. Results: Our results showed that the rats exposed in 10 mW/cm<sup>2</sup> and 50 mW/cm<sup>2</sup> microwave displayed significant deficits in spatial learning and memory at 6 h, 1 d and 3 d after exposure. Decreased PS amplitudes were also found after 10 mW/cm<sup>2</sup> and 50 mW/cm<sup>2</sup> microwave exposure. In addition, varying degrees of degeneration of hippocampal neurons, decreased synaptic vesicles and blurred synaptic clefts were observed in the rats exposed in 10 mW/cm<sup>2</sup> and 50 mW/cm<sup>2</sup> microwave. Compared with the sham group, the rats exposed in 5 mW/cm<sup>2</sup> microwave showed no difference in the above experiments. Conclusions: This study suggested that impairment of LTP induction and the damages of hippocampal structure, especially changes of synapses, might contribute to cognitive impairment after microwave exposure.

### **Oksay T, Naziroğlu M, Doğan S, Güzel A, Gümral N, Koşar PA. Protective effects of melatonin against oxidative injury in rat testis induced by wireless (2.45 GHz) devices. Andrologia. 2012 Nov 12. doi: 10.1111/and.12044. [Epub ahead of print]**

Wireless devices have become part of everyday life and mostly located near reproductive organs while they are in use. The present study was designed to determine the possible protective effects of melatonin on oxidative stress-dependent testis injury induced by **2.45-GHz electromagnetic radiation (EMR)**. Thirty-two rats were equally divided into four different groups, namely cage control (A1), sham control (A2), 2.45-GHz EMR (B) and 2.45-GHz EMR+melatonin (C). Group B and C were exposed to 2.45-GHz EMR during 60 min day<sup>-1</sup> for 30 days. Lipid peroxidation levels were higher in Group B than in Group A1 and A2. Melatonin treatment prevented the increase in the lipid peroxidation induced by EMR. Also reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) levels in Group D were higher than that of exposure group. Vitamin A and E concentrations decreased in exposure group, and melatonin prevented the decrease in vitamin E levels. In conclusion, wireless (2.45 GHz) EMR caused oxidative damage in testis by increasing the levels of lipid peroxidation and decreasing in vitamin A and E levels. Melatonin supplementation prevented oxidative damage induced by EMR and also supported the antioxidant redox system in the testis.

### **Mortazavi S, Parsanezhad M, Kazempour M, Ghahramani P, Mortazavi A, Davari M. Male reproductive health under threat: Short term exposure to radiofrequency radiations emitted by common mobile jammers. J Hum Reprod Sci. 2013 Apr;6(2):124-**

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### 8. doi: 10.4103/0974-1208.117178.

BACKGROUND: Modern life prompted man to increasingly generate, transmit and use electricity that leads to exposure to different levels of electromagnetic fields (EMFs). Substantial evidence indicates that exposure to common sources of EMF such as mobile phones, laptops or wireless internet-connected laptops decreases human semen quality. In some countries, mobile jammers are occasionally used in offices, shrines, conference rooms and cinemas to block the signal. AIMS: To the best of our knowledge, this is the first study to investigate the effect of short term exposure of human sperm samples to radiofrequency (RF) radiations emitted by common mobile jammers. SUBJECTS AND METHODS: Fresh semen samples were collected by masturbation from 30 healthy donors who had referred to Infertility Treatment Center at the Mother and Child Hospital with their wives. Female problem was diagnosed as the reason for infertility in these couples. STATISTICAL ANALYSIS: T-test and analysis of variance were used to show statistical significance. RESULTS: The motility of sperm samples exposed to jammer RF radiation for 2 or 4 h were significantly lower than those of sham-exposed samples. These findings lead us to the conclusion that mobile jammers may significantly decrease sperm motility and the couples' chances of conception. CONCLUSION: Based on these results, it can be suggested that in countries that have not banned mobile jammer use, legislations should be urgently passed to restrict the use of these signal blocking devices in public or private places.

### **Meena R, Kumari K, Kumar J, Rajamani P, Verma HN, Kesari KK. Therapeutic approaches of melatonin in microwave radiations-induced oxidative stress-mediated toxicity on male fertility pattern of Wistar rats. Electromagn Biol Med. 2013 May 15. [Epub ahead of print]**

Abstract Microwave (MW) radiation produced by wireless telecommunications and a number of electrical devices used in household or in healthcare institutions may adversely affects the reproductive pattern. Present study aimed to investigate the protective effects of melatonin (is well known antioxidant that protects DNA, lipids and proteins from free radical damage) against oxidative stress-mediated testicular impairment due to long-term exposure of MWs. For this, 70-day-old male Wistar rats were divided into four groups (n = 6/group): Sham exposed, Melatonin (Mel) treated (2 mg/kg), **2.45 GHz MWs** exposed and MWs + Mel treated. Exposure took place in Plexiglas cages for 2 h a day for 45 days where, power density (0.21 mW/cm<sup>2</sup>) and specific absorption rate (SAR 0.14 W/Kg) were estimated. After the completion of exposure period, rats were sacrificed and various stress related parameters, that is LDH-X (lactate dehydrogenase isoenzyme) activity, xanthine oxidase (XO), ROS (reactive oxygen species), protein carbonyl content, DNA damage and MDA (malondialdehyde) were performed. Result shows that melatonin prevent oxidative damage biochemically by significant increase (p < 0.001) in the levels of testicular LDH-X, decreased (p < 0.001) levels of MDA and ROS in testis (p < 0.01). Meanwhile, it reversed the effects of MWs on XO, protein carbonyl content, sperm count, testosterone level and DNA fragmentation in testicular cells. These results concluded that the melatonin has strong antioxidative potential against MW induced oxidative stress mediated DNA damage in testicular cells.

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### Effects on Pregnancy

**Col-Araz N. Evaluation of factors affecting birth weight and preterm birth in southern Turkey. J Pak Med Assoc. 2013 Apr;63(4):459-62.**

**OBJECTIVE:** To identify factors affecting birth weight and pre-term birth, and to find associations with electromagnetic devices such as television, computer and mobile phones. **METHODS:** The study was conducted in Turkey at Gazintep University, Faculty of Medicine's Outpatient Clinic at the Paediatric Ward. It comprised 500 patients who presented at the clinic from May to December 2009. All participants were administered a questionnaire regarding their pregnancy history. SPSS 13 was used for statistical analysis. **RESULTS:** In the study, 90 (19%) patients had pre-term birth, and 64 (12.9%) had low birth weight rate Birth weight was positively correlated with maternal age and baseline maternal weight ( $r = 0.115$ ,  $p < 0.010$ ;  $r = 0.168$ ,  $p < 0.000$ , respectively). Pre-term birth and birth weight less than 2500g were more common in mothers with a history of disease during pregnancy ( $p < 0.046$  and  $p < 0.008$ , respectively). The habit of watching television and using mobile phones and computer by mothers did not demonstrate any relationship with birth weight. Mothers who used mobile phones or computers during pregnancy had more deliveries before 37 weeks ( $p < 0.018$ ,  $p < 0.034$ ; respectively). Similarly, pregnancy duration was shorter in mothers who used either mobile phone or computers during pregnancy ( $p < 0.005$ ,  $p < 0.048$ , respectively). **CONCLUSION:** Mobile phones and computers may have an effect on pre-term birth.

**Cabot E, Christ A, Bühlmann B, Zefferer M, Chavannes N, Bakker JF, van Rhoon GC, Kuster N. Quantification Of RF-exposure of the Fetus Using Anatomical CAD-Models in Three Different Gestational Stages. Health Phys. 107(5):369-381, 2014.**

This study analyzes the exposure of pregnant women and their fetuses in three different gestational stages to electromagnetic radiation in the radio frequency range in the near- and the far-field using numerical modeling. For far-field exposure, the power density at which the basic restriction for the whole body SAR is reached is calculated for both the mother and the fetus at whole body resonance and at frequencies between 450 MHz and **2,450 MHz**. The near-field exposure is assessed at 450 MHz, 900 MHz, and 2,450 MHz using half wavelength dipoles as generic sources located at different locations around the abdomen of the mother. For the investigated cases, the exposure of the mother is always below or on the order of magnitude of the basic restriction for exposure at the reference level. When applying the reference levels for the general public, the fetus is sufficiently shielded by the mother. However, the basic restrictions for general public exposure can be exceeded in the fetus when the mother is exposed at reference levels for occupational conditions. For plane wave exposure at occupational levels, the whole body SAR in the fetus can exceed the basic restrictions for the general population by at least 1.8 dB, and in the near-field of professional devices, the 10 g SAR can be non-compliant with the product standard for the general public by > 3.5 dB.

**Bellieni CV, Pinto I, Bogi A, Zoppetti N, Andreuccetti D, Buonocore G. Exposure to electromagnetic fields from laptop use of "laptop" computers. Arch Environ Occup**

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### **Health. 67(1):31-36, 2012.**

Portable computers are often used at tight contact with the body and therefore are called "laptop." The authors measured electromagnetic fields (EMFs) laptop computers produce and estimated the induced currents in the body, to assess the safety of laptop computers. The authors evaluated 5 commonly used laptop of different brands. They measured EMF exposure produced and, using validated computerized models, the authors exploited the data of one of the laptop computers (LTCs) to estimate the magnetic flux exposure of the user and of the fetus in the womb, when the laptop is used at close contact with the woman's womb. In the LTCs analyzed, EMF values (range 1.8-6  $\mu$ T) are within International Commission on Non-Ionizing Radiation (NIR) Protection (ICNIRP) guidelines, but are considerably higher than the values recommended by 2 recent guidelines for computer monitors magnetic field emissions, MPR II (Swedish Board for Technical Accreditation) and TCO (Swedish Confederation of Professional Employees), and those considered risky for tumor development. When close to the body, the laptop induces currents that are within 34.2% to 49.8% ICNIRP recommendations, but not negligible, to the adult's body and to the fetus (in pregnant women). On the contrary, the power supply induces strong intracorporal electric current densities in the fetus and in the adult subject, which are respectively 182-263% and 71-483% higher than ICNIRP 98 basic restriction recommended to prevent adverse health effects. Laptop is paradoxically an improper site for the use of a LTC, which consequently should be renamed to not induce customers towards an improper use.

**Çelik Ö, Kahya MC, Nazıroğlu M. Oxidative stress of brain and liver is increased by Wi-Fi (2.45GHz) exposure of rats during pregnancy and the development of newborns. J Chem Neuroanat. 2015 Oct 28. pii: S0891-0618(15)00074-5. doi:**

**10.1016/j.jchemneu.2015.10.005. [Epub ahead of print]**

An excessive production of reactive oxygen substances (ROS) and reduced antioxidant defence systems resulting from electromagnetic radiation (EMR) exposure may lead to oxidative brain and liver damage and degradation of membranes during pregnancy and development of rat pups. We aimed to investigate the effects of **Wi-Fi-induced EMR** on the brain and liver antioxidant redox systems in the rat during pregnancy and development. Sixteen pregnant rats and their 48 newborns were equally divided into control and EMR groups. The EMR groups were exposed to 2.45GHz EMR (1hour/day for 5 days/week) from pregnancy to 3 weeks of age. Brain cortex and liver samples were taken from the newborns between the first and third weeks. In the EMR groups, lipid peroxidation levels in the brain and liver were increased following EMR exposure; however, the glutathione peroxidase (GSH-Px) activity, and vitamin A, vitamin E and -carotene concentrations were decreased in the brain and liver. Glutathione (GSH) and vitamin C concentrations in the brain were also lower in the EMR groups than in the controls; however, their concentrations did not change in the liver. In conclusion, Wi-Fi-induced oxidative stress in the brain and liver of developing rats was the result of reduced GSH-Px, GSH and antioxidant vitamin concentrations. Moreover, the brain seemed to be more sensitive to oxidative injury compared to the liver in the development of newborns.

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**Yoshida Y, Seto T, Ohsu W, Hayashi S, Okazawa T, Nagase H, Yoshida M, Nakamura H, [Endocrine mechanism of placental circulatory disturbances induced by microwave in pregnant rats]. Nippon Sanka Fujinka Gakkai Zasshi 47(2):101-108, 1995. [Article in Japanese]**

Effects of microwaves on fetus and female genital organs remain to be elucidated. To demonstrate the placental circulatory disturbances induced by microwaves and to clarify the endocrine pathogenesis, placental blood flow and five endocrine indicators, i.e., corticosterone (CS), estradiol (E2), progesterone (P), prostaglandin E2 (PGE2) and prostaglandin F2 alpha (PGF2 alpha) were measured in rats exposed to whole-body microwaves with an intensity of 10 mW/cm<sup>2</sup> at a frequency of 2,450 MHz. The placental blood flow at 45-90 min after exposure was significantly decreased in the rats exposed to the microwaves. Placental blood flow at 15 and 30 min was increased by pretreatment with intraperitoneal administration of angiotensin II (All). In contrast, no significant change in placental blood flow was recognized in the All pretreated rats exposed to the microwaves. An increase in CS and a decrease in E2 were induced by the microwave exposure independent of pretreatment with All. P was increased by microwave exposure in the rats without pretreatment with All. PGE2 was not changed by the microwave exposure in the case of either nonpretreatment or pretreatment with All. PGF2 alpha was increased by the microwave exposure in the rats without pretreatment with All. The present results indicate that excessive exposure to whole-body microwave disorders pregnancy in terms of placental circulatory dysfunction. The data suggest the involvement of endocrine mechanisms in the decrease in placental blood flow which is induced via a detrimental effect of microwaves on PGF2 alpha and on pituitary functions such as general emotional stress.

**Cobb BL, Jauchem JR, Mason PA, Dooley MP, Miller SA, Ziriak JM, Murphy MR, Neural and behavioral teratological evaluation of rats exposed to ultra-wideband electromagnetic fields. Bioelectromagnetics 21(7):524-537, 2000.**

Several investigators have reported teratologic effects of electromagnetic field exposure. The majority of these studies have been performed at levels of exposure that could produce substantial heating of the animals. New and unique sources of ultra-wideband (UWB) electromagnetic fields are currently being developed and tested that are capable of generating nonthermalizing, high-peak-power, microwave(MW) pulses with nanosecond (ns) pulse widths, picosecond (ps) rise times, and an UWB of frequencies. Our study was performed to determine if teratological changes occur in rat pups as a result of (i) daily UWB exposures during gestation days 3-18, or (ii) as a result of both prenatal and postnatal (10 days) exposures. Dams were exposed either to (i) UWB irradiation from a Kentech system that emitted a 55 kV/m-peak E field, 300 ps rise time, and a 1.8 ns pulse width, average whole-body specific absorption rate 45 mW/kg; (ii) sham irradiation; or (iii) a positive control, lead (Pb) acetate solution (2000 mug/ml) continuously available in the drinking water. Offspring were examined for ontogeny (litter size, sex-ratios, weights, coat appearance, tooth-eruption, eye-opening, air-righting, and ultrasonic stress vocalizations). Male pups were tested on various

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performance measures (locomotor, water-maze learning, and fertilization capabilities). The pups postnatally exposed were examined for hippocampal morphology and operant behavior. Behavioral, functional, and morphological effects of UWB exposure were unremarkable with these exceptions: (i) The UWB-exposed pups emitted significantly more stress vocalizations than the sham-exposed pups; (ii) the medial-to-lateral length of the hippocampus was significantly longer in the UWB-exposed pups than in the sham-exposed animals; (iii) male offspring exposed in utero to UWB mated significantly less frequently than sham-exposed males, but when they did mate there was no difference in fertilization and offspring numbers from the sham group. There does not appear to be a unifying physiological or behavioral relationship among the significant differences observed, and our findings could be due to the expected spurious results derived when a large number of statistical comparisons are made. Significant effects found between our positive-controls and other groups on numerous measures indicates that the techniques used were sensitive enough to detect teratological effects.

## Cancer

**Richter E, Berman T, Ben-Michael E, Laster R, Westin JB, Cancer in Radar Technicians Exposed to Radiofrequency/Microwave Radiation: Sentinel Episodes. Int J Occup Environ Health 6(3):187-193, 2000.**

Controversy exists concerning the health risks from exposures to radiofrequency/microwave irradiation (RF/MW). The authors report exposure-effect relationships in sentinel patients and their co-workers, who were technicians with high levels of exposure to RF/MW radiation. Information about exposures of patients with sentinel tumors was obtained from interviews, medical records, and technical sources. One patient was a member of a cohort of 25 workers with six tumors. The authors estimated relative risks for cancer in this group and latency periods for a larger group of self-reported individuals. Index patients with melanoma of the eye, testicular cancer, nasopharyngioma, non-Hodgkin's lymphoma, and breast cancer were in the 20-37-year age group. Information about work conditions suggested prolonged exposures to high levels of RF/MW radiation that produced risks for the entire body. Clusters involved many different types of tumors. Latency periods were extremely brief in index patients and a larger self-reported group. The findings suggest that young persons exposed to high levels of RF/MW radiation for long periods in settings where preventive measures were lax were at increased risk for cancer. Very short latency periods suggest high risks from high-level exposures. Calculations derived from a linear model of dose-response suggest the need to prevent exposures in the range of 10-100  $\mu\text{W}/\text{cm}^2$ .

**Paulraj R, Behari J. The effect of low level continuous 2.45 GHz waves on enzymes of developing rat brain. Electromag Biol Med 21:221-231, 2002.**

The present work describes the effect of low level continuous microwaves (2.45 GHz) on developing rat brain. Some 35-day-old Wistar rats were used for this study. The animals were exposed 2 hr/day for 35 days at a power density of 0.34  $\text{mW}/\text{cm}^2$  [specific



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absorption rate (SAR), 0.1 W/kg] in a specially made anechoic chamber. After the exposure, the rats were sacrificed and the brain tissue was dissected out and used for various biochemical assays. A significant increase in calcium ion efflux and ornithine decarboxylase (ODC) activity was observed in the exposed group as compared to the control. Correspondingly, a significant decrease in the calcium-dependent protein kinase activity was observed. These results indicate that this type of radiation affects the membrane bound enzymes, which are associated with cell proliferation and differentiation, thereby pointing out its possible role as a tumor promoter.

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

In an attempt to determine whether electromagnetic field (EMF) exposure might lead to DNA damage, we exposed SnCl<sub>2</sub>-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.

**Singh N, Rudra N, Bansal P, Mathur R, Behari J, Nayar U, Poly ADP ribosylation as a possible mechanism of microwave--biointeraction. Indian J Physiol Pharmacol 38(3):181-184, 1994.**

Electromagnetic fields (EMFs) affect the metabolism of the body including the nervous, endocrine, cardiovascular, hematological as well as the reproductive system. EMFs are environmental pollutants, thus posing a health hazard which can cause steric changes in the molecule located at the cell surface. Microwaves are known to cause chromosomal aberrations and act as tumor promoters. The process involves a stream of signals from cell membrane to nucleus and other organelles. The present investigations aim to understand the mechanism of biological effects of **microwaves (2.45 GHz)**. The effect was studied on poly ADP-ribosylation, which is a post translational modification of chromatin protein catalysed by the enzyme poly ADPR polymerase using NAD<sup>+</sup> as the substrate. Poly ADP-ribosylation has been shown to be involved in several aspects of chromatin structure and function. Twenty-three days old rats weighing 42-48 gms were exposed at a microwave dose level of 1.0 mW/cm<sup>2</sup>. After exposure for sixty days the animals were sacrificed and an estimation of poly ADPR polymerase activity was undertaken in different organs of these animals. There was an increase of 20% in its activity in liver, 35% in testis, whereas brain showed a 53% decrease in diencephalon and 20% decrease in the cortex in the exposed animals as compared to their respective controls. There was no change in enzyme activity in spleen and kidney. This was accompanied by concomitant changes in NAD<sup>+</sup> levels. The above results may be cited as important events in carcinogenesis and tumor promotion related to microwave exposure

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and the signal transduction mechanism involved. The goal is to shed light on complex ecogenetic interactions leading to cancer modulation of gene expression by epigenetic mechanism.

**Balcer-Kubiczek EK, Harrison GH. Neoplastic transformation of C3H/10T1/2 cells following exposure to 120-Hz modulated 2.45-GHz microwaves and phorbol ester tumor promoter. Radiat Res 126(1):65-72, 1991.**

Some recent epidemiological studies have shown a positive association between cancer incidence and exposure to electromagnetic (EM) fields. Evidence from in vitro studies indicates that this effect could be due to synergistic interaction between EM fields and tumor promoters. However, no dose-response data related directly to carcinogenesis have been published. In this study, actively growing cultures of C3H/10T1/2 cells were exposed for 24 h to **2.45-GHz microwaves pulse-modulated** at 120 Hz. Conditions of EM-field exposure were designed to simulate low-field exposures (specific absorption rate 0.1, 1, or 4.4 W/kg; the corresponding peak amplitudes were electric field 18, 56, or 120 V/m, magnetic field 0.09, 0.27, or 0.56  $\mu$ T, respectively). In separate experiments, a 24-h EM-field exposure at 4.4 W/kg was preceded or followed by X irradiation at 0.5, 1, or 1.5 Gy. Cells were assayed for cell survival and neoplastic transformation with or without post-treatment administration of 0.1 micrograms/ml of 12-O-tetradecanoylphorbol-13-acetate (TPA) for the duration of the assay. The EM fields alone had no effect on cell survival or induction of neoplastic transformation. However, enhancement of transformation due to EM fields plus TPA was highly significant and ranged up to a level equivalent to that produced by 1.5 Gy of X rays. The frequency of neoplastic transformation was dependent on the level of EM exposure and was additive with doses of X rays given as a cocarcinogen.

**Zhu W, Zhang W, Li Y, Xu J, Luo J, Jiang Y, Lu X, Lü S. [Inhibitory effect of microwave radiation on proliferation of human pancreatic cancer JF305 cells and its mechanism]. Wei Sheng Yan Jiu. 42(6):1008-1011, 2013.**

**[Article in Chinese]**

**OBJECTIVE:** To investigate on the proliferation effect of different intensities **2450 MHz microwave radiation on human pancreatic cancer JF305 cells** and its possible mechanism. **METHODS:** JF305 cells were radiated by intensity of 2.5, 5.0, 10.0, 15.0 and 20.0 mW/cm<sup>2</sup> microwave for 20 min. The proliferation capacity of JF305 was measured by MTT assays, Annexin V-FITC and PI staining was used for detecting cell apoptosis. The activity of Caspase-3 was examined. The expressions of Caspase-3 and HSP 70 protein after the cell treatment with microwave were detected by Western blotting. **RESULTS:** After microwave radiation, the proliferation inhibition rates of JF305 cells were significantly higher compared with control group. Annexin V-FITC and PI staining result showed that microwave radiation could induce cell apoptosis. Caspase-3 increased after radiated by microwave, compared with control group (P < 0.05). Results of Western blotting showed that the expression of Caspase-3 and HSP 70 protein increased significantly in different dosage radiation group. **CONCLUSION:** Microwave radiation can

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inhibit the proliferation of JF305 cells, the possible mechanism may be related with inducing cell apoptosis by changing of stress level.

**Johnson EH, Chima SC, Muirhead DE, A cerebral primitive neuroectodermal tumor in a squirrel monkey (*Saimiri sciureus*). *J Med Primatol* 28(2):91-96, 1999.**

An adult squirrel monkey with a history of long-term exposure to microwave radiation was found at necropsy to have a malignant tumor of the right cerebral cortex. Gross examination revealed a mass with expanding borders in the right frontoparietal cortex with compression of the adjacent lateral ventricle. Microscopy revealed a tumor composed of sheets of moderate-sized cells, resembling an oligodendroglioma, with clear cytoplasm and central nuclei interrupted by delicate vasculature. Malignant features were present in the form of marked nuclear pleomorphism, frequent mitotic figures, and focal necrosis. A neuronal cell origin for this tumor was supported by immunohistochemical analysis, which revealed immunopositivity for neurofilament proteins and neuron-specific enolase. Staining for vimentin and glial fibrillary acid protein was negative, except in reactive astrocytes at the tumor margins and adjacent to intratumoral blood vessels. Antibody activity against Ki-67 antigen, a marker of rapidly proliferating tumor cells, and p53 oncoprotein was strongly positive, indicative of the aggressive and malignant nature of this tumor. The tumor was diagnosed as a cerebral primitive neuroectodermal tumor.

## Effects on Children

**Sangun O, Dundar B, Darici H, Comlekci S, Doguc DK, Celik S. The effects of long-term exposure to a 2450 MHz electromagnetic field on growth and pubertal development in female Wistar rats. *Electromagn Biol Med*. 2014 Jan 24. [Epub ahead of print]**

The aim of this study was to investigate the effects of a **2450 MHz electromagnetic field (EMF) (wireless internet frequency)** on the growth and development of female Wistar rats. The study was conducted on three groups of rats. The prenatal and postnatal groups were exposed to EMF 1 h/day beginning from intrauterine and postnatal periods, respectively. The third group was the sham-exposed group. Growth, nutrition and vaginal opening (VO) were regularly monitored. Serum and tissue specimens were collected at puberty. Histological examinations, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) measurements in ovary and brain tissues and also immunohistochemical staining of the hypothalamus were performed besides the determination of serum FSH, LH, E2 and IGF-1 values. Birth masses of the groups were similar ( $p > 0.05$ ). Mass gain per day was significantly lower and the puberty was significantly later in the prenatal group. Brain and ovary TOS and OSI values in the prenatal group were significantly increased ( $p < 0.05$ ) compared to the control group. Serum LH levels of the prenatal and postnatal groups were increased, although serum FSH, and E2 values did not differ among the groups ( $p > 0.05$ ). Histological examinations of the specimens revealed no statistically significant difference between the groups

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( $p > 0.05$ ). Exposure to 2450 MHz EMF, particularly in the prenatal period, resulted in postnatal growth restriction and delayed puberty in female Wistar rats. Increased TOS and OSI values in the brain and ovary tissues can be interpreted as a sign of chronic stress induced by EMF. This is the first longitudinal study which investigates the effects of EMF induced by wireless internet on pubertal development beside growth.

## Hormones

**Sinha RK. Chronic non-thermal exposure of modulated 2450 MHz microwave radiation alters thyroid hormones and behavior of male rats. *Int J Radiat Biol.* 84(6):505-513, 2008.**

Purpose: The purpose of this investigation was to analyze the effects of leakage microwave (**2450 MHz**) irradiation on thyroid hormones and behavior of male rats. Materials and methods: Experiments were carried out on two groups of male rats (exposure and control, respectively). Radio-immuno assay (RIA) methods were used for estimation of 3,5,3'-triiodothyronine (T(3)), thyroxine (T(4)) and thyrotrophin or thyroid stimulating hormone (TSH). The assessments of behavioral changes were performed in Open-Field (OF) and Elevated Plus-Maze (EPM) apparatuses. Results: Following chronic microwave exposure, rats were found hyperactive and aggressive on the 16th and 21st days. Behavioral changes in OF were analyzed and found to be significantly changed from controls ( $p < 0.05$ ) for immobilization, rearing and ambulation behavior. In EPM, rats showed increased activity with decreased time spent in the open arm and more time spent in the center on the 11th ( $p < 0.05$ ), 16th ( $p < 0.05$ ) and 21st day ( $p < 0.01$ ) after irradiation. Changes in behavioral parameters are also correlated with the trend of changes, compared to control animals, in hormonal blood levels of T3 (decreased on the 16th day,  $p < 0.05$  and 21st day,  $p < 0.01$ ) and T4 (increased on the 21st day,  $p < 0.05$ ). Conclusion: Low energy microwave irradiation may be harmful as it is sufficient to alter the levels of thyroid hormones as well as the emotional reactivity of the irradiated compared to control animals.

**Koveshnikova IV, Antipenko EN, [The participation of thyroid hormones in modifying the mutagenic effect of microwaves]. *Radiobiologiya* 31(1):147-149, 1991. [Article in Russian] The mutagenic effect of microwaves (2,450 or 2,750 MHz, 500 microW/cm<sup>2</sup>, 30 days, 7 h a day) increases with both low and high thyroid hormone content in rats. This indicates that normal functioning of the thyroid gland is an important condition for the stabilization of chromosome integrity under the effect of nonionizing radiation of microwaves.**

**Eskander EF, Estefan SF, Abd-Rabou AA. How does long term exposure to base stations and mobile phones affect human hormone profiles? *Clin Biochem.* 45(1-2):157-161, 2012**

**OBJECTIVES:** This study is concerned with assessing the role of exposure to radio frequency radiation (RFR) emitted either from mobiles or base stations and its relations

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with human's hormone profiles. **DESIGN AND METHODS:** All volunteers' samples were collected for hormonal analysis. **RESULTS:** This study showed significant decrease in volunteers' ACTH, cortisol, thyroid hormones, prolactin for young females, and testosterone levels. **CONCLUSION:** The present study revealed that high RFR effects on pituitary-adrenal axis.

## DNA Damage

**Sarkar S, Ali S, Behari J, Effect of low power microwave on the mouse genome: a direct DNA analysis. Mutat Res 320(1-2):141-147, 1994.**

The potential mutagenic effect of low power microwave at the DNA sequence level in the mouse genome was evaluated by direct DNA analysis. Animals were exposed to microwave at a power density of 1 mW/cm<sup>2</sup> for 2 h/day at a frequency of **2.45 GHz** over a period of 120, 150 and 200 days. HinfI digested DNA samples from testis and brain of control and exposed animals were hybridized with a synthetic oligo probe (OAT 36) comprising nine repeats of 5'-GACA-3'. As compared to control animals, band patterns in exposed animals were found to be distinctly altered in the range of 7-8 kb which was also substantiated by densitometric analysis. Though the mechanism of this rearrangement is not yet clear, the results obtained at the present dose are of significance. This dose, which has been set as the safe limit for general public exposure by the Non-Ionizing Radiation Committee of the International Radiation Protection Association, may imply a need for (re)evaluation of the mutagenic potential of microwaves at the prescribed safe limit for the personnel and people who are being exposed.

**Lee S, Johnson D, Dunbar K, Dong H, Ge X, Kim YC, Wing C, Jayathilaka N, Emmanuel N, Zhou CQ, Gerber HL, Tseng CC, Wang SM. 2.45GHz radiofrequency fields alter gene expression in cultured human cells. FEBS Lett. 579(21):4829-4836, 2005.**

The biological effect of radiofrequency (RF) fields remains controversial. We address this issue by examining whether RF fields can cause changes in gene expression. We used the pulsed RF fields at a frequency of **2.45GHz** that is commonly used in telecommunication to expose cultured human HL-60 cells. We used the serial analysis of gene expression (SAGE) method to measure the RF effect on gene expression at the genome level. We observed that 221 genes altered their expression after a 2-h exposure. The number of affected genes increased to 759 after a 6-h exposure. Functional classification of the affected genes reveals that apoptosis-related genes were among the upregulated ones and the cell cycle genes among the downregulated ones. We observed no significant increase in the expression of heat shock genes. These results indicate that the RF fields at 2.45GHz can alter gene expression in cultured human cells through non-thermal mechanism.

**Lai H, Singh NP, Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. Int J Radiat Biol 69(4):513-521, 1996.**

We investigated the effects of acute (2-h) exposure to pulsed (2-micros pulse width, 500

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pulses s(-1)) and continuous wave **2450-MHz radiofrequency** electromagnetic radiation on DNA strand breaks in brain cells of rat. The spatial averaged power density of the radiation was 2mW/cm<sup>2</sup>, which produced a whole-body average-specific absorption rate of 1.2W/kg. Single- and double-strand DNA breaks in individual brain cells were measured at 4h post-exposure using a microgel electrophoresis assay. An increase in both types of DNA strand breaks was observed after exposure to either the pulsed or continuous-wave radiation, No significant difference was observed between the effects of the two forms of radiation. We speculate that these effects could result from a direct effect of radiofrequency electromagnetic energy on DNA molecules and/or impairment of DNA-damage repair mechanisms in brain cells. Our data further support the results of earlier in vitro and in vivo studies showing effects of radiofrequency electromagnetic radiation on DNA.

### **Lai H, Singh NP, Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. Bioelectromagnetics 16(3):207-210, 1995.**

Levels of DNA single-strand break were assayed in brain cells from rats acutely exposed to low-intensity **2450 MHz microwaves** using an alkaline microgel electrophoresis method. Immediately after 2 h of exposure to pulsed (2 microseconds width, 500 pulses/s) microwaves, no significant effect was observed, whereas a dose rate-dependent [0.6 and 1.2 W/kg whole body specific absorption rate (SAR)] increase in DNA single-strand breaks was found in brain cells of rats at 4 h postexposure. Furthermore, in rats exposed for 2 h to continuous-wave 2450 MHz microwaves (SAR 1.2 W/kg), increases in brain cell DNA single-strand breaks were observed immediately as well as at 4 h postexposure.

### **Hatice Ş. Gürler, Birşen Bilgici, Ayşegül K. Akar, Leman Tomak & Abdülkerim Bedir. Increased DNA oxidation (8-OHdG) and protein oxidation (AOPP) by low level electromagnetic field (2.45 GHz) in rat brain and protective effect of garlic. International Journal of Radiation Biology. Posted online on August 4, 2014.**

*Purpose:* To investigate the oxidative damage and protective effect of garlic on rats exposed to low level of electromagnetic fields (EMF) at 2.45 GHz Microwave radiation (MWR). *Methods:* Thirty-six Wistar rats were divided into three groups. Group I was the control group and not exposed to EMF. Group II and III were exposed to low level EMF ( $3.68 \pm 0.36$  V/m) at **2.45 GHz MWR** for 1 hour/day for 30 consecutive days. Daily 500 mg/kg garlic was given to Group III during the study period. At the end of the study, thiobarbituric acid reactive substances (TBARS), advanced oxidation protein products (AOPP) and 8-hydroxydeoxyguanosine (8-OHdG) levels were investigated in brain tissue and blood samples. *Results:* Exposure to low level of EMF increased 8-OHdG level in both plasma and brain tissue whereas it increased AOPP level only in plasma. Garlic prevented the increase of 8-OHdG level in brain tissue and plasma AOPP levels. *Conclusions:* It may be concluded that low level EMF at 2.45 GHz MWR increases the DNA damage in both brain tissues and plasma of the rats whereas it increases protein oxidation only in plasma. It may also be argued that the use of garlic decreases these effects.

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**Maes A, Verschaeve L, Arroyo A, De Wagter C, Vercruyssen L, In vitro cytogenetic effects of 2450 MHz waves on human peripheral blood lymphocytes. *Bioelectromagnetics* 14(6):495-501, 1993.**

Cytogenetic analyses were performed on human peripheral blood lymphocytes exposed to **2450 MHz microwaves** during 30 and 120 min at a constant temperature of 36.1 degrees C (body temperature). The temperature was kept constant by means of a temperature probe put in the blood sample which gives feedback to a microcomputer that controls the microwave supply. We found a marked increase in the frequency of chromosome aberrations (including dicentric chromosomes and acentric fragments) and micronuclei. On the other hand the microwave exposure did not influence the cell kinetics nor the sister chromatid exchange (SCE) frequency.

**Lakshmi NK, Tiwari R, BhargavaSC, Ahuja YR Investigations on DNA damage and frequency of micronuclei in occupational exposure to electromagnetic fields (EMFs) emitted from video display terminals (VDTs). *Gen Mol Biol* 33(1): 154-158, 2010.**

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

**Fucic A, Garaj-Vrhovac V, Skara M, Dimitrovic B, X-rays, microwaves and vinyl chloride monomer: their clastogenic and aneugenic activity, using the micronucleus assay on human lymphocytes. *Mutat Res* 282(4):265-271, 1992.**

Chromosome aberration assays, sister-chromatid exchange techniques and micronucleus assays are commonly used methods for biomonitoring genetic material damaged by chemical or physical agents. On the other hand, their aneugenic activity, which can lead to hypoploidy and may also be associated with carcinogenesis, has not been thoroughly investigated. In our study we chose the micronucleus assay with a new mathematical approach to separate clastogenic from aneugenic activity of three well-known mutagens (vinyl chloride monomer, X-rays and microwaves) on the genome of human somatic cells. The comparison of frequencies of size distribution of micronuclei in the lymphocytes of humans exposed to each of these three mutagens showed that X-rays and microwaves

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were preferentially clastogens while vinyl chloride monomer showed aneugenic activity as well. Microwaves possess some mutagenic characteristics typical of chemical mutagens.

### Effects on the Heart

**Türker Y, Nazıroğlu M, Gümral N, Celik O, Saygın M, Cömlekçi S, Flores-Arce M. Selenium and L-carnitine reduce oxidative stress in the heart of rat induced by 2.45-GHz radiation from wireless devices. Biol Trace Elem Res. 143(3):1640-1650, 2011.**

The aim of this study was to investigate the possible protective role of selenium and L-carnitine on oxidative stress induced by **2.45-GHz** radiation in heart of rat. For this purpose, 30 male Wistar Albino rats were equally divided into five groups namely controls, sham controls, radiation-exposed rats, radiation-exposed rats treated with intraperitoneal injections of sodium selenite at a dose of 1.5 mg/kg/day, and radiation-exposed rats treated with intraperitoneal injections of L-carnitine at a dose of 1.5 mg/kg/day. Except for the controls and sham controls, the animals were exposed to 2.45-GHz radiation during 60 min/day for 28 days. The lipid peroxidation (LP) levels were higher in the radiation-exposed groups than in the control and sham control groups. The lipid peroxidation level in the irradiated animals treated with selenium and L-carnitine was lower than in those that were only exposed to 2.45-GHz radiation. The concentrations of vitamins A, C, and E were lower in the irradiated-only group relative to control and sham control groups, but their concentrations were increased in the groups treated with selenium- and L-carnitine. The activity of glutathione peroxidase was higher in the selenium-treated group than in the animals that were irradiated but received no treatment. The erythrocyte-reduced glutathione and  $\beta$ -carotene concentrations did not change in any of the groups. In conclusion, 2.45-GHz electromagnetic radiation caused oxidative stress in the heart of rats. There is an apparent protective effect of selenium and L-carnitine by inhibition of free radical formation and support of the antioxidant redox system.

**Seaman RL, DeHaan RL, Inter-beat intervals of cardiac-cell aggregates during exposure to 2.45 GHz CW, pulsed, and square-wave-modulated microwaves. Bioelectromagnetics 14(1):41-55, 1993.**

Inter-beat intervals of aggregated cardiac cells from chicken embryos were studied during 190 s exposures to **2.45 GHz microwaves** in an open-ended coaxial device. Averaged specific-absorption rates (SARs) and modulation conditions were 1.2-86.9 W/kg continuous-wave (CW), 1.2-12.2 W/kg pulse modulation (PW, duty cycle approximately 11%), and 12.0-43.5 W/kg square-wave modulation (duty cycle = 50%). The inter-beat interval decreased during microwave exposures at 42.0 W/kg and higher when CW or square-wave modulation was used, which is consistent with established effects of elevated temperatures. However, increases in the inter-beat interval during CW exposures at 1.2-12.2 W/kg, and decreases in the inter-beat interval after PW exposures at 8.4-12.2 W/kg, are not consistent with simple thermal effects. Analysis of variance



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indicated that SAR, modulation, and the modulation-SAR interaction were all significant factors in altering the inter-beat interval. The latter two factors indicated that the cardiac cells were affected by athermal as well as thermal effects of microwave exposure.

### Inflammation

**Trosic I. Multinucleated giant cell appearance after whole body microwave irradiation of rats. Int J Hyg Environ Health. 204(2-3):133-138, 2001.**

Multinucleated giant cells are common for some chronic inflammatory processes in the lung. These cells are formed by fusion of macrophages, but how the process relates to the kinetics of alveolar macrophage generation is not clear. This study investigated the influence of **2450 MHz microwave irradiation** on alveolar macrophage kinetics and formation of multinucleated giant cells after whole body irradiation of rats. The range of electromagnetic radiation was selected as 2450 MHz microwaves at a power density of 5-15 mW/cm<sup>2</sup>. 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. Free lung cell population was obtained by bronchoalveolar lavage. Cell response to the selected irradiation level was followed quantitatively, qualitatively and morphologically using standard laboratory methods. Total cell number retrieved by lavage slightly decreased in treated animals showing time- and dose-dependence. Cell viability did not significantly change in the irradiated animal group (G2) as compared with the control group (G1). Multinucleated cells significantly increased ( $p < 0.01$ ) in treated animals. The elevation of the 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, that is three and more nucleoli in a single cell, was frequently observed after 13 or 22 treatments. Binucleation and multinucleation of alveolar macrophages were sensitive time- and dose-dependent morphological indicators of pulmonary stress.

### Effects on Blood

**Cleary SF, Liu LM, Merchant RE, In vitro lymphocyte proliferation induced by radio-frequency electromagnetic radiation under isothermal conditions. Bioelectromagnetics 11(1):47-56, 1990.**

Whole human blood was exposed or sham-exposed in vitro for 2 h to 27 or **2,450 MHz radio-frequency electromagnetic (RF) radiation** under isothermal conditions (i.e., 37 +/- 0.2 degrees C). Immediately after exposure, mononuclear cells were separated from blood by Ficoll density-gradient centrifugation and cultured for 3 days at 37 degrees C with or without mitogenic stimulation by phytohemagglutinin (PHA). Lymphocyte proliferation was assayed at the end of the culture period by 6 h of pulse labeling with 3H-thymidine (3H-TdR). Exposure to radiation at either frequency at specific absorption rates (SARs) below 50 W/kg resulted in a dose-dependent, statistically significant

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increase of 3H-TdR uptake in PHA-activated or unstimulated lymphocytes. Exposure at 50 W/kg or higher suppressed 3H-TdR uptake relative to that of sham-exposed cells. There were no detectable effects of RF radiation on lymphocyte morphology or viability. Notwithstanding the characteristic temperature dependence of lymphocyte activation in vitro, the isothermal exposure conditions of this study warrant the conclusion that the biphasic, dose-dependent effects of the radiation on lymphocyte proliferation were not dependent on heating.

**Czerska EM, Elson EC, Davis CC, Swicord ML, Czerski P, Effects of continuous and pulsed 2450-MHz radiation on spontaneous lymphoblastoid transformation of human lymphocytes in vitro. *Bioelectromagnetics* 13(4):247-259, 1992.**

Normal human lymphocytes were isolated from the peripheral blood of healthy donors. One-ml samples containing (10<sup>6</sup>) cells in chromosome medium 1A were exposed for 5 days to conventional heating or to **continuous wave (CW)** or **pulsed wave (PW) 2450-MHz radiation** at non-heating (37 degrees C) and various heating levels (temperature increases of 0.5, 1.0, 1.5, and 2 degrees C). The pulsed exposures involved 1-microsecond pulses at pulse repetition frequencies from 100 to 1,000 pulses per second at the same average SAR levels as the CW exposures. Actual average SARs ranged to 12.3 W/kg. Following termination of the incubation period, spontaneous lymphoblastoid transformation was determined with an image analysis system. The results were compared among each of the experimental conditions and with sham-exposed cultures. At non-heating levels, CW exposure did not affect transformation. At heating levels both conventional and CW heating enhanced transformation to the same extent and correlate with the increases in incubation temperature. PW exposure enhanced transformation at non-heating levels. This finding is significant (P less than .002). At heating levels PW exposure enhanced transformation to a greater extent than did conventional or CW heating. This finding is significant at the .02 level. We conclude that PW 2450-MHz radiation acts differently on the process of lymphoblastoid transformation in vitro compared with CW 2450-MHz radiation at the same average SARs.

**Trosic I, Matausicpisl M, Radalj Z, Prlic I, Animal study on electromagnetic field biological potency. *Arh Hig Rada Toksikol* 50(1):5-11, 1999.**

This recent basic research study used an animal model protocol to assess specific biomarkers of the effect of non-ionising, non-thermal radiation (**2450 MHz microwave radiation** at 5-15 mW/cm<sup>2</sup>) on bone marrow, peripheral blood, and bronchoalveolar free cell populations. Of 40 male Wistar rats taken in the study, 20 animals of the experimental group were irradiated for 2 hours a day, 5 days a week, and subsequently killed on days 1, 8, 16, and 30 of the experiment. The remaining 20 rats served as control. All animals were previously intratracheally instilled with biologically inert microspheres to see the influence of irradiation on lung retention kinetics. The cell response to chosen electromagnetic irradiation was followed quantitatively and qualitatively using the standard laboratory methods. The results of peripheral blood cell response suggested a decreasing tendency in total leukocyte count and in relative lymphocyte count in the treated group. A slight increase was also observed in granulocyte count and in the

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absolute count of peripheral blood erythrocytes over control animals.

**Trosic I, Busljeta I, Pavicic I. Blood-forming system in rats after whole-body microwave exposure; reference to the lymphocytes. Toxicol Lett. 154(1-2):125-132, 2004.**

The influence of 2.45GHz microwave (RF/MW) irradiation on blood-forming cells after whole-body irradiation of rats was investigated. The exposures were conducted with a field power density of 5-10mW/cm(2), and whole-body average specific absorption rate (SAR) of 1-2W/kg. Four experimental subgroups were created and irradiated 2, 8, 15 or 30 days, for 2h a day, 7 days a week. Concurrent sham-exposed rats were also included in the study. The cell response was assessed by number and type of the bone marrow nuclear cells and peripheral blood white cells using standard laboratory methods. Significant decrease in lymphoblast count was obtained at 15 and 30th experimental day (P < 0.05), whereas other examined parameters did not significantly differed in comparison to the sham-exposed controls. The findings point out at stress response in blood-forming system in rats after selected microwave exposure, which could be considered rather as sign of adaptation than malfunction.

**Grigoriev YG, Grigoriev OA, Ivanov AA, Lyaginskaya AM, Merkulov AV, Shagina NB, Maltsev VN, Lévêque P, Ulanova AM, Osipov VA, Shafirkin AV. Confirmation studies of Soviet research on immunological effects of microwaves: Russian immunology results. Bioelectromagnetics. 31(8):589-602, 2010.**

This paper presents the results of a replication study performed to investigate earlier Soviet studies conducted between 1974 and 1991 that showed immunological and reproductive effects of long-term low-level exposure of rats to radiofrequency (RF) electromagnetic fields. The early studies were used, in part, for developing exposure standards for the USSR population and thus it was necessary to confirm the Russian findings. In the present study, the conditions of RF exposure were made as similar as possible to those in the earlier experiments: Wistar rats were exposed in the far field to **2450 MHz** continuous wave RF fields with an incident power density in the cages of 5 W/m<sup>2</sup> for 7 h/day, 5 days/week for a total of 30 days, resulting in a whole-body SAR of 0.16 W/kg. Effects of the exposure on immunological parameters in the brain and liver of rats were evaluated using the complement fixation test (CFT), as in the original studies, and an additional test, the more modern ELISA test. Our results, using CFT and ELISA, partly confirmed the findings of the early studies and indicated possible effects from non-thermal RF exposure on autoimmune processes. The RF exposure resulted in minor increases in formation of antibodies in brain tissue extract and the exposure did not appear to be pathological. In addition, a study was conducted to replicate a previous Soviet study on effects from the injection of blood serum from RF-exposed rats on pregnancy and foetal and offspring development of rats, using a similar animal model and protocol. Our results showed the same general trends as the earlier study, suggesting possible adverse effects of the blood serum from exposed rats on pregnancy and foetal development of intact rats, however, application of these results in developing exposure standards is limited.

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**Zotti-Martelli L, Peccatori M, Scarpato R, Migliore L, Induction of micronuclei in human lymphocytes exposed in vitro to microwave radiation. *Mutat Res* 472(1-2):51-58, 2000.**

Increasing applications of electromagnetic fields are of great concern with regard to public health. Several in vitro studies have been conducted to detect effects of microwave exposure on the genetic material leading to negative or questionable results. The micronucleus (MN) assay which is proved to be a useful tool for the detection of radiation exposure-induced cytogenetic damage was used in the present study to investigate the genotoxic effect of microwaves in human peripheral blood lymphocytes in vitro exposed in G(0) to electromagnetic fields with different frequencies (2.45 and 7.7GHz) and power density (10, 20 and 30mW/cm<sup>2</sup>) for three times (15, 30 and 60min). The results showed for both radiation frequencies an induction of micronuclei as compared to the control cultures at a power density of 30mW/cm<sup>2</sup> and after an exposure of 30 and 60min. Our study would indicate that microwaves are able to cause cytogenetic damage in human lymphocytes mainly for both high power density and long exposure time.

## Oxidative Stress

**Naziroğlu M, Ciğ B, Doğan S, Uğuz AC, Dilek S, Faouzi D. 2.45-Gz wireless devices induce oxidative stress and proliferation through cytosolic Ca<sup>2+</sup> influx in human leukemia cancer cells. *Int J Radiat Biol.* 88(6):449-456, 2012.**

PURPOSE: Electromagnetic radiation from wireless devices may affect biological systems by increasing free radicals. The present study was designed to determine the effects of 2.45 GHz radiation on the antioxidant redox system, calcium ion signaling, cell count and viability in human leukemia 60 cells. MATERIALS AND METHODS: Twelve cell cultures were equally divided into two main groups as controls (n = 6) and irradiated (n = 6) and then subdivided into four different subgroups depending on the duration of exposure, namely 1, 2, 12 and 24 hours. The samples were analyzed immediately after the experimental period. RESULTS: The extent of lipid peroxidation, cytosolic free Ca<sup>2+</sup> and cell numbers were higher in 2.45 GHz groups than in the controls. The increase of cytosolic free Ca<sup>2+</sup> concentrations was radiation time-dependent and was highest at 24-h exposure. The reduced glutathione, glutathione peroxidase, vitamin C and cell viability values did not show any changes in any of the experimental groups. 2-aminoethyl diphenylborinate inhibits Ca<sup>2+</sup> ions influx by blockage of the transient receptor potential melastatin 2. CONCLUSIONS: 2.45 GHz electromagnetic radiation appears to induce proliferative effects through oxidative stress and Ca<sup>2+</sup> influx although blocking of transient receptor potential melastatin 2 channels by 2-aminoethyl diphenylborinate seems to counteract the effects on Ca<sup>2+</sup> ions influx.

**Kim MJ, Rhee SJ. Green tea catechins protect rats from microwave-induced oxidative damage to heart tissue. *J Med Food.* 7(3):299-304, 2004.**

We investigated the effects of greentea catechin on oxidative damage in microwave-exposed rats. The microwave-exposed rats received one of three diets: catechin-free

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(MW-0C), 0.25% catechin (MW-0.25C), or 0.5% catechin (MW-0.5C). Rats were sacrificed 6 days after microwave irradiation (2.45 GHz, 15 minutes). Cytochrome P(450) levels in the MW-0C group was increased by 85% compared with normal, but was 11% and 14% lower in the MW-0.25C and MW-0.5C groups than in the MW-0C group. NADPH-cytochrome P(450) reductase activity in the MW-0C group was increased by 29%, compared with the normal group, but was significantly less in the MW-0.25C and MW-0.5C groups. Superoxide dismutase activity in the MW-0C group was decreased by 34%, compared with the normal group, but in the MW-0.25C and MW-0.5C groups was 19% and 25% higher. The activity of glutathione peroxidase in the MW-0C group was decreased by 28% but remained near normal with catechin supplements. Superoxide radical concentrations in the MW-0C group were increased by 35%, compared with the normal group. However, superoxide radicals in the MW-0.25C and MW-0.5C groups were 11% and 12% lower, respectively, compared with the MW-0C group. Microwave irradiation significantly increased levels of thiobarbituric acid-reactive substances, carbonyl values, and lipofuscin contents, but **greentea** catechin partially overcame the effects of the microwave irradiation. In conclusion, the mixed function oxidase system was activated, the formation of superoxide radical, lipid peroxide, oxidized protein, and lipofuscin was increased, and the antioxidative defense system was weakened in heart tissue of microwave-exposed rats, but the oxidative damage was significantly reduced by catechin supplementation.

**Aweda MA, Gbenebitse S, Meidinyo RO. Effects of 2.45 GHz microwave exposures on the peroxidation status in Wistar rats. Niger Postgrad Med J. 10(4):243-246, 2003.**

One of the consequences of exposures to microwave (MW) radiations is the enhanced production of free O<sub>2</sub>, free radicals, peroxides and superoxides. The effects on the **lipid peroxidation status (LPS)** of whole body irradiation of 120 Wistar rats with **2.45 GHz MW** at a power density of 6mWcm<sup>-2</sup> have been studied using the MW generator model ER6660E from Toshiba UK Ltd. The LPS in the rats was monitored for a period of 8 weeks post irradiation using thiobarbituric acid (TRA) method. The MW exposures caused an increase in the LPS from the mean control value of 4.18 x 10<sup>-6</sup>g 1<sup>-1</sup> to a maximum of 6.50 x 10<sup>-6</sup> g 1<sup>-1</sup> within the first 24 hrs, and then gradually reduced to control value after about a week. 1mg kg<sup>-1</sup> of ascorbic acid administered before irradiation caused a decrease in the LPS from the control value to a minimum of 2.86 x 10<sup>-6</sup>g 1<sup>-1</sup> within the first week. The value then gradually rose to a maximum of 3.96 x 10<sup>-6</sup>g 1<sup>-1</sup> within the monitoring period. 1 mg kg<sup>-1</sup> of α-tocopherol also administered before irradiation also caused a decrease in the LPS from the control value to a minimum of 2.10 x 10<sup>-6</sup> g 1<sup>-1</sup> within the first week. The value then gradually rose to a maximum of 3.94 x 10<sup>-6</sup> g 1<sup>-1</sup> within the monitoring period. The results obtained from this study demonstrate that MW exposures cause significant increase in the LPS and there are protective effects of the anti-oxidants ascorbic acid and alpha-tocopherol.

**Aynali G, Nazıroğlu M, Celik O, Doğan M, Yarıktaş M, Yasan H. Modulation of wireless (2.45 GHz)-induced oxidative toxicity in laryngotracheal mucosa of rat by melatonin. Eur Arch Otorhinolaryngol. 270(5):1695-1700, 2013.**

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It is well known that oxidative stress induces larynx cancer, although antioxidants induce modulator role on etiology of the cancer. It is well known that electromagnetic radiation (EMR) induces oxidative stress in different cell systems. The aim of this study was to investigate the possible protective role of melatonin on oxidative stress induced by **Wi-Fi (2.45 GHz)** EMR in laryngotracheal mucosa of rat. For this purpose, 32 male rats were equally categorized into four groups, namely controls, sham controls, EMR-exposed rats, EMR-exposed rats treated with melatonin at a dose of 10 mg/kg/day. Except for the controls and sham controls, the animals were exposed to 2.45 GHz radiation during 60 min/day for 28 days. The lipid peroxidation levels were significantly ( $p < 0.05$ ) higher in the radiation-exposed groups than in the control and sham control groups. The lipid peroxidation level in the irradiated animals treated with melatonin was significantly ( $p < 0.01$ ) lower than in those that were only exposed to Wi-Fi radiation. The activity of glutathione peroxidase was lower in the irradiated-only group relative to control and sham control groups but its activity was significantly ( $p < 0.05$ ) increased in the groups treated with melatonin. The reduced glutathione levels in the mucosa of rat did not change in the four groups. There is an apparent protective effect of melatonin on the Wi-Fi-induced oxidative stress in the laryngotracheal mucosa of rats by inhibition of free radical formation and support of the glutathione peroxidase antioxidant system.

**Ceyhan AM, Akkaya VB, Güleçol ŞC, Ceyhan BM, Özgüner F, Chen W. Protective effects of  $\beta$ -glucan against oxidative injury induced by 2.45-GHz electromagnetic radiation in the skin tissue of rats. Arch Dermatol Res. 304(7):521-527, 2012.**

In recent times, there is widespread use of 2.45-GHz irradiation-emitting devices in industrial, medical, military and domestic application. The aim of the present study was to investigate the effect of **2.45-GHz** electromagnetic radiation (EMR) on the oxidant and antioxidant status of skin and to examine the possible protective effects of  $\beta$ -glucans against the oxidative injury. Thirty-two male Wistar albino rats were randomly divided into four equal groups: control; sham exposed; EMR; and EMR +  $\beta$ -glucan. A 2.45-GHz EMR emitted device from the experimental exposure was applied to the EMR group and EMR +  $\beta$ -glucan group for 60 min daily, respectively, for 4 weeks.  $\beta$ -glucan was administered via gavage at a dose of 50 mg/kg/day before each exposure to radiation in the treatment group. The activities of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), as well as the concentration of malondialdehyde (MDA) were measured in tissue homogenates of the skin. Exposure to 2.45-GHz EMR caused a significant increase in MDA levels and CAT activity, while the activities of SOD and GSH-Px decreased in skin tissues. Systemic  $\beta$ -glucan significantly reversed the elevation of MDA levels and the reduction of SOD activities.  $\beta$ -glucan treatment also slightly enhanced the activity of CAT and prevented the depletion of GSH-Px activity caused by EMR, but not statistically significantly. The present study demonstrated the role of oxidative mechanisms in EMR-induced skin tissue damages and that  $\beta$ -glucan could ameliorate oxidative skin injury via its antioxidant properties.

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## Wellbeing

**Kowall B, Breckenkamp J, Heyer K, Berg-Beckhoff G. German wide cross sectional survey on health impacts of electromagnetic fields in the view of general practitioners. Int J Public Health.55(5):507-512, 2010.**

**OBJECTIVES:** The proportion of **general practitioners (GPs)** in Germany who assume health impacts of electromagnetic fields (EMF) is assessed. Moreover, factors associated with this risk perception are examined. **METHODS:** A 7% random sample was drawn from online lists of all the GPs working in Germany. 1,867 doctors received a long version of a self-administered postal questionnaire about EMF and health (response rate 23.3%), 928 doctors received a short version (response rate 49.1%). **RESULTS:** 37.3% of responders to the short and 57.5% of responders to the long questionnaire agreed "that there are persons whose health complaints are caused by EMF when legal limit values are met". A late responder analysis for the survey with the short questionnaire led to a still lower estimate of 29% for GPs believing in health-relevant effects of EMF. **CONCLUSION:** About a third of German GPs associate EMF with health complaints and thus deviate considerably from current scientific knowledge. To avoid a strong selection bias in the surveys of the perception of EMF risks, use of short questionnaires and late responder analysis are recommended.

**Kato Y, Johansson O. Reported functional impairments of electrohypersensitive Japanese: A questionnaire survey. Pathophysiology.19(2) 95-100, 2012.**

An increasing number of people worldwide complain that they have become **electromagnetic hypersensitive (EHS)**. We conducted a questionnaire survey of EHS persons in Japan. The aim was to identify electromagnetic fields (EMF) and plausible EMF sources that caused their symptoms. Postal questionnaires were distributed via a self-help group, and 75 participants (95% women) responded. Reported major complaints were "fatigue/tiredness" (85%), "headache", "concentration, memory, and thinking" difficulty (81%, respectively). Seventy-two per cent used some form of complementary/alternative therapy. The most plausible trigger of EHS onset was a mobile phone base station or personal handy-phone system (37%). Sixty-five percent experienced health problems to be due to the radiation from other passengers' mobile phones in trains or buses, and 12% reported that they could not use public transportation at all. Fifty-three percent had a job before the onset, but most had lost their work and/or experienced a decrease in income. Moreover, 85.3% had to take measures to protect themselves from EMF, such as moving to low EMF areas, or buying low EMF electric appliances. EHS persons were suffering not only from their symptoms, but also from economical and social problems.

**Bergdahl J, Tillberg A, Stenman E. Odontologic survey of referred patients with symptoms allegedly caused by electricity or visual display units. Acta Odontol Scand. 56(5):303-307, 1998.**

Twenty-eight consecutive patients with symptoms allegedly caused by electricity or

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visual display units were odontologically investigated according to a specially designed registration form including an anamnestic interview and a clinical protocol. The most common oral and general symptoms reported were burning mouth, craniomandibular dysfunction symptoms, skin complaints, and fatigue. Oral symptoms such as craniomandibular dysfunction and general symptoms such as eye complaints and dizziness scored highest on a visual analog scale regarding mean symptom intensity. The patients reported various numbers of medical diagnoses, such as allergic rhinitis or asthma and hypothyroidism. Various dental diseases were found; the most common were temporomandibular joint and masticatory muscle dysfunctions, lesions in the oral mucosa, and periodontal diseases. Urinary-Hg (U-Hg) analysis showed a mean U-Hg concentration of 8.5 nmol Hg/L urine, and none of the patients exceeded the limit of 50 nmol Hg/L urine. The U-Hg concentration was positively correlated with the number of amalgam fillings ( $P < 0.01$ ) and craniomandibular disorders ( $P < 0.05$ ). No or low secretion of the minor mucous glands was found in 43% of the patients. One patient showed hypersensitivity to gold and cobalt. The present study showed that various odontologic factors might be involved in some of these patients' suffering. Thus, it is important that professionals from other disciplines collaborate with dentistry if these patients are to be properly investigated.

## Whole Body

**Kühn S, Jennings W, Christ A, Kuster N. Assessment of induced radio-frequency electromagnetic fields in various anatomical human body models. *Phys Med Biol.* 54(4):875-890, 2009.**

The reference levels for testing compliance of human exposure with radio-frequency (RF) safety limits have been derived from very simplified models of the human. In order to validate these findings for anatomical models, we investigated the absorption characteristics for various anatomies ranging from 6 year old child to large adult male by numerical modeling. We address the exposure to plane-waves incident from all major six sides of the humans with two orthogonal polarizations each. Worst-case scattered field exposure scenarios have been constructed in order to test the implemented procedures of current in situ compliance measurement standards (spatial averaging versus peak search). Our findings suggest that the reference levels of current electromagnetic (EM) safety guidelines for demonstrating compliance as well as some of the current measurement standards are not consistent with the basic restrictions and need to be revised.

## Bone Marrow

**Vijayalaxmi, Frei, MR, Dusch, SJ, Guel, V, Meltz, ML, Jauchem, JR, Frequency of micronuclei in the peripheral blood and bone marrow of cancer-prone mice chronically exposed to 2450 MHz radiofrequency radiation. *Radiat Res* 147(4):495-500, 1997.**



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C3H/HeJ mice, which are prone to mammary tumors, were exposed for 20 h/day, 7 days/week, over 18 months to continuous-wave **2450 MHz radiofrequency (RF)** radiation in circularly polarized wave guides at a whole-body average specific absorption rate of 1.0 W/kg. Sham-exposed mice were used as controls. The positive controls were the sentinel mice treated with mitomycin C during the last 24 h before necropsy. At the end of the 18 months, all mice were necropsied. Peripheral blood and bone marrow smears were examined for the extent of genotoxicity as indicated by the presence of micronuclei in polychromatic erythrocytes (PCEs). The results indicate that the incidence of micronuclei/1,000 PCEs was not significantly different between groups exposed to RF radiation (62 mice) and sham-exposed groups (58 mice), and the mean frequencies were 4.5 +/- 1.23 and 4.0 +/- 1.12 in peripheral blood and 6.1 +/- 1.78 and 5.7 +/- 1.60 in bone marrow, respectively. In contrast, the positive controls (7 mice) showed a significantly elevated incidence of micronuclei/1,000 PCEs in peripheral blood and bone marrow, and the mean frequencies were 50.9 +/- 6.18 and 55.2 +/- 4.65, respectively. When the animals with mammary tumors were considered separately, there were no significant differences in the incidence of micronuclei/1,000 PCEs between the group exposed to RF radiation (12 mice) and the sham-exposed group (8 mice), and the mean frequencies were 4.6 +/- 1.03 and 4.1 +/- 0.89 in peripheral blood and 6.1 +/- 1.76 and 5.5 +/- 1.51 in bone marrow, respectively. Thus there was no evidence for genotoxicity in mice prone to mammary tumors that were exposed chronically to 2450 MHz RF radiation compared with sham-exposed controls. **A correction was published in a subsequent issue of the journal, stating that there was actually a significant increase in micronucleus formation in peripheral blood and bone marrow cells after chronic exposure to the radiofrequency radiation. "Vijayalaxmi, Frei, MR, Dusch, SJ, Guel, V, Meltz, ML, Jauchem, JR, Correction of an error in calculation in the article "Frequency of micronuclei in the peripheral blood and bone marrow of cancer-prone mice chronically exposed to 2450 MHz radiofrequency radiation" (Radiat. Res. 147, 495-500, 1997). Radiat Res 149(3):308, 1998 "**

**Busljeta I, Trosic I, Milkovic-Kraus S. Erythropoietic changes in rats after 2.45 GJz nonthermal irradiation. Int J Hyg Environ Health.207(6):549-554, 2004.**

The purpose of this study was to observe the erythropoietic changes in rats subchronically exposed to radiofrequency microwave (RF/MW) irradiation at nonthermal level. Adult male Wistar rats (N=40) were exposed to **2.45 GHz** continuous RF/MW fields for 2 hours daily, 7 days a week, at 5-10 mW/cm<sup>2</sup>. Exposed animals were divided into four subgroups (n=10 animals in each subgroup) in order to be irradiated for 2, 8, 15 and 30 days. Animals were sacrificed on the final irradiation day of each treated subgroup. Unexposed rats were used as control (N=24). Six animals were included into the each control subgroup. Bone marrow smears were examined to determine absolute counts of anuclear cells and erythropoietic precursor cells. The absolute erythrocyte count, haemoglobin and haematocrit values were observed in the peripheral blood by an automatic cell counter. The bone marrow cytogenetic analysis was accomplished by micronucleus (MN) tests. In the exposed animals erythrocyte count, haemoglobin and haematocrit were increased in peripheral blood on irradiation days 8 and 15.

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Concurrently, anuclear cells and erythropoietic precursor cells were significantly decreased ( $p < 0.05$ ) in the bone marrow on day 15, but micronucleated cells' frequency was increased. In the applied experimental condition, RF/MW radiation might cause disturbance in red cell maturation and proliferation, and induce micronucleus formation in erythropoietic cells.

### **Trosic I, Busljeta I, Kasuba V, Rozgaj R. Micronucleus induction after whole-body microwave irradiation of rats. *Mutat Res* 521(1-2):73-79, 2002.**

Adult male Wistar rats were exposed for 2h a day, 7 days a week for up to 30 days to continuous **2450MHz radiofrequency microwave** (rf/MW) radiation at a power density of 5-10mW/cm<sup>2</sup>. Sham-exposed rats were used as controls. After ether anesthesia, experimental animals were euthanized on the final irradiation day for each treated group. Peripheral blood smears were examined for the extent of genotoxicity, as indicated by the presence of micronuclei in polychromatic erythrocytes (PCEs). The results for the time-course of PCEs indicated significant differences ( $P < 0.05$ ) for the 2nd, the 8th and the 15th day between control and treated subgroups of animals. Increased influx of immature erythrocytes into the peripheral circulation at the beginning of the experiment revealed that the proliferation and maturation of nucleated erythropoietic cells were affected by exposure to the 2450MHz radiofrequency radiation. Such findings are indicators of radiation effects on bone-marrow erythropoiesis and their subsequent effects in circulating red cells. The incidence of micronuclei/1000 PCEs in peripheral blood was significantly increased ( $P < 0.05$ ) in the subgroup exposed to rf/MW radiation after eight irradiation treatments of 2h each in comparison with the sham-exposed control group. It is likely that an adaptive mechanism, both in erythrocytopoiesis and genotoxicity appeared in the rat experimental model during the subchronic irradiation treatment.

### **Trosic I, Busljeta I, Modlic B. Investigation of the genotoxic effect of microwave irradiation in rat bone marrow cells: in vivo exposure. *Mutagenesis*. 19(5):361-364, 2004.**

An in vivo mammalian cytogenetic test (the erythrocyte micronucleus assay) was used to investigate the extent of genetic damage in bone marrow red cells of rats exposed to radiofrequency/microwave (RF/MW) radiation. Wistar rats ( $n = 40$ ) were exposed to a **2.45 GHz** continuous RF/MW field for 2 h daily, 7 days a week, at a power density of 5-10 mW/cm<sup>2</sup>. The whole body average specific absorption rate (SARs) was calculated to be 1.25 +/- 0.36 (SE) W/kg. Four subgroups were irradiated for 4, 16, 30 and 60 h. Sham-exposed controls ( $n = 24$ ) were included in the study. The animals of each treated subgroup were killed on the final day of irradiation. Bone marrow smears were examined to determine the extent of genotoxicity after particular treatment times. The results were statistically evaluated using non-parametric Mann-Whitney and Kruskal-Wallis tests. In comparison with the sham-exposed subgroups, the findings of polychromatic erythrocytes (PCE) revealed significant differences ( $P < 0.05$ ) for experimental days 8 and 15. The frequency of micronucleated PCEs was also significantly increased on experimental day 15 ( $P < 0.05$ ). Pair-wise comparison of data obtained after 2, 8 and 30

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irradiation treatments did not reveal statistically significant differences between sham-exposed and treated subgroups. Under the applied experimental conditions the findings revealed a transient effect on proliferation and maturation of erythropoietic cells in the rat bone marrow and the sporadic appearance of micronucleated immature bone marrow red cells.

### **Trosic I, Busljeta I. Frequency of micronucleated erythrocytes in rat bone marrow exposure to 2.45 GHz radiation. *Physica Scripta T118: 168-170, 2005.***

Wistar rats were exposed to **2.45GHz** continuous, radiofrequency microwave (RF/MW) field 2 hours daily, 7 days weekly, at power density 5–10mW/cm<sup>2</sup>. Four subgroups were created in order to be irradiated 4, 16, 30 and 60 hours. Sham exposed controls were included in the study. Animals were euthanized on the final irradiation day of each treated subgroup. Bone marrow smears were examined to determine the extent of genotoxicity after the particular treatment time. Mann-Whitney test was used for statistical evaluation of data. In comparison to the sham exposed subgroups, the findings of polychromatic erythrocytes revealed significant differences for the 8th and 15th experimental day. Bone marrow erythrocyte maturation and/or proliferation initiated by subthermogenic RF/MW irradiation showed temporary disturbance. Thereafter, the frequency of micronucleated bone marrow red cells was significantly increased after 15 irradiation treatments. Comparison of micronucleus frequency data obtained after 2, 8 and 30 irradiation treatments did not reveal statistically significant differences between sham and treated subgroups. Under the applied experimental conditions, RF/MW irradiation initiates transitory cytogenetic effect manifested with micronucleus formation in erythropoietic cells.

### **Trosic I, Busljeta I. Erythropoietic dynamic equilibrium in rats maintained after microwave irradiation. *Exp Toxicol Pathol. 57(3):247-251, 2006.***

The aim of study was to define influence of radiofrequency microwave (RF/MW) radiation on erythropoiesis in rats. The kinetics of polychromatic erythrocytes (PCEs) and micronucleated (MN) PCEs in the bone marrow (BM) and peripheral blood (PB) of rats during the intermittent subchronic experiment was followed. Rats were exposed 2h/day, 7 days/week to RF/MW of **2.45GHz** and whole-body specific absorption rate (SAR) of 1.25+/-0.36W/kg. Control animals were included in the study. Each exposed and control group was killed on the final day of irradiation. Acridine-orange stained BM and blood smears were examined by fluorescence microscope. PCEs were obtained by inspection of 2000BM and 1000PB erythrocytes/slides. BMMNs and PBMNs frequency was obtained by observation of 1000PCEs/slides. BMPCEs were increased on day 8 and 15, and PBPCEs were elevated on days 2 and 8 (p<0.05). The BMMN frequency was increased on experimental day 15, and MNPCEs in the PB was increased on day 8 (p<0.05). Findings of BM and PBPCEs or MNPCEs declined nearly to the control values until the end of the experiment. Such findings are considered to be indicators of radiation effects on BM erythropoiesis consequently reflected in the PB. Rehabilitated dynamic haemopoietic equilibrium in rats by the end of experiment indicates possibility of activation adaptation process in rats to the selected experimental conditions of subchronic RF/MW exposure.

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### Effects on Insulin

**Salah MB, Abdelmelek H, Abderraba M. Effects of olive leaf extract on metabolic disorders and oxidative stress induced by 2.45 GHz WiFi signals. Environ Toxicol Pharmacol. 36(3):826-834, 2013.**

We investigated the effect of olive leaves extract administration on glucose metabolism and oxidative response in liver and kidneys of rats exposed to radio frequency (RF). **The exposure of rats to RF (2.45 GHz, 1h/day during 21 consecutive days) induced a diabetes-like status.** Moreover, RF decreased the activities of glutathione peroxidase (GPx, -33.33% and -49.40%) catalase (CAT, -43.39% and -39.62%) and the superoxide dismutase (SOD, -59.29% and -68.53%) and groups thiol amount (-62.68% and -34.85%), respectively in liver and kidneys. Indeed, exposure to RF increased the malondialdehyde (MDA, 29.69% and 51.35%) concentration respectively in liver and kidneys. Olive leaves extract administration (100 mg/kg, ip) in RF-exposed rats prevented glucose metabolism disruption and restored the activities of GPx, CAT and SOD and thiol group amount in liver and kidneys. Moreover, olive leaf extract administration was able to bring down the elevated levels of MDA in liver but not in kidneys. Our investigations suggested that RF exposure induced a diabetes-like status through alteration of oxidative response. Olive leaves extract was able to correct glucose metabolism disorder by minimizing oxidative stress induced by RF in rat tissues.

### Cell Effects

**Phelan AM, Lange DG, Kues HA, Luty GA, Modification of membrane fluidity in melanin-containing cells by low-level microwave radiation. Bioelectromagnetics13(2):131-146, 1992.**

The treatment of a B16 melanoma cell line with **2.45-GHz pulsed microwaves** (10 mW/cm<sup>2</sup>, 10-microseconds pulses at 100 pps, 1-h exposure; SAR, 0.2 W/kg) resulted in changes of membrane ordering as measured by EPR (electron paramagnetic resonance) reporter techniques. The changes reflected a shift from a more fluid-like phase to a more solid (ordered) state of the cell membrane. Exposure of artificially prepared liposomes that were reconstituted with melanin produced similar results. In contrast, neither B16 melanoma cells treated with 5-Bromo-2-Deoxyuridine (3 micrograms/day x 7 days) to render them amelanotic, nor liposomes prepared without melanin, exhibited the microwave-facilitated increase of ordering. Inhibition of the ordering was achieved by the use of superoxide dismutase (SOD), which strongly implicates oxygen radicals as a cause of the membrane changes. The data indicate that a significant, specific alteration of cell-membrane ordering followed microwave exposure. This alteration was unique to melanotic membranes and was due, at least in part, to the generation of oxygen radicals.

**Misa Agustiño MJ, Leiro JM, Jorge Mora MT, Rodríguez-González JA, Jorge Barreiro FJ, Ares-Pena FJ, López-Martín E. Electromagnetic fields at 2.45 GHz trigger changes in heat**

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**shock proteins 90 and 70 without altering apoptotic activity in rat thyroid gland. Biol Open. 1(9):831-838, 2012.**

Non-ionizing radiation at 2.45 GHz may modify the expression of genes that codify heat shock proteins (HSP) in the thyroid gland. Using the enzyme-linked immunosorbent assay (ELISA) technique, we studied levels of HSP-90 and HSP-70. We also used hematoxylin eosin to look for evidence of lesions in the gland and applied the DAPI technique of fluorescence to search for evidence of chromatin condensation and nuclear fragmentation in the thyroid cells of adult female Sprague-Dawley rats. Fifty-four rats were individually exposed for 30 min to 2.45 GHz radiation in a Gigahertz transverse electromagnetic (GTEM) cell at different levels of non-thermal specific absorption rate (SAR), which was calculated using the finite difference time domain (FDTD) technique. Ninety minutes after radiation, HSP-90 and HSP-70 had decreased significantly ( $P < 0.01$ ) after applying a SAR of  $0.046 \pm 1.10$  W/Kg or  $0.104 \pm 5.10(-3)$  W/Kg. Twenty-four hours after radiation, HSP-90 had partially recovered and HSP-70 had recovered completely. There were few indications of lesions in the glandular structure and signs of apoptosis were negative in all radiated animals. The results suggest that acute sub-thermal radiation at 2.45 GHz may alter levels of cellular stress in rat thyroid gland without initially altering their anti-apoptotic capacity.

**Lee SS, Kim HR, Kim MS, Park S, Yoon ES, Park SH, Kim DW. Influence of Smartphone Wi-Fi Signals on Adipose-Derived Stem Cells. J Craniofac Surg. 2014 Aug 5. [Epub ahead of print].**

The use of smartphones is expanding rapidly around the world, thus raising the concern of possible harmful effects of radiofrequency generated by smartphones. We hypothesized that Wi-Fi signals from smartphones may have harmful influence on adipose-derived stem cells (ASCs). An in vitro study was performed to assess the influence of Wi-Fi signals from smartphones. The ASCs were incubated under a smartphone connected to a **Wi-Fi network**, which was uploading files at a speed of 4.8 Mbps for 10 hours a day, for a total of 5 days. We constructed 2 kinds of control cells, one grown in 37°C and the other grown in 39°C. After 5 days of Wi-Fi exposure from the smartphone, the cells underwent cell proliferation assay, apoptosis assay, and flow cytometry analysis. Three growth factors, vascular endothelial growth factor, hepatocyte growth factor, and transforming growth factor- $\beta$ , were measured from ASC-conditioned media. Cell proliferation rate was higher in Wi-Fi-exposed cells and 39°C control cells compared with 37°C control cells. Apoptosis assay, flow cytometry analysis, and growth factor concentrations showed no remarkable differences among the 3 groups. We could not find any harmful effects of Wi-Fi electromagnetic signals from smartphones. The increased proliferation of ASCs under the smartphone, however, might be attributable to the thermal effect.

**Somogyi Z, Thurucz G, Kubasova T, Kovacs J, Szabo LD, Effects of modulated and continuous microwave irradiation on the morphology and cell surface negative charge of 3T3 fibroblasts. Scanning Microsc 5(4):1145-1155, 1991**

Mouse embryo 3T3 cells were irradiated with **2450 MHz** continuous and low frequency

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(16 Hz) square modulated waves of absorbed energy ranging from 0.0024 to 2.4 mW/g. The low frequency modulated microwave irradiation yielded more morphological cell changes than did the continuous microwave fields of the same intensity. The amount of free negative charges (cationized ferritin binding) on cell surfaces decreased following irradiation by modulated waves but remained unchanged under the effect of a continuous field of the same dose. Modulated waves of 0.024 mW/g dose increased the ruffling activity of the cells, and caused ultrastructural alteration in the cytoplasm. Similar effects were experienced by continuous waves at higher (0.24 and 2.4 mW/g) doses.

### **Cleary, SF, Du, Z, Cao, G, Liu, LM, McCrady, C, Effect of isothermal radiofrequency radiation on cytolytic T lymphocytes. FASEB J 10(8):913-919. 1996.**

Previous in vitro studies provide evidence that RF electromagnetic radiation modulates proliferation of human glioma, lymphocytes, and other cell types. The mechanism of RF radiation cell proliferation modulation, as well as mechanisms for effects on other cell physiologic endpoints, are not well understood. To obtain insight regarding interaction mechanisms, we investigated effects of RF radiation exposure on interleukin 2 (IL-2) - dependent proliferation of cytolytic T lymphocytes (CTL-2). After exposure to RF radiation in the presence or absence of IL-2 cells were cultured at various physiological concentrations of IL-2. Treatment effects on CTL-2 proliferation were determined by tritiated thymidine incorporation immediately or 24 h after exposure. Exposure to **2450 MHz** RIF radiation at specific absorption rates (SARs) of greater than 25 W/kg (induced E-field strength 98.4 V/m) induced a consistent, statistically significant reduction in CTL-2 proliferation, especially at low IL-2 concentrations. At lower SARs, 2450 MHz exposure increased CTL-2 proliferation immediately after exposure but reduced 24 h postexposure proliferation. RF radiation effects depended on the mitotic state of the cells at the time of exposure. Comparison of the effects of temperature elevation and RF radiation indicated significant qualitative and quantitative differences.

### **Obukhan KI, [The effect of ultrahigh-frequency radiation on adaptation thresholds and the damages to blood system cells]. Lik Sprava (7):71-73, 1998. [Article in Ukrainian]**

Cytologic investigations designed to study bone marrow, peripheral blood, spleen, and thymus of albino rats irradiated by an electromagnetic field, 2375, **2450**, and 3000 MEGS, revealed structural and functional changes in populations of megakaryocytes, immunocompetent cells as well as of undifferentiated cells, and of other types of cells that are dependent on the intensity of irradiation and permit establishing the probability-threshold levels of exposure taking account of reactions of perception and physiologic adaptation together with compensatory and regenerative processes and the injury sustained. It is shown that changes in bone marrow cells differentiation and reproduction rather than integral shifts in the peripheral blood that acquire the utmost significance. Subjected to a particular scrutiny in the paper are blast cells, which cells' repopulation was noted to be getting increased in low-intensity exposure as were disturbances in their mitosis pattern.

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**Margaritis LH, Manta AK, Kokkaliaris CD, Schiza D, Alimisis K, Barkas G, Georgiou E, Giannakopoulou O, Kollia I, Kontogianni G, Kourouzidou A, Myari A, Roumelioti F, Skouroliakou A, Sykioti V, Varda G, Xenos K, Ziomas K. Drosophila oogenesis as a biomarker responding to EMF sources. Electromagn Biol Med. 2013 Aug 5. [Epub ahead of print]**

The model biological organisms *Drosophila melanogaster* and *Drosophila virilis* have been utilized to assess effects on apoptotic cell death of follicles during oogenesis and reproductive capacity (fecundity) decline. A total of 280 different experiments were performed using newly emerged flies exposed for short time daily for 3-7 d to various EMF sources including: GSM 900/1800 MHz mobile phone, 1880-1900 MHz DECT wireless base, DECT wireless handset, mobile phone-DECT handset combination, **2.44 GHz wireless network (Wi-Fi)**, 2.44 GHz blue tooth, 92.8 MHz FM generator, 27.15 MHz baby monitor, 900 MHz CW RF generator and microwave oven's 2.44 GHz RF and magnetic field components. Mobile phone was used as a reference exposure system for evaluating factors considered very important in dosimetry extending our published work with *D. melanogaster* to the insect *D. virilis*. Distance from the emitting source, the exposure duration and the repeatability were examined. All EMF sources used created statistically significant effects regarding fecundity and cell death-apoptosis induction, even at very low intensity levels (0.3 V/m blue tooth radiation), well below ICNIRP's guidelines, suggesting that *Drosophila oogenesis* system is suitable to be used as a biomarker for exploring potential EMF bioactivity. Also, there is no linear cumulative effect when increasing the duration of exposure or using one EMF source after the other (i.e. mobile phone and DECT handset) at the specific conditions used. The role of the average versus the peak E-field values as measured by spectrum analyzers on the final effects is discussed.

**Marjanović AM, Pavičić I, Trošić I Biological indicators in response to radiofrequency/microwave exposure. Arh Hig Rada Toksikol. 2012 Sep 25;63(3):407-416, 2012.**

Over the years, due to rapid technological progress, radiation from man-made sources exceeded that of natural origin. There is a general concern regarding a growing number of appliances that use radiofrequency/ microwave (RF/MW) radiation with particular emphasis on mobile communication systems. Since nonthermal biological effects and mechanisms of RF/MW radiation are still uncertain, laboratory studies on animal models, tissues, cells, and cell free system are of extraordinary importance in bioelectromagnetic research. We believe that such investigations play a supporting role in public risk assessment. Cellular systems with the potential for a clear response to RF/MW exposures should be used in those studies. It is known that organism is a complex electrochemical system where processes of oxidation and reduction regularly occur. One of the plausible mechanisms is connected with generation of reactive oxygen species (ROS). Depending on concentration, ROS can have both beneficial and deleterious effects. Positive effects are connected with cell signalling, defence against infectious agents, and proliferative cell ability. On the other hand, excessive production, which overloads antioxidant defence mechanism, leads to cellular damage with serious potential for disease development.

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ROS concentration increase within the cell caused by RF/MW radiation seems to be a biologically relevant hypothesis to give clear insight into the RF/MW action at non-thermal level of radiation. In order to better understand the exact mechanism of action and its consequences, further research is needed in the field. We would like to present current knowledge on possible biological mechanisms of RF/MW actions.

**Peinnequin A, Piriou A, Mathieu J, Dabouis V, Sebbah C, Malabiau R, Debouzy JC Non-thermal effects of continuous 2.45 GHz microwaves on Fas-induced apoptosis in human Jurkat T-cell line. Bioelectrochemistry 51(2):157-161, 2000.**

Non-thermal effects of microwaves (MWs) are one of the main issues studied for revising standards. The effects of MW exposure on apoptosis at non-thermal level (48 h, **2.45 GHz**, 5 mW/cm<sup>2</sup>) have been studied. Results obtained assess non-thermal MW effects on Fas, but neither on butyrate- nor on ceramide-induced apoptosis in human Jurkat T-cell line. These data show that MW interacts either with Fas pathway between receptor and caspase-3 activation or on membrane proteins (i.e. Fas receptor or neurosphingomyelinase).

**Koyama S, Narita E, Suzuki Y, Taki M, Shinohara N, Miyakoshi J. Effect of a 2.45-GHz radiofrequency electromagnetic field on neutrophil chemotaxis and phagocytosis in differentiated human HL-60 cells. J Radiat Res. 2014 Sep 5. pii: rru075. [Epub ahead of print]**

The potential public health risks of radiofrequency (RF) fields have been discussed at length, especially with the use of mobile phones spreading extensively throughout the world. In order to investigate the properties of RF fields, we examined the effect of **2.45-GHz RF** fields at the specific absorption rate (SAR) of 2 and 10 W/kg for 4 and 24 h on neutrophil chemotaxis and phagocytosis in differentiated human HL-60 cells. Neutrophil chemotaxis was not affected by RF-field exposure, and subsequent phagocytosis was not affected either compared with that under sham exposure conditions. These studies demonstrated an initial immune response in the human body exposed to 2.45-GHz RF fields at the SAR of 2 W/kg, which is the maximum value recommended by the International Commission for Non-Ionizing Radiation Protection (ICNIRP) guidelines. The results of our experiments for RF-field exposure at an SAR under 10 W/kg showed very little or no effects on either chemotaxis or phagocytosis in neutrophil-like human HL-60 cells.

**Koyama S, Isozumi Y, Suzuki Y, Taki M, Miyakoshi J. Effects of 2.45-GHz electromagnetic fields with a wide range of SARs on micronucleus formation in CHO-K1 cells. ScientificWorldJournal 4 Suppl 2:29-40, 2004.**

There has been considerable discussion about the influence of high-frequency electromagnetic fields (HFEMF) on the human body. In particular, HFEMF used for mobile phones may be of great concern for human health. In order to investigate the properties of HFEMF, we have examined the effects of **2.45-GHz EMF** on micronucleus (MN) formation in Chinese hamster ovary (CHO)-K1 cells. MN formation is induced by chromosomal breakage or inhibition of spindles during cell division and leads to cell



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damage. We also examined the influence of heat on MN formation, since HFEMF exposure causes a rise in temperature. CHO-K1 cells were exposed to HFEMF for 2 h at average specific absorption rates (SARs) of 5, 10, 20, 50, 100, and 200 W/kg, and the effects on these cells were compared with those in sham-exposed control cells. The cells were also treated with bleomycin alone as a positive control or with combined treatment of HFEMF exposure and bleomycin. Heat treatment was performed at temperatures of 37, 38, 39, 40, 41, and 42 degrees C. The MN frequency in cells exposed to HFEMF at a SAR of lower than 50 W/kg did not differ from the sham-exposed controls, while those at SARs of 100 and 200 W/kg were significantly higher when compared with the sham-exposed controls. There was no apparent combined effect of HFEMF exposure and bleomycin treatment. On heat treatment at temperatures from 38-42 degrees C, the MN frequency increased in a temperature-dependent manner. We also showed that an increase in SAR causes a rise in temperature and this may be connected to the increase in MN formation generated by exposure to HFEMF.

**Inoue S, Motoda H, Koike Y, Kawamura K, Hiragami F, Kano Y. Microwave irradiation induces neurite outgrowth in PC12m3 cells via the p38 mitogen-activated protein kinase pathway. *Neurosci Lett.*432(1):35-39,2008.**

The increasing use of mobile phone communication has raised concerns about possible health hazard effects of microwave irradiation. We investigated damage and differentiation caused by microwave irradiation on drug-hypersensitive PC12 cell line (PC12m3). These cells showed enhancement of neurite outgrowth to various stimulants. The frequency of neurite outgrowth induced by 2.45GHz (200W) of microwave irradiation was approximately 10-fold greater than that of non-irradiated control cells. Incubation of PC12m3 cells with SB203580, a specific inhibitor of p38 MAPK, resulted in marked inhibition of the microwave radiation-induced neurite outgrowth. Also, activation of the transcription factor CREB induced by microwave irradiation was inhibited by SB203580. Heat shock treatment at 45 degrees C had a strong toxic effect on PC12m3 cells, whereas microwave treatment had no toxic effect on PC12m3 cells. These findings indicate that p38 MAPK is responsible for the survival of PC12m3 cells and might induce neurite outgrowth via a CREB signaling pathway when subjected to microwave irradiation.

## Effects on Behavior

**Thomas S, Heinrich S, von Kries R, Radon K. Exposure to radio-frequency electromagnetic fields and behavioural problems in Bavarian children and adolescents. *Eur J Epidemiol.* 25(2):135-141, 2010.**

Only few studies have so far investigated possible health effects of radio-frequency electromagnetic fields (RF EMF) in children and adolescents, although experts discuss a potential higher vulnerability to such fields. We aimed to investigate a possible association between measured exposure to RF EMF fields and behavioural problems in children and adolescents. 1,498 children and 1,524 adolescents were randomly selected

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from the population registries of four Bavarian (South of Germany) cities. During an Interview data on participants' mental health, socio-demographic characteristics and potential confounders were collected. Mental health behaviour was assessed using the German version of the Strengths and Difficulties Questionnaire (SDQ). Using a personal dosimeter, we obtained radio-frequency EMF exposure profiles over 24 h. Exposure levels over waking hours were expressed as mean percentage of the reference level. Overall, exposure to radiofrequency electromagnetic fields was far below the reference level. Seven percent of the children and 5% of the adolescents showed an abnormal mental behaviour. In the multiple logistic regression analyses measured exposure to RF fields in the highest quartile was associated to overall behavioural problems for adolescents (OR 2.2; 95% CI 1.1-4.5) but not for children (1.3; 0.7-2.6). These results are mainly driven by one subscale, as the results showed an association between exposure and conduct problems for adolescents (3.7; 1.6-8.4) and children (2.9; 1.4-5.9). As this is one of the first studies that investigated an association between exposure to mobile telecommunication networks and mental health behaviour more studies using personal dosimetry are warranted to confirm these findings.

**Yuan K, Qin W, Wang G, Zeng F, Zhao L, Yang X, Liu P, Liu J, Sun J, von Deneen KM, Gong Q, Liu Y, Tian J. Microstructure abnormalities in adolescents with internet addiction disorder. PLoS One.6(6):e20708, 2011.**

**BACKGROUND:** Recent studies suggest that internet addiction disorder (IAD) is associated with structural abnormalities in brain gray matter. However, few studies have investigated the effects of internet addiction on the microstructural integrity of major neuronal fiber pathways, and almost no studies have assessed the microstructural changes with the duration of internet addiction. **METHODOLOGY/PRINCIPAL FINDINGS:** We investigated the morphology of the brain in adolescents with IAD (N = 18) using an optimized voxel-based morphometry (VBM) technique, and studied the white matter fractional anisotropy (FA) changes using the diffusion tensor imaging (DTI) method, linking these brain structural measures to the duration of IAD. We provided evidences demonstrating the multiple structural changes of the brain in IAD subjects. VBM results indicated the decreased gray matter volume in the bilateral dorsolateral prefrontal cortex (DLPFC), the supplementary motor area (SMA), the orbitofrontal cortex (OFC), the cerebellum and the left rostral ACC (rACC). DTI analysis revealed the enhanced FA value of the left posterior limb of the internal capsule (PLIC) and reduced FA value in the white matter within the right parahippocampal gyrus (PHG). Gray matter volumes of the DLPFC, rACC, SMA, and white matter FA changes of the PLIC were significantly correlated with the duration of internet addiction in the adolescents with IAD.**CONCLUSIONS:** Our results suggested that long-term internet addiction would result in brain structural alterations, which probably contributed to chronic dysfunction in subjects with IAD. The current study may shed further light on the potential brain effects of IAD.

**Hirata A, Kojima M, Kawai H, Yamashiro Y, Watanabe S, Sasaki H, Fujiwara O. Acute dosimetry and estimation of threshold inducing behavioral signs of thermal stress in rabbits at 2.45-GHz microwave exposure. IEEE Trans Biomed Eng. 57(5):1234-42, 2010.**

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In the current international guidelines and standards for human exposure to microwaves, the basic restriction is determined by the whole-body average specific absorption rate (SAR). The basis for the guidelines is the adverse effect such as work stoppage in animals for whole-body average SARs above a certain level. Although it is known that absorbed microwave energy causes the behavioral sign of thermal stress, the relationship of whole-body average SAR with temperature/temperature elevation has not been sufficiently investigated. In the present study, we performed experiments on rabbits exposed to 2.45-GHz microwaves. A total of 24 measurements were conducted for power densities from approximately 100 to 1,000 W/m<sup>2</sup>. Our computational code for electromagnetic-thermal dosimetry was used to set the exposure time duration and incident power density. Our experimental results suggest that a core temperature elevation of 1°C is an estimate of the threshold inducing complex behavioral signs of microwave-induced thermal stress in rabbits for different whole-body average SARs and exposure time durations. The whole-body average SAR required for microwave-induced behavioral sign in rabbits was estimated as approximately 1.3 W/kg for 2.45-GHz microwaves.

**Duan L, Shan Y, Yu X, [Observations of changes in neurobehavioral functions in workers exposed to high-frequency radiation]. Chung Hua Yu Fang I Hsueh Tsa Chih 32(2):109-111, 1998.** [Article in Chinese]

OBJECTIVE: To study the effects of exposure to high-frequency radiation on neurobehavioral function of the exposed workers and its measurement in evaluating occupational hazards caused by it. METHODS: Four neurobehavioral functions were tested for the workers exposed to high-frequency radiation with Neurobehavioral Core Tests Battery recommended by WHO. RESULTS: Scores for various indicators in exposed workers were significantly lower than those in controls, and correlated to the detection of neurasthenia in the exposed workers, to certain extent. CONCLUSION: Changes in neurobehavioral function in workers exposed to high-frequency radiation can reflect its important adverse effects.

## Immune Function

**Li CY, Liao MH, Lin CW, Tsai WS, Huang CC, Tang TK. Inhibitory Effects of Microwave Radiation on LPS-Induced NFκB Expression in THP-1 Monocytes. Chin J Physiol. 55(6):421-427, 2012.**

Microwave radiations can be encountered regularly in daily lives. When WHO announced that microwave radiations were a kind of environmental energy which interfere with the physiological functions of the human body, great concerns have been raised over the damages microwave frequencies can do to human physiology. The immunological performance and the activities of the cellular inflammatory factor NFκB have been closely related in monocyte. Due to the effect of phorbol 12-myristate 13-acetate (PMA) on THP-1 monocytes, THP-1 monocytes would differentiate into macrophages and would then react with lipopolysaccharides (LPS), and the amount of NFκB increased in the THP-

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1 monocytes. Expression of cytokine is affected when cells are exposed to a frequency of **2450 MHz** and at 900 W. Thus, in our experiments, an observation was made when THP-1 monocytes were stimulated with PMA and LPS to differentiate into macrophage, the amount of NFκB in cells increased exponentially, and the levels of NFκB expression were decreased by the exposure of microwave radiation. In conclusion, microwave radiations were found to inhibit the activity functions of THP-1 monocytes stimulated with PMA and LPS.

**Elekes, E, Thuroczy, G, Szabo, LD, Effect on the immune system of mice exposed chronically to 50 Hz amplitude-modulated 2.45 GHz microwaves. *Bioelectromagnetics* 17(3):246-248, 1996.**

The effect of continuous (CW; **2.45 GHz** carrier frequency) or amplitude-modulated (AM; 50 Hz square wave) microwave radiation on the immune response was tested. CW exposures (6 days, 3 h/day) induced elevations of the number of antibody-producing cells in the spleen of male Balb/c mice (+37%). AM microwave exposure induced elevation of the spleen index (+15%) and antibody-producing cell number (+55%) in the spleen of male mice. No changes were observed in female mice. It is concluded that both types of exposure conditions induced moderate elevation of antibody production only in male mice.

## Effects on Protein

**George DF, Bilek MM, McKenzie DR. Non-thermal effects in the microwave induced unfolding of proteins observed by chaperone binding. *Bioelectromagnetics* 29(4):324-330, 2008.**

We study the effect of microwaves at **2,450 MHz** on protein unfolding using surface plasmon resonance sensing. Our experimental method makes use of the fact that unfolding proteins tend to bind to chaperones on their unfolding pathway and this attachment is readily monitored by surface plasmon resonance. We use the protein citrate synthase (CS) for this study as it shows strong binding to the chaperone alpha crystallin when stressed by exposure to excess temperature. The results of microwave heating are compared with the effect of ambient heating and a combination of ambient and microwave heating to the same final temperature. We study the temperature distributions during the heating process. We show that microwaves cause a significantly higher degree of unfolding than conventional thermal stress for protein solutions heated to the same maximum temperature.

## Electromagnetic Hypersensitivity

**Nordin S, Neely G, Olsson D, Sandström M. Odor and Noise Intolerance in Persons with Self-Reported Electromagnetic Hypersensitivity. *Int J Environ Res Public Health*. 11(9):8794-8805, 2014.**

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Lack of confirmation of symptoms attributed to electromagnetic fields (EMF) and triggered by EMF exposure has highlighted the role of individual factors. Prior observations indicate intolerance to other types of environmental exposures among persons with electromagnetic hypersensitivity (EHS). This study assessed differences in odor and noise intolerance between persons with EHS and healthy controls by use of subscales and global measures of the Chemical Sensitivity Scale (CSS) and the Noise Sensitivity Scale (NSS). The EHS group scored significantly higher than the controls on all CSS and NSS scales. Correlation coefficients between CSS and NSS scores ranged from 0.60 to 0.65 across measures. The findings suggest an association between EHS and odor and noise intolerance, encouraging further investigation of individual factors for understanding EMF-related symptoms.

**Landgrebe M, Hauser S, Langguth B, Frick U, Hajak G, Eichhammer P. Altered cortical excitability in subjectively electrosensitive patients: results of a pilot study. J Psychosom Res. 62(3):283-288, 2007.**

**OBJECTIVE:** Hypersensitivity to electromagnetic fields is frequently claimed to be linked to a variety of unspecific somatic and/or neuropsychological complaints. Whereas provocation studies often failed to demonstrate a causal relationship between electromagnetic field exposure and symptom formation, neurophysiological examinations highlight baseline deviations in people claiming to be electrosensitive.

**METHODS:** To elucidate a potential role of dysfunctional cortical regulations in mediating hypersensitivity to electromagnetic fields, cortical excitability parameters were measured by transcranial magnetic stimulation in subjectively electrosensitive patients (n=23) and two control groups (n=49) differing in their level of unspecific health complaints.

**RESULTS:** Electrosensitive patients showed reduced intracortical facilitation as compared to both control groups, while motor thresholds and intracortical inhibition were unaffected.

**CONCLUSIONS:** This pilot study gives additional evidence that altered central nervous system function may account for symptom manifestation in subjectively electrosensitive patients as has been postulated for several chronic multisymptom illnesses sharing a similar clustering of symptoms.

**Hagström M, Auranen J, Ekman R. Electromagnetic hypersensitive Finns: Symptoms, perceived sources and treatments, a questionnaire study. Pathophysiology. 2013 Apr 1. pii: S0928-4680(13)00002-3.**

The aim was to analyze the subjective experiences of Finns who describe themselves as suffering from electromagnetic hypersensitivity (EHS), their symptoms, self-perceived sources of the health complaints and the effectiveness of medical and complementary alternative therapies. A total of 395 questionnaires were mailed to self-diagnosed EHS persons. Of the participants 345 belonged to a Finnish self-help group and 50 came from outside of the group. The return rate of the study was 52.1% (206) and 80.9% of the respondents were women. Before the onset of EHS the most common health complaints were different types of allergies (35.1%, 68). During the acute phase of EHS the most common symptoms were nervous system related: "stress" (60.3%, 117), "sleeping disorders" (59.3%, 115) and "fatigue" (57.2%, 111). The sources that were most often

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reported to have triggered EHS were: "personal computers" (50.8%, 94) and "mobile phones" (47.0%, 87). The same devices were also claimed to cause the most symptoms during the acute phase. After the acute phase of EHS had passed, the respondents still claimed to react to these same digital and wireless devices while their reactions to basic electrical appliances were reduced. According to 76% of 157 respondents the reduction or avoidance of electromagnetic fields (EMF) helped in their full or partial recovery. The best treatments for EHS were given as: "dietary change" (69.4%), "nutritional supplements" (67.8%) and "increased physical exercise" (61.6%). The official treatment recommendations of psychotherapy (2.6%) and medication (-4.2%) were not significantly helpful. According to the present results the official treatment protocols should take better account the EHS person's own experiences. The avoidance of electromagnetic radiation and fields effectively removed or lessened the symptoms in EHS persons.

**Landgrebe M, Frick U, Hauser S, Langguth B, Rosner R, Hajak G, Eichhammer P.**

**Cognitive and neurobiological alterations in electromagnetic hypersensitive patients: results of a case-control study. Psychol Med. 38(12):1781-1791, 2008.**

BACKGROUND: Hypersensitivity to electromagnetic fields (EMF) is frequently claimed to be linked to a variety of non-specific somatic and neuropsychological complaints. Whereas provocation studies often failed to demonstrate a causal relationship between EMF exposure and symptom formation, recent studies point to a complex interplay of neurophysiological and cognitive alterations contributing to symptom manifestation in electromagnetic hypersensitive patients (EHS). However, these studies have examined only small sample sizes or have focused on selected aspects. Therefore this study examined in the largest sample of EHS EMF-specific cognitive correlates, discrimination ability and neurobiological parameters in order to get further insight into the pathophysiology of electromagnetic hypersensitivity. METHOD: In a case-control design 89 EHS and 107 age- and gender-matched controls were included in the study. Health status and EMF-specific cognitions were evaluated using standardized questionnaires. Perception thresholds following single transcranial magnetic stimulation (TMS) pulses to the dorsolateral prefrontal cortex were determined using a standardized blinded measurement protocol. Cortical excitability parameters were measured by TMS. RESULTS: Discrimination ability was significantly reduced in EHS (only 40% of the EHS but 60% of the controls felt no sensation under sham stimulation during the complete series), whereas the perception thresholds for real magnetic pulses were comparable in both groups (median 21% versus 24% of maximum pulse intensity). Intra-cortical facilitation was decreased in younger and increased in older EHS. In addition, typical EMF-related cognitions (aspects of rumination, symptom intolerance, vulnerability and stabilizing self-esteem) specifically differentiated EHS from their controls. CONCLUSIONS: These results demonstrate significant cognitive and neurobiological alterations pointing to a higher genuine individual vulnerability of electromagnetic hypersensitive patients.

**Landgrebe M, Frick U, Hauser S, Hajak G, Langguth B. Association of tinnitus and electromagnetic hypersensitivity: hints for a shared pathophysiology? PLoS One.4(3):e5026, 2009.**

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**BACKGROUND:** Tinnitus is a frequent condition with high morbidity and impairment in quality of life. The pathophysiology is still incompletely understood. Electromagnetic fields are discussed to be involved in the multi-factorial pathogenesis of tinnitus, but data proving this relationship are very limited. Potential health hazards of electromagnetic fields (EMF) have been under discussion for long. Especially, individuals claiming themselves to be electromagnetic hypersensitive suffer from a variety of unspecific symptoms, which they attribute to EMF-exposure. The aim of the study was to elucidate the relationship between EMF-exposure, electromagnetic hypersensitivity and tinnitus using a case-control design. **METHODOLOGY:** Tinnitus occurrence and tinnitus severity were assessed by questionnaires in 89 electromagnetic hypersensitive patients and 107 controls matched for age-, gender, living surroundings and workplace. Using a logistic regression approach, potential risk factors for the development of tinnitus were evaluated. **FINDINGS:** Tinnitus was significantly more frequent in the electromagnetic hypersensitive group (50.72% vs. 17.5%) whereas tinnitus duration and severity did not differ between groups. Electromagnetic hypersensitivity and tinnitus were independent risk factors for sleep disturbances. However, measures of individual EMF-exposure like e.g. cell phone use did not show any association with tinnitus. **CONCLUSIONS:** Our data indicate that tinnitus is associated with subjective electromagnetic hypersensitivity. An individual vulnerability probably due to an over activated cortical distress network seems to be responsible for, both, electromagnetic hypersensitivity and tinnitus. Hence, therapeutic efforts should focus on treatment strategies (e.g. cognitive behavioral therapy) aiming at normalizing this dysfunctional distress network.

## Effects on Critical Organs

**Phelan AM, Neubauer CF, Timm R, Neirenberg J, Lange DG, Athermal alterations in the structure of the canalicular membrane and ATPase activity induced by thermal levels of microwave radiation. Radiat Res 137(1):52-58, 1994.**

Sprague-Dawley rats (200-250 g) were exposed 30 min/day for 4 days to thermogenic levels (rectal temperature increase of 2.2 degrees C) of microwave radiation [2.45 GHz, 80 mW/cm<sup>2</sup>, continuous-wave mode (CW)] or to a radiant heat source resulting in an equivalent increase in body temperature of 2.2 degrees C. On the fifth day after the 4 days of exposure to microwave radiation, the animals were sacrificed and their livers removed. The canalicular membranes were isolated and evaluated for adenosinetriphosphatase (ATPase) activity, total fatty acid composition and membrane fluidity characteristics. Mg(++)-ATPase activity (V<sub>max</sub>) decreased by 48.5% in the group exposed to microwave radiation, with no significant change in the group exposed to radiant heat. The decrease in Mg(++)-ATPase was partially compensated by a concomitant increase in Na<sup>+</sup>/K<sup>+</sup>-ATPase activity (170% increase in V<sub>max</sub> over control) in animals exposed to microwave radiation, while no change occurred in the group exposed to radiant heat. This alteration in ATPase activity in the group exposed to microwave radiation is associated with a large decrease in the ratio of saturated to unsaturated fatty acids. Conversely, the group exposed to radiant heat had an increase in the ratio of

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saturated to unsaturated fatty acids. The most dramatic changes were found in the levels of arachidonic acid. Finally, the electron paramagnetic resonance (EPR) spin label technique used to measure the fluidity of the canalicular membranes of the animals in the three groups (sham, microwave radiation and radiant heat) indicated that the results were different in the three groups, reflecting the changes found in their fatty acid composition. The physiological response to "equivalent" thermal loads in rats is expressed differently for different types of energy sources. Possible mechanisms producing these divergent thermogenic responses are discussed.

**Lange DG, D'Antuono ME, Timm RR, Ishii TK, Fujimoto JM. Differential response of the permeability of the rat liver canalicular membrane to sucrose and mannitol following in vivo acute single and multiple exposures to microwave radiation (2.45 GHz) and radiant-energy thermal stress. Radiat Res 134(1):54-62, 1993.**

Both acute and chronic exposures to microwave radiation altered the function of the rat canalicular membrane. A single acute exposure to microwave radiation [80 mW/cm<sup>2</sup>, **2.45 GHz**, continuous wave, 30 min exposure (SAR approximately equal to 72 W/kg)] or a matched radiant-energy thermal load, both designed to raise core body temperature approximately 3 degrees C, decreased the permeability of the canalicular membrane of male Sprague-Dawley rats to sucrose. The change in canalicular membrane permeability was demonstrated by a significant increase in the percentage of [3H]sucrose recovered in bile following its administration by a segmented retrograde intrabiliary injection. Similar acute exposures to microwave and radiant-energy thermal sources produced no significant alterations in canalicular membrane permeability to [14C]mannitol. In both acute exposure protocols, a rapidly reversible increase in bile flow rate was observed. Four exposures (30 min/day x 4 days) to either microwave radiation (80 mW/cm<sup>2</sup>) or a matched radiant-energy thermal load resulted in a significant depression in bile flow rate at normothermic temperatures. Animals receiving multiple exposures to microwave radiation had significant decreases in canalicular membrane permeability to both [3H]sucrose and [14C]mannitol, while similar exposure to radiant-energy thermal load alone altered canalicular membrane permeability to [3H]sucrose. An examination of the hepatic clearance of sucrose and mannitol following acute microwave exposure demonstrated no significant differences. Thus acute single exposure to microwave and radiant-energy thermal loads produced similar alterations in canalicular membrane permeability. Conversely, multiple exposures produced nonreversible changes in bile flow rate and canalicular membrane permeability, with microwave exposure producing greater alterations in the function of the canalicular membrane than an equivalent radiant-energy thermal load.

**Somosy Z, Thuroczy G, Kovacs J, Effects of modulated and continuous microwave irradiation on pyroantimonate precipitable calcium content in junctional complex of mouse small intestine. Scanning Microsc 7(4):1255-1261, 1993.**

The pyroantimonate precipitable calcium content of intestinal epithelial cells was investigated in mice following total body irradiation with **2450 MHz** continuous and low frequency (16 Hz) square modulated waves. In the control animals the reaction products



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appeared in the intercellular space of adjacent cells including intermediate junctions and desmosomes and were absent in the area of tight junctions. Immediately after low frequency modulated microwave irradiation at 0.5 and 1 mW/cm<sup>2</sup> power densities, a rapid distribution of pyroantimonate precipitable calcium content was observed. The pyroantimonate deposits were located on the cytoplasmic side of lateral membrane, in the area of junctional complex, including tight junction, and in other parts of lateral plasma membrane. These changes were reversible and 24 hours after the irradiation the distribution of pyroantimonate deposits was similar to the control. Continuous waves with same energy not altered the distribution of precipitable calcium. We conclude the low frequency modulated microwave irradiation can modify the calcium distribution without heat effects.

## Effects on Sleep

**Liu H, Chen G, Pan Y, Chen Z, Jin W, Sun C, Chen C, Dong X, Chen K, Xu Z, Zhang S, Yu Y. (2014) Occupational Electromagnetic Field Exposures Associated with Sleep Quality: A Cross-Sectional Study. PLoS ONE 9(10): e110825. doi:10.1371/journal.pone.0110825.**

BACKGROUND: Exposure to electromagnetic field (EMF) emitted by mobile phone and other machineries concerns half the world's population and raises the problem of their impact on human health. The present study aims to explore the effects of electromagnetic field exposures on sleep quality and sleep duration among workers from electric power plant. METHODS: A cross-sectional study was conducted in an electric power plant of Zhejiang Province, China. A total of 854 participants were included in the final analysis. The detailed information of participants was obtained by trained investigators using a structured questionnaire, which including socio-demographic characteristics, lifestyle variables, sleep variables and electromagnetic exposures. Physical examination and venous blood collection were also carried out for every study subject. RESULTS: After grouping daily occupational electromagnetic exposure into three categories, subjects with long daily exposure time had a significantly higher risk of poor sleep quality in comparison to those with short daily exposure time. The adjusted odds ratios were 1.68 (95%CI: 1.18, 2.39) and 1.57 (95%CI: 1.10, 2.24) across tertiles. Additionally, among the subjects with long-term occupational exposure, the longer daily occupational time apparently increased the risk of poor sleep quality (OR (95%CI): 2.12 (1.23~3.66) in the second tertile; 1.83 (1.07~3.15) in the third tertile). There was no significant association of long-term occupational exposure duration, monthly electric fee or years of mobile-phone use with sleep quality or sleep duration. CONCLUSIONS: The findings showed that daily occupational EMF exposure was positively associated with poor sleep quality. It implies EMF exposure may damage human sleep quality rather than sleep duration.

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### Synergistic Health Effects – RF and Other Agents

**Zhang MB, He JL, Jin LF, Lu DQ. Study of low-intensity 2450-MHz microwave exposure enhancing the genotoxic effects of mitomycin C using micronucleus test and comet assay in vitro. Biomed Environ Sci 15(4):283-290, 2002.**

**OBJECTIVE:** To determine the interaction between **2450-MHz microwaves (MW) radiation and mitomycin C (MMC)**. **METHODS:** The synergistic genotoxic effects of low-intensity 2450-MHz microwave and MMC on human lymphocytes were studied using single cell gel electrophoresis (SCGE) assay (comet assay) and cytokinesis-blocked micronucleus (CBMN) test in vitro. The whole blood cells from a male donor and a female donor were either only exposed to 2450-MHz microwaves (5.0 mW/cm<sup>2</sup>) for 2 h or only exposed to MMC (0.0125 microgram/mL, 0.025 microgram/mL and 0.1 microgram/mL) for 24 h; and the samples were exposed to MMC for 24 h after exposure to MW for 2 h. **RESULTS:** In the comet assay, the comet lengths (29.1 microns and 25.9 microns) of MW were not significantly longer than those (26.3 microns and 24.1 microns) of controls ( $P > 0.05$ ). The comet lengths (57.4 microns, 68.9 microns, 91.4 microns, 150.6 microns, 71.7 microns, 100.1 microns, 145.1 microns) of 4 MMC groups were significantly longer than those of controls ( $P < 0.01$ ). The comet lengths (59.1 microns, 92.3 microns, 124.5 microns, 182.7 microns and 57.4 microns, 85.5 microns, 137.5 microns, 178.3 microns) of 4 MW plus MMC groups were significantly longer than those of controls too ( $P < 0.01$ ). The comet lengths of MW plus MMC groups were significantly longer than those of the corresponding MMC doses ( $P < 0.05$  or  $P < 0.01$ ) when the doses of MMC were  $>$  or  $=$  0.025 microgram/mL. In the CBMN, the micronucleated cell (MNC) rates of MW were 5@1000 and 6@1000, which showed no difference compared with those (4@1000 and 4@1000) of controls ( $P > 0.05$ ). The MNC rates of 4 MMC groups were 8@1000, 9@1000, 14@1000, 23@1000 and 8@1000, 8@1000, 16@1000, 30@1000 respectively. When the doses of MMC were  $>$  or  $=$  0.05 microgram/mL, MNC rates of MMC were higher than those of controls ( $P < 0.05$ ). MNC rates of 4 MW plus MMC groups were 12@1000, 13@1000, 20@1000, 32@1000 and 8@1000, 9@1000, 23@1000, 40@1000. When the doses of MMC were  $>$  or  $=$  0.05 microgram/mL, MNC rates of MW plus MMC groups were much higher than those of controls ( $P < 0.01$ ). MNC rates of 4 MW plus MMC groups were not significantly higher than those of the corresponding MMC doses. **CONCLUSION:** The low-intensity 2450-MHz microwave radiation can not induce DNA and chromosome damage, but can increase DNA damage effect induced by MMC in comet assay.

**Zhang MB, Jin LF, He JL, Hu J, Zheng W. [Effects of 2,450 MHz microwave on DNA damage induced by three chemical mutagens in vitro] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 21(4):266-269, 2003. [Article in Chinese]**

**OBJECTIVE:** To study the combined damage-effects of low-intensity **2,450 MHz** microwave (MW) with three chemical mutagens on human lymphocyte DNA. **METHODS:** DNA damage of lymphocytes exposed to microwave and(or) with chemical mutagens were observed at different incubation time (0 h or 21 h) with comet assay in vitro. Three

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combination-exposure ways of MW with chemicals were used: MW irradiation before chemical exposures, simultaneously exposed to MW and chemicals and MW irradiation after chemical exposures. The three chemical mutagens were mitomycin C (MMC, DNA crosslinker), bleomycin (BLM, radiometric agent), methyl methanesulfonate (MMS, alkylating agent). The exposure time of MW and chemical mutagens were 2 h and 3 h respectively. RESULTS: The differences of comet tail length between MW group and control group were not significant when lymphocytes were incubated for 0 h or 21 h ( $P > 0.05$ ). However, when lymphocytes were incubated for 21 h with 30.00 micro mol/L of MMC, the comet tail lengths of MW + MMC group, MW-MMC group and MMC + MW group were (18.00 +/- 5.96), (21.79 +/- 11.47) and (22.32 +/- 8.10) micro m respectively; while with 3.00 micro mol/L of MMC, the comet tail lengths were (8.99 +/- 3.75), (12.40 +/- 5.35) and (14.00 +/- 5.38) micro m respectively, which were significantly higher than those of corresponding MMC groups [(9.42 +/- 3.34) and (6.50 +/- 2.89) micro m,  $P < 0.01$  or  $P < 0.05$ ]. The DNA damage of MW plus BLM groups and MW plus MMS groups were not significantly different from the corresponding BLM and MMS groups ( $P < 0.05$ ). CONCLUSION: 2 450 MHz MW (5 mW/cm<sup>2</sup>) did not induce DNA damage directly, but could enhance the DNA damage effects induced by MMC. The synergistic effects of 2 450 MHz MW with BLM and MMS were not obvious.

**Nelson BK, Conover DL, Shaw PB, Snyder DL, Edwards RM, Interactions of radiofrequency radiation on 2-methoxyethanol teratogenicity in rats. *J Appl Toxicol* 17(1):31-39, 1997.**

Concurrent exposures to chemical and physical agents occur in the workplace; exposed workers include those involved with microelectronics industry, plastic sealers and electrosurgical units. Previous animal research indicates that hyperthermia induced by an elevation in ambient temperature can potentiate the toxicity and teratogenicity of some chemical agents. We previously demonstrated that combined exposure to radiofrequency (r.f.; 10 MHz) radiation, which also induces hyperthermia and is teratogenic to exposed animals, and the industrial solvent 2-methoxyethanol (2ME) produces enhanced teratogenicity in rats. A subsequent study replicated and extended that research by investigating the interactive dose-related teratogenicity of r.f. radiation (sham exposure or maintaining colonic temperatures at 42.0 degrees C for 0, 10, 20 or 30 min by r.f. radiation absorption) and 2ME (0, 75, 100, 125 or 150 mg/kg) on gestation days 9 or 13 of rats. The purpose of the present research is to determine the effects of r.f. radiation (sufficient to maintain colonic temperatures at 42.0 degrees C for 10 min) on a range of doses of 2ME (0, 20, 40, 60, 80, 100, 120 and 140 mg kg<sup>-1</sup>) administered on gestation day 13 of rats. Focusing on characterizing the dose-response pattern of interactions, this research seeks to determine the lowest interactive effect level. Day 20 fetuses were examined for external and skeletal malformations. The results are consistent with previous observations. Dose-related developmental toxicity was observed for 2ME both in the presence and absence of r.f. radiation. However, concurrent RF radiation exposure changed the shape of the dose-effect curve of 2ME. These data indicate that combined exposure effects should be considered when developing exposure guidelines and intervention strategies.

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**Nelson BK, Conover DL, Krieg EF Jr, Snyder DL, Edwards RM, Interactions of radiofrequency radiation-induced hyperthermia and 2-methoxyethanol teratogenicity in rats. *Bioelectromagnetics*. 18(5):349-359, 1997.**

Radiofrequency (RF) radiation is used in a variety of workplaces. In addition to RF radiation, many workers are concurrently exposed to numerous chemicals; exposed workers include those involved with the microelectronics industry, plastic sealers, and electrosurgical units. The developmental toxicity of RF radiation is associated with the degree and duration of hyperthermia induced by the exposure. Previous animal research indicates that hyperthermia induced by an elevation in ambient temperature can potentiate the toxicity and teratogenicity of some chemical agents. We previously demonstrated that combined exposure to RF radiation (10 MHz) and the industrial solvent, 2-methoxyethanol (2ME), produces enhanced teratogenicity in rats. The purpose of the present research is to determine the effects of varying the degree and duration of hyperthermia induced by RF radiation (sufficient to maintain colonic temperatures at control [38.5], 39.0, 40.0, or 41.0 degrees C for up to 6 h) and 2ME (100 mg/kg) administered on gestation day 13 of rats. Focusing on characterizing the dose-response pattern of interactions, this research seeks to determine the lowest interactive effect level. Day 20 fetuses were examined for external and skeletal malformations. The results are consistent with previous observations. Significant interactions were observed between 2ME and RF radiation sufficient to maintain colonic temperatures at 41 degrees C for 1 h, but no consistent interactions were seen at lower temperatures even with longer durations. These data indicate that combined exposure effects should be considered when developing both RF radiation and chemical exposure guidelines and intervention strategies.

## Effects on the Environment

**Soran ML, Stan M, Niinemets U, Copolovici L. Influence of microwave frequency electromagnetic radiation on terpene emission and content in aromatic plants. *J Plant Physiol*. 171(15):1436-1443, 2014**

Influence of environmental stress factors on both crop and wild plants of nutritional value is an important research topic. The past research has focused on rising temperatures, drought, soil salinity and toxicity, but the potential effects of increased environmental contamination by human-generated electromagnetic radiation on plants have little been studied. Here we studied the influence of microwave irradiation at bands corresponding to **wireless router (WLAN) and mobile devices (GSM)** on leaf anatomy, essential oil content and volatile emissions in *Petroselinum crispum*, *Apium graveolens* and *Anethum graveolens*. Microwave irradiation resulted in thinner cell walls, smaller chloroplasts and mitochondria, and enhanced emissions of volatile compounds, in particular, monoterpenes and green leaf volatiles (GLV). These effects

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were stronger for WLAN-frequency microwaves. Essential oil content was enhanced by GSM-frequency microwaves, but the effect of WLAN-frequency microwaves was inhibitory. There was a direct relationship between microwave-induced structural and chemical modifications of the three plant species studied. These data collectively demonstrate that human-generated microwave pollution can potentially constitute a stress to the plants.

### Miscellaneous Effects

**Ramundo-Orlando A, Liberti M, Mossa G, D'Inzeo G. Effects of 2.45 GHz microwave fields on liposomes entrapping glycoenzyme ascorbate oxidase: evidence for oligosaccharide side chain involvement. Bioelectromagnetics. 25(5):338-345, 2004.**

Previous observations reported by our group indicate that 2.45 GHz microwave fields at specific absorption rate (SAR) of 5.6 W/kg reduce the enzyme activity rate of ascorbate oxidase (AO) trapped in liposomes. In this study, we report dose-response studies on these AO containing liposomes irradiated at different SAR values (1.4, 2.8, 4.2, and 5.6 W/kg). No response was observed for SAR below 5.6 W/kg. Liposomes entrapping functional AO in its deglycated form (AO-D) were also used. In this case, no MW related enzyme activity changes were observed, demonstrating a direct involvement of oligosaccharide chains of AO. Furthermore, the catalytic properties of both AO and AO-D were not impaired by MW irradiation, neither in homogeneous solution nor loaded in liposomes, excluding possible changes in the conformation of enzyme as a mechanism. Our results suggest that the oligosaccharide chains of AO are critical to elicit the microwave observed effects on lipid membrane.

**Saalman E, Norden B, Arvidsson L, Hamnerius Y, Hojevik P, Connell KE, Kurucsev T, Effect of 2.45 GHz microwave radiation on permeability of unilamellar liposomes to 5(6)-carboxyfluorescein. Evidence of non-thermal leakage. Biochim Biophys Acta 1064(1):124-130, 1991.**

The influence of **2.45 GHz microwave radiation** on the membrane permeability of unilamellar liposomes was studied using the marker 5(6)-carboxyfluorescein trapped in phosphatidylcholine liposomes. The release of the fluorescent marker was followed by spectrofluorimetry after an exposure of 10 minutes to either microwave radiation or to heat alone of the liposome solutions. A significant increase of the permeability of carboxyfluorescein through the membrane was observed for the microwave-exposed samples compared to those exposed to normal heating only. Exposure to 2.45 GHz microwave radiation of liposomes has been previously found to produce increased membrane permeability as compared with heating. However, in contrast to previous studies, the observations reported here were made above the phase transition temperature of the lipid membrane. The experimental setup included monitoring of the temperature during microwave exposure simultaneously at several points in the solution volume using a fiberoptic thermometer. Possible mechanisms to explain the observations

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are discussed.

**Panagopoulos, D. J., Johansson O. & Carlo G.L. Polarization: A Key Difference between Man-made and Natural Electromagnetic Fields, in regard to Biological Activity. *Sci. Rep.* 5, 14914; doi: 10.1038/srep14914 (2015). Published online Oct 12, 2015.**

In the present study we analyze the role of polarization in the biological activity of Electromagnetic Fields (EMFs)/Electromagnetic Radiation (EMR). All types of man-made EMFs/EMR - in contrast to natural EMFs/EMR - are polarized. Polarized EMFs/EMR can have increased biological activity, due to: 1) Ability to produce constructive interference effects and amplify their intensities at many locations. 2) Ability to force all charged/polar molecules and especially free ions within and around all living cells to oscillate on parallel planes and in phase with the applied polarized field. Such ionic forced-oscillations exert additive electrostatic forces on the sensors of cell membrane electro-sensitive ion channels, resulting in their irregular gating and consequent disruption of the cell's electrochemical balance. These features render man-made EMFs/EMR more bioactive than natural non-ionizing EMFs/EMR. This explains the increasing number of biological effects discovered during the past few decades to be induced by man-made EMFs, in contrast to natural EMFs in the terrestrial environment which have always been present throughout evolution, although human exposure to the latter ones is normally of significantly higher intensities/energy and longer durations. Thus, polarization seems to be a trigger that significantly increases the probability for the initiation of biological/health effects.

**Nelson BK, Conover DL, Krieg EF Jr, Snyder DL, Edwards RM, Effect of environmental temperature on the interactive developmental toxicity of radiofrequency radiation and 2-methoxyethanol in rats. *Int Arch Occup Environ Health* 71(6):413-423, 1998.**

OBJECTIVE: This research was conducted to determine if altered environmental temperatures would affect the interactive developmental toxicity of radiofrequency (RF) radiation and the industrial solvent, 2-methoxyethanol (2ME). This is important because RF radiation is used in a variety of workplaces that have poorly controlled environmental temperatures, and many workers are concurrently exposed to various chemicals. Furthermore, we have previously demonstrated that combined exposure to RF radiation (10 MHz) and 2ME produces enhanced teratogenicity in rats. METHODS: RF radiation sufficient to maintain colonic temperatures at the control value (38degrees ), 39.0degrees or 40.0 degrees C for 2 or 4 h combined with either 0 or 100 mg/ kg 2ME at environmental temperatures of 18 degrees , 24 degrees and 30 degrees C (65 degrees , 75 degrees , and 85 degrees F) were given on gestation day 13 to Sprague-Dawley rats. Dams were killed on gestation day 20, and the fetuses were examined for external malformations. RESULTS AND CONCLUSIONS: Environmental temperature does affect the specific absorption rate (SAR) necessary to maintain a specific colonic temperature but does not affect the interactive developmental toxicity of RF radiation and 2ME in rats. These results, consistent with the literature, add to the evidence that the developmental toxicity of RF radiation (combined or alone) is associated with colonic temperature, not with SAR.

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**Nelson BK, Snyder DL, Shaw PB, Developmental toxicity interactions of methanol and radiofrequency radiation or 2-methoxyethanol in rats. *Int J Toxicol* 20(2):89-100, 2001.**

This research was undertaken to determine potential interactions among chemical and physical agents. Radiofrequency (RF) radiation is used in numerous workplaces, and many workers are concurrently exposed to RF radiation and various chemicals. The developmental toxicity of RF radiation is associated with the degree and duration of hyperthermia induced by the exposure. Previous animal research indicates that hyperthermia induced by an elevation in ambient temperature can potentiate the toxicity and teratogenicity of some chemical agents. We previously demonstrated that combined exposure to RF radiation (10 MHz) and the industrial solvent, 2-methoxyethanol (2ME), enhanced teratogenicity in rats. Interactions were noted at even the lowest levels of 2ME tested, but only at hyperthermic levels of RF radiation. The purpose of the present research is to investigate if the interactive effects noted for RF radiation and 2ME are unique to these agents, or if similar interactions might be seen with other chemicals. Because methanol is widely used as a solvent as well as fuel additive, and, at high levels, is teratogenic in animals, we selected methanol as a chemical to address generalizability. Based on the literature and our pilot studies, 0, 2, or 3 g/kg methanol (twice, at 6-hour intervals) were administered on gestation day 9 or 13 to groups of 10 Sprague-Dawley rats. Dams treated on day 9 were given methanol and exposed to RF radiation sufficient to maintain colonic temperature at 41 degrees C for 60 minutes (or sham). Those treated on day 13 were given methanol plus either 0 or 100 mg/kg 2ME. Because we observed that methanol produced hypothermia, some groups were given the initial dose of methanol concurrently with the RF or 2ME, and others were given the first dose of methanol 1.5 hours prior to RF or 2ME. Dams were sacrificed on gestation day 20, and the fetuses were examined for external malformations. The results indicate that RF radiation or methanol on day 9 increased the incidence of resorbed fetuses, but no interactive effects were observed. The resorptions were highest in groups given the experimental treatments 1.5 hours apart. The higher dose of methanol also reduced fetal weights. Administration of 2ME or methanol on day 13 increased the rate of malformations, and there was evidence of a positive interaction between 2ME and methanol. Fetal weights were reduced by 2ME and methanol alone, but no interaction was observed. Also, separation of the dosing with the teratogens did not affect the results. These results point out that interactions in developmental toxicology, such as those of RF radiation, 2ME, and methanol that we have studied, are complex, and such interactions cannot be fully understood or predicted without more research. It is important that combined exposure effects be considered when developing both physical agent and chemical agent exposure guidelines and intervention strategies.

**Solomentsev GY, English NJ, Mooney DA. Effects of external electromagnetic fields on the conformational sampling of a short alanine peptide. *J Comput Chem*. 33(9):917-923, 2012.**

Non-equilibrium molecular dynamics simulations of a solvated 21-residue polyalanine (A21) peptide, featuring a high propensity for helix formation, have been performed at

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300 K and 1 bar in the presence of external electromagnetic (e/m) fields in the microwave region (**2.45 GHz**) and an r.m.s. electric field intensity range of 0.01-0.05 V/Å. To investigate how the field presence affects transitions between the conformational states of a protein, we report 16 independent 40 ns-trajectories of A21 starting from both extended and fully folded states. We observe folding-behavior of the peptide consistent with prior simulation and experimental studies. The peptide displays a natural tendency to form stable elements of secondary structure which are stabilized by tertiary interactions with proximate regions of the peptide. Consistent with our earlier work, the presence of external e/m fields disrupts this behavior, involving a mechanism of localized dipolar alignment which serves to enhance intra-protein perturbations in hydrogen bonds (English, et al., J. Chem. Phys. 2010, 133, 091105), leading to more frequent transitions between shorter-lifetime states.

**Somosi Z, Thuroczy G, Koteles GJ, Kovacs J, Effects of modulated microwave and X-ray irradiation on the activity and distribution of Ca(2+)-ATPase in small intestine epithelial cells. Scanning Microsc 8(3):613-619; discussion 619-620, 1994.**

The distribution and activity of Ca(2+)-ATPase were investigated by histochemical methods in small intestine epithelial cells of mice following total body 2450 MHz low frequency (16 Hz) microwave and X-ray irradiation. In the control animals, enzyme activities were found in the brush border and on lateral membranes, including junctional areas of the cells. The enzyme activity of lateral membranes was inhibited by quercetin, a specific inhibitor of Ca(2+)-ATPase. Immediately after square modulated (16 Hz) 2450 MHz microwave irradiation at 1 mW/cm<sup>2</sup> power densities, we observed a decreased activity of Ca(2+)-ATPase on the lateral membrane regions. The X-ray irradiation (1 Gy) induced a similar decrease of Ca(2+)-ATPase activity which was reversible within 24 hours. "5 Gy" doses resulted in a decrease of enzyme activities on both apical and lateral membrane areas persisting up to 24 hours following irradiation.

**Takashima Y, Hirose H, Koyama S, Suzuki Y, Taki M, Miyakoshi J. Effects of continuous and intermittent exposure to RF fields with a wide range of SARs on cell growth, survival, and cell cycle distribution. Bioelectromagnetics.27(5):392-400, 2006.**

To examine the biological effects of radio frequency (RF) electromagnetic fields in vitro, we have examined the fundamental cellular responses, such as cell growth, survival, and cell cycle distribution, following exposure to a wide range of specific absorption rates (SAR). Furthermore, we compared the effects of continuous and intermittent exposure at high SARs. An RF electromagnetic field exposure unit operating at a frequency of **2.45 GHz** was used to expose cells to SARs from 0.05 to 1500 W/kg. When cells were exposed to a continuous RF field at SARs from 0.05 to 100 W/kg for 2 h, cellular growth rate, survival, and cell cycle distribution were not affected. At 200 W/kg, the cell growth rate was suppressed and cell survival decreased. When the cells were exposed to an intermittent RF field at 300 W/kg(pk), 900 W/kg(pk) and 1500 W/kg(pk) (100 W/kg(mean)), no significant differences were observed between these conditions and intermittent wave exposure at 100 W/kg. When cells were exposed to a SAR of 50 W/kg for 2 h, the temperature of the medium around cells rose to 39.1 degrees C, 100 W/kg



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exposure increased the temperature to 41.0 degrees C, and 200 W/kg exposure increased the temperature to 44.1 degrees C. Exposure to RF radiation results in heating of the medium, and the thermal effect depends on the mean SAR. Hence, these results suggest that the proliferation disorder is caused by the thermal effect.