20-1025 (Lead); 20-1138 (Consolidated)

UNITED STATES COURT OF APPEALS
FOR THE DISTRICT OF COLUMBIA CIRCUIT

ENVIRONMENTAL HEALTH TRUST; CONSUMERS FOR SAFE CELL PHONES; ELIZABETH BARRIS; THEODORA SCARATO

CHILDREN'S HEALTH DEFENSE; MICHELE HERTZ; PETRA BROKKEN; DR. DAVID O. CARPENTER; DR. PAUL DART; DR. TORIL H. JELTER; DR. ANN LEE; VIRGINIA FARVER, JENNIFER BARAN; PAUL STANLEY, M.Ed.
Petitioners

v.

FEDERAL COMMUNICATIONS COMMISSION;
UNITED STATES OF AMERICA
Respondents

Petition for Review of Order Issued by the
Federal Communications Commission

DEFERRED JOINT APPENDIX

VOLUME 10

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BioInitiative-Mechanisms of Harm; Percent Comparison Showing Effect vs No Effect, DNA (Comet Assay), 2017 and Free Radical (Oxidative Stress), 2019

Percent Comparison Showing Effect vs No Effect in Comet Assay and Free Radical (Oxidative Effects) Studies (RFR and Static Field/ELF-EMF)

BioInitiative Report Research Summaries Updates,

December 2017 and April, 2019

Chapter 6, Genotoxic Effects

RFR Comet Assay (December 2017 Update)

Of 76 total studies: (E= 49 (64%); NE= 27 (36%)

ELF EMF Comet Assay (December 2017 Update)

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

RFR - Free Radical (Oxidative Effect) April 19. 2019 Update

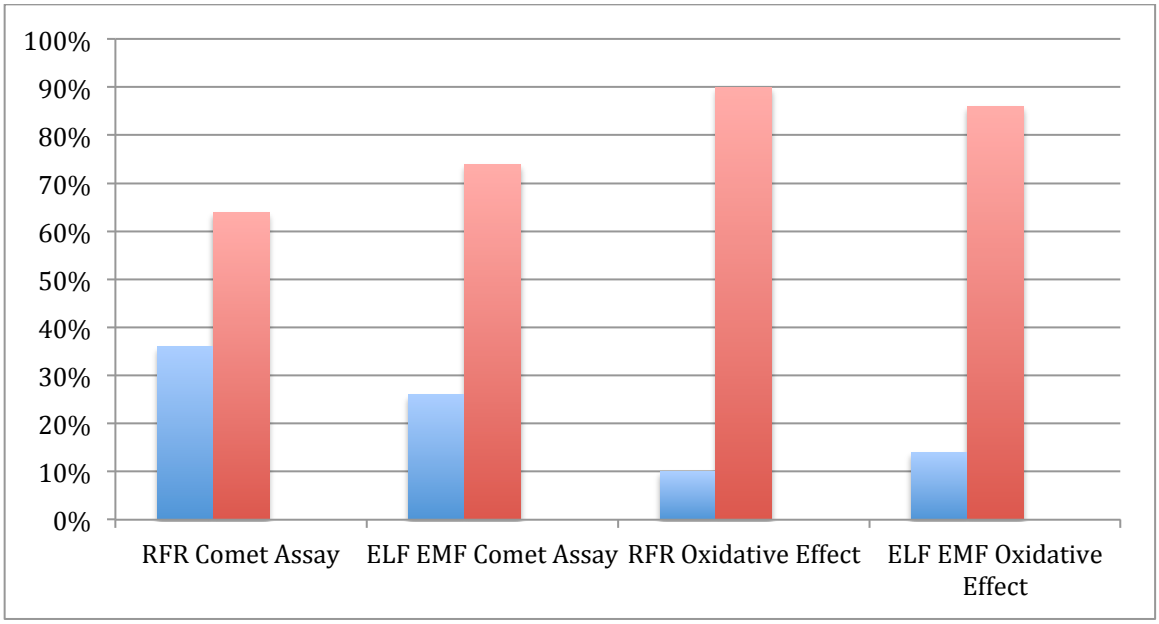
Of 225 total studies: (E=203 (90%); NE=22 (10%)

Static Field/ELF-EMF Free Radical (Oxidative Effect) April 19, 2019 Update

Of 229 total studies: **(E= 203** (89%)**; NE= 26** (**11**%)

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

Percent Comparison Showing Effect (Red) vs No Effect (Blue)



BioInitiative - Mechanisms of Harm; Research Summaries, DNA (Comet Assay) Studies; 76 Studies, 2017

April 1, 2017

RFR Comet Assay Studies

Of 76 total studies: **(E= 49 (64%); NE= 27 (36%)**

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

	E	NE	
in vitro	25	21	46
in vivo	24	6	30
	49 (64%)	27 (36%)	76

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

(NE) (VO) Akdag MZ, Dasdag S, Canturk F, Karabulut D, Caner Y, Adalier N. Does prolonged radiofrequency radiation emitted from Wi-Fi devices induce DNA damage in various tissues of rats? J Chem Neuroanat. 75(ptB):116-122, 2016.

Wireless Internet (Wi-Fi) providers have become essential in our daily lives, as wireless technology is evolving at a dizzying pace. Although there are different frequency generators, one of the most commonly used Wi-Fi devices are 2.4GHz frequency generators. These devices are heavily used in all areas of life but the effect of radiofrequency (RF) radiation emission on users is generally ignored. Yet, an increasing share of the public expresses concern on this issue. Therefore, this study intends to respond to the growing public concern. The purpose of this study is to reveal whether long term exposure of 2.4GHz frequency RF radiation will cause DNA damage of different tissues such as brain, kidney, liver, and skin tissue and testicular tissues of rats. The study was conducted on 16 adult male Wistar-Albino rats. The rats in the experimental group (n=8) were exposed to 2.4GHz frequency radiation for over a year. The rats in the sham control group (n=8) were subjected to the same experimental conditions except the Wi-Fi generator was turned off. After the exposure period was complete the possible DNA damage on the rat's brain, liver, kidney, skin, and testicular tissues was detected through the single cell gel electrophoresis assay (comet) method. The amount of DNA damage was measured as% tail DNA value. Based on the DNA damage results determined by the single cell gel electrophoresis (Comet) method, it was found that the% tail DNA values of the brain, kidney, liver, and skin tissues of the rats in the experimental group increased more than those in the control group. The increase of the DNA damage in all tissues was not significant ($p > 0.05$). However the increase of the DNA damage in rat testes tissue was significant ($p < 0.01$). In conclusion, long-

term exposure to 2.4GHz RF radiation (Wi-Fi) does not cause DNA damage of the organs investigated in this study except testes. The results of this study indicated that testes are more sensitive organ to RF radiation.

(E) (VI) Baohong Wang, Jiliang H, Lifen J, Deqiang L, Wei Z, Jianlin L, Hongping D. Studying the synergistic damage effects induced by 1.8 GHz radiofrequency field radiation (RFR) with four chemical mutagens on human lymphocyte DNA using comet assay in vitro. Mutat Res 578:149-57, 2005.

The aim of this investigation was to study the synergistic DNA damage effects in human lymphocytes induced by 1.8GHz radiofrequency field radiation (RFR, SAR of 3W/kg) with four chemical mutagens, i.e. mitomycin C (MMC, DNA crosslinker), bleomycin (BLM, radiomimetic agent), methyl methanesulfonate (MMS, alkylating agent), and 4-nitroquinoline-1-oxide (4NQO, UV-mimetic agent). The DNA damage of lymphocytes exposed to RFR and/or with chemical mutagens was detected at two incubation time (0 or 21h) after treatment with comet assay in vitro. Three combinative exposure ways were used. Cells were exposed to RFR and chemical mutagens for 2 and 3h, respectively. Tail length (TL) and tail moment (TM) were utilized as DNA damage indexes. The results showed no difference of DNA damage indexes between RFR group and control group at 0 and 21h incubation after exposure (P>0.05). There were significant difference of DNA damage indexes between MMC group and RFR+MMC co-exposure group at 0 and 21h incubation after treatment (P<0.01). Also the significant difference of DNA damage indexes between 4NQO group and RFR+4NQO co-exposure group at 0 and 21h incubation after treatment was observed (P<0.05 or P<0.01). The DNA damage in RFR+BLM co-exposure groups and RFR+MMS co-exposure groups was not significantly increased, as compared with corresponding BLM and MMS groups (P>0.05). The experimental results indicated 1.8GHz RFR (SAR, 3W/kg) for 2h did not induce the human lymphocyte DNA damage effects in vitro, but could enhance the human lymphocyte DNA damage effects induced by MMC and 4NQO. The synergistic DNA damage effects of 1.8GHz RFR with BLM or MMS were not obvious.

(E) (VI) Baohong W, Lifen J, Lanjuan L, Jianlin L, Deqiang L, Wei Z, Jiliang H.Evaluating the combinative effects on human lymphocyte DNA damage induced by ultraviolet ray C plus 1.8GHz microwaves using comet assay in vitro. Toxicology. 232(3):311-316, 2007.

The objective of this study was to observe whether 1.8GHz microwaves (MW) (SAR, 3 W/kg) exposure can influence human lymphocyte DNA damage induced by ultraviolet ray C (UVC). The lymphocytes, which were from three young healthy donors, were exposed to 254 nm UVC at the doses of 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 J m(-2), respectively. The lymphocytes were irradiated by 1.8GHz MW (SAR, 3 W/kg) for 0, 1.5 and 4 h. The combinative exposure of UVC plus MW was conducted. The treated cells were incubated for 0, 1.5 and 4 h. Finally, comet assay was used to measure DNA damage of above treated lymphocytes. The results indicated that the difference of DNA damage induced between MW group and control group was not significant (P>0.05). The MTLs induced by UVC were 1.71+/-0.09, 2.02+/-0.08, 2.27+/-0.17, 2.27+/-0.06, 2.25+/-0.12, 2.24+/-0.11 microm, respectively, which were significantly higher than that (0.96+/-0.05 microm) of control (P<0.01). MTLs of some sub-groups in combinative

exposure groups at 1.5-h incubation were significantly lower that those of corresponding UVC sub-groups (P<0.01 or P<0.05). However, MTLs of some sub-groups in combinative exposure groups at 4-h incubation were significantly higher that those of corresponding UVC sub-groups (P<0.01 or P<0.05). In this experiment it was found that 1.8GHz (SAR, 3 W/kg) MW exposure for 1.5 and 4 h did not enhance significantly human lymphocyte DNA damage, but could reduce and increase DNA damage of human lymphocytes induced by UVC at 1.5-h and 4-h incubation, respectively.

(E) (VO) Cam ST, Seyhan N. Single-strand DNA breaks in human hair root cells exposed to mobile phone radiation. Int J Radiat Biol 88(5):420-424, 2012.

Purpose: To analyze the short term effects of radiofrequency radiation (RFR) exposure on genomic deoxyribonucleic acid (DNA) of human hair root cells. Subjects and methods: Hair samples were collected from 8 healthy human subjects immediately before and after using a 900-MHz GSM (Global System for Mobile Communications) mobile phone for 15 and 30 minutes. Single-strand DNA breaks of hair root cells from the samples were determined using the 'comet assay'. Results: The data showed that talking on a mobile phone for 15 or 30 minutes significantly increased (p< .05) single-strand DNA breaks in cells of hair roots close to the phone. Comparing the 15-min and 30-min data using the paired t-test also showed that significantly more damages resulted after 30 minutes than after 15 minutes of phone use. Conclusions: A short-term exposure (15 and 30 minutes) to RFR (900-MHz) from a mobile phone caused a significant increase in DNA single-strand breaks in human hair root cells located around the ear which is used for the phone calls.

(E) (VO) Chaturvedi CM, Singh VP, Singh P, Basu P, Singaravel M, Shukla RK, Dhawan A, Pati AK, Gangwar RK, and Singh SP, Progress In Electromagnetics Research B, Vol. 29, 23-42, 2011.

Present study examines biological effects of 2.45 GHz microwave radiation in Parkes strain mice. Forty-day-old mice were exposed to CW (continuous wave) microwave radiation (2 h/day for 30 days). Locomotor activity was recorded on running wheel for 12 days prior to microwave exposure (pre-exposure), 7 days during the first week of exposure (short-term exposure) and another 7-day spell during the last week of the 30-day exposure period (long-term exposure). Morris water maze test was performed from 17th to 22nd day of exposure. At the termination of the exposure, blood was processed for hematological parameters, brain for comet assay, epididymis for sperm count and motility and serum for SGOT (serum glutamate oxaloacetate transaminase) and SGPT (serum glutamate pyruvate transaminase). The results show that long-term radiation-exposed group exhibited a positive ψ□(phase angle difference) for the onset of activity with reference to lights-off timing and most of the activity occurred within the light fraction of the LD (light: dark) cycle. Microwave radiation caused an increase in erythrocyte and leukocyte counts, a significant DNA strand break in brain cells and the loss of spatial memory in mice. This report for the first time provides experimental evidence that continuous exposure to low intensity microwave radiation may have an adverse effect on the brain function by altering circadian system and rate of DNA damage.

(E) (VO) 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.

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 × 10(-4) W/kg, Group III: Animals exposed to 1800 MHz at SAR 5.835 × 10(-4) W/kg and Group IV: Animals exposed to 2450 MHz at SAR 6.672 × 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.

(E) (VO) Deshmukh PS, Nasare N, Megha K, Banerjee BD, Ahmed RS, Singh D, Abegaonkar MP, Tripathi AK, Mediratta PK. Cognitive Impairment and Neurogenotoxic Effects in Rats Exposed to Low-Intensity Microwave Radiation. Int J Toxicol. 2015 Mar 5. pii: 1091581815574348. [Epub ahead of print]

The health hazard of microwave radiation (MWR) has become a recent subject of interest as a result of the enormous increase in mobile phone usage. The present study aimed to investigate the effects of chronic low-intensity microwave exposure on cognitive function, heat shock protein 70 (HSP70), and DNA damage in rat brain. Experiments were performed on male Fischer rats exposed to MWR for 180 days at 3 different frequencies, namely, 900, 1800 MHz, and 2450 MHz. Animals were divided into 4 groups: group I: sham exposed; group II: exposed to MWR at 900 MHz, specific absorption rate (SAR) 5.953×10^{-4} W/kg; group III: exposed to 1800 MHz, SAR 5.835×10^{-4} W/kg; and group IV: exposed to 2450 MHz, SAR 6.672×10^{-4} W/kg. All the rats were tested for cognitive function at the end of the exposure period and were subsequently sacrificed to collect brain. Level of HSP70 was estimated by enzyme-linked immunotarget assay and DNA damage was assessed using alkaline comet assay in all the groups. The results showed declined cognitive function, elevated HSP70 level, and DNA damage in the brain of microwave-exposed animals. The results indicated that, chronic low-intensity microwave exposure in the frequency range of 900 to 2450 MHz may cause hazardous effects on the brain.

(E) (VO) Deshmukh PS, Megha K, Nasare N, Banerjee BD, Ahmed RS, Abegaonkar MP, Tripathi AK, Mediratta PK. Effect of Low Level Subchronic Microwave Radiation on Rat Brain. Biomed Environ Sci. 29(12):858-867, 2016.

OBJECTIVE: The present study was designed to investigate the effects of subchronic low level microwave radiation (MWR) on cognitive function, heat shock protein 70 (HSP70) level and DNA damage in brain of Fischer rats. METHODS: Experiments were performed on male Fischer rats exposed to microwave radiation for 90 days at three different frequencies: 900, 1800, and 2450 MHz. Animals were divided into 4 groups: Group I: Sham exposed, Group II: animals exposed to microwave radiation at 900 MHz and specific absorption rate (SAR) 5.953 × 10-4 W/kg, Group III: animals exposed to 1800 MHz at SAR 5.835 × 10-4 W/kg and Group IV: animals exposed to 2450 MHz at SAR 6.672 × 10-4 W/kg. All the animals were tested for cognitive function using elevated plus maze and Morris water maze at the end of the exposure period and subsequently sacrificed to collect brain tissues. HSP70 levels were estimated by ELISA and DNA damage was assessed using alkaline comet assay. RESULTS: Microwave exposure at 900-2450 MHz with SAR values as mentioned above lead to decline in cognitive function, increase in HSP70 level and DNA damage in brain. CONCLUSION: The results of the present study suggest that low level microwave exposure at frequencies 900, 1800, and 2450 MHz may lead to hazardous effects on brain.

(E) (VI) Diem E, Schwarz C, Adlkofer F, Jahn O, Rudiger H. Non-thermal DNA breakage by mobile-phone radiation (1800MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro. Mutat Res. 583:178-183, 2005.

Cultured human diploid fibroblasts and cultured rat granulosa cells were exposed to intermittent and continuous radiofrequency electromagnetic fields (RF-EMF) used in mobile phones, with different specific absorption rates (SAR) and different mobile-phone modulations. DNA strand breaks were determined by means of the alkaline and neutral comet assay. RF-EMF exposure (1800MHz; SAR 1.2 or 2W/kg; different modulations; during 4, 16 and 24h; intermittent 5min on/10min off or continuous wave) induced DNA single- and double-strand breaks. Effects occurred after 16h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect in the comet assay than continuous exposure. Therefore we conclude that the induced DNA damage cannot be based on thermal effects.

(E) (VI) Duan W, Liu C, Zhang L, He M, Xu S, Chen C, Pi H, Gao P, Zhang Y, Zhong M, Yu Z, Zhou Z. Comparison of the Genotoxic Effects Induced by 50 Hz Extremely Low-Frequency Electromagnetic Fields and 1800 MHz Radiofrequency Electromagnetic Fields in GC-2 Cells. Radiat Res. 183:305-314, 2015. [Epub ahead of print]

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

(E) (VI) Franzellitti S, Valbonesi P, Ciancaglini N, Biondi C, Contin A, Bersani F, Fabbri E. Transient DNA damage induced by high-frequency electromagnetic fields (GSM 1.8 GHz) in the human trophoblast HTR-8/SVneo cell line evaluated with the alkaline comet assay. Mutat Res 683(1-2):35-42, 2010.

One of the most controversial issue regarding high-frequency electromagnetic fields (HF-EMF) is their putative capacity to affect DNA integrity. This is of particular concern due to the increasing use of HF-EMF in communication technologies, including mobile phones. Although epidemiological studies report no detrimental effects on human health, the possible disturbance generated by HF-EMF on cell physiology remains controversial. In addition, the question remains as to whether cells are able to compensate their potential effects. We have previously reported that a 1-h exposure to amplitude-modulated 1.8 GHz sinusoidal waves (GSM-217 Hz, SAR=2 W/kg) largely used in mobile telephony did not cause increased levels of primary DNA damage in human trophoblast HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations were considered of interest. In the present work, HTR-8/SVneo cells were exposed for 4, 16 or 24h to 1.8 GHz continuous wave (CW) and different GSM signals, namely GSM-217 Hz and GSM-Talk (intermittent exposure: 5 min field on, 10 min field off). The alkaline comet assay was used to evaluate primary DNA damages and/or strand breaks due to uncompleted repair processes in HF-EMF exposed samples. The amplitude-modulated signals GSM-217 Hz and GSM-Talk induced a significant increase in comet parameters in trophoblast cells after 16 and 24h of exposure, while the un-modulated CW was ineffective. However, alterations were rapidly recovered and the DNA integrity of HF-EMF exposed cells was similar to that of sham-exposed cells within 2h of recovery in the absence irradiation. Our data suggest

that HF-EMF with a carrier frequency and modulation scheme typical of the GSM signal may affect the DNA integrity.

(NE) (VO) Furtado-Filho OV, Borba JB, Maraschin T, Souza LM, Jose JA, Moreira CF, Saffi J. Effects of chronic exposure to 950 MHz ultra-high-frequency electromagnetic radiation on reactive oxygen species metabolism in the right and left cerebral cortex of young rats of different ages. Int J Radiat Biol. 2015 Aug 14:1-17. [Epub ahead of print]

PURPOSE: To assess the effect of 950 MHz ultra-high-frequency electromagnetic radiation (UHF-EMR) on biomarkers of oxidative damage to DNA, proteins and lipids in the left cerebral cortex (LCC) and right cerebral cortex (RCC) of neonate and 6-day-old rats. MATERIALS AND METHODS: Twelve rats were equally divided into two groups as controls (CR) and exposed (ER), for each age (0 and 6 days). The LCC and RCC were examined in ER and CR after exposure. Radiation exposure lasted half an hour per day for up to 27 days (throughout pregnancy and 6 days postnatal). The specific absorption rate ranged from 1.32 - 1.14 W/kg. The damage to lipids, proteins and DNA was verified by thiobarbituric acid reactive substances, carbonylated proteins (CP) and comets, respectively. The concentration of glucose in the peripheral blood of the rats was measured by the Accu-Chek Active Kit due to increased CP in RCC. RESULTS: In neonates, no modification of the biomarkers tested was detected. On the other hand, there was an increase in the levels of CP in the RCC of the 6-day-old ER. Interestingly, the concentration of blood glucose was decreased in this group. CONCLUSIONS: Our results indicate that there is no genotoxicity and oxidative stress in neonates and 6 days rats. However, the RCC had the highest concentration of CP that do not seem to be a consequence of oxidative stress. This study is the first to demonstrate the use of UHF-EMR causes different damage responses to proteins in the LCC and RCC.

(E) (VI) Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (Apismellifera) against 915-MHz microwave radiation-induced DNA damage in wistar rat lymphocytes: in vitro study. Int J Toxicol 28:88-98, 2009.

The aim of this study is to investigate the radioprotective effect of bee venom against DNA damage induced by 915-MHz microwave radiation (specific absorption rate of 0.6 W/kg) in Wistar rats. Whole blood lymphocytes of Wistar rats are treated with 1 microg/mL bee venom 4 hours prior to and immediately before irradiation. Standard and formamidopyrimidine-DNA glycosylase (Fpg)-modified comet assays are used to assess basal and oxidative DNA damage produced by reactive oxygen species. Bee venom shows a decrease in DNA damage compared with irradiated samples. Parameters of Fpg-modified comet assay are statistically different from controls, making this assay more sensitive and suggesting that oxidative stress is a possible mechanism of DNA damage induction. Bee venom is demonstrated to have a radioprotective effect against basal and oxidative DNA damage. Furthermore, bee venom is not genotoxic and does not produce oxidative damage in the low concentrations used in this study.

(E) (VO) Gandhi G, Anita Genetic damage in mobile phone users: some preliminary findings. Ind J Hum Genet 11(2): 99-104, 2005.

BACKGROUND: The impact of microwave (MW)/radio frequency radiation (RFR) on important biological parameters is probably more than a simply thermal one. Exposure to radio frequency (RF) signals generated by the use of cellular telephones have increased dramatically and reported to affect physiological, neurological, cognitive and behavioural changes and to induce, initiate and promote carcinogenesis. Genotoxicity of RFR has also been reported in various test systems after in vitro and/or in vivo exposure but none in mobile phone users. AIMS: In the present study, DNA and chromosomal damage investigations were carried out on the peripheral blood lymphocytes of individuals using mobile phones, being exposed to MW frequency ranging from 800 to 2000 MHz. METHODS: DNA damage was assessed using the single cell gel electrophoresis assay and aneugenic and clastogenic damage by the in vivo capillary blood micronucleus test (MNT) in a total of 24 mobile phone users. RESULTS: Mean comet tail length (26.76 ± 0.054 mm; 39.75% of cells damaged) in mobile phone users was highly significant from that in the control group. The in vivo capillary blood MNT also revealed highly significant (0.25) frequency of micronucleated (MNd) cells. CONCLUSIONS: These results highlight a correlation between mobile phone use (exposure to RFR) and genetic damage and require interim public health actions in the wake of widespread use of mobile telephony.

(E) (VO) Gandhi G, Kaur G, Nisar U. A cross-sectional case control study on genetic damage in individuals residing in the vicinity of a mobile phone base station. Electromagn Biol Med. 34(4):344-354, 2015.

Mobile phone base stations facilitate good communication, but the continuously emitting radiations from these stations have raised health concerns. Hence in this study, genetic damage using the single cell gel electrophoresis (comet) assay was assessed in peripheral blood leukocytes of individuals residing in the vicinity of a mobile phone base station and comparing it to that in healthy controls. The power density in the area within 300 m from the base station exceeded the permissive limits and was significantly ($p = 0.000$) higher compared to the area from where control samples were collected. The study participants comprised 63 persons with residences near a mobile phone tower, and 28 healthy controls matched for gender, age, alcohol drinking and occupational sub-groups. Genetic damage parameters of DNA migration length, damage frequency (DF) and damage index were significantly ($p = 0.000$) elevated in the sample group compared to respective values in healthy controls. The female residents ($n = 25$) of the sample group had significantly ($p = 0.004$) elevated DF than the male residents ($n = 38$). The linear regression analysis further revealed daily mobile phone usage, location of residence and power density as significant predictors of genetic damage. The genetic damage evident in the participants of this study needs to be addressed against future disease-risk, which in addition to neurodegenerative disorders, may lead to cancer.

(E) (VO) Garaj-Vrhovac V, Gajski G, Pažanin S, Sarolić A, Domijan AM, Flajs D, Peraica M. Assessment of cytogenetic damage and oxidative stress in personnel occupationally exposed to the pulsed microwave radiation of marine radar equipment. Int J Hyg Environ Health. **4(1):59-65, 2011.**

Due to increased usage of microwave radiation, there are concerns of its adverse effect in today's society. Keeping this in view, study was aimed at workers occupationally exposed to pulsed microwave radiation, originating from marine radars. Electromagnetic field strength was measured at assigned marine radar frequencies (3 GHz, 5.5 GHz and 9.4 GHz) and corresponding specific absorption rate values were determined. Parameters of the comet assay and micronucleus test were studied both in the exposed workers and in corresponding unexposed subjects. Differences between mean tail intensity (0.67 vs. 1.22) and moment (0.08 vs. 0.16) as comet assay parameters and micronucleus test parameters (micronuclei, nucleoplasmic bridges and nuclear buds) were statistically significant between the two examined groups, suggesting that cytogenetic alterations occurred after microwave exposure. Concentrations of glutathione and malondialdehyde were measured spectrophotometrically and using high performance liquid chromatography. The glutathione concentration in exposed group was significantly lower than in controls (1.24 vs. 0.53) whereas the concentration of malondialdehyde was significantly higher (1.74 vs. 3.17), indicating oxidative stress. Results suggests that pulsed microwaves from working environment can be the cause of genetic and cell alterations and that oxidative stress can be one of the possible mechanisms of DNA and cell damage.

(E) (VO) Gulati S, Yadav A, Kumar N, Kanupriya, Aggarwal NK, Kumar R, Gupta R. Effect of GSTM1 and GSTT1 Polymorphisms on Genetic Damage in Humans Populations Exposed to Radiation From Mobile Towers. Arch Environ Contam Toxicol. 2015 Aug 5. [Epub ahead of print]

All over the world, people have been debating about associated health risks due to radiation from mobile phones and mobile towers. The carcinogenicity of this nonionizing radiation has been the greatest health concern associated with mobile towers exposure until recently. The objective of our study was to evaluate the genetic damage caused by radiation from mobile towers and to find an association between genetic polymorphism of GSTM1 and GSTT1 genes and DNA damage. In our study, 116 persons exposed to radiation from mobile towers and 106 control subjects were genotyped for polymorphisms in the GSTM1 and GSTT1 genes by multiplex polymerase chain reaction method. DNA damage in peripheral blood lymphocytes was determined using alkaline comet assay in terms of tail moment (TM) value and micronucleus assay in buccal cells (BMN). There was a significant increase in BMN frequency and TM value in exposed subjects (3.65 ± 2.44 and 6.63 ± 2.32) compared with control subjects (1.23 ± 0.97 and 0.26 ± 0.27). However, there was no association of GSTM1 and GSTT1 polymorphisms with the level of DNA damage in both exposed and control groups.

(NE) (VI) Hook GJ, Zhang P, Lagroye I, Li L, Higashikubo R, Moros EG, Straube WL, Pickard WF, Baty JD, Roti Roti JL. Measurement of DNA damage and apoptosis in molt-4 cells after in vitro exposure to radiofrequency radiation. Radiat Res. 161(2): 193-200, 2004.

To determine whether exposure to radiofrequency (RF) radiation can induce DNA damage or apoptosis, Molt-4 T lymphoblastoid cells were exposed with RF fields at frequencies and modulations of the type used by wireless communication devices. Four types of

frequency/modulation forms were studied: 847.74 MHz code-division multiple-access (CDMA), 835.62 MHz frequency-division multiple-access (FDMA), 813.56 MHz iDEN(R) (iDEN), and 836.55 MHz time-division multiple-access (TDMA). Exponentially growing cells were exposed to RF radiation for periods up to 24 h using a radial transmission line (RTL) exposure system. The specific absorption rates used were 3.2 W/kg for CDMA and FDMA, 2.4 or 24 mW/kg for iDEN, and 2.6 or 26 mW/kg for TDMA. The temperature in the RTLs was maintained at 37 degrees C +/- 0.3 degrees C. DNA damage was measured using the single-cell gel electrophoresis assay. The annexin V affinity assay was used to detect apoptosis. No statistically significant difference in the level of DNA damage or apoptosis was observed between sham-treated cells and cells exposed to RF radiation for any frequency, modulation or exposure time. Our results show that exposure of Molt-4 cells to CDMA, FDMA, iDEN or TDMA modulated RF radiation does not induce alterations in level of DNA damage or induce apoptosis.

(E) (VO) Ji S, Oh E, Sul D, Choi JW, Park H, Lee E. DNA Damage of Lymphocytes in Volunteers after 4 hours Use of Mobile Phone. J Prev Med Public Health. 37(4):373-380, 2004.

OBJECTIVES: There has been gradually increasing concern about the adverse health effects of electromagnetic radiation originating from cell phones which are widely used in modern life. Cell phone radiation may affect human health by increasing free radicals of human blood cells. This study has been designed to identify DNA damage of blood cells by electromagnetic radiation caused by cell phone use. **METHODS:** This study investigated the health effect of acute exposure to commercially available cell phones on certain parameters such as an indicator of DNA damage for 14 healthy adult volunteers. Each volunteer during the experiment talked over the cell phone with the keypad facing the right side of the face for 4 hours. The single cell gel electrophoresis assay (Comet assay), which is very sensitive in detecting the presence of DNA strand-breaks and alkali-labile damage in individual cells, was used to assess peripheral blood cells (T-cells, B-cells, granulocytes) from volunteers before and after exposure to cell phone radiation. The parameters of Comet assay measured were Olive Tail Moment and Tail DNA %. **RESULTS:** The Olive Tail Moment of B-cells and granulocytes and Tail DNA % of B-cells and granulocytes were increased by a statistically significant extent after 4- hour use of a cell phone compared with controls. **CONCLUSIONS:** It is concluded that cell phone radiation caused the DNA damage during the 4 hours of experimental condition. Nonetheless, this study suggested that cell phone use may increase DNA damage by electromagnetic radiation and other contributing factors.

(E) (VI) Ji Y, He Q, Sun Y, Tong J, Cao Y. Adaptive response in mouse bone-marrow stromal cells exposed to 900-MHz radiofrequency fields: Gamma-radiation-induced DNA strand breaks and repair. J Toxicol Environ Health A. 79(9-10):419-426, 2016.

The aim of this study was to examine whether radiofrequency field (RF) preexposure induced adaptive responses (AR) in mouse bone-marrow stromal cells (BMSC) and the mechanisms underlying the observed findings. Cells were preexposed to 900-MHz radiofrequency fields (RF) at 120 µW/cm(2) power intensity for 4 h/d for 5 d. Some cells were subjected to 1.5 Gy γ-radiation (GR) 4 h following the last RF exposure. The intensity of strand breaks in the DNA was assessed immediately at 4 h. Subsequently, some BMSC were examined at 30, 60, 90, or 120 min utilizing the alkaline comet assay and γ-H2AX foci technique. Data showed no significant differences in number and intensity of strand breaks in DNA between RF-exposed and control cells. A significant increase in number and intensity of DNA strand breaks was noted in cells exposed to GR exposure alone. RF followed by GR exposure significantly decreased number of strand breaks and resulted in faster kinetics of repair of DNA strand breaks compared to GR alone. Thus, data suggest that RF preexposure protected cells from damage induced by GR. Evidence indicates that in RF-mediated AR more rapid repair kinetics occurs under conditions of GR-induced damage, which may be attributed to diminished DNA strand breakage.

(E) (VO) Jiang B, Nie J, Zhou Z, Zhang J, Tong J, Cao Y. Adaptive response in mice exposed to 900 MHz radiofrequency fields: primary DNA damage. PLoS One. 7(2):e32040, 2012.

The phenomenon of adaptive response (AR) in animal and human cells exposed to ionizing radiation is well documented in scientific literature. We have examined whether such AR could be induced in mice exposed to non-ionizing radiofrequency fields (RF) used for wireless communications. Mice were pre-exposed to 900 MHz RF at 120 µW/cm(2) power density for 4 hours/day for 1, 3, 5, 7 and 14 days and then subjected to an acute dose of 3 Gy γ-radiation. The primary DNA damage in the form of alkali labile base damage and single strand breaks in the DNA of peripheral blood leukocytes was determined using the alkaline comet assay. The results indicated that the extent of damage in mice which were pre-exposed to RF for 1 day and then subjected to γ-radiation was similar and not significantly different from those exposed to γ-radiation alone. However, mice which were pre-exposed to RF for 3, 5, 7 and 14 days showed progressively decreased damage and was significantly different from those exposed to γ-radiation alone. Thus, the data indicated that RF pre-exposure is capable of inducing AR and suggested that the pre-exposure for more than 4 hours for 1 day is necessary to elicit such AR.

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

Purpose: To investigate the effect of 2.45 GHz microwave radiation on rat brain of male wistar strain. Material and methods: Male rats of wistar strain (35 days old with 130 +/- 10 g body weight) were selected for this study. Animals were divided into two groups: Sham exposed and experimental. Animals were exposed for 2 h a day for 35 days to 2.45 GHz frequency at 0.34 mW/cm power density. The whole body specific absorption rate (SAR) was estimated to be 0.11 W/Kg. Exposure took place in a ventilated Plexiglas cage and kept in anechoic chamber in a far field configuration from the horn antenna. After the completion of exposure period, rats were sacrificed and the whole brain tissue was dissected and used for study of double strand DNA (Deoxyribonucleic acid) breaks by micro gel electrophoresis and the statistical analysis was

carried out using comet assay (IV-2 version software). Thereafter, antioxidant enzymes and histone kinase estimation was also performed. Results: A significant increase was observed in comet head ($P < 0.002$), tail length ($P < 0.0002$) and in tail movement ($P < 0.0001$) in exposed brain cells. An analysis of antioxidant enzymes glutathione peroxidase ($P < 0.005$), and superoxide dismutase ($P < 0.006$) showed a decrease while an increase in catalase ($P < 0.006$) was observed. A significant decrease ($P < 0.023$) in histone kinase was also recorded in the exposed group as compared to the control (sham-exposed) ones. One-way analysis of variance (ANOVA) method was adopted for statistical analysis. Conclusion: The study concludes that the chronic exposure to these radiations may cause significant damage to brain, which may be an indication of possible tumour promotion (Behari and Paulraj 2007).

(E) (VI) Kim JY, Hong SY, Lee YM, Yu SA, Koh WS, Hong JR, Son T, Chang SK, Lee M.In vitro assessment of clastogenicity of mobile-phone radiation (835 MHz) using the alkaline comet assay and chromosomal aberration test. Environ Toxicol 23:319-327, 2008.

Recently we demonstrated that 835-MHz radiofrequency radiation electromagnetic fields (RF-EMF) neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro. Here, two kinds of cytogenetic endpoints were further investigated on mammalian cells exposed to 835-MHz RF-EMF (the most widely used communication frequency band in Korean CDMA mobile phone networks) alone and in combination with model clastogens: in vitro alkaline comet assay and in vitro chromosome aberration (CA) test. No direct cytogenetic effect of 835-MHz RF-EMF was found in the in vitro CA test. The combined exposure of the cells to RF-EMF in the presence of ethylmethanesulfonate (EMS) revealed a weak and insignificant cytogenetic effect when compared to cells exposed to EMS alone in CA test. Also, the comet assay results to evaluate the ability of RF-EMF alone to damage DNA were nearly negative, although showing a small increase in tail moment. However, the applied RF-EMF had potentiation effect in comet assay when administered in combination with model clastogens (cyclophosphamide or 4-nitroquinoline 1-oxide). Thus, our results imply that we cannot confidently exclude any possibility of an increased risk of genetic damage, with important implications for the possible health effects of exposure to 835-MHz electromagnetic fields.

(NE) (VI) Kumar G, McIntosh RL, Anderson V, McKenzie RJ, Wood AW. A genotoxic analysis of the hematopoietic system after mobile phone type radiation exposure in rats. Int J Radiat Biol. 91(8):664-672, 2015.

PURPOSE: In our earlier study we reported that 900 MHz continuous wave (CW) radiofrequency radiation (RFR) exposure (2 W/kg specific absorption rate [SAR]) had no significant effect on the hematopoietic system of rats. In this paper we extend the scope of the previous study by testing for possible effects at: (i) different SAR levels; (ii) both 900 and 1800 MHz, and; (iii) both CW and pulse modulated (PM) RFR. MATERIALS AND METHODS: Excised long bones from rats were placed in medium and RFR exposed in (i) a Transverse Electromagnetic (TEM) cell or (ii) a waveguide. Finite-difference time-domain (FDTD) numerical analyses were used to estimate forward power needed to produce nominal SAR levels of 2/10 and 2.5/12.4 W/kg in the bone marrow. After exposure, the lymphoblasts were extracted and assayed for proliferation rate, and genotoxicity. RESULTS: Our data did not indicate any significant change in these end points

for any combination of CW/PM exposure at 900/1800 MHz at SAR levels of nominally 2/10 W/kg or 2.5/12.4 W/kg. CONCLUSIONS: No significant changes were observed in the hematopoietic system of rats after the exposure of CW/PM wave 900 MHz/1800 MHz RF radiations at different SAR values.

(NE) (VO) Lagroye I, Anane R, Wettring BA, Moros EG, Straube WL, Laregina M, Niehoff M, Pickard WF, Baty J, Roti JL. Measurement of DNA damage after acute exposure to pulsed-wave 2450 MHz microwaves in rat brain cells by two alkaline comet assay methods. Int J Radiat Biol. 80(1):11-20, 2004.

Purpose: To investigate the effect of 2450 MHz pulsed-wave microwaves on the induction of DNA damage in brain cells of exposed rats and to discover whether proteinase K is needed to detect DNA damage in the brain cells of rats exposed to 2450 MHz microwaves. Materials and methods: Sprague-Dawley rats were exposed to 2450 MHz pulsed-wave microwaves and sacrificed 4 h after a 2-h exposure. Rats irradiated whole-body with 1 Gy (137)Cs were included as positive controls. DNA damage was assayed by two variants of the alkaline comet assay on separate aliquots of the same cell preparation. Results: Significant DNA damage was observed in the rat brain cells of rats exposed to gamma-rays using both versions of the alkaline comet assay independent of the presence or absence of proteinase K. However, neither version of the assay could detect any difference in comet length and/or normalized comet moment between sham- and 2450 MHz pulsed-wave microwave-exposed rats, regardless of the inclusion or omission of proteinase K in the comet assay. Conclusions: No DNA damage in brain cells was detected following exposure of rats to 2450 MHz microwaves pulsed-wave at a specific absorption rate of 1.2 W kg(-1) regardless of whether or not proteinase K was included in the assay. Thus, the results support the conclusion that low-level 2450 MHz pulsed-wave microwave exposures do not induce DNA damage detectable by the alkaline comet assay.

(NE) (VI) Lagroye I, Hook GJ, Wettring BA, Baty JD, Moros EG, Straube WL, Roti Roti JL. Measurements of alkali-labile DNA damage and protein-DNA crosslinks after 2450 MHz microwave and low-dose gamma irradiation In vitro. Radiat Res. 161(2): 201-214, 2004.

In vitro experiments were performed to determine whether 2450 MHz microwave radiation induces alkali-labile DNA damage and/or DNA-protein or DNA-DNA crosslinks in C3H 10T(1/2) cells. After a 2-h exposure to either 2450 MHz continuous-wave (CW) microwaves at an SAR of 1.9 W/kg or 1 mM cisplatinum (CDDP, a positive control for DNA crosslinks), C3H 10T(1/2) cells were irradiated with 4 Gy of gamma rays ((137)Cs). Immediately after gamma irradiation, the single-cell gel electrophoresis assay was performed to detect DNA damage. For each exposure condition, one set of samples was treated with proteinase K (1 mg/ml) to remove any possible DNA-protein crosslinks. To measure DNA-protein crosslinks independent of DNA-DNA crosslinks, we quantified the proteins that were recovered with DNA after microwave exposure, using CDDP and gamma irradiation, positive controls for DNA-protein crosslinks. Ionizing

radiation (4 Gy) induced significant DNA damage. However, no DNA damage could be detected after exposure to 2450 MHz CW microwaves alone. The crosslinking agent CDDP significantly reduced both the comet length and the normalized comet moment in C3H 10T(1/2) cells irradiated with 4 Gy gamma rays. In contrast, 2450 MHz microwaves did not impede the DNA migration induced by gamma rays. When control cells were treated with proteinase K, both parameters increased in the absence of any DNA damage. However, no additional effect of proteinase K was seen in samples exposed to 2450 MHz microwaves or in samples treated with the combination of microwaves and radiation. On the other hand, proteinase K treatment was ineffective in restoring any migration of the DNA in cells pretreated with CDDP and irradiated with gamma rays. When DNA-protein crosslinks were specifically measured, we found no evidence for the induction of DNA-protein crosslinks or changes in amount of the protein associated with DNA by 2450 MHz CW microwave exposure. Thus 2-h exposures to 1.9 W/ kg of 2450 MHz CW microwaves did not induce measurable alkali-labile DNA damage or DNA-DNA or DNA-protein crosslinks.

(E) (VO) 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.

(E) (VO) 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 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/cm2, 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.

(E) (VO) 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/cm2, 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-phenylnitrone (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.

(E) (VO) 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^2, 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.

(E) (VO) 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 □s pulses, 500 pps) RFR at a power density of 2 mW/cm2 (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.

(E) (VO) Lakshmi NK, Tiwari R, Bhargava SC, Ahuja YR. Investigations on DNA damage and frequency of micronuclei in occupational exposure to electromagnetic fields (EMFs) emitted from video display terminals (VDTs). Gen MolBiol 33, 154-158, 2010.

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.

(NE) (VI) Li L, Bisht KS, LaGroye I, Zhang P, Straube WL, Moros EG, Roti Roti JL.Measurement of DNA damage in mammalian cells exposed in vitro to radiofrequency fields at sars of 3-5 w/kg. Radiat Res 156:328-332, 2001.

In the present study, we determined whether exposure of mammalian cells to 3.2-5.1 W/kg specific absorption rate (SAR) radiofrequency fields could induce DNA damage in murine C3H 10T(1/2) fibroblasts. Cell cultures were exposed to 847.74 MHz code-division multiple access (CDMA) and 835.62 frequency-division multiple access (FDMA) modulated radiations in radial transmission line (RTL) irradiators in which the temperature was regulated to 37.0 +/- 0.3 degrees C. Using the alkaline comet assay to measure DNA damage, we found no statistically significant differences in either comet moment or comet length between sham-exposed cells and those exposed for 2, 4 or 24 h to CDMA or FDMA radiations in either exponentially growing or plateau-phase cells. Further, a 4-h incubation after the 2-h exposure resulted in no significant

changes in comet moment or comet length. Our results show that exposure of cultured C3H 10T(1/2) cells at 37 degrees C CDMA or FDMA at SAR values of up to 5.1 W/kg did not induce measurable DNA damage.

(E) (VI) Liu C, Duan W, Xu S, Chen C, He M, Zhang L, Yu Z, Zhou Z. Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line. Toxicol Lett 218(1): 2-9, 2013a.

Whether exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from mobile phones can induce DNA damage in male germ cells remains unclear. In this study, we conducted a 24 h intermittent exposure (5 min on and 10 min off) of a mouse spermatocyte-derived GC-2 cell line to 1800 MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at specific absorption rates (SAR) of 1 W/kg, 2 W/kg or 4 W/kg. Subsequently, through the use of formamidopyrimidine DNA glycosylase (FPG) in a modified comet assay, we determined that the extent of DNA migration was significantly increased at a SAR of 4 W/kg. Flow cytometry analysis demonstrated that levels of the DNA adduct 8-oxoguanine (8-oxoG) were also increased at a SAR of 4 W/kg. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS); these phenomena were mitigated by co-treatment with the antioxidant α-tocopherol. However, no detectable DNA strand breakage was observed by the alkaline comet assay. Taking together, these findings may imply the novel possibility that RF-EMR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells.

(E) (VI) Liu C, Gao P, Xu SC, Wang Y, Chen CH, He MD, Yu ZP, Zhang L, Zhou Z. Mobile phone radiation induces mode-dependent DNA damage in a mouse spermatocyte-derived cell line: a protective role of melatonin. Int J Radiat Biol. 2013b Aug 19. [Epub ahead of print]

Purpose: To evaluate whether exposure to mobile phone radiation (MPR) can induce DNA damage in male germ cells. Materials and methods: A mouse spermatocyte-derived GC-2 cell line was exposed to a commercial mobile phone handset once every 20 minutes in standby, listen, dialed or dialing modes for 24 h. DNA damage was determined using an alkaline comet assay. Results: The levels of DNA damage were significantly increased following exposure to MPR in the listen, dialed and dialing modes. Moreover, there were significantly higher increases in the dialed and dialing modes than in the listen mode. Interestingly, these results were consistent with the radiation intensities of these modes. However, the DNA damage effects of MPR in the dialing mode were efficiently attenuated by melatonin pretreatment. Conclusions: These results regarding mode-dependent DNA damage have important implications for the safety of inappropriate mobile phone use by males of reproductive age and also suggest a simple preventive measure, keeping our body from mobile phones as far away as possible, not only during conversations but during "dialed" and "dialing" operation modes as well. Since the "dialed" mode is actually part of the standby mode, mobile phones should be kept at a safe

distance from our body even during standby operation. Furthermore, the protective role of melatonin suggests that it may be a promising pharmacological candidate for preventing mobile phone use-related reproductive impairments.

(E) (VI) Lixia S, Yao K, Kaijun W, Deqiang L, Huajun H, Xiangwei G, Baohong W, Wei Z, Jianling L, Wei W. Effects of 1.8GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells. Mutat Res.602(1-2):135-142, 2006.

To investigate the DNA damage, expression of heat shock protein 70 (Hsp70) and cell proliferation of human lens epithelial cells (hLEC) after exposure to the 1.8GHz radiofrequency field (RF) of a global system for mobile communications (GSM). An Xc-1800 RF exposure system was used to employ a GSM signal at 1.8GHz (217Hz amplitude-modulated) with the output power in the specific absorption rate (SAR) of 1, 2 and 3W/kg. After 2h exposure to RF, the DNA damage of hLEC was accessed by comet assay at five different incubation times: 0, 30, 60, 120 and 240min, respectively. Western blot and RT-PCR were used to determine the expression of Hsp70 in hLECs after RF exposure. The proliferation rate of cells was evaluated by bromodeoxyuridine incorporation on days 0, 1 and 4 after exposure. The results show that the difference of DNA-breaks between the exposed and sham-exposed (control) groups induced by 1 and 2W/kg irradiation were not significant at any incubation time point ($P>0.05$). The DNA damage caused by 3W/kg irradiation was significantly increased at the times of 0 and 30min after exposure ($P<0.05$), a phenomenon that could not be seen at the time points of 60, 120 or 240min ($P>0.05$). Detectable mRNA as well as protein expression of Hsp70 was found in all groups. Exposure at SARs of 2 and 3W/kg for 2h exhibited significantly increased Hsp70 protein expression ($P<0.05$), while no change in Hsp70 mRNA expression could be found in any of the groups ($P>0.05$). No difference of the cell proliferation rate between the sham-exposed and exposed cells was found at any exposure dose tested ($P>0.05$). The results indicate that exposure to non-thermal dosages of RF for wireless communications can induce no or repairable DNA damage and the increased Hsp70 protein expression in hLECs occurred without change in the cell proliferation rate. The non-thermal stress response of Hsp70 protein increase to RF exposure might be involved in protecting hLEC from DNA damage and maintaining the cellular capacity for proliferation.

(NE) (VI) Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL, Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation. Radiat Res 148(6):608-617, 1997.

Recent reports suggest that exposure to 2450 MHz electromagnetic radiation causes DNA single-strand breaks (SSBs) and double-strand breaks (DSBs) in cells of rat brain irradiated in vivo (Lai and Singh, Bioelectromagnetics 16, 207-210, 1995; Int. J. Radiat. Biol. 69, 513-521, 1996). Therefore, we endeavored to determine if exposure of cultured mammalian cells in vitro

to 2450 MHz radiation causes DNA damage. The alkaline comet assay (single-cell gel electrophoresis), which is reportedly the most sensitive method to assay DNA damage in individual cells, was used to measure DNA damage after in vitro 2450 MHz irradiation. Exponentially growing U87MG and C3H 10T1/2 cells were exposed to 2450 MHz continuous-wave (CW) radiation in specially designed radial transmission lines (RTLs) that provided relatively uniform microwave exposure. Specific absorption rates (SARs) were calculated to be 0.7 and 1.9 W/kg. Temperatures in the RTLs were measured in real time and were maintained at 37 +/- 0.3 degrees C. Every experiment included sham exposure(s) in an RTL. Cells were irradiated for 2 h, 2 h followed by a 4-h incubation at 37 degrees C in an incubator, 4 h and 24 h. After these treatments samples were subjected to the alkaline comet assay as described by Olive et al. (Exp. Cell Res. 198, 259-267, 1992). Images of comets were digitized and analyzed using a PC-based image analysis system, and the "normalized comet moment" and "comet length" were determined. No significant differences were observed between the test group and the controls after exposure to 2450 MHz CW irradiation. Thus 2450 MHz irradiation does not appear to cause DNA damage in cultured mammalian cells under these exposure conditions as measured by this assay.

(NE) (VO) Malyapa RS, Ahern EW, Bi C, Straube WL, LaRegina M, Pickard WF, Roti RotiJL, DNA damage in rat brain cells after in vivo exposure to 2450 MHz electromagnetic radiation and various methods of euthanasia. Radiat Res 149(6):637-645, 1998.

The present study was done to confirm the reported observation that low-intensity acute exposure to 2450 MHz radiation causes DNA single-strand breaks (Lai and Singh, Bioelectromagnetics 16, 207-210, 1995). Male Sprague-Dawley rats weighing approximately 250 g were irradiated with 2450 MHzcontinuous-wave (CW) microwaves for 2 h at a specific absorption rate of 1.2 W/kg in a cylindrical waveguide system (Guy et al., Radio Sci. 14, 63-74, 1979). There was no associated rise in the core body temperature of the rats. After the irradiation or sham treatments, rats were euthanized by either CO2 asphyxia or decapitation by guillotine (eight pairs of animals per euthanasia group). After euthanasia the brains were removed and immediately immersed in cold Ames medium and the cells of the cerebral cortex and the hippocampus were dissociated separately and subjected to the alkaline comet assay. Irrespective of whether the rats were euthanized by CO2 asphyxia or decapitated by guillotine, no significant differences were observed between either the comet length or the normalized comet moment of cells from either the cerebral cortex or the hippocampus of sham-treated rats and those from the irradiated rats. However, the data for the rats asphyxiated with CO2 showed more intrinsic DNA damage and more experiment-to-experiment variation than did the data for rats euthanized by guillotine. Therefore, the guillotine method of euthanasia is the most appropriate in studies relating to DNA damage. Furthermore, we did not confirm the observation that DNA damage is produced in cells of the rat cerebral cortex or the

hippocampus after a 2-h exposure to 2450 MHz CW microwaves or at 4 h after the exposure.

(NE) (VI) Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL. Measurement of DNA damage after exposure to electromagnetic radiation in the cellular phone communication frequency band (835.62 and 847.74 MHz). Radiat Res 148(6):618-627, 1997.

Mouse C3H 10T1/2 fibroblasts and human glioblastoma U87MG cells were exposed to cellular phone communication frequency radiations to investigate whether such exposure produces DNA damage in in vitro cultures. Two types of frequency modulations were studied: frequency-modulated continuous-wave (FMCW), with a carrier frequency of 835.62 MHz, and code-division multiple-access (CDMA) centered on 847.74 MHz. Exponentially growing (U87MG and C3H 10T1/2 cells) and plateau-phase (C3H 10T1/2 cells) cultures were exposed to either FMCW or CDMA radiation for varying periods up to 24 h in specially designed radial transmission lines (RTLs) that provided relatively uniform exposure with a specific absorption rate (SAR) of 0.6 W/kg. Temperatures in the RTLs were monitored continuously and maintained at 37 +/- 0.3 degrees C. Sham exposure of cultures in an RTL (negative control) and 137Cs gamma-irradiated samples (positive control) were included with every experiment. The alkaline comet assay as described by Olive et al. (Exp. Cell Res. 198, 259-269, 1992) was used to measure DNA damage. No significant differences were observed between the test group exposed to FMCW or CDMA radiation and the sham-treated negative controls. Our results indicate that exposure of cultured mammalian cells to cellular phone communication frequencies under these conditions at an SAR of 0.6 W/kg does not cause DNA damage as measured by the alkaline comet assay.

(NE) (VI) McNamee JP, Bellier PV, Gajda GB, Miller SM, Lemay EP, Lavallee BF, Marro L, Thansandote A. DNA damage and micronucleus induction in human leukocytes after acute in vitro exposure to a 1.9 GHz continuous-wave radiofrequency field. Radiat Res 158(4):523-533, 2002.

Human blood cultures were exposed to a 1.9 GHz continuous-wave (CW) radiofrequency (RF) field for 2 h using a series of six circularly polarized, cylindrical waveguides. Mean specific absorption rates (SARs) of 0.0, 0.1, 0.26, 0.92, 2.4 and 10 W/kg were achieved, and the temperature within the cultures during a 2-h exposure was maintained at 37.0 +/- 0.5 degrees C. Concurrent negative (incubator) and positive (1.5 Gy (137)Cs gamma radiation) control cultures were run for each experiment. DNA damage was quantified immediately after RF-field exposure using the alkaline comet assay, and four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. No evidence of increased primary DNA damage was detected by any parameter for RF-field-exposed cultures at any SAR tested. The formation of micronuclei in the RF-field-exposed blood cell cultures was assessed using the cytokinesis-block micronucleus assay. There was no significant difference in

the binucleated cell frequency, incidence of micronucleated binucleated cells, or total incidence of micronuclei between any of the RF-field-exposed cultures and the sham-exposed controls at any SAR tested. These results do not support the hypothesis that acute, nonthermalizing 1.9 GHz CW RF-field exposure causes DNA damage in cultured human leukocytes.

(NE) (VI) McNamee JP, Bellier PV, Gajda GB, Lavallee BF, Lemay EP, Marro L, Thansandote A. DNA Damage in human leukocytes after acute in vitro exposure to a 1.9 GHz pulse-modulated radiofrequency field. Radiat Res 158(4):534-537, 2002.

Blood cultures from human volunteers were exposed to an acute 1.9 GHz pulse-modulated radiofrequency (RF) field for 2 h using a series of six circularly polarized, cylindrical waveguides. Mean specific absorption rates (SARs) ranged from 0 to 10 W/kg, and the temperature within the cultures during the exposure was maintained at 37.0 +/- 0.5 degrees C. DNA damage was quantified in leukocytes by the alkaline comet assay and the cytokinesis-block micronucleus assay. When compared to the sham-treated controls, no evidence of increased primary DNA damage was detected by any parameter for any of the RF-field-exposed cultures when evaluated using the alkaline comet assay. Furthermore, no significant differences in the frequency of binucleated cells, incidence of micronucleated binucleated cells, or total incidence of micronuclei were detected between any of the RF-field-exposed cultures and the sham-treated control at any SAR tested. These results do not support the hypothesis that acute, nonthermalizing 1.9 GHz pulse-modulated RF-field exposure causes DNA damage in cultured human leukocytes.

(NE) (VI) McNamee, J. P., Bellier, P. V., Gajda, G. B., Lavallee, B. F., Marro, L., Lemay, E. and Thansandote, A. No Evidence for Genotoxic Effects from 24 h Exposure of Human Leukocytes to 1.9 GHz Radiofrequency Fields. Radiat Res 159:693-697, 2003.

The current study extends our previous investigations of 2-h radiofrequency (RF)-field exposures on genotoxicity in human blood cell cultures by examining the effect of 24-h continuous-wave (CW) and pulsed-wave (PW) 1.9 GHz RF-field exposures on both primary DNA damage and micronucleus induction in human leukocyte cultures. Mean specific absorption rates (SARs) ranged from 0 to 10 W/kg, and the temperature within the cultures was maintained at 37.0 +/- 1.0 degrees C for the duration of the 24-h exposure period. No significant differences in primary DNA damage were observed between the sham-treated controls and any of the CW or PW 1.9 GHz RF-field-exposed cultures when processed immediately after the exposure period by the alkaline comet assay. Similarly, no significant differences were observed in the incidence of micronuclei, incidence of micronucleated binucleated cells, frequency of binucleated cells, or proliferation index between the sham-treated controls and any of the CW or PW 1.9 GHz RF-field-exposed cultures. In conclusion, the current study found no evidence of 1.9 GHz RF-field-induced genotoxicity in human blood cell

cultures after a 24-h exposure period.

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

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

(E) (VI) Luukkonen J, Hakulinen P, Mäki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872MHz radiofrequency radiation. Mutat Res 662:54-58, 2009.

The objective of the study was to investigate effects of 872 MHz radiofrequency (RF) radiation on intracellular reactive oxygen species (ROS) production and DNA damage at a relatively high SAR value (5W/kg). The experiments also involved combined exposure to RF radiation and menadione, a chemical inducing intracellular ROS production and DNA damage. The production of ROS was measured using the fluorescent probe dichlorofluorescein and DNA damage was evaluated by the Comet assay. Human SH-SY5Y neuroblastoma cells were exposed to RF radiation for 1h with or without menadione. Control cultures were sham exposed. Both continuous waves (CW) and a pulsed signal similar to that used in global system for mobile communications (GSM) mobile phones were used. Exposure to the CW RF radiation increased DNA breakage ($p<0.01$) in comparison to the cells exposed only to menadione. Comparison of the same groups also showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60 min after the end of exposure ($p<0.05$ and $p<0.01$, respectively). No effects of the

GSM signal were seen on either ROS production or DNA damage. The results of the present study suggest that 872MHz CW RF radiation at 5W/kg might enhance chemically induced ROS production and thus cause secondary DNA damage. However, there is no known mechanism that would explain such effects from CW RF radiation but not from GSM modulated RF radiation at identical SAR.

(NE) (VI) Luukkonen J, Juutilainen J, Naarala J. Combined effects of 872 MHz radiofrequency radiation and ferrous chloride on reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells. Bioelectromagnetics 31:417-424, 2010.

The aim of the present study was to investigate possible cooperative effects of radiofrequency (RF) radiation and ferrous chloride (FeCl) on reactive oxygen species (ROS) production and DNA damage. In order to test intracellular ROS production as a possible underlying mechanism of DNA damage, we applied the fluorescent probe DCFH-DA. Integrity of DNA was quantified by alkaline comet assay. The exposures to 872 MHz RF radiation were conducted at a specific absorption rate (SAR) of 5 W/kg using continuous waves (CW) or a modulated signal similar to that used in Global System for Mobile Communications (GSM) phones. Four groups were included: Sham exposure (control), RF radiation, Chemical treatment, Chemical treatment, and RF radiation. In the ROS production experiments, human neuroblastoma (SH-SY5Y) cells were exposed to RF radiation and 10 microg/ml FeCl for 1 h. In the comet assay experiments, the exposure time was 3 h and an additional chemical (0.015% diethyl maleate) was used to make DNA damage level observable. The chemical treatments resulted in statistically significant responses, but no effects from either CW or modulated RF radiation were observed on ROS production, DNA damage or cell viability.

(NE) (VO) Maes A, Van Gorp U, Verschaeve L. Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation. Mutagenesis 21:139-42, 2006.

Nowadays, virtually everybody is exposed to radiofrequency radiation (RFR) from mobile phone base station antennas or other sources. At least according to some scientists, this exposure can have detrimental health effects. We investigated cytogenetic effects in peripheral blood lymphocytes from subjects who were professionally exposed to mobile phone electromagnetic fields in an attempt to demonstrate possible RFR-induced genetic effects. These subjects can be considered well suited for this purpose as their RFR exposure is 'normal' though rather high, and definitely higher than that of the 'general population'. The alkaline comet assay, sister chromatid exchange (SCE) and chromosome aberration tests revealed no evidence of RFR-induced genetic effects. Blood cells were also exposed to the well known chemical mutagen mitomycin C in order to investigate possible combined effects of RFR and the chemical. No cooperative action was found between the electromagnetic field exposure and the mutagen using either the comet assay or SCE test.

(E) **Pandey N, Giri S, Das S, Upadhaya P. Radiofrequency radiation (900 MHz)-induced DNA damage and cell cycle arrest in testicular germ cells in swiss albino mice. Toxicol Ind Health. 2016 Oct 13. pii: 0748233716671206. [Epub ahead of print]**

Even though there are contradictory reports regarding the cellular and molecular changes induced by mobile phone emitted radiofrequency radiation (RFR), the possibility of any biological effect cannot be ruled out. In view of a widespread and extensive use of mobile phones, this study evaluates alterations in male germ cell transformation kinetics following RFR exposure and after recovery. Swiss albino mice were exposed to RFR (900 MHz) for 4 h and 8 h duration per day for 35 days. One group of animals was terminated after the exposure period, while others were kept for an additional 35 days post-exposure. RFR exposure caused depolarization of mitochondrial membranes resulting in destabilized cellular redox homeostasis. Statistically significant increases in the damage index in germ cells and sperm head defects were noted in RFR-exposed animals. Flow cytometric estimation of germ cell subtypes in mice testis revealed 2.5-fold increases in spermatogonial populations with significant decreases in spermatids. Almost fourfold reduction in spermatogonia to spermatid turnover (1C:2C) and three times reduction in primary spermatocyte to spermatid turnover (1C:4C) was found indicating arrest in the premeiotic stage of spermatogenesis, which resulted in loss of post-meiotic germ cells apparent from testis histology and low sperm count in RFR-exposed animals. Histological alterations such as sloughing of immature germ cells into the seminiferous tubule lumen, epithelium depletion and maturation arrest were also observed. However, all these changes showed recovery to varied degrees following the post-exposure period indicating that the adverse effects of RFR on mice germ cells are detrimental but reversible. To conclude, RFR exposure-induced oxidative stress causes DNA damage in germ cells, which alters cell cycle progression leading to low sperm count in mice.

(E) (VO) 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.

(E) (VI) Phillips, J.L., Ivaschuk, O., Ishida-Jones, T., Jones, R.A., Campbell-Beachler, M. and Haggren, W. DNA damage in Molt-4 T- lymphoblastoid cells exposed to cellular telephone radiofrequency fields in vitro. Bioelectrochem. Bioenerg. 45:103-110, 1998.

Molt-4 T-lymphoblastoid cells have been exposed to pulsed signals at cellular telephone frequencies of 813.5625 MHz (iDEN signal) and 836.55 MHz (TDMA signal). These studies were performed at low SAR (average = 2.4 and 24 microwatt/g for iDEN and 2.6 and 26 microwatt/g for TDMA) in studies designed to look for athermal RF effects. The alkaline comet, or single cell

gel electrophoresis, assay was employed to measure DNA single-strand breaks in cell cultures exposed to the radiofrequency (RF) signal as compared to concurrent sham-exposed cultures. Tail moment and comet extent were calculated as indicators of DNA damage. Statistical differences in the distribution of values for tail moment and comet extent between exposed and control cell cultures were evaluated with the SKolmogorov-Smirnoff distribution test. Data points for all experiments of each exposure condition were pooled and analyzed as single groups. It was found that: 1) exposure of cells to the iDEN signal at an SAR of 2.4 microwatt/g for 2 h or 21 h significantly decreased DNA damage; 2) exposure of cells to the TDMA signal at an SAR of 2.6 microwatt/g for 2 h and 21 h significantly decreased DNA damage; 3) exposure of cells to the iDEN signal at an SAR of 24 microwatt/g for 2 h and 21 h significantly increased DNA damage; 4) exposure of cells to the TDMA signal at an SAR of 26 microwatt/g for 2 h significantly decreased DNA damage. The data indicate a need to study the effects of exposure to RF signals on direct DNA damage and on the rate at which DNA damage is repaired.

(NE) (VI) Sakuma N, Komatsubara Y, Takeda H, Hirose H, Sekijima M, Nojima T, Miyakoshi J. DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. Bioelectromagnetics 27:51-57, 2006.

We conducted a large-scale in vitro study focused on the effects of low level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system in order to test the hypothesis that modulated RF fields may act as a DNA damaging agent. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced different levels of DNA damage. Human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs were exposed to mobile communication frequency radiation to investigate whether such exposure produced DNA strand breaks in cell culture. A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg and CW radiation at 80 mW/kg for 2 and 24 h, while IMR-90 cells were exposed to both W-CDMA and CW radiations at a SAR of 80 mW/kg for the same time periods. Under the same RF field exposure conditions, no significant differences in the DNA strand breaks were observed between the test groups exposed to W-CDMA or CW radiation and the sham exposed negative controls, as evaluated immediately after the exposure periods by alkaline comet assays. Our results confirm that low level exposures do not act as a genotoxicant up to a SAR of 800 mW/kg.

(NE) (VI) Sannino A, Di Costanzo G, Brescia F, Sarti M, Zeni O, Juutilainen J, Scarfì MR. Human fibroblasts and 900 MHz radiofrequency radiation: evaluation of DNA damage after exposure and co-exposure to 3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-furanone (MX). Radiat Res 171:743-751, 2009.

The aim of this study was to investigate DNA damage in human dermal fibroblasts from a healthy subject and from a subject affected by Turner's syndrome that were exposed for 24 h to radiofrequency (RF) radiation at 900 MHz. The RF-radiation exposure was carried out alone or in combination with 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a well-known environmental mutagen and carcinogen produced during the chlorination of drinking water. Turner's syndrome fibroblasts were also exposed for a shorter time (1 h). A signal similar to that emitted by Global System for Mobile Communications (GSM) mobile phones was used at a specific absorption rate of 1 W/kg under strictly controlled conditions of temperature and dosimetry. To evaluate DNA damage after RF-radiation exposure alone, the alkaline comet assay and the cytokinesis-block micronucleus assay were used. In the combined-exposure experiments, MX was given at a concentration of 25 microM for 1 h immediately after the RF-radiation exposure, and the effects were evaluated by the alkaline comet assay. The results revealed no genotoxic and cytotoxic effects from RF radiation alone in either cell line. As expected, MX treatment induced an increase in DNA migration in the comet assay, but no enhancement of the MX-induced DNA damage was observed in the cells exposed to RF radiation.

(E) (VI) Schwarz C, Kratochvil E, Pilger A, Kuster N, Adlkofer F, Rüdiger HW. Radiofrequency electromagnetic fields (UMTS, 1,950 MHz) induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes. Int Arch Occup Environ Health 81:755-767, 2008.

OBJECTIVE: Universal Mobile Telecommunication System (UMTS) was recently introduced as the third generation mobile communication standard in Europe. This was done without any information on biological effects and genotoxic properties of these particular high-frequency electromagnetic fields. This is discomforting, because genotoxic effects of the second generation standard Global System for Mobile Communication have been reported after exposure of human cells in vitro. METHODS: Human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures were exposed to 1,950 MHz UMTS below the specific absorption rate (SAR) safety limit of 2 W/kg. The alkaline comet assay and the micronucleus assay were used to ascertain dose and time-dependent genotoxic effects. Five hundred cells per slide were visually evaluated in the comet assay and comet tail factor (CTF) was calculated. In the micronucleus assay 1,000 binucleated cells were evaluated per assay. The origin of the micronuclei was determined by fluorescence labeled anticentromere antibodies. All evaluations were performed under blinded conditions. RESULTS: UMTS exposure increased the CTF and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way. Incubation for 24 h at a SAR of 0.05 W/kg generated a statistically significant rise in both CTF and MN ($P = 0.02$). At a SAR of 0.1 W/kg the CTF was significantly increased after 8 h of incubation ($P = 0.02$), the number of MN after 12 h ($P = 0.02$). No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with Phytohemagglutinin. CONCLUSION: UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

(E) (VO) Shahin S, Singh VP, Shukla RK, Dhawan A, Gangwar RK, Singh SP, Chaturvedi CM. 2.45 GHz microwave irradiation-induced oxidative stress affects implantation or pregnancy in mice, Mus musculus. Appl Biochem Biotechnol. 169(5):1727-1751, 2013.

The present experiment was designed to study the 2.45 GHz low-level microwave (MW) irradiation-induced stress response and its effect on implantation or pregnancy in female mice. Twelve-week-old mice were exposed to MW radiation (continuous wave for 2 h/day for 45 days, frequency 2.45 GHz, power density=0.033549 mW/cm(2), and specific absorption rate=0.023023 W/kg). At the end of a total of 45 days of exposure, mice were sacrificed, implantation sites were monitored, blood was processed to study stress parameters (hemoglobin, RBC and WBC count, and neutrophil/lymphocyte (N/L) ratio), the brain was processed for comet assay, and plasma was used for nitric oxide (NO), progesterone and estradiol estimation. Reactive oxygen species (ROS) and the activities of ROS-scavenging enzymes- superoxide dismutase, catalase, and glutathione peroxidase-were determined in the liver, kidney and ovary. We observed that implantation sites were affected significantly in MW-irradiated mice as compared to control. Further, in addition to a significant increase in ROS, hemoglobin ($p<0.001$), RBC and WBC counts ($p<0.001$), N/L ratio ($p<0.01$), DNA damage ($p<0.001$) in brain cells, and plasma estradiol concentration ($p<0.05$), a significant decrease was observed in NO level ($p<0.05$) and antioxidant enzyme activities of MW-exposed mice. Our findings led us to conclude that a low level of MW irradiation-induced oxidative stress not only suppresses implantation, but it may also lead to deformity of the embryo in case pregnancy continues. We also suggest that MW radiation-induced oxidative stress by increasing ROS production in the body may lead to DNA strand breakage in the brain cells and implantation failure/resorption or abnormal pregnancy in mice.

(NE) (VI) Speit G, Schütz P, Hoffmann H. Genotoxic effects of exposure to radiofrequency electromagnetic fields (RF-EMF) in cultured mammalian cells are not independently reproducible. Mutat Res. 626(1-2):42-47, 2007.

Conflicting results have been published regarding the induction of genotoxic effects by exposure to radiofrequency electromagnetic fields (RF-EMF). Using the comet assay, the micronucleus test and the chromosome aberration test with human fibroblasts (ES1 cells), the EU-funded "REFLEX" project (Risk Evaluation of Potential Environmental Hazards From Low Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods) reported clearly positive effects for various exposure conditions. Because of the ongoing discussion on the biological significance of the effects observed, it was the aim of the present study to independently repeat the results using the same cells, the same equipment and the same exposure conditions. We therefore exposed ES1 cells to RF-EMF (1800 MHz; SAR 2 W/kg, continuous wave with intermittent exposure) for different time periods and then performed the alkaline (pH>13) comet assay and the micronucleus test (MNT). For both tests, clearly negative results were obtained in independently repeated experiments. We also performed these experiments with V79 cells, a sensitive Chinese hamster cell line that is frequently used in genotoxicity testing, and also did not measure any genotoxic effect in the comet assay and the MNT. Appropriate measures of quality control were considered to exclude variations in the test performance, failure of the RF-EMF exposure or an evaluation bias. The reasons for the difference between the results reported by the REFLEX project and our experiments remain unclear.

(NE) (VI) Stronati L, Testa A, Moquet J, Edwards A, Cordelli E, Villani P, Marino C, Fresegna AM, Appolloni M, Lloyd D. 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes. Int J Radiat Biol. 82(5):339-346, 2006.

Purpose: The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with x-rays was investigated in vitro using several assays on human lymphocytes. The chosen specific absorption rate (SAR) values are near the upper limit of actual energy absorption in localized tissue when persons use some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen.Methods: Blood specimens from 14 donors were exposed continuously for 24 h to a Global System for Mobile Communications (GSM) basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in vitro cell cycling.Results: By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did not modify any measured effects of the x-radiation.Conclusions: This study has used several standard in vitro tests for chromosomal and DNA damage in Go human lymphocytes exposed in vitro to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic per se or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed.

(E) (VI) Sun C, Wei X, Fei Y, Su L, Zhao X, Chen G, Xu Z. Mobile phone signal exposure triggers a hormesis-like effect in Atm+/+ and Atm-/- mouse embryonic fibroblasts. Sci Rep. 2016 Nov 18;6:37423. doi: 10.1038/srep37423.

Radiofrequency electromagnetic fields (RF-EMFs) have been classified by the International Agency for Research on Cancer as possible carcinogens to humans; however, this conclusion is based on limited epidemiological findings and lacks solid support from experimental studies. In particular, there are no consistent data regarding the genotoxicity of RF-EMFs. Ataxia telangiectasia mutated (ATM) is recognised as a chief guardian of genomic stability. To address the debate on whether RF-EMFs are genotoxic, we compared the effects of 1,800 MHz RF-EMF exposure on genomic DNA in mouse embryonic fibroblasts (MEFs) with proficient ($Atm^{+/+}$) or deficient ($Atm^{-/-}$) ATM. In $Atm^{+/+}$ MEFs, RF-EMF exposure for 1 h at an average special absorption rate of 4.0 W/kg induced significant DNA single-strand breaks (SSBs) and activated the SSB repair mechanism. This effect reduced the DNA damage to less than that of the

background level after 36 hours of exposure. In the $Atm^{-/-}$ MEFs, the same RF-EMF exposure for 12 h induced both SSBs and double-strand breaks and activated the two repair processes, which also reduced the DNA damage to less than the control level after prolonged exposure. The observed phenomenon is similar to the hormesis of a toxic substance at a low dose. To the best of our knowledge, this study is the first to report a hormesis-like effect of an RF-EMF.

(E) (VI) Sun LX, Yao K, He JL, Lu DQ, Wang KJ, Li HW.[Effect of acute exposure to microwave from mobile phone on DNA damage and repair of cultured human lens epithelial cells in vitro.] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing ZaZhi. 24:465-467, 2006. [Article in Chinese]

OBJECTIVE: To investigate the DNA damage of human lens epithelial cells (LECs) caused by acute exposure to low-power 217 Hz modulated 1.8 GHz microwave radiation and DNA repair. METHODS: Cultured LECs were exposed to 217 Hz modulated 1.8 GHz microwave radiation at SAR (specific absorption rate) of 0, 1, 2, 3 and 4 W/kg for 2 hours in an sXc-1800 incubator and irradiate system. The DNA single strand breaks were detected with comet assay in sham-irradiated cells and irradiated cells incubated for varying periods: 0, 30, 60, 120 and 240 min after irradiation. Images of comets were digitized and analyzed using an Imagine-pro plus software, and the indexes used in this study were tail length (TL) and tail moment (TM). RESULTS: The difference in DNA-breaks between the exposure and sham exposure groups induced by 1 and 2 W/kg irradiation was not significant at every detect time ($P > 0.05$). As for the dosage of 3 and 4 W/kg there was difference in both groups immediately after irradiation ($P < 0.01$). At the time of 30 min after irradiation the difference went on at both group ($P < 0.01$). However, the difference disappeared after one hour's incubation in 3 W/kg group ($P > 0.05$), and existed in 4 W/kg group. CONCLUSION: No or repairable DNA damage was observed after 2 hour irradiation of 1.8 GHz microwave on LECs when SAR </= 3 W/kg. The DNA damages caused by 4 W/kg irradiation were irreversible.

(E) (VI) Sun LX, Yao K, Jiang H, He JL, Lu DQ, Wang KJ, Li HW [DNA damage and repair induced by acute exposure of microwave from mobile phone on cultured human lens epithelial cells] Zhonghua Yan Ke Za Zhi. 42(12):1084-1088, 2006. [Article in Chinese]

OBJECTIVE: To investigate the effects of acute exposure of low-power 217 Hz modulated 1. 8 GHz microwave radiation on the DNA damage of human lens epithelial cells (hLECs) and repair. METHODS: Cultured hLECs were exposed to 217 Hz modulated 1. 8 GHz microwave radiation at SAR (specific absorption rate) of 1. 0, 2. 0, 3. O0 and 4. 0 W/kg for 2 hours in an sXc-1800 incubator and irradiate system, the DNA single strand breaks were detected with comet assay (single-cell gel electrophoresis) in sham-irradiated cells and irradiated cells incubated for varying periods: 0, 30 and 60 minutes after irradiation. Images of comets were digitized and analyzed using an Imagine-pro plus software, and the indexes used in this study were tail length (TL) and tail moment (TM). BrdU was added into the medium with additional one hour incubation after radiation, the cell proliferation rate was determined using a BrdU-kit. RESULTS: The difference of DNA-breaks between the exposure and sham exposure groups induced by 1.0 and 2.0 W/kg irradiation were not significant in each time points ($P > 0.05$) ; there were significant difference

in both groups at the exposure dose of 3. 0 and 4. 0 W/kg immediately and at the time of 30 minutes after irradiation (P <0. 01) ; if the radiation exposure time was beyond one hour no differences were be able to detected in 3.0 W/kg group (P > 0. 05) compared with control, but the evidence of significant DNA damage still existed in 4. 0 W/kg group at the same time point. Cell proliferation rate had no significant difference when the application of SAR was < or = 3. 0 W/kg (P >0. 05) , however the cell proliferation was decreased significantly at the dose of 4. 0 W/kg irradiation (P < 0. 01). CONCLUSIONS: No effective DNA damage was induced using comet assay after 2 hours irradiation of 1. 8 GHz microwave on hLECs at the dose SAR < or = 3.0 W/kg. 4.0 W/kg irradiation caused significantly DNA damage and inhibition of hLECs proliferation.

(NE) (VI) Tice RR, Hook GG, Donner M, McRee DI, Guy AW. Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. Bioelectromagnetics 23:113-126, 2002.

As part of a comprehensive investigation of the potential genotoxicity of radiofrequency (RF) signals emitted by cellular telephones, in vitro studies evaluated the induction of DNA and chromosomal damage in human blood leukocytes and lymphocytes, respectively. The signals were voice modulated 837 MHz produced by an analog signal generator or by a time division multiple access (TDMA) cellular telephone, 837 MHz generated by a code division multiple access (CDMA) cellular telephone (not voice modulated), and voice modulated 1909.8 MHz generated by a global system of mobile communication (GSM)-type personal communication systems (PCS) cellular telephone. DNA damage (strand breaks/alkali labile sites) was assessed in leukocytes using the alkaline (pH>13) single cell gel electrophoresis (SCG) assay. Chromosomal damage was evaluated in lymphocytes mitogenically stimulated to divide postexposure using the cytochalasin B-binucleate cell micronucleus assay. Cells were exposed at 37±1°C, for 3 or 24 h at average specific absorption rates (SARs) of 1.0-10.0 W/kg. Exposure for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes, nor did exposure for 3 h induce a significant increase in micronucleated cells among lymphocytes. However, exposure to each of the four RF signal technologies for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase in the frequency of micronucleated lymphocytes. The magnitude of the response (approximately four fold) was independent of the technology, the presence or absence of voice modulation, and the frequency (837 vs. 1909.8 MHz). This research demonstrates that, under extended exposure conditions, RF signals at an average SAR of at least 5.0 W/kg are capable of inducing chromosomal damage in human lymphocytes.

(E) (VI) Tiwari R, Lakshmi NK, Surender V, Rajesh AD, Bhargava SC, Ahuja YR. Combinative exposure effect of radio frequency signals from CDMA mobile phones and aphidicolin on DNA integrity. Electromagn Biol Med 27:418-425, 2008.

The aim of present study is to assess DNA integrity on the effect of exposure to a radio frequency (RF) signal from Code Division Multiple Access (CDMA) mobile phones. Whole blood samples from six healthy male individuals were exposed for RF signals from a CDMA mobile phone for 1 h. Alkaline comet assay was performed to assess the DNA damage. The

combinative exposure effect of the RF signals and APC at two concentrations on DNA integrity was studied. DNA repair efficiency of the samples was also studied after 2 h of exposure. The RF signals and APC (0.2 microg/ml) alone or in synergism did not have any significant DNA damage as compared to sham exposed. However, univariate analysis showed that DNA damage was significantly different among combinative exposure of RF signals and APC at 0.2 microg/ml ($p < 0.05$) and at 2 microg/ml ($p < 0.02$). APC at 2 microg/ml concentration also showed significant damage levels ($p < 0.05$) when compared to sham exposed. DNA repair efficiency also varied in a significant way in combinative exposure sets ($p < 0.05$). From these results, it appears that the repair inhibitor APC enhances DNA breaks at 2 microg/ml concentration and that the damage is possibly repairable. Thus, it can be inferred that the in vitro exposure to RF signals induces reversible DNA damage in synergism with APC.

(E) (VO) Tkalec M, Stambuk A, Srut M, Malarić K, Klobučar GI. Oxidative and genotoxic effects of 900 MHz electromagnetic fields in the earthworm Eisenia fetida. Ecotoxicol Environ Saf. 90:7-12, 2013.

Accumulating evidence suggests that exposure to radiofrequency electromagnetic field (RF-EMF) can have various biological effects. In this study the oxidative and genotoxic effects were investigated in earthworms Eisenia fetida exposed in vivo to RF-EMF at the mobile phone frequency (900MHz). Earthworms were exposed to the homogeneous RF-EMF at field levels of 10, 23, 41 and 120Vm(-1) for a period of 2h using a Gigahertz Transversal Electromagnetic (GTEM) cell. At the field level of 23Vm(-1) the effect of longer exposure (4h) and field modulation (80% AM 1kHz sinusoidal) was investigated as well. All exposure treatments induced significant genotoxic effect in earthworms coelomocytes detected by the Comet assay, demonstrating DNA damaging capacity of 900MHz electromagnetic radiation. Field modulation additionally increased the genotoxic effect. Moreover, our results indicated the induction of antioxidant stress response in terms of enhanced catalase and glutathione reductase activity as a result of the RF-EMF exposure, and demonstrated the generation of lipid and protein oxidative damage. Antioxidant responses and the potential of RF-EMF to induce damage to lipids, proteins and DNA differed depending on the field level applied, modulation of the field and duration of E. fetida exposure to 900MHz electromagnetic radiation. Nature of detected DNA lesions and oxidative stress as the mechanism of action for the induction of DNA damage are discussed.

(E) (VO) Trosić I, Pavicić I, Milković-Kraus S, Mladinić M, Zeljezić D. Effect of electromagnetic radiofrequency radiation on the rats' brain, liver and kidney cells measured by comet assay. Coll Antropol 35:1259-1264, 2011.

The goal of study was to evaluate DNA damage in rat's renal, liver and brain cells after in vivo exposure to radiofrequency/microwave (Rf/Mw) radiation of cellular phone frequencies range. To determine DNA damage, a single cell gel electrophoresis/comet assay was used. Wistar rats (male, 12 week old, approximate body weight 350 g) (N = 9) were exposed to the carrier frequency of 915 MHz with Global System Mobile signal modulation (GSM), power density of 2.4 W/m2, whole body average specific absorption rate SAR of 0.6 W/kg. The animals were

irradiated for one hour/day, seven days/week during two weeks period. The exposure set-up was Gigahertz Transversal Electromagnetic Mode Cell (GTEM--cell). Sham irradiated controls (N = 9) were apart of the study. The body temperature was measured before and after exposure. There were no differences in temperature in between control and treated animals. Comet assay parameters such as the tail length and tail intensity were evaluated. In comparison with tail length in controls (13.5 +/- 0.7 microm), the tail was slightly elongated in brain cells of irradiated animals (14.0 +/- 0.3 microm). The tail length obtained for liver (14.5 +/- 0.3 microm) and kidney (13.9 +/- 0.5 microm) homogenates notably differs in comparison with matched sham controls (13.6 +/- 0.3 microm) and (12.9 +/- 0.9 microm). Differences in tail intensity between control and exposed animals were not significant. The results of this study suggest that, under the experimental conditions applied, repeated 915 MHz irradiation could be a cause of DNA breaks in renal and liver cells, but not affect the cell genome at the higher extent compared to the basal damage.

(NE) (VO) Verschaeve L, Heikkinen P, Verheyen G, Van Gorp U, Boonen F, Vander Plaetse F, Maes A, Kumlin T, Maki-Paakkanen J, Puranen L, Juutilainen J. Investigation of co-genotoxic effects of radiofrequency electromagnetic fields in vivo. Radiat Res 165:598-607, 2006.

We investigated the possible combined genotoxic effects of radiofrequency (RF) electromagnetic fields (900 MHz, amplitude modulated at 217 Hz, mobile phone signal) with the drinking water mutagen and carcinogen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). Female rats were exposed to RF fields for a period of 2 years for 2 h per day, 5 days per week at average whole-body specific absorption rates of 0.3 or 0.9 W/kg. MX was given in the drinking water at a concentration of 19 mug/ml. Blood samples were taken at 3, 6 and 24 months of exposure and brain and liver samples were taken at the end of the study (24 months). DNA damage was assessed in all samples using the alkaline comet assay, and micronuclei were determined in erythrocytes. We did not find significant genotoxic activity of MX in blood and liver cells. However, MX induced DNA damage in rat brain. Co-exposures to MX and RF radiation did not significantly increase the response of blood, liver and brain cells compared to MX exposure only. In conclusion, this 2-year animal study involving long-term exposures to RF radiation and MX did not provide any evidence for enhanced genotoxicity in rats exposed to RF radiation.

(NE) (VI) Vijayalaxmi, Leal BZ, Szilagyi M, Prihoda TJ, Meltz ML, Primary DNA Damage in Human Blood Lymphocytes Exposed In Vitro to 2450 MHz Radiofrequency Radiation. Radiat Res 153(4):479-486, 2000.

Human peripheral blood samples collected from three healthy human volunteers were exposed in vitro to pulsed-wave 2450 MHz radiofrequency (RF) radiation for 2 h. The RF radiation was generated with a net forward power of 21 W and transmitted from a standard gain rectangular antenna horn in a vertically downward direction. The average power density at the position of the cells in the flask was 5 mW/cm(2). The mean specific absorption rate, calculated by finite difference time domain analysis, was 2.135 (+/-0.005 SE) W/kg. Aliquots of whole blood that were sham-exposed or exposed in vitro to 50 cGy of ionizing radiation from a (137)Cs gamma-ray source were used as controls. The lymphocytes were examined to determine the extent of

primary DNA damage (single-strand breaks and alkali-labile lesions) using the alkaline comet assay with three different slide-processing schedules. The assay was performed on the cells immediately after the exposures and at 4 h after incubation of the exposed blood at 37 +/- 1 degrees C to allow time for rejoining of any strand breaks present immediately after exposure, i.e. to assess the capacity of the lymphocytes to repair this type of DNA damage. At either time, the data indicated no significant differences between RF-radiation- and sham-exposed lymphocytes with respect to the comet tail length, fluorescence intensity of the migrated DNA in the tail, and tail moment. The conclusions were similar for each of the three different comet assay slide-processing schedules examined. In contrast, the response of lymphocytes exposed to ionizing radiation was significantly different from RF-radiation- and sham-exposed cells. Thus, under the experimental conditions tested, there is no evidence for induction of DNA single-strand breaks and alkali-labile lesions in human blood lymphocytes exposed in vitro to pulsed-wave 2450 MHz radiofrequency radiation, either immediately or at 4 h after exposure.

(NE) (VI) Waldmann P, Bohnenberger S, Greinert R, Hermann-Then B, Heselich A, Klug SJ, Koenig J, Kuhr K, Kuster N, Merker M, Murbach M, Pollet D, Schadenboeck W, Scheidemann-Wesp U, Schwab B, Volkmer B, Weyer V, Blettner M. Influence of GSM Signals on Human Peripheral Lymphocytes: Study of Genotoxicity. Radiat Res. 2013 Jan 14. [Epub ahead of print]

Exposure to radiofrequency (RF) electromagnetic fields (EMF) is continuously increasing worldwide. Yet, conflicting results of a possible genotoxic effect of RF EMF continue to be discussed. In the present study, a possible genotoxic effect of RF EMF (GSM, 1,800 MHz) in human lymphocytes was investigated by a collaboration of six independent institutes (institutes a, b, c, d, e, h). Peripheral blood of 20 healthy, nonsmoking volunteers of two age groups (10 volunteers 16-20 years old and 10 volunteers 50-65 years old) was taken, stimulated and intermittently exposed to three specific absorption rates (SARs) of RF EMF (0.2 W/kg, 2 W/kg, 10 W/kg) and sham for 28 h (institute a). The exposures were performed in a setup with strictly controlled conditions of temperature and dose, and randomly and automatically determined waveguide SARs, which were designed and periodically maintained by ITIS (institute h). Four genotoxicity tests with different end points were conducted (institute a): chromosome aberration test (five types of structural aberrations), micronucleus test, sister chromatid exchange test and the alkaline comet assay (Olive tail moment and % DNA). To demonstrate the validity of the study, positive controls were implemented. The genotoxicity end points were evaluated independently by three laboratories blind to SAR information (institute c = laboratory 1; institute d = laboratory 2; institute e = laboratory 3). Statistical analysis was carried out by institute b. Methods of primary statistical analysis and rules to adjust for multiple testing were specified in a statistical analysis plan based on a data review before unblinding. A linear trend test based on a linear mixed model was used for outcomes of comet assay and exact permutation test for linear trend for all other outcomes. It was ascertained that only outcomes with a significant SAR trend found by at least two of three analyzing laboratories indicated a substantiated suspicion of an exposure effect. On the basis of these specifications, none of the nine end points tested for SAR trend showed a significant and reproducible exposure effect. Highly significant differences between sham exposures and positive controls were detected by

each analyzing laboratory, thus validating the study. In conclusion, the results show no evidence of a genotoxic effect induced by RF EMF (GSM, 1,800 MHz).

(E) (VI) Wang X, Liu C, Ma Q, Feng W, Yang L, Lu Y, Zhou Z, Yu Z, Li W, Zhang L. 8-oxoG DNA Glycosylase-1 Inhibition Sensitizes Neuro-2a Cells to Oxidative DNA Base Damage Induced by 900 MHz Radiofrequency Electromagnetic Radiation. Cell Physiol Biochem. 2015, 37(3):1075-1088. [Epub ahead of print]

BACKGROUND/AIMS: The purpose of this study was to explore the in vitro putative genotoxicity during exposure of Neuro-2a cells to radiofrequency electromagnetic fields (RF-EMFs) with or without silencing of 8-oxoG DNA glycosylase-1 (OGG1). METHODS: Neuro-2a cells treated with or without OGG1 siRNA were exposed to 900 MHz Global System for Mobile Communication (GSM) Talk signals continuously at a specific absorption rate (SAR) of 0, 0.5, 1 or 2 W/kg for 24 h. DNA strand breakage and DNA base damage were measured by the alkaline comet assay and a modified comet assay using formamidopyrimidine DNA glycosylase (FPG), respectively. Reactive oxygen species (ROS) levels and cell viability were monitored using the non-fluorescent probe 2, 7-dichlorofluorescein diacetate (DCFH-DA) and CCK-8 assay. RESULTS: Exposure to 900 MHz RF-EMFs with insufficient energy could induce oxidative DNA base damage in Neuro-2a cells. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS). Without OGG1 siRNA, 2 W/kg RF-EMFs induced oxidative DNA base damage in Neuro-2a cells. Interestingly, with OGG1 siRNA, RF-EMFs could cause DNA base damage in Neuro-2a cells as low as 1 W/kg. However, neither DNA strand breakage nor altered cell viability was observed. CONCLUSION: Even if further studies remain conducted we support the hypothesis that OGG1 is involved in the process of DNA base repair and may play a pivotal role in protecting DNA bases from RF-EMF induced oxidative damage.

(E) (VI) Wu W, Yao K, Wang KJ, Lu DQ, He JL, Xu LH, Sun WJ. [Blocking 1800 MHz mobile phone radiation-induced reactive oxygen species production and DNA damage in lens epithelial cells by noise magnetic fields.]Zhejiang Da XueXueBao Yi Xue Ban 37:34-38, 2008. [Article in Chinese]

OBJECTIVE: To investigate whether the exposure to the electromagnetic noise can block reactive oxygen species (ROS) production and DNA damage of lens epithelial cells induced by 1800 MHz mobile phone radiation. METHODS: The DCFH-DA method and comet assay were used respectively to detect the intracellular ROS and DNA damage of cultured human lens epithelial cells induced by 4 W/kg 1800 MHz mobile phone radiation or/and 2microT electromagnetic noise for 24 h intermittently. RESULT: 1800 MHz mobile phone radiation at 4 W/kg for 24 h increased intracellular ROS and DNA damage significantly ($P<0.05$). However, the ROS level and DNA damage of mobile phone radiation plus noise group were not significant enhanced ($P>0.05$) as compared to sham exposure group. Conclusion: Electromagnetic noise can block intracellular ROS production and DNA damage of human lens epithelial cells induced by 1800 MHz mobile phone radiation.

(E) (VI) Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, Ye J, Sun L. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol Vis 14:964-969, 2008.

PURPOSE: The goal of this study was to investigate whether superposing of electromagnetic noise could block or attenuate DNA damage and intracellular reactive oxygen species (ROS) increase of cultured human lens epithelial cells (HLECs) induced by acute exposure to 1.8 GHz radiofrequency field (RF) of the Global System for Mobile Communications (GSM). METHODS: An sXc-1800 RF exposure system was used to produce a GSM signal at 1.8 GHz (217 Hz amplitude-modulated) with the specific absorption rate (SAR) of 1, 2, 3, and 4 W/kg. After 2 h of intermittent exposure, the ROS level was assessed by the fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DNA damage to HLECs was examined by alkaline comet assay and the phosphorylated form of histone variant H2AX (gammaH2AX) foci formation assay. RESULTS: After exposure to 1.8 GHz RF for 2 h, HLECs exhibited significant intracellular ROS increase in the 2, 3, and 4 W/kg groups. RF radiation at the SAR of 3 W/kg and 4 W/kg could induce significant DNA damage, examined by alkaline comet assay, which was used to detect mainly single strand breaks (SSBs), while no statistical difference in double strand breaks (DSBs), evaluated by gammaH2AX foci, was found between RF exposure (SAR: 3 and 4 W/kg) and sham exposure groups. When RF was superposed with 2 muT electromagnetic noise could block RF-induced ROS increase and DNA damage. CONCLUSIONS: DNA damage induced by 1.8 GHz radiofrequency field for 2 h, which was mainly SSBs, may be associated with the increased ROS production. Electromagnetic noise could block RF-induced ROS formation and DNA damage.

(E) (VO) Ye W, Wang F, Zhang W, Fang N, Zhao W, Wang J. Effect of Mobile Phone Radiation on Cardiovascular Development of Chick Embryo. Anat Histol Embryol. 2015 Jul 14. doi: 10.1111/ahe.12188. [Epub ahead of print]

The biological effects on cardiovascular development of chicken embryos were examined after radiation exposure using mobile phone (900 MHz; specific absorption rate˜1.07 W/kg) intermittently 3 h per day during incubation. Samples were selected by morphological and histological methods. The results showed the rate of embryonic mortality and cardiac deformity increased significantly in exposed group ($P < 0.05$). No any histological pathological changes were observed on Day 5-7 (D5-D7) of incubation. A higher distribution of lipid droplets was unexpectedly present in myocardial tissue from the exposure groups on D10-D13. Soon afterwards, myofilament disruption, atrioventricular valve focal necrosis, mitochondria vacuolization and atrial natriuretic peptide (ANP) decrease appeared on D15-D21 of incubation. Comet assay data showed the haemocyte mean tail in the exposed group was significantly larger than that of the control ($P < 0.01$). The arterial vascular wall of exposed group was thicker ($P < 0.05$) than that of the control on D13, which was reversed to normal in later stages. Our findings suggest that long-term exposure of MPR may induce myocardium pathological changes, DNA damage and increased mortality; however, there was little effect on vascular development.

(NE) (VI) Zeni O, Romano M, Perrotta A, Lioi MB, Barbieri R, d'Ambrosio G, Massa R, Scarfi MR. Evaluation of genotoxic effects in human peripheral blood leukocytes following an acute in vitro exposure to 900 MHz radiofrequency fields. Bioelectromagnetics. 26(4):258-265, 2005.

Human peripheral blood leukocytes from healthy volunteers have been employed to investigate the induction of genotoxic effects following 2 h exposure to 900 MHz radiofrequency radiation. The GSM signal has been studied at specific absorption rates (SAR) of 0.3 and 1 W/kg. The exposures were carried out in a waveguide system under strictly controlled conditions of both dosimetry and temperature. The same temperature conditions (37.0 ± 0.1 degrees C) were realized in a second waveguide, employed to perform sham exposures. The induction of DNA damage was evaluated in leukocytes by applying the alkaline single cell gel electrophoresis (SCGE)/comet assay, while structural chromosome aberrations and sister chromatid exchanges were evaluated in lymphocytes stimulated with phytohemagglutinin. Alterations in kinetics of cell proliferation were determined by calculating the mitotic index. Positive controls were also provided by using methyl methanesulfonate (MMS) for comet assay and mitomycin-C (MMC), for chromosome aberration, or sister chromatid exchange tests. No statistically significant differences were detected in exposed samples in comparison with sham exposed ones for all the parameters investigated. On the contrary, the positive controls gave a statistically significant increase in DNA damage in all cases, as expected. Thus the results obtained in our experimental conditions do not support the hypothesis that 900 MHz radiofrequency field exposure induces DNA damage in human peripheral blood leukocytes in this range of SAR.

(NE) (VI) Zeni O, Schiavoni A, Perrotta A, Forigo D, Deplano M, Scarfi MR. Evaluation of genotoxic effects in human leukocytes after in vitro exposure to 1950 MHz UMTS radiofrequency field. Bioelectromagnetics 29:177-184, 2008.

In the present study the third generation wireless technology of the Universal Mobile Telecommunication System (UMTS) signal was investigated for the induction of genotoxic effects in human leukocytes. Peripheral blood from six healthy donors was used and, for each donor, intermittent exposures (6 min RF on, 2 h RF off) at the frequency of 1950 MHz were conducted at a specific absorption rate of 2.2 W/kg. The exposures were performed in a transverse electro magnetic (TEM) cell hosted in an incubator under strictly controlled conditions of temperature and dosimetry. Following long duration intermittent RF exposures (from 24 to 68 h) in different stages of the cell cycle, micronucleus formation was evaluated by applying the cytokinesis block micronucleus assay, which also provides information on cell division kinetics. Primary DNA damage (strand breaks/alkali labile sites) was also investigated following 24 h of intermittent RF exposures, by applying the alkaline single cell gel electrophoresis (SCG)/comet assay. Positive controls were included by treating cell cultures with Mitomycin-C and methylmethanesulfonate for micronucleus and comet assays, respectively. The results obtained indicate that intermittent exposures of human lymphocytes

in different stages of cell cycle do not induce either an increase in micronucleated cells, or change in cell cycle kinetics; moreover, 24 h intermittent exposures also fail to affect DNA structure of human leukocytes soon after the exposures, likely indicating that repairable DNA damage was not induced.

(E) (VI) Zhang DY, Xu ZP, Chiang H, Lu DQ, Zeng QL. [Effects of GSM 1800 MHz radiofrequency electromagnetic fields on DNA damage in Chinese hamster lung cells.] Zhonghua Yu Fang Yi Xue Za Zhi. 40(3):149-152, 2006. [Article in Chinese]

OBJECTIVE: To study the effects of GSM 1800 MHz radiofrequency electromagnetic fields (RF EMF) on DNA damage in Chinese hamster lung (CHL) cells. METHODS: The cells were intermittently exposed or sham-exposed to GSM 1800 MHz RF EMF (5 minutes on/10 minutes off) at a special absorption rate (SAR) of 3.0 W/kg for 1 hour or 24 hours. Meanwhile, cells exposed to 2-acetaminofluorene, a DNA damage agent, at a final concentration of 20 mg/L for 2 hours were used as positive control. After exposure, cells were fixed by using 4% paraformaldehyde and processed for phosphorylated form of H2AX (gammaH2AX) immunofluorescence measurement. The primary antibody used for immunofluorescence was mouse monoclonal antibody against gammaH2AX and the secondary antibody was fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG. Nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI). The gammaH2AX foci and nuclei were visualized with an Olympus AX70 fluorescent microscope. Image Pro-Plus software was used to count the gammaH2AX foci in each cell. For each exposure condition, at least 50 cells were selected to detect gammaH2AX foci. Cells were classified as positive when more than five foci were detected. The percentage of gammaH2AX foci positive cells was adopted as the index of DNA damage. RESULTS: The percentage of gammaH2AX foci positive cell of 1800 MHz RF EMF exposure for 24 hours (37.9 +/- 8.6)% or 2-acetylaminofluorene exposure (50.9 +/- 9.4)% was significantly higher compared with the sham-exposure (28.0 +/- 8.4)%. However, there was no significant difference between the sham-exposure and RF EMF exposure for 1 hour (31.8 +/- 8.7)%. CONCLUSION: 1800 MHz RF EMF (SAR, 3.0 W/kg) for 24 hours might induce DNA damage in CHL cells.

(E) (VI) 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/cm2) 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.

(E) (VI) Zhijian C, Xiaoxue L, Yezhen L, Shijie C, Lifen J, Jianlin L, Deqiang L, Jiliang H. Impact of 1.8-GHz radiofrequency radiation (RFR) on DNA damage and repair induced by doxorubicin in human B-cell lymphoblastoid cells. Mutat Res. 695(1-2):16-21, 2010.

In the present in vitro study, a comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR, SAR of 2W/kg) can influence DNA repair in human B-cell lymphoblastoid cells exposed to doxorubicin (DOX) at the doses of 0microg/ml, 0.05microg/ml, 0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml. The combinative exposures to RFR with DOX were divided into five categories. DNA damage was detected at 0h, 6h, 12h, 18h and 24h after exposure to DOX via the comet assay, and the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage. The results demonstrated that (1) RFR could not directly induce DNA damage of human B-cell lymphoblastoid cells; (2) DOX could significantly induce DNA damage of human B-cell lymphoblastoid cells with the dose-effect relationship, and there were special repair characteristics of DNA damage induced by DOX; (3) E-E-E type (exposure to RFR for 2h, then simultaneous exposure to RFR and DOX, and exposure to RFR for 6h, 12h, 18h and 24h after exposure to DOX) combinative exposure could obviously influence DNA repair at 6h and 12h after exposure to DOX for four DOX doses (0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml) in human B-cell lymphoblastoid cells.

(NE) (VI) Zhijian C, Xiaoxue L, Yezhen L, Deqiang L, Shijie C, Lifen J, Jianlin L, Jiliang H. Influence of 1.8-GHz (GSM) radiofrequency radiation (RFR) on DNA damage and repair induced by X-rays in human leukocytes in vitro. Mutat Res. 677(1-2):100-104, 2009.

In the present study, the in vitro comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR) can influence DNA repair in human leukocytes exposed to X-rays. The specific energy absorption rate (SAR) of 2 W/kg (the current European safety limit) was applied. The leukocytes from four young healthy donors were intermittently exposed to RFR for 24 h (fields on for 5 min, fields off for 10 min), and then irradiated with X-rays at doses of 0.25, 0.5, 1.0 and 2.0 Gy. DNA damage to human leukocytes was detected using the comet assay at 0, 15, 45, 90, 150 and 240 min after exposure to X-rays. Using the comet assay, the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage; the DNA repair percentage (DRP) served as the indicator of the DNA repair speed. The results demonstrated that (1) the DNA repair speeds of human leukocytes after X-ray exposure exhibited individual differences among the four donors; (2) the intermittent exposures of 1.8-GHz RFR at the SAR of 2 W/kg for 24 h did not directly induce DNA damage or exhibit synergistic effects with X-rays on human leukocytes.

(NE) (VI) Zuo WQ, Hu YJ, Yang Y, Zhao XY, Zhang YY, Kong W, Kong WJ. Sensitivity of spiral ganglion neurons to damage caused by mobile phone **electromagnetic radiation will increase in lipopolysaccharide-induced inflammation in vitro model. J Neuroinflammation. 2015 May 29;12(1):105. [Epub ahead of print]**

BACKGROUND: With the increasing popularity of mobile phones, the potential hazards of radiofrequency electromagnetic radiation (RF-EMR) on the auditory system remain unclear. Apart from RF-EMR, humans are also exposed to various physical and chemical factors. We established a lipopolysaccharide (LPS)-induced inflammation in vitro model to investigate whether the possible sensitivity of spiral ganglion neurons to damage caused by mobile phone electromagnetic radiation (at specific absorption rates: 2, 4 W/kg) will increase. METHODS: Spiral ganglion neurons (SGN) were obtained from neonatal (1- to 3-day-old) Sprague Dawley® (SD) rats. After the SGN were treated with different concentrations (0, 20, 40, 50, 100, 200, and 400 μg/ml) of LPS, the Cell Counting Kit-8 (CCK-8) and alkaline comet assay were used to quantify cellular activity and DNA damage, respectively. The SGN were treated with the moderate LPS concentrations before RF-EMR exposure. After 24 h intermittent exposure at an absorption rate of 2 and 4 W/kg, DNA damage was examined by alkaline comet assay, ultrastructure changes were detected by transmission electron microscopy, and expression of the autophagy markers LC3-II and Beclin1 were examined by immunofluorescence and confocal laser scanning microscopy. Reactive oxygen species (ROS) production was quantified by the dichlorofluorescin-diacetate assay. RESULTS: LPS (100 μg/ml) induced DNA damage and suppressed cellular activity ($P < 0.05$). LPS (40 μg/ml) did not exhibit cellular activity changes or DNA damage ($P > 0.05$); therefore, 40 μg/ml was used to pretreat the concentration before

exposure to RF-EMR. RF-EMR could not directly induce DNA damage. However, the 4 W/kg combined with LPS (40 μg/ml) group showed mitochondria vacuoles, karyopyknosis, presence of lysosomes and autophagosome, and increasing expression of LC3-II and Beclin1. The ROS values significantly increased in the 4 W/kg exposure, 4 W/kg combined with LPS (40 μg/ml) exposure, and H_2O_2 groups ($P < 0.05, 0.01$). CONCLUSIONS: Short-term exposure to radiofrequency electromagnetic radiation could not directly induce DNA damage in normal spiral ganglion neurons, but it could cause the changes of cellular ultrastructure at special SAR 4.0 W/kg when cells are in fragile or micro-damaged condition. It seems that the sensitivity of SGN to damage caused by mobile phone electromagnetic radiation will increase in a lipopolysaccharide-induced inflammation in vitro model.

BioInitiative - Mechanisms of Harm; Research Summaries,
Free Radicals (Oxidative Stress Effects), 225 studies, 2019

April 19, 2019

RFR - Free Radical (oxidative effects) (E=203 (90%); NE=22 (10%)) (E= reported effect; NE= reported no significant effect).

(E) Abu Khadra KM, Khalil AM, Abu Samak M, Aljaberi A. Evaluation of selected biochemical parameters in the saliva of young males using mobile phones. Electromagn Biol Med. 2014 Feb 5. [Epub ahead of print]

Abstract The biochemical status in the saliva of 12 males before/after using mobile phone has been evaluated. Radio frequency signals of 1800 MHz (continuous wave transmission, 217 Hz modulate and Global System for Mobile Communications [GSM - non-DTX]) with 1.09 w/kg specific absorption rate (SAR) value were used for 15 and 30 min. Cell phone radiation induced a significant increase of superoxide dismutase (SOD); there was a statistically significant effect of talking time on the levels of SOD, $F(2, 33) = 8.084$, $p < 0.05$, $\omega = 0.53$. The trend analysis suggests a significant quadratic trend, $F(1, 33) = 4.891$, $p < 0.05$; indicating that after 15 min of talking the levels of SOD increased, but as talking time increased the SOD activity started to drop. In contrast to this, there was no statistically significant effect of talking time on the level of salivary albumin, cytochrome c, catalase or uric acid. Results suggest that exposure to electromagnetic radiation may exert an oxidative stress on human cells as evidenced by the increase in the concentration of the superoxide radical anion released in the saliva of cell phone users.

(E) Achudume A, Onibere B, Aina F, Tchokossa P. Induction of oxidative stress in male rats subchronically exposed to electromagnetic fields at non-thermal intensities. J Electromagnetic Analysis and Applications 2(8), 482-487, 2010. (LI)

To investigate the oxidative stress-inducing potential of non-thermal electromagnetic fields in rats. Male Wister rats were exposed to electrical field intensity of 2.3 ± 0.82 µV/m . Exposure was in three forms: continuous waves, or modulated at 900 MHz or modulated GSM-nonDTX. The radio frequency radiation (RFR) was 1800 MHz, specific absorption radiation (SAR) (0.95-3.9 W/kg) for 40 and/or 60 days continuously. Control animals were located > 300 m from base station, while sham control animals were located in a similar environmental conditions, but in the vicinity of a non-functional base station. The rats were assessed for thiobarbituric and reactive species (TBARS), reduced glutathione (GSH) content, catalase activity, glutathione reductase (GR) and glucose residue after 40 and 60 days of exposure. At 40 days, electromagnetic radiation failed to induce any significant alterations. However, at 60 days of exposure various attributes evaluated decreased. The respective decreases in both nicotinamide adenine dinucleotide phosphate (NADPH) and Ascorbate- linked lipid peroxidation (LPO) with concomitant diminution in enzymatic antioxidative defense systems resulted in decreased glucose residue. The present studies showed some biochemical changes that may be associated with a prolong exposure to electromagnetic fields and its relationship to the activity of antioxidant system in rat Regular assessment and early detection of antioxidative defense system among people working around the base stations are recommended.

(E) Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. Fertil Steril. 92(4) 1318-1325, 2009.

OBJECTIVE: To evaluate effects of cellular phone radiofrequency electromagnetic waves (RF-EMW) during talk mode on unprocessed (neat) ejaculated human semen. DESIGN: Prospective pilot study. SETTING: Center for reproductive medicine laboratory in tertiary hospital setting. SAMPLES: Neat semen samples from normal healthy donors (n = 23) and infertile patients (n = 9). INTERVENTION(S): After liquefaction, neat semen samples were divided into two aliquots. One aliquot (experimental) from each patient was exposed to cellular phone radiation (in talk mode) for 1 h, and the second aliquot (unexposed) served as the control sample under identical conditions. MAIN OUTCOME MEASURE(S): Evaluation of sperm parameters (motility, viability), reactive oxygen species (ROS), total antioxidant capacity (TAC) of semen, ROS-TAC score, and sperm DNA damage. RESULT(S): Samples exposed to RF-EMW showed a significant decrease in sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score. Levels of TAC and DNA damage showed no significant differences from the unexposed group. CONCLUSION(S): Radiofrequency electromagnetic waves emitted from cell phones may lead to oxidative stress in human semen. We speculate that keeping the cell phone in a trouser pocket in talk mode may negatively affect spermatozoa and impair male fertility.

(E) Ahmed NA, Radwan NM, Aboul Ezz HS, Salama NA. The antioxidant effect of Green Tea Mega EGCG against electromagnetic radiation-induced oxidative stress in the hippocampus and striatum of rats. Electromagn Biol Med. 2017;36(1):63-73, 2017.

Electromagnetic radiation (EMR) of cellular phones may affect biological systems by increasing free radicals and changing the antioxidant defense systems of tissues, eventually leading to oxidative stress. Green tea has recently attracted significant attention due to its health benefits in a variety of disorders, ranging from cancer to weight loss. Thus, the aim of the present study was to investigate the effect of EMR (frequency 900 MHz modulated at 217 Hz, power density 0.02 mW/cm^2, SAR 1.245 W/kg) on different oxidative stress parameters in the hippocampus and striatum of adult rats. This study also extends to evaluate the therapeutic effect of green tea mega EGCG on the previous parameters in animals exposed to EMR after and during EMR exposure. The experimental animals were divided into four groups: EMR-exposed animals, animals treated with green tea mega EGCG after 2 months of EMR exposure, animals treated with green tea mega EGCG during EMR exposure and control animals. EMR exposure resulted in oxidative stress in the hippocampus and striatum as evident from the disturbances in oxidant and antioxidant parameters. Co-administration of green tea mega EGCG at the beginning of EMR exposure for 2 and 3 months had more beneficial effect against EMR-induced oxidative stress than oral administration of green tea mega EGCG after 2 months of exposure. This

recommends the use of green tea before any stressor to attenuate the state of oxidative stress and stimulate the antioxidant mechanism of the brain.

(E) Akbari A, Jelodar G, Nazifi S. Vitamin C protects rat cerebellum and encephalon from oxidative stress following exposure to radiofrequency wave generated by a BTS antenna model. Toxicol Mech Methods. 24(5):347-352, 2014.

Radio frequency wave (RFW) generated by base transceiver station has been reported to produce deleterious effects on the central nervous system function, possibly through oxidative stress. This study was conducted to evaluate the effect of RFW-induced oxidative stress in the cerebellum and encephalon and the prophylactic effect of vitamin C on theses tissues by measuring the antioxidant enzymes activity, including: glutathione peroxidase, superoxide dismutase, catalase, and malondialdehyde (MDA). Thirty-two adult male Sprague-Dawley rats were randomly divided into four equal groups. The control group; the control-vitamin C group received L-ascorbic acid (200 mg/kg of body weight/day by gavage) for 45 days. The RFW group was exposed to RFW and the RFW+ vitamin C group was exposed to RFW and received vitamin C. At the end of the experiment, all groups were killed and encephalon and cerebellum of all rats were removed and stored at -70 °C for measurement of antioxidant enzymes activity and MDA. The results indicate that exposure to RFW in the test group decreased antioxidant enzymes activity and increased MDA compared with the control groups ($p < 0.05$). The protective role of vitamin C in the treated group improved antioxidant enzymes activity and reduced MDA compared with the test group ($p < 0.05$). It can be concluded that RFW causes oxidative stress in the brain and vitamin C improves the antioxidant enzymes activity and decreases MDA.

(E) Akimoto T, Umemura M, Nagasako A, Ohtake M, Fujita T, Yokoyama U, Eguchi H, Yamamoto T, Ishikawa Y. Alternating magnetic field enhances cytotoxicity of Compound C. Cancer Sci. 109(11):3483-3493, 2018.

We previously reported the efficacy of anti-cancer therapy with hyperthermia using an alternating magnetic field (AMF) and a magnetic compound. In the course of the study, unexpectedly, we found that an AMF enhances the cytotoxicity of Compound C, an activated protein kinase (AMPK) inhibitor, although this compound is not magnetic. Therefore, we examined the cellular mechanism of AMF-induced cytotoxicity of Compound C in cultured human glioblastoma (GB) cells. An AMF (280 kHz, 250 Arms) for 30 minutes significantly enhanced the cytotoxicity of Compound C and promoted apoptosis towards several human GB cell lines in vitro. The AMF also increased Compound C-induced cell-cycle arrest of GB cells at the G2 phase and, thus, inhibited cell proliferation. The AMF increased Compound C-induced reactive oxygen species production. Furthermore, the AMF decreased ERK phosphorylation in the presence of Compound C and suppressed the protective autophagy induced by this compound. The application of an AMF in cancer chemotherapy may be a simple and promising method, which might reduce the doses of drugs used in future cancer treatment and, therefore, the associated side effects.

(E) Akoev IG, Pashovkina MS, Dolgacheva LP, Semenova TP, Kalmykov VL. [Enzymatic activity of some tissues and blood serum from animals and humans exposed to microwaves and hypothesis on the possible role of free radical processes in the nonlinear effects and modification of emotional behavior of animals]. [Article in Russian] Radiats Biol Radioecol. 42(3):322-330, 2002. (LI)

The dependence of activities of actomyosin ATPase, alkaline phosphatase, aspartataminotranspherase, monoaminoxidase and that of affective rat behavior on frequency of modulation of microwaves (0.8-10 microW/cm2) was explored at short-time actions. Series of nonlinear phenomenons, inexplicable from positions of the energy approaches are revealed, The working hypothesis explaining opportunity of high performance of weak and super-weak microwaves and other revealed phenomena by resonance interaction of such electromagnetic radiofrequency radiation with paramagnetic molecules of biological tissues was proposed. This resonance interaction activate free radicals and initiate auto-supporting and auto-intensifying of chain chemical reactions. The spontaneous autocatalytic oxidation of catecholamines enlarges a common pool of free radicals, capable to participate in such enhanced generating. The protective role of monoaminoxidase is postulated. Monoaminoxidase is basically located on an outer surface of mitochondrias and it is deaminating monoamines. The deaminating prevents penetration of catecholamines inside of mitochondrias and their quinoid oxidation there with formation of free-radical semi-quinons, capable to destroy system of ATP synthesis. These inferences are obliquely confirmed by the experimentally revealed correlation between activity of monoaminoxidase and integrative activity of the rat brain.

(E) Alkis ME, Bilgin HM, Akpolat V, Dasdag S, Yegin K, Yavas MC, Akdag MZ. Effect of 900-, 1800-, and 2100-MHz radiofrequency radiation on DNA and oxidative stress in brain. Electromagn Biol Med. 38(1):32-47, 2019.

Ubiquitous and ever increasing use of mobile phones led to the growing concern about the effects of radiofrequency radiation (RFR) emitted by cell phones on biological systems. The aim of this study is to explore whether long-term RFR exposure at different frequencies affects DNA damage and oxidant-antioxidant parameters in the blood and brain tissue of rats. 28 male Sprague Dawley rats were randomly divided into four equal groups (n = 7). They were identified as Group 1: sham-control, Group 2: 900 MHz, Group 3: 1800 MHz, and Group 4: 2100 MHz. Experimental groups of rats were exposed to RFR 2 h/day for 6 months. The sham-control group of rats was subjected to the same experimental condition but generator was turned off. Specific absorption rates (SARs) at brain with 1 g average were calculated as 0.0845 W/kg, 0.04563 W/kg, and 0.03957, at 900 MHz, 1800 MHz, and 2100 MHz, respectively. Additionally, malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), total antioxidant status (TAS), and total oxidant status (TOS) analyses were conducted in the brain tissue samples. Results of the study showed that DNA damage and oxidative stress indicators were found higher in the RFR exposure groups than in the sham-control group. In conclusion, 900-, 1800-, and 2100-MHz RFR emitted from mobile phones may cause oxidative damage, induce increase in lipid peroxidation, and increase oxidative DNA damage formation

in the frontal lobe of the rat brain tissues. Furthermore, 2100-MHz RFR may cause formation of DNA single-strand breaks.

(cancer) **Anghileri LJ, Mayayo E, Domingo JL. Iron-radiofrequency synergism in lymphomagenesis. Immunopharmacol Immunotoxicol. 28(1):175-183, 2006.**

The parenteral **iron** administration effects on the acceleration of lymphomagenesis by radiofrequency exposure were investigated using an animal model that develops spontaneous lymphomas with ageing. Complementary studies of the in vivo uptake of 59Fe-labeled ferric gluconate and ferric-ATP complex showed differences ob absorption and excretion between both **iron** compounds. In vitro assays of their effects on calcium cellular uptake using a cell model and tissues homogenates showed a molecular structure-dependence. The current results (mortality, clinical and histopathological examinations) demonstrated a synergism between radiofrequency and ferric gluconate, and the increased risk of radiofrequency exposure when it is simultaneous to parenteral **iron** administration.

(E) Arbabi-Kalati F, Salimi S, Vaziry-Rabiee A, Noraeei M. Effect of mobile phone usage time on total antioxidant capacity of saliva and salivary immunoglobulin a. Iran J Public Health. 43(4):480-484, 2014.

BACKGROUND: Nowadays mobile phone is very popular, causing concern about the effect it has on people's health. Parotid salivary glands are in close contact to cell phone while talking with the phone and the possibility of being affected by them. Limited studies have evaluated the effect of cell phone use on the secretions of these glands; so this study was designed to investigate the effects of duration of mobile phone use on the total antioxidant capacity of saliva. METHODS: Unstimulated saliva from 105 volunteers without oral lesions collected. The volunteers based on daily usage of mobile phones were divided into three groups then total antioxidant capacity of saliva was measured by Ferric Reducing Ability of Plasma (FRAP) method. Data were analyzed by SPSS software version 19. ANOVA was used to compare 3 groups and post-hoc Tukey test to compare between two groups. RESULTS: Average total antioxidant capacities of saliva in 3 groups were 657.91 µmol/lit, 726.77 µm/lit and 560.17 µmol/lit, respectively. The two groups had statistically significant different (P = 0.039). CONCLUSION: Over an hour talking with a cell phone decreases total antioxidant capacity of saliva in comparison with talking less than twenty minutes.

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

OBJECTIVE: To investigate effects on rat testes of radiofrequency radiation emitted from indoor Wi-Fi Internet access devices using 802.11.g wireless standards. METHODS: Ten Wistar albino male rats were divided into experimental and control groups, with five rats per group. Standard wireless gateways communicating at 2.437 GHz were used as radiofrequency wave sources. The experimental group was exposed to radiofrequency energy for 24 h a day for 20 weeks. The rats were sacrificed at the end of the study. Intracardiac blood was sampled for serum 8-hydroxy-2'-deoxyguanosine levels. Testes were removed and examined

histologically and immunohistochemically. Testis tissues were analyzed for malondialdehyde levels and prooxidant-antioxidant enzyme activities. RESULTS: We observed significant increases in serum 8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine staining in the testes of the experimental group indicating DNA damage due to exposure ($p < 0.05$). We also found decreased levels of catalase and glutathione peroxidase activity in the experimental group, which may have been due to radiofrequency effects on enzyme activity ($p < 0.05$). CONCLUSIONS: These findings raise questions about the safety of radiofrequency exposure from Wi-Fi Internet access devices for growing organisms of reproductive age, with a potential effect on both fertility and the integrity of germ cells.

(E) *Avci B, Akar A, Bilgici B, Tunçel ÖK. Oxidative stress induced by 1.8 GHz radio frequency electromagnetic radiation and effects of garlic extract in rats. Int J Radiat Biol. 88(11):799-805, 2012.

PURPOSE: We aimed to study the oxidative damage induced by radiofrequency electromagnetic radiation (RF-EMR) emitted by mobile telephones and the protective effect of garlic extract used as an anti-oxidant against this damage. MATERIALS AND METHODS: A total of 66 albino Wistar rats were divided into three groups. The first group of rats was given 1.8 GHz, 0.4 W/kg specific absorption rate (SAR) for 1 h a day for three weeks. The second group was given 500 mg/kg garlic extract in addition to RF-EMR. The third group of rats was used as the control group. At the end of the study, blood and brain tissue samples were collected from the rats. RESULTS: After the RF-EMR exposed, the advanced oxidation protein product (AOPP) levels of brain tissue increased compared with the control group ($p < 0.001$). Garlic administration accompanying the RF-EMR, on the other hand, significantly reduced AOPP levels in brain tissue ($p < 0.001$). The serum nitric oxide (NO) levels significantly increased both in the first and second group ($p < 0.001$). However, in the group for which garlic administration accompanied that of RF-EMR, there was no difference in serum NO levels compared with the RF-EMR exposed group ($p > 0.05$). There was no significant difference among the groups with respect to malondialdehyde (MDA) levels in brain tissue and blood samples ($p > 0.05$). Similarly, no difference was detected among the groups regarding serum paroxonase (PON) levels ($p > 0.05$). We did not detect any PON levels in the brain tissue. CONCLUSIONS: The exposure of RF-EMR similar to 1.8 GHz Global system for mobile communication (GSM) leads to protein oxidation in brain tissue and an increase in serum NO. We observed that garlic administration reduced protein oxidation in brain tissue and that it did not have any effects on serum NO levels.

(E) 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 O2, 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(-2) 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(-6)g 1(-1)to a

maximum of 6.50 x 10(-6) g 1(-1) within the first 24 hrs, and then gradually reduced to control value after about a week. 1mg kg(-1) of ascorbic acid administered before irradiation caused a decrease in the LPS from the control value to a minimum of 2.86 x 10(-6)g 1(-1) within the first week. The value then gradually rose to a maximum of 3.96 x 10(-6)g 1(-1) within the monitoring period. 1 mg kg(-1) of a-tocopherol also administered before irradiation also caused a decrease in the LPS from the control value to a minimum of 2.10 x 10(-6) g 1(-1) within the first week. The value then gradually rose to a maximum of 3.94 x 10(-6) g 1(-1) 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.

(E) Ayata A, Mollaoglu H, Yilmaz HR, Akturk O, Ozguner F, Altuntas I. Oxidative stress-mediated skin damage in an experimental mobile phone model can be prevented by melatonin. J Dermatol. 31(11):878-883, 2004.

Most mobile phones emit 900 MHz of radiation that is mainly absorbed by the external organs. The effects of 900 MHz of radiation on fibrosis, lipid peroxidation, and anti-oxidant enzymes and the ameliorating effects of melatonin (Mel) were evaluated in rat skin. Thirty Wistar-Albino rats were used in the study. The experimental groups were the control group, the irradiated group (IR), and the irradiated+Mel treated group (IR+Mel). A dose of 900 MHz, 2 W radiation was applied to the IR group every day for 10 days (30 min/day). The IR+Mel group received 10 mg/kg/day melatonin in tap water for 10 days before the irradiation. At the end of the 10th day, a skin specimen was excised from the thoracoabdominal area. The levels of malondialdehyde (MDA) and hydroxypyroline and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were studied in the skin samples. MDA and hydroxyproline levels and activities of CAT and GSH-Px were increased significantly in the IR group compared to the control group ($p<0.05$) and decreased significantly in the IR+Mel group ($p<0.05$). SOD activity was decreased significantly in the IR group and this decrease was not prevented by the Mel treatment. These results suggest that rats irradiated with 900 MHz suffer from increased fibrosis and lipid peroxidation (LPO). Mel treatment can reduce the fibrosis and LPO caused by radiation.

(E) *Aydin B, Akar A. Effects of a 900-MHz electromagnetic field on oxidative stress parameters in rat lymphoid organs, polymorphonuclear leukocytes and plasma. Arch Med Res. 42(4):261-267, 2011.

BACKGROUND AND AIMS: The present study investigated the effects of a 900-MHz electromagnetic field (EMF) for 2 h/day for 45 days on lymphoid organs (spleen, thymus, bone marrow), polymorphonuclear leukocytes (PMNs) and plasma of rats, focusing on changes in the enzymatic and nonenzymatic antioxidant system. We determined whether there is any difference between immature and mature rats in terms of oxidative damage caused by EMF and tested recovery groups to determine whether EMF-induced damage is reversible in immature and mature rats. METHODS: Twenty four immature and 24 mature rats were divided randomly and equally into six groups as follows: two control groups, immature (2 weeks old) and mature (10 weeks old); two groups were exposed to 900 MHz (28.2 ± 2.1 V/m) EMF for 2 h/day for 45 days. Two recovery groups were kept for 15 days after EMF exposure. RESULTS: Substantial, deleterious biochemical changes were observed in

oxidative stress metabolism after EMF exposure. Antioxidant enzyme activity, glutathione levels in lymphoid organs and the antioxidant capacity of the plasma decreased, but lipid peroxidation and nitric oxide levels in PMNs and plasma and also myeloperoxidase activity in PMNs increased. Oxidative damage was tissue specific and improvements seen after the recovery period were limited, especially in immature rats. CONCLUSIONS: In the present study, much higher levels of irreversible oxidative damage were observed in the major lymphoid organs of immature rats than in mature rats.

(E) *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. (LI)

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.

(E) Bahreyni Toossi MH, Sadeghnia HR, Mohammad Mahdizadeh Feyzabadi M, Hosseini M, Hedayati M, Mosallanejad R, Beheshti F, Alizadeh Rahvar Z. Exposure to mobile phone **(900-1800 MHz) during pregnancy: tissue oxidative stress after childbirth. J Matern Fetal Neonatal Med. 31(10):1298-1303, 2018.**

BACKGROUND: The present study has investigated the effects of **mobile phone** (900-1800 MHz)-induced electromagnetic radiation on redox status in the heart, liver, kidney, cerebellum, and hippocampus of dams and the offspring mice.MATERIALS AND METHODS: Pregnant Balb/C were divided into two groups including the control and the experimental group. The experimental group was exposed to **mobile phone** (900-1800 MHz), during pregnancy (2 h/d for 20 d). The dams and the offspring of both groups were sacrificed and tissues of interest were harvested immediately after delivery. Malondialdehyde (MDA) concentration, total thiol groups (TTG) content, superoxide dismutase (SOD), and catalase (CAT) activities were determined in the tissues.RESULTS: In the experimental groups, MDA levels were significantly increased, while TTG, SOD, and CAT were significantly decreased

in the total tissues of dams and their offspring.CONCLUSION: Exposure to **mobile phone** (900-1800 MHz) during pregnancy induced oxidative stress in tissues of dams and their offspring.

(E) Balci M, Devrim E, Durak I. Effects of mobile phones on oxidant/antioxidant balance in cornea and lens of rats. Curr Eye Res. 32(1):21-25, 2007.

Purpose: To investigate the effects of mobile-phone-emitted radiation on the oxidant/antioxidant balance in corneal and lens tissues and to observe any protective effects of vitamin C in this setting. Methods: Forty female albino Wistar rats were assigned to one of four groups containing 10 rats each. One group received a standardized daily dose of mobile phone radiation for 4 weeks. The second group received this same treatment along with a daily oral dose of vitamin C (250 mg/kg). The third group received this dose of vitamin C alone, while the fourth group received standard laboratory care and served as a control. In corneal and lens tissues, malondialdehyde (MDA) levels and activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were measured with spectrophotometric methods. Results: In corneal tissue, MDA level and CAT activity significantly increased in the mobile phone group compared with the mobile phone plus vitamin C group and the control group ($p < 0.05$), whereas SOD activity was significantly decreased ($p < 0.05$). In the lens tissues, only the MDA level significantly increased in the mobile phone group relative to mobile phone plus vitamin C group and the control groups ($p < 0.05$). In lens tissue, significant differences were not found between the groups in terms of SOD, GSH-Px, or CAT ($p > 0.05$). Conclusions: The results of this study suggest that mobile telephone radiation leads to oxidative stress in corneal and lens tissues and that antioxidants such as vitamin C can help to prevent these effects.

(E) 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.

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.

(E) Barteri M, De Carolis R, Marinelli F, Tomassetti G, Montemiglio LC. Effects of microwaves (900 MHz) on peroxidase systems: a comparison between lactoperoxidase and horseradish peroxidase. Electromagn Biol Med. 2015 Jan 12:1-7. [Epub ahead of print]

This work shows the effects of exposure to an electromagnetic field at 900 MHz on the catalytic activity of the enzymes lactoperoxidase (LPO) and horseradish peroxidase (HRP). Experimental evidence that irradiation causes conformational changes of the active sites and influences the formation and stability of the intermediate free radicals is documented by measurements of enzyme kinetics, circular dichroism spectroscopy (CD) and cyclic voltammetry.

(E) *Bilgici B, Akar A, Avci B, Tuncel OK. Effect of 900 MHz radıofrequency radıatıon on oxıdatıve stress in rat brain and serum. Electromagn Biol Med. 32:20-29, 2013.

The increasing use of mobile telephones raises the question of possible adverse effects of the electromagnetic fields (EMF) that these phones produce. In this study, we examined the oxidative stress in the brain tissue and serum of rats that resulted from exposure to a 900-MHz EMF at a whole body average specific absorption rate (SAR) of 1.08 W/kg for 1 h/day for 3 weeks. We also examined the antioxidant effect of garlic powder (500 mg/kg/day) given orally to EMF-exposed rats. We found that malondialdehyde (MDA) ($p < 0.001$) and advanced oxidation protein product (AOPP) ($p < 0.05$) increased in rat brain tissue exposed to the EMF and that garlic reduced these effects ($p < 0.05$). There was no significant difference in the nitric oxide (NO) levels in the brain. Paraoxonase (PON) was not detected in the brain. There was a significant increase in the levels of NO ($p < 0.001$) detected in the serum after EMF exposure, and garlic intake did not affect this increase in NO. Our results suggest that there is a significant increase in brain lipid and protein oxidation after electromagnetic radiation (EMR) exposure and that garlic has a protective effect against this oxidative stress.

(E) Bin-Meferij MM, El-Kott AF. The radioprotective effects of Moringa oleifera against mobile phone electromagnetic radiation-induced infertility in rats.Int J Clin Exp Med. 2015 Aug 15;8(8):12487-97. eCollection 2015.

The present study has investigated the effects of mobile phone electromagnetic radiation (EMR) on fertility in rats. The purpose of this study was to explore the capability of polyphenolic-rich Moringa oleifera leaf extract in protecting rat testis against EMR-induced impairments based on evaluation of sperm count, viability, motility, sperm cell morphology, anti-oxidants (SOD & CAT), oxidative stress marker, testis tissue histopathology and PCNA immunohistochemistry. The sample consisted of sixty male Wistar rats which were divided

into four equal groups. The first group (the control) received only standard diet while the second group was supplemented daily and for eight weeks with 200 mg/kg aqueous extract of Moringa leaves. The third group was exposed to 900 MHz fields for one hour a day and for (7) days a week. As for the fourth group, it was exposed to mobile phone radiation and received the Moringa extract. The results showed that the EMR treated group exhibited a significantly decrease sperm parameters. Furthermore, concurrent exposure to EMR and treated with MOE significantly enhanced the sperm parameters. However, histological results in EMR group showed irregular seminiferous tubules, few spermatogonia, giant multinucleated cells, degenerated spermatozoa and the number of Leydig cells was significantly reduced. PCNA labeling indices were significant in EMR group versus the control group. Also, EMR affects spermatogenesis and causes to apoptosis due to the heat and other stress-related EMR in testis tissue. This study concludes that chronic exposure to EMR marked testicular injury which can be prevented by Moringa oleifera leaf extract.

(E) *Bodera P, Stankiewicz W, Zawada K, Antkowiak B, Paluch M, Kieliszek J, Kalicki B, Bartosiński A, Wawer I. Changes in antioxidant capacity of blood due to mutual action of electromagnetic field (1800 MHz) and opioid drug (tramadol) in animal model of persistent inflammatory state. Pharmacol Rep. 65(2):421-428, 2013.

Background: The biological effects and health implications of electromagnetic field (EMF) associated with cellular mobile telephones and related wireless systems and devices have become a focus of international scientific interest and world-wide public concern. It has also been proved that EMF influences the production of reactive oxygen species (ROS) in different tissues. Methods: Experiments were performed in healthy rats and in rats with persistent inflammatory state induced by Complete Freund's Adjuvant (CFA) injection, which was given 24 h before EMF exposure and drug application. Rats were injected with CFA or the same volume of paraffin oil into the plantar surface of the left hind paw. Animals were exposed to the far-field range of an antenna at 1800 MHz with the additional modulation which was identical to that generated by mobile phone GSM 1800. Rats were given 15 min exposure, or were sham-exposed with no voltage applied to the field generator in control groups. Immediately before EMF exposure, rats were injected intraperitoneally with tramadol in the 20 mg/kg dose or vehicle in the 1 ml/kg volume. Results: Our study revealed that single EMF exposure in 1800 MHz frequency significantly reduced antioxidant capacity both in healthy animals and those with paw inflammation. A certain synergic mode of action between applied electromagnetic fields and administered tramadol in rats treated with CFA was observed. Conclusions: The aim of the study was to examine the possible, parallel/combined effects of electromagnetic radiation, artificially induced inflammation and a centrally-acting synthetic opioid analgesic drug, tramadol, (used in the treatment of severe pain) on the antioxidant capacity of blood of rats. The antioxidant capacity of blood of healthy rats was higher than that of rats which received only tramadol and were exposed to electromagnetic fields.

(E) Bodera P, Stankiewicz W, Antkowiak B, Paluch M, Kieliszek J, Sobiech J, Niemcewicz M. Influence of electromagnetic field (1800 MHz) on lipid peroxidation in brain, blood, liver and kidney in rats. Int J Occup Med Environ Health. 28(4):751-759, 2015.

OBJECTIVES: The aim of this study is the evaluation of the influence of repeated (5 times for 15 min) exposure to electromagnetic field (EMF) of 1800 MHz frequency on tissue lipid peroxidation (LPO) both in normal and inflammatory state, combined with analgesic treatment. MATERIAL AND METHODS: The concentration of malondialdehyde (MDA) as the end-product of the lipid peroxidation (LPO) was estimated in blood, liver, kidneys, and brain of Wistar rats, both healthy and those with complete Freund's adjuvant (CFA)-induced persistent paw inflammation. RESULTS: The slightly elevated levels of the MDA in blood, kidney, and brain were observed among healthy rats in electromagnetic field (EMF)-exposed groups, treated with tramadol (TRAM/EMF and exposed to the EMF). The malondialdehyde remained at the same level in the liver in all investigated groups: the control group (CON), the exposed group (EMF), treated with tramadol (TRAM) as well as exposed to and treated with tramadol (TRAM/EMF). In the group of animals treated with the complete Freund's adjuvant (CFA) we also observed slightly increased values of the MDA in the case of the control group (CON) and the exposed groups (EMF and TRAM/EMF). The MDA values concerning kidneys remained at the same levels in the control, exposed, and not-exposed group treated with tramadol. Results for healthy rats and animals with inflammation did not differ significantly. CONCLUSIONS: The electromagnetic field exposure (EMF), applied in the repeated manner together with opioid drug tramadol (TRAM), slightly enhanced lipid peroxidation level in brain, blood, and kidneys.

(E) Bourdineaud JP, Šrut M, Štambuk A, Tkalec M, Brèthes D, Malarić K, Klobučar GIV. Electromagnetic fields at a mobile phone frequency (900 MHz) trigger the onset of general stress response along with DNA modifications in Eisenia fetida earthworms. Arh Hig Rada Toksikol. 68(2):142-152, 2017.

Eisenia fetida earthworms were exposed to electromagnetic field (EMF) at a mobile phone frequency (900 MHz) and at field levels ranging from 10 to 120 V m-1 for a period of two hours (corresponding to specific absorption rates ranging from 0.13 to 9.33 mW kg-1). Potential effects of longer exposure (four hours), field modulation, and a recovery period of 24 h after two hours of exposure were addressed at the field level of 23 V m-1. All exposure treatments induced significant DNA modifications as assessed by a quantitative random amplified polymorphic DNA-PCR. Even after 24 h of recovery following a two hour-exposure, the number of probe hybridisation sites displayed a significant two-fold decrease as compared to untreated control earthworms, implying a loss of hybridisation sites and a persistent genotoxic effect of EMF. Expression of genes involved in the response to general stress (HSP70 encoding the 70 kDa heat shock protein, and MEKK1 involved in signal transduction), oxidative stress (CAT, encoding catalase), and chemical and immune defence (LYS, encoding lysenin, and MYD, encoding a myeloid differentiation factor) were up-regulated after exposure to 10 and modulated 23 V m-1 field levels. Western blots showing an increased quantity of HSP70 and MTCO1 proteins confirmed this stress response. HSP70 and LYS genes were up-regulated after 24 h of recovery following a two hour-exposure, meaning that the effect of EMF exposure lasted for hours. Exposure to mobile phone (900-1800 MHz) during pregnancy: tissue oxidative stress after childbirth.

(NE) Brescia F, Sarti M, Massa R, Calabrese ML, Sannino A, Scarfi MR. Reactive oxygen species formation is not enhanced by exposure to UMTS 1950 MHz radiation and co-exposure to ferrous ions in Jurkat cells. Bioelectromagnetics. 30:525-535, 2009.

This study was designed to assess if radiofrequency (RF) radiation induces oxidative stress in cultured mammalian cells when given alone or in combination with ferrous ions (FeSO(4)). For this purpose the production of reactive oxygen species (ROS) was measured by flow cytometry in human lymphoblastoid cells exposed to 1950 MHz signal used by the third generation wireless technology of the Universal Mobile Telecommunication System (UMTS) at Specific Absorption Rate of 0.5 and 2.0 W/kg. Short (5-60 min) or long (24 h) duration exposures were carried out in a waveguide system under strictly controlled conditions of both dosimetry and environment. Cell viability was also measured after 24 h RF exposure using the Resazurin and Neutral Red assays. Several co-exposure protocols were applied to test if RF radiation is able to alter ROS formation induced by FeSO(4) (RF given before or concurrently to FeSO(4)). The results obtained indicate that non-thermal RF exposures do not increase spontaneous ROS formation in any of the experimental conditions investigated. Consistent with the lack of ROS production, no change in cell viability was observed in Jurkat cells exposed to RF radiation for 24 h. Similar results were obtained when co-exposures were considered: combined exposures to RF radiation and FeSO(4) did not increase ROS formation induced by the chemical treatment alone. In contrast, in cultures treated with FeSO(4) as positive control, a dose-dependent increase in ROS formation was recorded, validating the sensitivity of the method employed.

(E) *Burlaka A, Tsybulin O, Sidorik E, Lukin S, Polishuk V, Tsehmistrenko S, Yakymenko I. Overproduction of free radical species in embryonal cells exposed to low intensity radiofrequency radiation. Exp Oncol. 35(3):219-225, 2013. (LI)

Aim: Long-term exposure of humans to low intensity radiofrequency electromagnetic radiation (RF-EMR) leads to a statistically significant increase in tumor incidence. Mechanisms of such the effects are unclear, but features of oxidative stress in living cells under RF-EMR exposure were previously reported. Our study aims to assess a production of initial free radical species, which lead to oxidative stress in the cell. Materials and Methods: Embryos of Japanese quails were exposed in ovo to extremely low intensity RF-EMR of GSM 900 MHz (0.25 μW/cm2) during 158-360 h discontinuously (48 c - ON, 12 c - OFF) before and in the initial stages of development. The levels of superoxide (O2·-), nitrogen oxide (NO·), thiobarbituric acid reactive substances (TBARS), 8-oxo-2'-deoxyguanosine (8-oxo-dG) and antioxidant enzymes' activities were assessed in cells/tissues of 38-h, 5- and 10-day RF-EMR exposed and unexposed embryos. Results: The exposure resulted in a significant persistent overproduction of superoxide and nitrogen oxide in embryo cells during all period of analyses. As a result, significantly increased levels of TBARS and 8-oxo-dG followed by significantly decreased levels of superoxide dismutase and catalase activities were developed in the exposed embryo cells. Conclusion: Exposure of developing quail embryos to extremely low intensity RF-EMR of GSM 900 MHz during at least one hundred and fifty-eight hours leads to a significant overproduction of free radicals/reactive oxygen species and oxidative damage of DNA in embryo cells. These oxidative changes may lead to pathologies up to oncogenic transformation of cells.

(E) Burlaka A, Selyuk M, Gafurov M, Lukin S, Potaskalova V, Sidorik E. Changes in mitochondrial functioning with electromagnetic radiation of ultra high frequency as revealed by electron paramagnetic resonance methods. Int J Radiat Biol. 90:357-362, 2014.

Purpose: To study the effects of electromagnetic radiation (EMR) of ultra high frequency (UHF) in the doses equivalent to the maximal permitted energy load for the staffs of the radar stations on the biochemical processes that occur in the cell organelles. Materials and Methods: Liver, cardiac and aorta tissues from the male rats exposed to non-thermal UHF EMR in pulsed and continuous modes were studied during 28 days after the irradiation by the electron paramagnetic resonance (EPR) methods including a spin trapping of superoxide radicals. Results: The qualitative and quantitative disturbances in electron transport chain (ETC) of mitochondria are registered. A formation of the iron-nitrosyl complexes of nitric oxide (NO) radicals with the iron-sulphide (FeS) proteins, the decreased activity of FeS-protein N2 of NADH-ubiquinone oxidoreductase complex and flavo ubisemiquinone growth combined with the increased rates of superoxide production are obtained. Conclusions: (1) Abnormalities in the mitochondrial ETC of liver and aorta cells are more pronounced for animals radiated in a pulsed mode. (2) The alterations in the functioning of the mitochondrial ETC cause increase of superoxide radicals generation rate in all samples, formation of cellular hypoxia, and intensification of the oxide-initiated metabolic changes. (3) Electron paramagnetic resonance methods could be used to track the qualitative and quantitative changes in the mitochondrial ETC caused by the UHF EMR.

(E) Campisi A, Gulino M, Acquaviva R, Bellia P, Raciti G, Grasso R, Musumeci F, Vanella A, Triglia A. Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. Neurosci Lett. 473(1):52-55, 2010.

The exposure of primary rat neocortical astroglial cell cultures to acute electromagnetic fields (EMF) in the microwave range was studied. Differentiated astroglial cell cultures at 14 days in vitro were exposed for 5, 10, or 20min to either 900MHz continuous waves or 900MHz waves modulated in amplitude at 50Hz using a sinusoidal waveform and 100% modulation index. The strength of the electric field (rms value) at the sample position was 10V/m. No change in cellular viability evaluated by MTT test and lactate dehydrogenase release was observed. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated EMF for 20min. No evident effects were detected when shorter time intervals or continuous waves were used. The irradiation conditions allowed the exclusion of any possible thermal effect. Our data demonstrate, for the first time, that even acute exposure to low intensity EMF induces ROS production and DNA fragmentation in astrocytes in primary cultures, which also represent the principal target of modulated EMF. Our findings also suggest the hypothesis that the effects could be due to hyperstimulation of the glutamate receptors, which play a crucial role in acute and chronic brain damage. Furthermore, the results show the importance of the amplitude modulation in the interaction between EMF and neocortical astrocytes.

(E) Cao H, Qin F, Liu X, Wang J, Cao Y, Tong J, Zhao H. Circadian Rhythmicity of Antioxidant Markers in Rats Exposed to 1.8 GHz Radiofrequency Fields. Int J Environ Res Public Health. 12(2):2071-2087, 2015.

BACKGROUND: The potential health risks of exposure to Radiofrequency Fields (RF) emitted by mobile phones are currently of considerable public interest, such as the adverse effects on the circadian rhythmicities of biological systems. To determine whether circadian rhythms of the plasma antioxidants (Mel, GSH-Px and SOD) are affected by RF, we performed a study on male Sprague Dawley rats exposed to the 1.8 GHz RF. METHODS: All animals were divided into seven groups. The animals in six groups were exposed to 1.8 GHz RF (201.7 μW/cm2 power density, 0.05653 W/kg specific absorption rate) at a specific period of the day (3, 7, 11, 15, 19 and 23 h GMT, respectively), for 2 h/day for 32 consecutive days. The rats in the seventh group were used as sham-exposed controls. At the end of last RF exposure, blood samples were collected from each rat every 4 h (total period of 24 h) and also at similar times from sham-exposed animals. The concentrations of three antioxidants (Mel, GSH-Px and SOD) were determined. The data in RF-exposed rats were compared with those in sham-exposed animals. RESULTS: circadian rhythms in the synthesis of Mel and antioxidant enzymes, GSH-Px and SOD, were shifted in RF-exposed rats compared to sham-exposed animals: the Mel, GSH-Px and SOD levels were significantly decreased when RF exposure was given at 23 and 3 h GMT. CONCLUSION: The overall results indicate that there may be adverse effects of RF exposure on antioxidant function, in terms of both the daily antioxidative levels, as well as the circadian rhythmicity.

(cancer) (E) Cao Y, Zhang W, Lu MX, Xu Q, Meng QQ, Nie JH, Tong J. 900-MHz microwave radiation enhances gamma-ray adverse effects on SHG44 cells. J Toxicol Environ Health A. 72(11):727-732, 2009.

Mobile phones are widely used globally. However, the biological effects due to exposure to electromagnetic fields (EMF) produced by mobile phones are largely unknown. Environmental and occupational exposure of humans to gamma-rays is a biologically relevant phenomenon. Consequently studies were undertaken to examine the interactions between gamma-rays and EMF on human health. In this study, exposure to 900-MHz EMF expanded gamma-ray damage to SHG44 cells. Preexposure EMF enhanced the decrease in cell proliferation induced by gamma-ray irradiation and the rate of apoptosis. The combination of EMF and gamma-ray exposure resulted in a synergistic effect by triggering stress response, which increased reactive oxygen species, but the expression of hsp70 at both mRNA and protein levels remained unaltered. Data indicate that the adverse effects of gamma-rays on cellular functions are strengthened by EMF.

(cancer) (E) Castello PR, Hill I, Sivo F, Portelli L, Barnes F, Usselman R, Martino CF. Inhibition of cellular proliferation and enhancement of hydrogen peroxide production in fibrosarcoma cell line by weak radio frequency magnetic fields. Bioelectromagnetics. 35(8):598-602, 2014.

This study presents experimental data for the effects of weak radio frequency (RF) magnetic fields on hydrogen peroxide (H2O2) production and cellular growth rates of fibrosarcoma HT1080 cells in vitro. Cells were exposed either to 45 μT static magnetic fields (SMFs)-oriented vertical to the plane of growth or to SMFs combined with weak 5 and 10 MHz RF magnetic fields of 10 μTRMS intensity perpendicular to the static field. Cell numbers were reduced up to 30% on Day 2 for the cells exposed to the combination of SMF and a 10 MHz RF magnetic field compared with the SMF control cells. In addition, cells exposed to 10 MHz RF magnetic fields for 8 h increased H2O2 production by 55%. The results demonstrate an overall magnetic field-induced biological effect that shows elevated H2O2 levels with accompanying decrease in cellular growth rates.

(E) Cetin H, Nazıroğlu M, Celik O, Yüksel M, Pastacı N, Ozkaya MO. Liver antioxidant stores protect the brain from electromagnetic radiation (900 and 1800 MHz)-induced oxidative stress in rats during pregnancy and the development of offspring. J Matern Fetal Neonatal Med. 2014 Mar 3. [Epub ahead of print]

Objectives: The present study determined the effects of mobile phone (900 and 1800 MHz)-induced electromagnetic radiation (EMR) exposure on oxidative stress in the brain and liver as well as the element levels in growing rats from pregnancy to 6 weeks of age. Methods: Thirty-two rats and their offspring were equally divided into 3 different groups: the control, 900 MHz, and 1800 MHz groups. The 900 MHz and 1800 MHz groups were exposed to EMR for 60 min/day during pregnancy and neonatal development. At the 4th, 5th, and 6th weeks of the experiment, brain samples were obtained. Results: Brain and liver glutathione peroxidase (GSH-Px) activities, as well as liver vitamin A and β-carotene concentrations decreased in the EMR groups, although brain iron, vitamin A, and β-carotene concentrations increased in the EMR groups. In the 6th week, selenium concentrations in the brain decreased in the EMR groups. There were no statistically significant differences in glutathione, vitamin E, chromium, copper, magnesium, manganese, and zinc concentrations between the 3 groups. Conclusion: EMR-induced oxidative stress in the brain and liver was reduced during the development of offspring. Mobile phone-induced EMR could be considered as a cause of oxidative brain and liver injury in growing rats.

(E) *Ceyhan AM, Akkaya VB, Güleçol ŞC, Ceyhan BM, Özgüner F, Chen W. Protective effects of β-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 β-glucans against the oxidative injury. Thirty-two male Wistar albino rats were randomly divided into four equal groups: control; sham exposed; EMR; and EMR + β-glucan. A 2.45-GHz EMR emitted device from the experimental exposure was applied to the EMR group and EMR + β-glucan group for 60 min daily, respectively, for 4 weeks. β-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 β-glucan significantly reversed the elevation of MDA levels and the reduction of SOD activities. β-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 β-glucan could ameliorate oxidative skin injury via its antioxidant properties.

(E) Chandel S, Kaur S, Singh HP, Batish DR, Kohli RK. Exposure to 2100 MHz electromagnetic field radiations induces reactive oxygen species generation in Allium cepa roots. Journal of Microscopy and Ultrastructure, 5(4):225-229, 2017.

During the last few decades there has been an enormous increase in the usage of cell phones as these are one of the most convenient gadgets and provide excellent mode of communication without evoking any hindrance to movement. However, these are significantly adding to the electromagnetic field radiations (EMF-r) in the environment and thus, are required to be analysed for their impacts on living beings. The present study investigated the role of cell phone EMF-r in inciting oxidative damage in onion (Allium cepa) roots at a frequency of 2100 MHz. Onion roots were exposed to continuous wave homogenous EMF-r for 1, 2 and 4 h for single day and generation of reactive oxygen species (ROS) in terms of malondialdehyde (MDA), hydrogen peroxide (H2O2) and superoxide anion (O2−) content and changes in the activities of antioxidant enzymes- superoxide dismutases (SOD) and catalases (CAT) were measured. The results showed that EMF-r exposure enhanced the content of MDA, H2O2 and O2−. Also, there was an upregulation in the activity of antioxidant enzymes− SOD and CAT− in onion roots. The study concluded that 2100 MHz cell phone EMF-r incite oxidative damage in onion roots by altering the oxidative metabolism.

(E) Chauhan P, Verma HN, Sisodia R, Kesari KK. Microwave radiation (2.45 GHz)-induced oxidative stress: Whole-body exposure effect on histopathology of Wistar rats. Electromagn Biol Med. 36(1):20-30, 2017.

Man-made microwave and radiofrequency (RF) radiation technologies have been steadily increasing with the growing demand of electronic appliances such as microwave oven and cell phones. These appliances affect biological systems by increasing free radicals, thus leading to oxidative damage. The aim of this study was to explore the effect of 2.45 GHz microwave radiation on histology and the level of lipid peroxide (LPO) in Wistar rats. Sixty-day-old male Wistar rats with 180 ± 10 g body weight were used for this study. Animals were divided into two groups: sham exposed (control) and microwave exposed. These animals were exposed for 2 h a day for 35 d to 2.45 GHz microwave radiation (power density, 0.2 mW/cm^2). The whole-body specific absorption rate (SAR) was estimated to be 0.14 W/kg. After completion of the exposure period, rats were sacrificed, and brain, liver, kidney, testis and spleen were stored/preserved for determination of LPO and histological parameters. Significantly high level of LPO was observed in

the liver ($p < 0.001$), brain ($p < 0.004$) and spleen ($p < 0.006$) in samples from rats exposed to microwave radiation. Also histological changes were observed in the brain, liver, testis, kidney and spleen after whole-body microwave exposure, compared to the control group. Based on the results obtained in this study, we conclude that exposure to microwave radiation 2 h a day for 35 d can potentially cause histopathology and oxidative changes in Wistar rats. These results indicate possible implications of such exposure on human health.

(E) *Chen YB, Li J, Liu JY, Zeng LH, Wan Y, Li YR, Ren D, Guo GZ. Effect of Electromagnetic Pulses (EMP) on associative learning in mice and a preliminary study of mechanism. Int J Radiat Biol. 87(12):1147-1154, 2011.

PURPOSE: To investigate the effects of electromagnetic pulses (EMP) on associative learning in mice and test a preliminary mechanism for these effects. MATERIALS AND METHODS: A tapered parallel plate gigahertz transverse electromagnetic (GTEM) cell with a flared rectangular coaxial transmission line was used to expose male BALB/c mice to EMP (peak-intensity 400 kV/m, rise-time 10 ns, pulse-width 350 ns, 0.5 Hz and total 200 pulses). Concurrent sham-exposed mice were used as a control. Associative learning, oxidative stress in the brain, serum chemistry and the protective action of tocopherol monoglucoside (TMG) in mice were measured, respectively. RESULTS: (1) Twelve hour and 1 day post EMP exposure associative learning was reduced significantly compared with sham control ($p<0.05$) but recovered at 2 d post EMP exposure. (2) Compared with the sham control, lipid peroxidation of brain tissue and chemiluminescence (CL) intensity increased significantly ($p<0.05$), while the activity of the antioxidant enzymes Superoxide Dismutase [SOD], Glutathione [GSH], Glutathione Peroxidase [GSH-Px], Catalase [CAT]) decreased significantly ($p<0.05$) at 3 h, 6 h, 12 h and 1 d post EMP exposure. All these parameters recovered at 2 d post EMP exposure. (3) No significant differences between the sham control group and EMP exposed group were observed in serum cholesterol and triglycerides. (4) Pretreatment of mice with TMG showed protective effects to EMP exposure. CONCLUSIONS: EMP exposure significantly decreased associative learning in mice and TMG acted as an effective protective agent from EMP exposure. This mechanism could involve an increase of oxidative stress in brain by EMP exposure.

(cancer) (E) Çiğ B, Nazıroğlu M. Investigation of the effects of distance from sources on apoptosis, oxidative stress and cytosolic calcium accumulation via TRPV1 channels induced by mobile phones and Wi-Fi in breast cancer cells. Biochim Biophys Acta. 2015 Feb 19. pii: S0005-2736(15)00053-X. doi: 10.1016/j.bbamem.2015.02.013. [Epub ahead of print]

TRPV1 is a Ca^{2+} permeable channel and gated by noxious heat, oxidative stress and capsaicin (CAP). Some reports have indicated that non-ionized electromagnetic radiation (EMR)-induces heat and oxidative stress effects. We aimed to investigate the effects of distance from sources on calcium signaling, cytosolic ROS production, cell viability, apoptosis, plus caspase-3 and -9 values induced by mobile phones and Wi-Fi in breast cancer cells MCF-7 human breast cancer cell lines were divided into A, B, C and D groups as control, 900, 1800 and 2450MHz groups, respectively. Cells in Group A were

used as control and were kept in cell culture conditions without EMR exposure. Groups B, C and D were exposed to the EMR frequencies at different distances (0cm, 1cm, 5cm, 10cm, 20cm and 25cm) for 1h before CAP stimulation. The cytosolic ROS production, Ca^{2+} concentrations, apoptosis, caspase-3 and caspase-9 values were higher in groups B, C and D than in A group at 0cm, 1cm and 5cm distances although cell viability (MTT) values were increased by the distances. There was no statistically significant difference in the values between control, 20 and 25cm. Wi-Fi and mobile phone EMR placed within 10cm of the cells induced excessive oxidative responses and apoptosis via TRPV1-induced cytosolic Ca^{2+} accumulation in the cancer cells. Using cell phones and Wi-Fi sources which are farther away than 10cm may provide useful protection against oxidative stress, apoptosis and overload of intracellular Ca^{2+}. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

(E) Comelekoglu U, Aktas S, Demirbag B, Karagul MI, Yalin S, Yildirim M, Akar A, Korunur Engiz B, Sogut F, Ozbay E. Effect of low-level 1800 MHz radiofrequency radiation on the rat sciatic nerve and the protective role of paricalcitol. Bioelectromagnetics. 39(8):631-643, 2018.

The nervous system is an important target of radiofrequency (RF) radiation exposure since it is the excitable component that is potentially able to interact with electromagnetic fields. The present study was designed to investigate the effects of 1,800 MHz RF radiation and the protective role of paricalcitol on the rat sciatic nerve. Rats were divided into four groups as control, paricalcitol, RF, and RF + paricalcitol. In RF groups, the rats were exposed to 1,800 MHz RF for 1 h per day for 4 weeks. Control and paricalcitol rats were kept under the same conditions without RF application. In paricalcitol groups, the rats were given 0.2 μg/kg/day paricalcitol, three times per week for 4 weeks. Amplitude and latency of nerve compound action potentials, catalase activities, malondialdehyde (MDA) levels, and ultrastructural changes of sciatic nerve were evaluated. In the RF group, a significant reduction in amplitude, prolongation in latency, an increase in the MDA level, and an increase in catalase activity and degeneration in the myelinated nerve fibers were observed. The electrophysiological and histological findings were consistent with neuropathy, and the neuropathic changes were partially ameliorated with paricalcitol administration.

(E) Dasdag S, Akdag MZ, Ulukaya E, Uzunlar AK, Ocak AR. Effect of mobile phone exposure on apoptotic glial cells and status of oxidative stress in rat brain. Electromagn Biol Med. 28(4):342-354, 2009.

The aim of this study was to investigate the effects of mobile phone exposure on glial cells in brain. The study carried out on 31 Wistar Albino adult male rats. The rat heads in a carousel exposed to 900 MHz microwave. For the study group (n:14), rats exposed to the radiation 2 h per day (7 days in a week) for 10 months. For the sham group (n:7), rats were placed into the carousel and the same procedure was applied except that the generator was turned off. For the cage control (n:10), nothing applied to rats in this group. In this study, rats were euthanized after 10 months of exposure periods and brains were removed. Brain tissues were immunohistochemically stained for the active

(cleaved) caspase-3, which is a well-known apoptosis marker, and p53. The expression of the proteins was evaluated by a semi-quantitative scoring system. However, total antioxidative capacity (TAC), catalase, total oxidant status (TOS), and oxidative stress index were measured in rat brain. Final score for apoptosis in the exposed group was significantly lower than the sham ($p < 0.001$) and the cage control groups ($p < 0.01$). p53 was not significantly changed by the exposure ($p > 0.05$). The total antioxidant capacity and catalase in the experimental group was found higher than that in the sham group ($p < 0.001$, $p < 0.05$). In terms of the TOS and oxidative stress index, there was no statistically significant difference between exposure and sham groups ($p > 0.05$). In conclusion, the final score for apoptosis, total antioxidant capacity and catalase in rat brain might be altered by 900 MHz radiation produced by a generator to represent exposure of global systems for mobile communication (GSM) cellular phones.

(E) De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile Phone Radiation Induces Reactive Oxygen Species Production and DNA Damage in Human Spermatozoa In Vitro. PLoS ONE 4(7): e6446, 2009. doi:10.1371/journal.pone.0006446

Background: In recent times there has been some controversy over the impact of electromagnetic radiation on human health. The significance of mobile phone radiation on male reproduction is a key element of this debate since several
studies have suggested a relationship between mobile phone use and semen quality. The potential mechanisms involved have not been established, however, human spermatozoa are known to be particularly vulnerable to oxidative stress by virtue of the abundant availability of substrates for free radical attack and the lack of cytoplasmic space to accommodate antioxidant enzymes. Moreover, the induction of oxidative stress in these cells not only perturbs their capacity for fertilization but also contributes to sperm DNA damage. The latter has, in turn, been linked with poor fertility, an increased incidence of miscarriage and morbidity in the offspring, including childhood cancer. In light of these associations, we have analyzed the influence of RF-EMR on the cell biology of human spermatozoa in vitro. Principal Findings: Purified human spermatozoa were exposed to radio-frequency electromagnetic radiation (RF-EMR) tuned to 1.8 GHz and covering a range of specific absorption rates (SAR) from 0.4 W/kg to 27.5 W/kg. In step with increasing SAR, motility and vitality were significantly reduced after RF-EMR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation were significantly elevated (P,0.001). Furthermore, we also observed highly significant relationships between SAR, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation after RF-EMR exposure. Conclusions: RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. These findings have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring.

(E) De Luca C, Chung Sheun Thai J, Raskovic D, Cesareo E, Caccamo D, Trukhanov A, Korkina L. Metabolic and genetic screening of electromagnetic hypersensitive subjects

as a feasible tool for diagnostics and intervention. Mediators Inflamm. 2014;2014:924184. doi: 10.1155/2014/924184. Epub 2014 Apr 9.

Growing numbers of "electromagnetic hypersensitive" (EHS) people worldwide self-report severely disabling, multiorgan, non-specific symptoms when exposed to low-dose electromagnetic radiations, often associated with symptoms of multiple chemical sensitivity (MCS) and/or other environmental "sensitivity-related illnesses" (SRI). This cluster of chronic inflammatory disorders still lacks validated pathogenetic mechanism, diagnostic biomarkers, and management guidelines. We hypothesized that SRI, not being merely psychogenic, may share organic determinants of impaired detoxification of common physic-chemical stressors. Based on our previous MCS studies, we tested a panel of 12 metabolic blood redox-related parameters and of selected drug-metabolizing-enzyme gene polymorphisms, on 153 EHS, 147 MCS, and 132 control Italians, confirming MCS altered ($P < 0.05$-0.0001) glutathione-(GSH), GSH-peroxidase/S-transferase, and catalase erythrocyte activities. We first described comparable-though milder-metabolic pro-oxidant/proinflammatory alterations in EHS with distinctively increased plasma coenzyme-Q10 oxidation ratio. Severe depletion of erythrocyte membrane polyunsaturated fatty acids with increased ω 6/ ω 3 ratio was confirmed in MCS, but not in EHS. We also identified significantly ($P = 0.003$) altered distribution-versus-control of the CYP2C19∗1/∗2 SNP variants in EHS, and a 9.7-fold increased risk (OR: 95% C.I. = 1.3-74.5) of developing EHS for the haplotype (null)GSTT1 + (null)GSTM1 variants. Altogether, results on MCS and EHS strengthen our proposal to adopt this blood metabolic/genetic biomarkers' panel as suitable diagnostic tool for SRI.

(NE) *Demirel S, Doganay S, Turkoz Y, Dogan Z, Turan B, Firat PG. Effects of third generation mobile phone-emitted electromagnetic radiation on oxidative stress parameters in eye tissue and blood of rats. Cutan Ocul Toxicol. 31(2):89-94, 2012.

Purpose: To investigate the effects of electromagnetic radiation (EMR) emitted by a third generation (3G) mobile phone on the antioxidant and oxidative stress parameters in eye tissue and blood of rats. Methods: Eighteen Wistar albino rats were randomly assigned into two groups: Group I (n = 9) received a standardized a daily dose of 3G mobile phone EMR for 20 days, and Group II served as the control group (n = 9), receiving no exposure to EMR. Glutathione peroxidase (GSH-Px) and catalase (CAT) levels were measured in eye tissues; in addition, malondialdehyde (MDA) and reduced GSH levels were measured in blood. Results: There was no significant difference between groups in GSH-Px ($p = 0.99$) and CAT ($p = 0.18$) activity in eye tissue. There was no significant difference between groups in MDA ($p = 0.69$) and GSH levels ($p = 0.83$) in blood. Conclusions: The results of this study suggest that under a short period of exposure, 3G mobile phone radiation does not lead to harmful effects on eye tissue and blood in rats.

(E) *Deshmukh PS, Banerjee BD, Abegaonkar MP, Megha K, Ahmed RS, Tripathi AK, Mediratta PK. Effect of low level microwave radiation exposure on cognitive function and oxidative stress in rats. Indian J Biochem Biophys. 50(2):114-119, 2013. (LI)

Use of wireless communicating devices is increasing at an exponential rate in present time and is raising serious concerns about possible adverse effects of microwave (MW) radiation emitted from these devices on human health. The present study aimed to evaluate the effects of 900 MHz MW radiation exposure on cognitive function and oxidative stress in blood of Fischer rats. Animals were divided into two groups (6 animals/group): Group I (MW-exposed) and Group II (Sham-exposed). Animals were subjected to MW exposure (Frequency 900 MHz; specific absorption rate 8.4738 x 10(-5) W/kg) in Gigahertz transverse electromagnetic cell (GTEM) for 30 days (2 h/day, 5 days/week). Subsequently, cognitive function and oxidative stress parameters were examined for each group. Results showed significant impairment in cognitive function and increase in oxidative stress, as evidenced by the increase in levels of MDA (a marker of lipid peroxidation) and protein carbonyl (a marker of protein oxidation) and unaltered GSH content in blood. Thus, the study demonstrated that low level MW radiation had significant effect on cognitive function and was also capable of leading to oxidative stress.

(NE) de Souza FT, Silva JF, Ferreira EF, Siqueira EC, Duarte AP, Gomez MV, Gomez RS, Gomes CC. Cell phone use and parotid salivary gland alterations: no molecular evidence. Cancer Epidemiol Biomarkers Prev. 2014 Apr 21. [Epub ahead of print]

Background The association between cell phone use and the development of parotid tumors is controversial. Because there is unequivocal evidence that the microenvironment is important for tumor formation, we investigated in the parotid glands whether cell phone use alters the expression of gene products related to cellular stress. Methods We used the saliva produced by the parotid glands of 62 individuals to assess molecular alterations compatible with cellular stress, comparing the saliva from the gland exposed to cell phone radiation (ipsilateral) to the saliva from the opposite, unexposed parotid gland (contralateral) of each individual. We compared salivary flow, total protein concentration, p53, p21, reactive oxygen species (ROS), and salivary levels of glutathione (GSH), heat shock proteins 27 and 70 and IgA between the ipsilateral and contralateral parotids. Results No difference was found for any of these parameters, even when grouping individuals by period of cell phone use in years or by monthly average calls in minutes. Conclusions and Impact We provide molecular evidence that the exposure of parotid glands to cell phone use does not alter parotid salivary flow, protein concentration or levels of proteins of genes that are directly or indirectly affected by heat-induced cellular stress.

(E) Devrim E, Ergüder IB, Kılıçoğlu B, Yaykaşlı E, Cetin R, Durak I. Effects of Electromagnetic Radiation Use on Oxidant/Antioxidant Status and DNA Turn-over Enzyme Activities in Erythrocytes and Heart, Kidney, Liver, and Ovary Tissues From Rats: Possible Protective Role of Vitamin C. Toxicol Mech Methods. 18(9):679-683, 2008.

In this study, the aim was to investigate possible effects of Electromagnetic Radiation (EMR) use on oxidant and antioxidant status in erythrocytes and kidney, heart, liver, and ovary tissues from rats, and possible protective role of vitamin C. For this aim, 40 Wistar albino female rats were used throughout the study. The treatment group was exposed to

EMR in a frequency of 900 MHz, the EMR plus vitamin C group was exposed to the same EMR frequency and given vitamin C (250 mg/kg/day) orally for 4 weeks. There were 10 animals in each group including control and vitamin C groups. At the end of the study period, blood samples were obtained from the animals to get erythrocyte sediments. Then the animals were sacrificed and heart, kidney, liver, and ovary tissues were removed. Malondialdehyde (MDA) levels and superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), xanthine oxidase (XO), and adenosine deaminase (ADA) enzyme activities were measured in the tissues and erythrocytes. It was observed that MDA level, XO, and GSH-Px activities significantly increased in the EMR group as compared with those of the control group in the erythrocytes. In the kidney tissues, it was found that MDA level and CAT activity significantly increased, whereas XO and ADA activities decreased in the cellular phone group as compared with those of the control group. However, in the heart tissues it was observed that MDA level, ADA, and XO activities significantly decreased in the cellular phone group as compared with those of the control group. The results suggest that EMR at the frequency generated by a cell phone causes oxidative stress and peroxidation in the erythrocytes and kidney tissues from rats. In the erythrocytes, vitamin C seems to make partial protection against the oxidant stress.

(E) Djordjevic B, Sokolovic D, Kocic G, Veljkovic A, Despotovic M, Basic J, Jevtovic-Stoimenov T, Sokolovic DM. The effect of melatonin on the liver of rats exposed to microwave radiation. Bratisl Lek Listy. 116(2):96-100, 2015.

OBJECTIVES: We aimed to clarify if melatonin treatment (2 mg/kg i.p.) may favorably impact the liver tissue in rats exposed to microwave radiation. The experiment was performed on 84 six-weeks-old Wistar male rats exposed for 4h a day, for 20, 40 and 60 days, respectively, to microwaves (900 MHz, 100-300 microT, 54-160 V/m). Rats were divided in to four groups: I (control) - rats treated with saline, II (Mel) - rats treated with melatonin, III (MWs) - microwave exposed rats, IV (MWs + Mel) - MWs exposed rats treated with melatonin. We evaluated oxidative stress parameters (malondialdehyde and carbonyl group content), catalase, xanthine oxidase, deoxyribonuclease I and II activity. BACKGROUND: Oxidative stress is the key mechanism of the microwave induced tissue injury. Melatonin, a lipophilic indoleamine primarily synthesized and released from the pineal gland is a powerful antioxidant. RESULTS: Exposure to microwaves caused an increase in malondialdehyde after 40 ($p < 0.01$), protein carbonyl content after 20 ($p < 0.05$), catalase ($p < 0.05$) and xantine oxidase activity ($p < 0.05$) after 40 days. Increase in deoxyribonuclease I activity was observed after 60 days ($p < 0.05$), while deoxyribonuclease II activity was unaffected. Melatonin treatment led to malondialdehyde decrease after 40 days ($p< 0.05$), but surprisingly had no effect on other analyzed parameters. CONCLUSION: Melatonin exerts certain antioxidant effects in the liver of rats exposed to microwaves, by diminishing the intensity of lipid peroxidation(Fig. 6, Ref. 32).

(E) Duan W, Liu C, Zhang L, He M, Xu S, Chen C, Pi H, Gao P, Zhang Y, Zhong M, Yu Z, Zhou Z. Comparison of the Genotoxic Effects Induced by 50 Hz Extremely Low-Frequency Electromagnetic Fields and 1800 MHz Radiofrequency Electromagnetic Fields in GC-2 Cells. Radiat Res. 183(3):305-314, 2015.

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

(E) Elhag MA, Nabil GM, Attia AM. Effects of electromagnetic field produced by mobile phones on the oxidant and antioxidant status of rats. Pak J Biol Sci. 10(23):4271-4274, 2007.

This study was designed to investigate the effect of EMR produced by GSM Mobile Phones (MP) on the oxidant and antioxidant status in rats. Rats were divided into three groups: (1) controls, (2) rats exposed to a fractionated dose of EMR (15 min day(-1) for four days) (EMR-F) and (3) rats exposed to an acute dose of EMR (EMR-A). A net drop in the plasma concentration of vitamin C (-47 and -59.8%) was observed in EMR-F and EMR-A groups, respectively, when compared to controls. While, a significant decrease in the levels of lypophilic antioxidant vitamins: vitamin E (-33 and -65.8%), vitamin A (-44.4 and -46.8%) was observed in EMR-F and EMR-A groups, respectively, when compared to controls. A net drop in plasma level of reduced glutathione (GSH) (-19.8 and -35.3%) was observed in EMR-F and EMR-A groups, respectively. EMR exposure of rats produced a significant decrease in catalase (CAT) and superoxide dismutase (SOD) activities, with the values of these activities for EMR-A group is significantly lower than those of EMR-F. These results indicate that the effects of acute doses of EMR produced by mobile phones on the rat's antioxidant status is significantly higher than those of fractionated doses of the same type of radiation. On the basis of present results, it can be concluded that exposure to acute doses of EMR produced by mobile phones is more hazardous than that produced by fractionated doses of the same type of radiation.

(E) Erdem Koç G, Kaplan S, Altun G, Gümüş H, Gülsüm Deniz Ö, Aydin I, Emin Onger M, Altunkaynak Z. Neuroprotective effects of melatonin and omega-3 on

hippocampal cells prenatally exposed to 900 MHz electromagnetic fields. Int J Radiat Biol. 2016 Jul 21:1-6. [Epub ahead of print]

PURPOSE: Adverse effects on human health caused by electromagnetic fields (EMF) associated with the use of mobile phones, particularly among young people, are increasing all the time. The potential deleterious effects of EMF exposure resulting from mobile phones being used in close proximity to the brain require particular evaluation. However, only a limited number of studies have investigated the effects of prenatal exposure to EMF in the development of the pyramidal cells using melatonin (MEL) and omega-3 (ω-3). MATERIALS AND METHODS: We established seven groups of pregnant rats consisting of three animals each; control (CONT), SHAM, EMF, EMF + MEL, MEL, EMF + ω-3 and ω-3 alone. The rats in the EMF, EMF + MEL, EMF + ω-3 groups were exposed to 900 MHz EMF for 60 min/day in an exposure tube during the gestation period. The CONT, MEL and ω-3 group rats were not placed inside the exposure tube or exposed to EMF during the study period. After delivery, only spontaneously delivered male rat pups were selected for the establishment of further groups. Each group of offspring consisted of six animals. The optical fractionator technique was used to determine total pyramidal neuron numbers in the rat hippocampal region. RESULTS: The total number of pyramidal cells in the cornu ammonis (CA) in the EMF group was significantly lower than in the CONT, SHAM, EMF + MEL, and EMF + ω-3 groups. No significant difference was observed between the EMF, MEL and ω-3 groups. No difference was also observed between any groups in terms of rats' body or brain weights. CONCLUSION: MEL and ω-3 can protect the cell against neuronal damage in the hippocampus induced by 900 MHz EMF. However, further studies are now needed to evaluate the chronic effects of 900 MHz EMF on the brain in the prenatal period.

(E) Ertilav K, Uslusoy F, Ataizi S, Nazıroğlu M.Long term exposure to cell phone frequencies (900 and 1800 MHz) induces apoptosis, mitochondrial oxidative stress and TRPV1 channel activation in the hippocampus and dorsal root ganglion of rats. Metab Brain Dis. 33(3):753-763, 2018. Jan 13.

Mobile phone providers use electromagnetic radiation (EMR) with frequencies ranging from 900 to 1800 MHz. The increasing use of mobile phones has been accompanied by several potentially pathological consequences, such as neurological diseases related to hippocampal (HIPPON) and dorsal root ganglion neuron (DRGN). The TRPV1 channel is activated different stimuli, including CapN, high temperature and oxidative stress. We investigated the contribution TRPV1 to mitochondrial oxidative stress and apoptosis in HIPPON and DRGN following long term exposure to 900 and 1800 MHz in a rat model. Twenty-four adult rats were equally divided into the following groups: (1) control, (2) 900 MHz, and (3) 1800 MHz exposure. Each experimental group was exposed to EMR for 60 min/ 5 days of the week during the one year. The 900 and 1800 MHz EMR exposure induced increases in TRPV1 currents, intracellular free calcium influx (Ca^{2+}), reactive oxygen species (ROS) production, mitochondrial membrane depolarization (JC-1), apoptosis, and caspase 3 and 9 activities in the HIPPON and DRGN. These deleterious processes were further increased in the 1800 MHz experimental group compared to the 900 MHz exposure group. In conclusion, mitochondrial oxidative stress, programmed cell death and Ca^{2+} entry pathway through TRPV1 activation in the HIPPON and DRGN of rats were increased in the rat model following exposure to 900

and 1800 MHz cell frequencies. Our results suggest that exposure to 900 and 1800 MHz EMR may induce a dose-associated, TRPV1-mediated stress response.

(E) *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, shrunken 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-1β 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.

(E) Esmekaya MA, Ozer C, Seyhan N. 900 MHz pulse-modulated radiofrequency radiation induces oxidative stress on heart, lung, testis and liver tissues. Gen Physiol Biophys. 30(1):84-89, 2011.

Oxidative stress may affect many cellular and physiological processes including gene expression, cell growth, and cell death. In the recent study, we aimed to investigate whether 900 MHz pulse-modulated radiofrequency (RF) fields induce oxidative damage on lung, heart and liver tissues. We assessed oxidative damage by investigating lipid peroxidation (malondialdehyde, MDA), nitric oxide (NOx) and glutathione (GSH) levels which are the indicators of tissue toxicity. A total of 30 male Wistar albino rats were used in this study. Rats were divided randomly into three groups; control group (n = 10), sham group (device off, n = 10) and 900 MHz pulsed-modulated RF radiation group (n = 10). The RF rats were exposed to 900 MHz pulsed modulated RF radiation at a specific absorption rate (SAR) level of 1.20 W/kg 20 min/day for three weeks. MDA and NOx levels were increased significantly in liver, lung, testis and heart tissues of the exposed group compared to sham and control groups ($p < 0.05$). Conversely GSH levels were significantly lower in exposed rat tissues ($p < 0.05$). No significantly difference was observed between sham and control groups. Results of our study showed that pulse-modulated RF radiation causes oxidative injury in liver, lung, testis and heart tissues mediated by lipid peroxidation, increased level of NOx and suppression of antioxidant defense mechanism.

(E) Esmekaya MA, Tuysuz MZ, Tomruk A, Canseven AG, Yücel E, Aktuna Z, Keskil S, Seyhan N. Effects of cell phone radiation on lipid peroxidation, glutathione and nitric oxide levels in mouse brain during epileptic seizure. J Chem Neuroanat. 75(pt.B):111-115, 2016.

The objective of the this study was to evaluate the effects of cellular phone radiation on oxidative stress parameters and oxide levels in mouse brain during pentylenetetrazole (PTZ) induced epileptic seizure. Eight weeks old mice were used in the study. Animals were distributed in the following groups: Group I: Control group treated with PTZ, Group II: 15min cellular phone radiation+PTZ treatment+30min cellular phone radiation, Group III: 30min cellular phone radiation+PTZ treatment+30min cellular phone radiation. The RF radiation was produced by a 900MHz cellular phone. Lipid peroxidation, which is the indicator of oxidative stress was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS). The glutathione (GSH) levels were determined by the Ellman method. Tissue total nitric oxide (NOx) levels were obtained using the Griess assay. Lipid peroxidation and NOx levels of brain tissue increased significantly in group II and III compared to group I. On the contrary, GSH levels were significantly lower in group II and III than group I. However, no statistically significant alterations in any of the endpoints were noted between group II and Group III. Overall, the experimental findings demonstrated that cellular phone radiation may increase the oxidative damage and NOx level during epileptic activity in mouse brain.

(NE) Fasseas MK, Fragopoulou AF, Manta AK, Skouroliakou A, Vekrellis K, Margaritis LH, Syntichaki P. Response of Caenorhabditis elegans to wireless devices radiation exposure. Int J Radiat Biol. 91(3):286-293, 2015.

Purpose: The aim of this study was to examine the impact of electromagnetic radiation, produced by GSM (Global System for Mobile communications) mobile phones, Wi-Fi (Wireless-Fidelity) routers and wireless DECT (Digital Enhanced Cordless Telecommunications) phones, on the nematode C. elegans. Materials and methods: We exposed synchronized populations, of different developmental stages, to these wireless devices at E-field levels below ICNIRP's (International Commission on Non-Ionizing Radiation Protection) guidelines for various lengths of time. WT (wild-type) and aging- or stress-sensitive mutant worms were examined for changes in growth, fertility, lifespan, chemotaxis, short-term memory, increased ROS (Reactive Oxygen Species) production and apoptosis by using fluorescent marker genes or qRT-PCR (quantitative Reverse Transcription-Polymerase Chain Reaction). Results: No statistically significant differences were found between the exposed and the sham/control animals in any of the experiments concerning lifespan, fertility, growth, memory, ROS, apoptosis or gene expression. Conclusions: The worm appears to be robust to this form of (pulsed) radiation, at least under the exposure conditions used.

(E) Fatma M. Ghoneim, Eetmad A. Arafat. Histological and histochemical study of the protective role of rosemary extract against harmful effect of cell phone electromagnetic radiation on the parotid glands. Acta Histochemica, Available online 4 May 2016.

Electromagnetic fields (EMFs) are a class of non-ionizing radiation (NIR) that is emitted from mobile phone. It may have hazardous effects on parotid glands. So, we aimed to investigate the histological and histochemical changes of the parotid glands of rats exposed to mobile phone and study the possible protective role of rosemary against its harmful effect. Forty adult male albino rats were used in this study. They were classified into 4 equal groups. Group I (control), group II (control receiving rosemary), group III (mobile phone exposed group) and group IV (mobile exposed, rosemary treated group). Parotid glands were dissected out for histological and histochemical study. Moreover, measurement of oxidative stress markers; malondialdehyde (MDA) and total antioxidant capacity (TAC) was done. The results of this study revealed that rosemary has protective effect through improving the histological and histochemical picture of the parotid gland in addition of its antioxidant effect. It could be concluded from the current study, that exposure of parotid gland of rat models to electromagnetic radiation of mobile phone resulted in structural changes at the level of light and electron microscopic examination which could be explained by oxidative stress effect of mobile phone. Rosemary could play a protective role against this harmful effect through its antioxidant activity.

(NE) 1 **Ferreira AR, Knakievicz T, de Bittencourt Pasquali MA, Gelain DP, Dal-Pizzol F, Fernandez CE, de Almeida de Salles AA, Ferreira HB, Moreira JC. Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring. Life Sci. 80(1)43-50, 2006.**

Mobile telephones and their base stations are an important ultra high frequency-electromagnetic field (UHF-EMF) source and their utilization is increasing all over the world. Epidemiological studies suggested that low energy UHF-EMF emitted from a cellular telephone may cause biological effects, such as DNA damage and changes on oxidative metabolism. An in vivo mammalian cytogenetic test, the micronucleus (MN) assay, was used to investigate the occurrence of chromosomal damage in erythrocytes from rat offspring exposed to a non-thermal UHF-EMF from a cellular phone during their embryogenesis; the irradiated group showed a significant increase in MN occurrence. In order to investigate if UHF-EMF could also alter oxidative parameters in the peripheral blood and in the liver - an important hematopoietic tissue in rat embryos and newborns - we also measured the activity of antioxidant enzymes, quantified total sulfhydryl content, protein carbonyl groups, thiobarbituric acid-reactive species and total non-enzymatic antioxidant defense. No significant differences were found in any oxidative parameter of offspring blood and liver. The average number of pups in each litter has also not been significantly altered. Our results suggest that, under our experimental conditions, UHF-EMF is able to induce a genotoxic response in hematopoietic tissue during the embryogenesis through an unknown mechanism.

(E) Friedman J, Kraus S, Hauptman Y, Schiff Y, Seger R. Mechanism of a short-term ERK activation by electromagnetic fields at mobile phone frequency. Biochem J. 405:559-568, 2007.

The exposure to non-thermal microwave electromagnetic field generated by mobile phones affects the expression of many proteins. This effect on transcription and protein stability can be mediated by the mitogen-activated protein kinase (MAPK) cascades, which serve as central signaling pathways, and govern essentially all stimulated cellular processes. Indeed, a long-term exposure of cells to mobile phone irradiation results in the activation of p38MAPKs as well as the ERK/MAPKs. Here we studied the immediate effect of irradiation on the MAPK cascades, and found that ERKs, but not stress related MAPKs are rapidly activated in response to various frequencies and intensities. Using signaling inhibitors we delineated the mechanism that is involved in this activation. We found that the first step is mediated in the plasma membrane by NADH oxidase, which rapidly generates reactive oxygen species (ROS). These ROS then directly stimulate matrix metalloproteinases and allow them to cleave and release heparin binding-EGF. This secreted factor, activates EGF receptor, which in turn further activates the ERK cascade. Thus, this study demonstrates for the first time a detailed molecular mechanism by which electromagnetic irradiation by mobile phones induces the activation of the ERK cascade and thereby induces transcription and other cellular processes.

(NE) *Furtado-Filho OV, Borba JB, Dallegrave A, Pizzolato TM, Henriques JA, Moreira JC, Saffi J. Effect of 950 MHz UHF electromagnetic radiation on biomarkers of oxidative damage, metabolism of UFA and antioxidants in the livers of young rats of different ages. Int J Radiat Biol. 90(2):159-168, 2014.

Purpose: To assess the effect of 950 MHz ultra-high-frequency electromagnetic radiation (UHF EMR) on biomarkers of oxidative damage, as well as to verify the concentration of unsaturated fatty acids (UFA) and the expression of the catalase in the livers of rats of different ages. Materials and methods: Twelve rats were equally divided into two groups as controls (CR) and exposed (ER), for each age (0, 6, 15 and 30 days). Radiation exposure lasted half an hour per day for up to 51 days (21 days of gestation and 6, 15 or 30 days of life outside the womb). The specific absorption rate (SAR) ranged from 1.3-1.0 W/kg. The damage to lipids, proteins and DNA was verified by thiobarbituric acid reactive substances (TBARS), protein carbonyls and comets, respectively. UFA were determined by gas chromatography with a flame ionization detector. The expression of catalase was by Western blotting. Results: The neonates had low levels of TBARS and concentrations of UFA after exposure. There was no age difference in the accumulation of protein carbonyls for any age. The DNA damage of ER 15 or 30 days was different. The exposed neonates exhibited lower expression of catalase. Conclusions: 950 MHz UHF EMR does not cause oxidative stress (OS), and it is not genotoxic to the livers of neonates or those of 6 and 15 day old rats, but it changes the concentrations of polyunsaturated fatty acid (PUFA) in neonates. For rats of 30 days, no OS, but it is genotoxic to the livers of ER to total body irradiation.

(NE) Furtado-Filho OV, Borba JB, Maraschin T, Souza LM, Jose JA, Moreira CF, Saffi J. Effects of chronic exposure to 950 MHz ultra-high-frequency electromagnetic radiation on reactive oxygen species metabolism in the right and left cerebral cortex of young rats of different ages. Int J Radiat Biol. 2015 Aug 14:1-17. [Epub ahead of print]

PURPOSE: To assess the effect of 950 MHz ultra-high-frequency electromagnetic radiation (UHF-EMR) on biomarkers of oxidative damage to DNA, proteins and lipids in the left cerebral cortex (LCC) and right cerebral cortex (RCC) of neonate and 6-day-old rats. MATERIALS AND METHODS: Twelve rats were equally divided into two groups as controls (CR) and exposed (ER), for each age (0 and 6 days). The LCC and RCC were examined in ER and CR after exposure. Radiation exposure lasted half an hour per day for up to 27 days (throughout pregnancy and 6 days postnatal). The specific absorption rate ranged from 1.32 - 1.14 W/kg. The damage to lipids, proteins and DNA was verified by thiobarbituric acid reactive substances, carbonylated proteins (CP) and comets, respectively. The concentration of glucose in the peripheral blood of the rats was measured by the Accu-Chek Active Kit due to increased CP in RCC. RESULTS: In neonates, no modification of the biomarkers tested was detected. On the other hand, there was an increase in the levels of CP in the RCC of the 6-day-old ER. Interestingly, the concentration of blood glucose was decreased in this group. CONCLUSIONS: Our results indicate that there is no genotoxicity and oxidative stress in neonates and 6 days rats. However, the RCC had the highest concentration of CP that do not seem to be a consequence of oxidative stress. This study is the first to demonstrate the use of UHF-EMR causes different damage responses to proteins in the LCC and RCC.

(E) Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (Apis mellifera) against 915-MHz microwave radiation-induced DNA damage in wistar rat lymphocytes: in vitro study. Int J Toxicol. 28(2):88-98, 2009.

The aim of this study is to investigate the radioprotective effect of bee venom against DNA damage induced by 915-MHz microwave radiation (specific absorption rate of 0.6 W/kg) in Wistar rats. Whole blood lymphocytes of Wistar rats are treated with 1 microg/mL bee venom 4 hours prior to and immediately before irradiation. Standard and formamidopyrimidine-DNA glycosylase (Fpg)-modified comet assays are used to assess basal and oxidative DNA damage produced by reactive oxygen species. Bee venom shows a decrease in DNA damage compared with irradiated samples. Parameters of Fpg-modified comet assay are statistically different from controls, making this assay more sensitive and suggesting that oxidative stress is a possible mechanism of DNA damage induction. Bee venom is demonstrated to have a radioprotective effect against basal and oxidative DNA damage. Furthermore, bee venom is not genotoxic and does not produce oxidative damage in the low concentrations used in this study.

(E) *Garaj-Vrhovac V, Gajski G, Pažanin S, Sarolić A, Domijan AM, Flajs D, Peraica M. Assessment of cytogenetic damage and oxidative stress in personnel occupationally

exposed to the pulsed microwave radiation of marine radar equipment. Int J Hyg Environ Health. 4(1):59-65, 2011.

Due to increased usage of microwave radiation, there are concerns of its adverse effect in today's society. Keeping this in view, study was aimed at workers occupationally exposed to pulsed microwave radiation, originating from marine radars. Electromagnetic field strength was measured at assigned marine radar frequencies (3 GHz, 5.5 GHz and 9.4 GHz) and corresponding specific absorption rate values were determined. Parameters of the comet assay and micronucleus test were studied both in the exposed workers and in corresponding unexposed subjects. Differences between mean tail intensity (0.67 vs. 1.22) and moment (0.08 vs. 0.16) as comet assay parameters and micronucleus test parameters (micronuclei, nucleoplasmic bridges and nuclear buds) were statistically significant between the two examined groups, suggesting that cytogenetic alterations occurred after microwave exposure. Concentrations of glutathione and malondialdehyde were measured spectrophotometrically and using high performance liquid chromatography. The glutathione concentration in exposed group was significantly lower than in controls (1.24 vs. 0.53) whereas the concentration of malondialdehyde was significantly higher (1.74 vs. 3.17), indicating oxidative stress. Results suggests that pulsed microwaves from working environment can be the cause of genetic and cell alterations and that oxidative stress can be one of the possible mechanisms of DNA and cell damage.

(E) *Ghanbari M[1], Mortazavi SB[1], Khavanin A[1], Khazaei M[2]. The Effects of Cell Phone Waves (900 MHz-GSM Band) on Sperm Parameters and Total Antioxidant Capacity in Rats. Int J Fertil Steril. 7(1):21-28, 2013.

BACKGROUND: There is tremendous concern regarding the possible adverse effects of cell phone microwaves. Contradictory results, however, have been reported for the effects of these waves on the body. In the present study, the effect of cell phone microwaves on sperm parameters and total antioxidant capacity was investigated with regard to the duration of exposure and the frequency of these waves. MATERIALS AND METHODS: This experimental study was performed on 28 adult male Wistar rats (200-250 g). The animals were randomly assigned to four groups (n=7): i. control; ii. two-week exposure to cell phone-simulated waves; iii. three-week exposure to cell phonesimulated waves; and iv. two-week exposure to cell phone antenna waves. In all groups, sperm analysis was performed based on standard methods and we determined the mean sperm total antioxidant capacity according to the ferric reducing ability of plasma (FRAP) method. Data were analyzed by one-way ANOVA followed by Tukey's test using SPSS version 16 software. RESULTS: The results indicated that sperm viability, motility, and total antioxidant capacity in all exposure groups decreased significantly compared to the control group ($p<0.05$). Increasing the duration of exposure from 2 to 3 weeks caused a statistically significant decrease in sperm viability and motility ($p<0.05$). CONCLUSION: Exposure to cell phone waves can decrease sperm viability and motility in rats. These waves can also decrease sperm total antioxidant capacity in rats and result in oxidative stress.

(E) 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. 2014 May 3. [Epub ahead of print]

Incidence rates of epilepsy and use of Wi-Fi worldwide have been increasing. TRPV1 is a 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 Ca^{2+} entry through activation of TRPV1 channel in epilepsy. Therefore, we tested the effects of Wi-Fi (2.45 GHz) exposure on 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 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 Ca^{2+} increase. However, pretreatment of the neurons with CPZ, results in a protection against epilepsy-induced Ca^{2+} influx, apoptosis and oxidative damages. In results of whole cell patch-clamp experiments, treatment of DRG with Ca^{2+} channel antagonists [thapsigargin, verapamil + diltiazem, 2-APB, MK-801] indicated that Wi-Fi exposure induced Ca^{2+} influx via the TRPV1 channels. In conclusion, epilepsy and Wi-Fi in our experimental model is involved in 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.

(NE) Gläser K, Rohland M, Kleine-Ostmann T, Schrader T, Stopper H, Hintzsche H. Effect of Radiofrequency Radiation on Human Hematopoietic Stem Cells. Radiat Res. 186(5):455-465, 2016.

Exposure to electromagnetic fields in the radiofrequency range is ubiquitous, mainly due to the worldwide use of mobile communication devices. With improving technologies and affordability, the number of cell phone subscriptions continues to increase. Therefore, the potential effect on biological systems at low-intensity radiation levels is of great interest. While a number of studies have been performed to investigate this issue, there has been no consensus reached based on the results. The goal of this study was to elucidate the extent to which cells of the hematopoietic system, particularly human hematopoietic stem cells (HSC), were affected by mobile phone radiation. We irradiated HSC and HL-60 cells at frequencies used in the major technologies, GSM (900 MHz), UMTS (1,950 MHz) and LTE (2,535 MHz) for a short period (4 h) and a long period (20 h/66 h), and with five different intensities ranging from 0 to 4 W/kg specific absorption rate (SAR). Studied end points included apoptosis, oxidative stress, cell cycle, DNA damage and DNA repair. In all but one of these end points, we detected no clear effect of mobile phone radiation; the only

alteration was found when quantifying DNA damage. Exposure of HSC to the GSM modulation for 4 h caused a small but statistically significant decrease in DNA damage compared to sham exposure. To our knowledge, this is the first published study in which putative effects (e.g., genotoxicity or influence on apoptosis rate) of radiofrequency radiation were investigated in HSC. Radiofrequency electromagnetic fields did not affect cells of the hematopoietic system, in particular HSC, under the given experimental conditions.

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

This paper presents the results of the study of the effects of long-term low-level exposure of rats to microwaves. Rats were exposed in far field to 2450 MHz continuous wave fields providing an incident power density at the cages of 500 microW/cm2 for 7 hours daily for a total of 30 days resulting in a whole-body SAR of 0.16 +/- 0.04 W/kg. Three groups ("EMF-exposure", "sham-exposure" and cage-control) were formed, each consisting of 16 rats. Circulating antibodies (IgA, IgG and IgM) directed against 16 chemical substances were evaluated in coded serum from each group of rats by enzyme multiplied analysis (ELISA test). An increased amount of compounds resulting from interaction of amino acids with nitric oxide (NO) or its derivatives (NO2-Tyrosine, NO-Arginine, NO-Cysteine + NO-Bovine Serum Albumin, NJ-Methionine + NO-Asparagine + No-Histidine, NO-BTrypnohan + NJ-Tyrosin), fatty acids with small chains, hydroxylated fatty acids, palmitic/myristic/oleic acid, AZE (product of oxidation of fatty acids) was found in blood serum from EMF-exposed rats. As a rule, antibodies to conjugated antigens were seen for IgM, rarely seen for IgG and were completely absent for IgA. The levels of antibodies were higher on day 7 after the exposure compared to those on day 14 after the exposure.

(E) Gulati S, Yadav A, Kumar N, Priya K, Aggarwal NK, Gupta R. Phenotypic and genotypic characterization of antioxidant enzyme system in human population exposed to radiation from mobile towers. Mol Cell Biochem. 2017 Aug 17. doi: 10.1007/s11010-017-3150-6. [Epub ahead of print]

In the present era, cellular phones have changed the life style of human beings completely and have become an essential part of their lives. The number of cell phones and cell towers are increasing in spite of their disadvantages. These cell towers transmit radiation continuously without any interruption, so people living within 100s of meters from the tower receive 10,000 to 10,000,000 times stronger signal than required for mobile communication. In the present study, we have examined superoxide dismutase (SOD) enzyme activity, catalase (CAT) enzyme activity, lipid peroxidation assay, and effect of functional polymorphism of SOD and CAT

antioxidant genes against mobile tower-induced oxidative stress in human population. From our results, we have found a significantly lower mean value of manganese superoxide dismutase (MnSOD) enzyme activity, catalase (CAT) enzyme activity, and a high value of lipid peroxidation assay in exposed as compared to control subjects. Polymorphisms in antioxidant MnSOD and CAT genes significantly contributed to its phenotype. In the current study, a significant association of genetic polymorphism of antioxidant genes with genetic damage has been observed in human population exposed to radiations emitted from mobile towers.

(E) Guler G, Tomruk A, Ozgur E, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. Gen Physiol Biophys. 29(1):59-66, 2010.

The concerns of people on possible adverse health effects of radiofrequency radiation (RFR) generated from mobile phones as well as their supporting transmitters (base stations) have increased markedly. RFR effect on oversensitive people, such as pregnant women and their developing fetuses, and older people is another source of concern that should be considered. In this study, oxidative DNA damage and lipid peroxidation levels in the brain tissue of pregnant and non-pregnant New Zealand White rabbits and their newborns exposed to RFR were investigated. Thirteen-month-old rabbits were studied in four groups as non-pregnant-control, non-pregnant-RFR exposed, pregnant-control and pregnant-RFR exposed. They were exposed to RFR (1800 MHz GSM; 14 V/m as reference level) for 15 min/day during 7 days. Malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were analyzed. MDA and 8-OHdG levels of non-pregnant and pregnant-RFR exposed animals significantly increased with respect to controls ($p < 0.001$, Mann-Whitney test). No difference was found in the newborns ($p > 0.05$, Mann-Whitney). There exist very few experimental studies on the effects of RFR during pregnancy. It would be beneficial to increase the number of these studies in order to establish international standards for the protection of pregnant women from RFR.

(E) *Güler G, Tomruk A, Ozgur E, Sahin D, Sepici A, Altan N, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in female and male infant rabbits. Int J Radiat Biol. 88(4):367-373, 2012.

PURPOSE: We aimed to design a prolonged radiofrequency (RF) radiation exposure and investigate in an animal model, possible bio-effects of RF radiation on the ongoing developmental stages of children from conception to childhood. **MATERIALS AND METHODS:** A total of 72 New Zealand female and male white rabbits aged one month were used. Females were exposed to RF radiation for 15 min/day during 7 days, whereas males were exposed to the same level of radiation for 15 min/day during 14 days. Thirty-six female and 36 male infant rabbits were randomly divided into four groups: Group I [Intrauterine (IU) exposure (-); Extrauterine (EU) exposure (-)]: Sham exposure which means rabbits were exposed to 1800 MHz Global System for Mobile Telecommunication (GSM)-like RF signals neither in the IU nor in the EU periods. Group II [IU exposure (-); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals when they reached one month of

age. Group III [IU exposure (+); EU exposure (-)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals in the IU period (between 15th and 22nd days of the gestational period). Group IV [IU exposure (+); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals both in the IU period (between 15th and 22nd days of the gestational period) and in the EU period when they reached one month of age. Biochemical analysis for lipid peroxidation and DNA damage were carried out in the livers of all rabbits. **RESULTS:** Lipid peroxidation levels in the liver tissues of female and male infant rabbits increased under RF radiation exposure. Liver 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of female rabbits exposed to RF radiation were also found to increase when compared with the levels of non-exposed infants. However, there were no changes in liver 8-OHdG levels of male rabbits under RF exposure. **CONCLUSION:** Consequently, it can be concluded that GSM-like RF radiation may induce biochemical changes by increasing free radical attacks to structural biomolecules in the rabbit as an experimental animal model.

(E) 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±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.

(E) Gulati S, Yadav A, Kumar N, Priya K, Aggarwal NK, Gupta R. Phenotypic and genotypic characterization of antioxidant enzyme system in human population exposed to radiation from mobile towers. Mol Cell Biochem. 2017 Aug 17. doi: 10.1007/s11010-017-3150-6. [Epub ahead of print]

In the present era, cellular phones have changed the life style of human beings completely and have become an essential part of their lives. The number of cell phones and cell towers are increasing in spite of their disadvantages. These cell towers transmit radiation continuously without any interruption, so people living within 100s of meters from the tower receive 10,000 to 10,000,000 times stronger signal than required for mobile communication. In the present study, we have examined superoxide dismutase (SOD) enzyme activity, catalase (CAT) enzyme activity, lipid peroxidation assay, and

effect of functional polymorphism of SOD and CAT antioxidant genes against mobile tower-induced oxidative stress in human population. From our results, we have found a significantly lower mean value of manganese superoxide dismutase (MnSOD) enzyme activity, catalase (CAT) enzyme activity, and a high value of lipid peroxidation assay in exposed as compared to control subjects. Polymorphisms in antioxidant MnSOD and CAT genes significantly contributed to its phenotype. In the current study, a significant association of genetic polymorphism of antioxidant genes with genetic damage has been observed in human population exposed to radiations emitted from mobile towers.

(E) Guney M, Ozguner F, Oral B, Karahan N, Mungan T. 900 MHz radiofrequency-induced histopathologic changes and oxidative stress in rat endometrium: protection by vitamins E and C. Toxicol Ind Health. 23(7):411-420, 2007.

There are numerous reports on the effects of electromagnetic radiation (EMR) in various cellular systems. Mechanisms of adverse effects of EMR indicate that reactive oxygen species (ROS) may play a role in the biological effects of this radiation. The aims of this study were to examine 900 MHz mobile phone-induced oxidative stress that promotes production of ROS and to investigate the role of vitamins E and C, which have antioxidant properties, on endometrial tissue against possible 900 MHz mobile phone-induced endometrial impairment in rats. The animals were randomly grouped (eight each) as follows: 1) Control group (without stress and EMR, Group I), 2) sham-operated rats stayed without exposure to EMR (exposure device off, Group II), 3) rats exposed to 900 MHz EMR (EMR group, Group III) and 4) a 900 MHz EMR exposed + vitamin-treated group (EMR + Vit group, Group IV). A 900 MHz EMR was applied to EMR and EMR + Vit group 30 min/day, for 30 days using an experimental exposure device. Endometrial levels of nitric oxide (NO, an oxidant product) and malondialdehyde (MDA, an index of lipid peroxidation), increased in EMR exposed rats while the combined vitamins E and C caused a significant reduction in the levels of NO and MDA. Likewise, endometrial superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities decreased in EMR exposed animals while vitamins E and C caused a significant increase in the activities of these antioxidant enzymes. In the EMR group histopathologic changes in endometrium, diffuse and severe apoptosis was present in the endometrial surface epithelial and glandular cells and the stromal cells. Diffuse eosinophilic leucocyte and lymphocyte infiltration were observed in the endometrial stroma whereas the combination of vitamins E and C caused a significant decrease in these effects of EMR. It is concluded that oxidative endometrial damage plays an important role in the 900 MHz mobile phone-induced endometrial impairment and the modulation of oxidative stress with vitamins E and C reduces the 900 MHz mobile phone-induced endometrial damage both at biochemical and histological levels.

(E) *Hamzany Y, Feinmesser R, Shpitzer T, Mizrachi A, Hilly O, Hod R, Bahar G, Otradnov I, Gavish M, Nagler RM. Is human saliva an indicator of the adverse health effects of using mobile phones? Antioxid Redox Signal. 18(6):622-627, 2013.

Increasing use of mobile phones creates growing concerns regarding harmful effects of radiofrequency nonionizing electromagnetic radiation on human tissues located close to the

ear, where phones are commonly held for long periods of time. We studied 20 subjects in the mobile-phone group who had a mean duration of mobile phone use of 12.5 years (range 8-15) and a mean time use of 29.6 h per month (range 8-100). Deaf individuals served as controls. We compared salivary outcomes (secretion, oxidative damage indices, flow rate, and composition) between mobile phone users and nonusers. We report a significant increase in all salivary oxidative stress indices studied in mobile phone users. Salivary flow, total protein, albumin, and amylase activity were decreased in mobile phone users. These observations lead to the hypothesis that the use of mobile phones may cause oxidative stress and modify salivary function.

(E) *Hancı H, Odacı E, Kaya H, Aliyazıcıoğlu Y, Turan I, Demir S, Colakoğlu S. The effect of prenatal exposure to 900-MHz electromagnetic field on the 21-old-day rat testicle. Reprod Toxicol. 42:203-209, 2013.

The aim of this study was to investigate the effect of exposure to a 900-MHz electromagnetic field (EMF) in the prenatal term on the 21-old-day rat testicle. Pregnant rats were divided into control (CG) and EMF (EMFG) groups. EMFG was exposed to 900-MHz EMF during days 13-21 of pregnancy. Newborn CG rats were obtained from the CG and newborn EMFG (NEMFG) rats from the EMFG. Testicles were extracted at postnatal day 21. Lipid peroxidation and DNA oxidation levels, apoptotic index and histopathological damage scores were compared. NEMFG rats exhibited irregularities in seminiferous tubule basal membrane and epithelium, immature germ cells in the lumen, and a decreased diameter in seminiferous tubules and thickness of epithelium. Apoptotic index, lipid peroxidation and DNA oxidation were higher in NEMFG rats than in NCG. 21-day-old rat testicles exposed to 900-MHz EMF in the prenatal term may be adversely affected, and this effect persists after birth.

(E) Hancı H, Türedi S, Topal Z, Mercantepe T, Bozkurt I, Kaya H, Ersöz Ş, Ünal B, Odacı E. Can prenatal exposure to a 900 MHz electromagnetic field affect the morphology of the spleen and thymus, and alter biomarkers of oxidative damage in 21-day-old male rats? Biotech Histochem. 2015 May 19:1-9. [Epub ahead of print]

We investigated the effects of a 900 Megahertz (MHz) electromagnetic field (EMF), applied during the prenatal period, on the spleen and thymus of 21-day-old male rat pups. Pregnant Sprague-Dawley rats were divided into control and EMF groups. We applied 900 MHz EMF for 1 h/day to the EMF group of pregnant rats. Newborn male rat pups were removed from their mothers and sacrificed on postnatal day 21. Spleen and thymus tissues were excised and examined. Compared to the control group, thymus tissue malondialdehyde levels were significantly higher in the group exposed to EMF, while glutathione levels were significantly decreased. Increased malondialdehyde and glutathione levels were observed in splenic tissue of rats exposed to EMF, while a significant decrease occurred in superoxide dismutase values compared to controls. Transmission electron microscopy showed pathological changes in cell morphology in the thymic and splenic tissues of newborn rats exposed to EMF. Exposure to 900 MHz EMF during the prenatal period can cause pathological and biochemical changes that may compromise the development of the male rat thymus and spleen.

(E) Hässig M, Jud F, Naegeli H, Kupper J, Spiess BM. Prevalence of nuclear cataract in Swiss veal calves and its possible association with mobile telephone antenna base stations. Schweiz Arch Tierheilkd. 151(10):471-478, 2009. (LI)

The purpose of this study was to valuate the prevalence of nuclear cataract in veal calves and to elucidate a possible impact by mobile phone base stations (MPBS). For this experiment a cohort study was conducted. A follow-up of the geographical location of each dam and its calf from conception through the fetal period up to slaughter was performed. The first trimester of gestation (organogenesis) was particularly emphasized. The activities of selected protective antioxidants (superoxide dismutase, catalase, glutathione peroxidase [GPx]) were assessed in aqueous humor of the eye to evaluate the redox status. Of 253 calves, 79 (32 %) had various degrees of nuclear cataract, but only 9 (3.6 %) calves had severe nuclear cataract. Results demonstrate a relation between the location of veals calves with nuclear cataracts in the first trimester of gestation and the strength of antennas. The number of antennas within 100 to 199 meters was associated with oxidative stress and there was an association between oxidative stress and the distance to the nearest MPBS. Oxidative stress was increased in eyes with cataract (OR per kilometer: 0.80, confidence interval 95 % 0.62,0.93). It has not been shown that the antennas actually affected stress. Hosmer-Lemeshow statistics showed an accuracy of 100 % in negative cases with low radiation, and only 11.11 % accuracy in positive cases with high radiation. This reflects, that there are a lot of other possibilities for nuclear cataract beside MPBS. Further studies on the influence of electromagnetic fields during embryonic development animal or person at risk are indicated.

(E) Hässig M, Wullschleger M, Naegeli HP, Kupper J, Spiess B, Kuster N, Capstick M, Murbach M. Influence of non ionizing radiation of base stations on the activity of redox proteins in bovines. BMC Vet Res. 2014 Jun 19;10(1):136. [Epub ahead of print]

BACKGROUND: The influence of electromagnetic fields on the health of humans and animals is still an intensively discussed and scientifically investigated issue (Prakt Tierarzt 11:15-20, 2003; Umwelt Medizin Gesellschaft 17:326-332, 2004; J Toxicol Environment Health, Part B 12:572-597, 2009). We are surrounded by numerous electromagnetic fields of variable strength, coming from electronic equipment and its power cords, from high-voltage power lines and from antennas for radio, television and mobile communication. Particularly the latter cause's controversy, as everyone likes to have good mobile reception at anytime and anywhere, whereas nobody wants to have such a base station antenna in their proximity. RESULTS: In this experiment, the non-ionizing radiation (NIR) has resulted in changes in the enzyme activities. Certain enzymes were disabled, others enabled by NIR. Furthermore, individual behavior patterns were observed. While certain cows reacted to NIR, others did not react at all, or even inversely. CONCLUSION: The present results coincide with the information from the literature, according to which NIR leads to changes in redox proteins, and that there are individuals who are sensitive to radiation and others that are not. However, the latter could not be distinctly attributed - there are cows that react clearly with one enzyme while they do not react with another enzyme at all, or even the inverse. The study approach of testing ten cows each ten times during three phases has proven to be appropriate. Future studies should however set the post-exposure phase later on.

(E) 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 ± 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.

(E) Hidisoglu E, Kantar Gok D, Er H, Akpinar D, Uysal F, Akkoyunlu G, Ozen S, Agar A, Yargicoglu P. 2100-MHz electromagnetic fields have different effects on visual evoked potentials and oxidant/antioxidant status depending on exposure duration. Brain Res. 2016 Jan 14. pii: S0006-8993(16)00031-7. doi: 10.1016/j.brainres.2016.01.018. [Epub ahead of print]

The purpose of the present study was to investigate the duration effects of 2100-MHz electromagnetic field (EMF) on visual evoked potentials (VEPs) and to assess lipid peroxidation (LPO), nitric oxide (NO) production and antioxidant status of EMF exposed rats. Rats were randomized to following groups: Sham rats (S1 and S10) and rats exposed to 2100-MHz EMF (E1 and E10) for 2h/day for 1 or 10 weeks, respectively. At the end of experimental periods, VEPs were recorded under anesthesia. Brain thiobarbituric acid reactive substances (TBARS) and 4-hydroxy-2-nonenal (4-HNE) levels were significantly decreased in the E1 whereas increased in the E10 compared with their control groups. While brain catalase (CAT), glutathione peroxidase (GSH-Px) activities and NO and glutathione (GSH) levels were significantly increased in the E1, reduction of superoxide dismutase (SOD) activity was detected in the same group compared with the S1. Conversely, decreased CAT, GSH-Px activities and NO levels were observed in the E10 compared with the S10. Latencies of all VEP components were shortened in the E1 compared with the S1, whereas latencies of all VEP components, except P1, were prolonged in the E10 compared with the S10. There was a positive correlation between all VEP latencies and brain TBARS and 4-HNE values. Consequently, it could be concluded that different effects of EMFs on VEPs depend on exposure duration. Additionally, our results indicated that short-term EMF could provide protective effects, while long-term EMF could have an adverse effect on VEPs and oxidant/antioxidant status.

(NE) Hong MN, Kim BC, Ko YG, Lee YS, Hong SC, Kim T, Pack JK, Choi HD, Kim N, Lee JS. Effects of 837 and 1950 MHz radiofrequency radiation exposure alone or combined on oxidative stress in MCF10A cells. Bioelectromagnetics. 33(7):604-611, 2012.

The aim of this study was to determine whether the exposure to either single or multiple radio-frequency (RF) radiation frequencies could induce oxidative stress in cell cultures. Exposures of human MCF10A mammary epithelial cells to either a single frequency (837 MHz alone or 1950 MHz alone) or multiple frequencies (837 and 1950 MHz) were conducted at specific absorption rate (SAR) values of 4 W/kg for 2 h. During the exposure period, the temperature in the exposure chamber was maintained isothermally. Intracellular levels of reactive oxygen species (ROS), the antioxidant enzyme activity of superoxide dismutase (SOD), and the ratio of reduced/oxidized glutathione (GSH/GSSG) showed no statistically significant alterations as the result of either single or multiple RF radiation exposures. In contrast, ionizing radiation-exposed cells, used as a positive control, showed evident changes in all measured biological endpoints. These results indicate that single or multiple RF radiation exposure did not elicit oxidative stress in MCF10A cells under our exposure conditions.

(NE) Hook, G. J., Spitz, D. R., Sim, J. E., Higashikubo, R., Baty, J. D., Moros, E. G. and Roti Roti, J. L. Evaluation of Parameters of Oxidative Stress after In Vitro Exposure to FMCW- and CDMA-Modulated Radiofrequency Radiation Fields. Radiat. Res. 162, 497–504, 2004.

The goal of this study was to determine whether radiofrequency (RF) radiation is capable of inducing oxidative stress or affecting the response to oxidative stress in cultured mammalian cells. The two types of RF radiation investigated were frequency-modulated continuous-wave with a carrier frequency of 835.62 MHz (FMCW) and code division multiple access centered on 847.74 MHz (CDMA). To evaluate the effect of RF radiation on oxidative stress, J774.16 mouse macrophage cells were stimulated with γ-interferon (IFN) and bacterial lipopolysaccharide (LPS) prior to exposure. Cell cultures were exposed for 20–22 h to a specific absorption rate of 0.8 W/kg at a temperature of 37.0 ± 0.3°C. Oxidative stress was evaluated by measuring oxidant levels, antioxidant levels, oxidative damage and nitric oxide production. Oxidation of thiols was measured by monitoring the accumulation of glutathione disulfide (GSSG). Cellular antioxidant defenses were evaluated by measuring superoxide dismutase activity (CuZnSOD and MnSOD) as well as catalase and glutathione peroxidase activity. The trypan blue dye exclusion assay was used to measure any changes in viability. The results of these studies indicated that FMCW- and CDMA-modulated RF radiation did not alter parameters indicative of oxidative stress in J774.16 cells. FMCW- and CDMA-modulated fields did not alter the level of intracellular oxidants, accumulation of GSSG or induction of antioxidant defenses in IFN/LPS-stimulated cells. Consistent with the lack of an effect on oxidative stress parameters, no change in toxicity was observed in J774.16 cells after either optimal (with or without inhibitors of nitric oxide synthase) or suboptimal stimulation.

(E) Hou Q, Wang M, Wu S, Ma X, An G, Liu H, Xie F. Oxidative changes and apoptosis induced by 1800-MHz electromagnetic radiation in NIH/3T3 cells. Electromagn Biol Med. 34(1):85-92, 2015.

To investigate the potential adverse effects of mobile phone radiation, we studied reactive oxygen species (ROS), DNA damage and apoptosis in mouse embryonic fibroblasts (NIH/3T3) after intermittent exposure (5 min on/10 min off, for various durations from 0.5 to 8 h) to an 1800-MHz GSM-talk mode electromagnetic radiation (EMR) at an average specific absorption rate of 2 W/kg. A 2',7'-dichlorofluorescin diacetate fluorescence probe was used to detect intracellular ROS levels, immunofluorescence was used to detect γH2AX foci as a marker for DNA damage, and flow cytometry was used to measure apoptosis. Our results showed a significant increase in intracellular ROS levels after EMR exposure and it reached the highest level at an exposure time of 1 h ($p < 0.05$) followed by a slight decrease when the exposure continued for as long as 8 h. No significant effect on the number of γH2AX was detected after EMR exposure. The percentage of late-apoptotic cells in the EMR-exposed group was significantly higher than that in the sham-exposed groups ($p < 0.05$). These results indicate that an 1800-MHz EMR enhances ROS formation and promotes apoptosis in NIH/3T3 cells.

(E) Houston BJ, Nixon B, King BV, De Iuliis GN, Aitken RJ. The effects of radiofrequency electromagnetic radiation on sperm function. Reproduction. 152(6):R263-R276, 2016.

Mobile phone usage has become an integral part of our lives. However, the effects of the radiofrequency electromagnetic radiation (RF-EMR) emitted by these devices on biological systems and specifically the reproductive systems are currently under active debate. A fundamental hindrance to the current debate is that there is no clear mechanism of how such non-ionising radiation influences biological systems. Therefore, we explored the documented impacts of RF-EMR on the male reproductive system and considered any common observations that could provide insights on a potential mechanism. Among a total of 27 studies investigating the effects of RF-EMR on the male reproductive system, negative consequences of exposure were reported in 21. Within these 21 studies, 11 of the 15 that investigated sperm motility reported significant declines, 7 of 7 that measured the production of reactive oxygen species (ROS) documented elevated levels and 4 of 5 studies that probed for DNA damage highlighted increased damage due to RF-EMR exposure. Associated with this, RF-EMR treatment reduced the antioxidant levels in 6 of 6 studies that discussed this phenomenon, whereas consequences of RF-EMR were successfully ameliorated with the supplementation of antioxidants in all 3 studies that carried out these experiments. In light of this, we envisage a two-step mechanism whereby RF-EMR is able to induce mitochondrial dysfunction leading to elevated ROS production. A continued focus on research, which aims to shed light on the biological effects of RF-EMR will allow us to test and assess this proposed mechanism in a variety of cell types.

(E) Houston BJ, Nixon B, King BV, Aitken RJ, De Iuliis GN. Probing the Origins of 1,800 MHz Radio Frequency Electromagnetic Radiation Induced Damage in Mouse Immortalized Germ Cells and Spermatozoa *in vitro*. Front Public Health. 6:270, 2018.

As the use of mobile phone devices is now highly prevalent, many studies have sought to evaluate the effects of the radiofrequency-electromagnetic radiation (RF-

EMR) on both human health and biology. While several such studies have shown RF-EMR is capable of inducing cellular stress, the physicobiological origin of this stress remains largely unresolved. To explore the effect of RF-EMR on the male reproductive system, we exposed cultured mouse spermatogonial GC1 and spermatocyte GC2 cell lines, as well as cauda epididymal spermatozoa to a waveguide generating continuous wave RF-EMR (1.8 GHz, 0.15 and 1.5 W/kg). This study demonstrated that a 4 h exposure is capable of inducing the generation of mitochondrial reactive oxygen species (ROS) in populations of GC1 (7 vs. 18%; $p < 0.001$) and GC2 cells (11.5 vs. 16 %; $p < 0.01$), identifying Complex III of the electron transport chain (ETC) as the potential source of electrons producing ROS. Assessing the generation of ROS in the presence of an antioxidant, penicillamine, as well as measuring lipid peroxidation via 4-hydroxynonenal levels, indicated that the elevated incidence of ROS generation observed under our exposure conditions did not necessarily induce an overt cellular oxidative stress response. However, exposure to RF-EMR at 0.15 W/kg for 3 h did induce significant DNA fragmentation in spermatozoa (that was no longer significant after 4 h), assessed by the alkaline comet assay ($p < 0.05$). Furthermore, this fragmentation was accompanied by an induction of oxidative DNA damage in the form of 8-hydroxy-2'-deoxyguanosine, which was significant ($p < 0.05$) after spermatozoa were exposed to RF-EMR for 4 h. At this exposure time point, a decline in sperm motility ($p < 0.05$) was also observed. This study contributes new evidence toward elucidating a mechanism to account for the effects of RF-EMR on biological systems, proposing Complex III of the mitochondrial ETC as the key target of this radiation.

(cancer) (E) Höytö A, Luukkonen J, Juutilainen J, Naarala J. Proliferation, oxidative stress and cell death in cells exposed to 872 MHz radiofrequency radiation and oxidants. Radiat. Res. 170(2):235-243, 2008. (WS)

Human SH-SY5Y neuroblastoma and mouse L929 fibroblast cells were exposed to 872 MHz radiofrequency (RF) radiation using continuous waves (CW) or a modulated signal similar to that emitted by GSM mobile phones at a specific absorption rate (SAR) of 5 W/kg in isothermal conditions. To investigate possible combined effects with other agents, menadione was used to induce reactive oxygen species, and tert-butylhydroperoxide (t-BOOH) was used to induce lipid peroxidation. After 1 or 24 h of exposure, reduced cellular glutathione levels, lipid peroxidation, proliferation, caspase 3 activity, DNA fragmentation and viability were measured. Two statistically significant differences related to RF radiation were observed: Lipid peroxidation induced by t-BOOH was increased in SH-SY5Y (but not in L929) cells, and menadione-induced caspase 3 activity was increased in L929 (but not in SH-SY5Y) cells. Both differences were statistically significant only for the GSM-modulated signal. The other end points were not significantly affected in any of the experimental conditions, and no effects were observed from exposure to RF radiation alone. The positive findings may be due to chance, but they may also reflect effects that occur only in cells sensitized by chemical stress. Further studies are required to investigate the reproducibility and dose response of the possible effects.

(E) Hu S, Peng R, Wang C, Wang S, Gao Y, Dong J, Zhou H, Su Z, Qiao S, Zhang S, Wang L, Wen X.Neuroprotective effects of dietary supplement Kang-fu-ling against high-power microwave through antioxidant action. Food Funct. 2014 Jul 24. [Epub ahead of print]

Kang-fu-ling (KFL) is a polybotanical dietary supplement with antioxidant properties. This study aimed to evaluate the potential protective effects of KFL on cognitive deficit induced by high-power microwave (HPM) and the underlying mechanism for this neuroprotection. The electron spin resonance technique was employed to evaluate the free radical scavenging activity of KFL in vitro and KFL exhibited scavenging hydroxyl radical activity. KFL at doses of 0.75, 1.5 and 3 g kg-1 and vehicle were administered orally once daily for 14 days to male Wistar rats after being exposed to 30 mW cm-2 HPM for 15 minutes. KFL reversed HPM-induced memory loss and the histopathological changes in hippocampus of rats. In addition, KFL displayed a protective effect against HPM-induced oxidative stress and activated the nuclear factor-E2-related factor 2 (Nrf2) and its target genes in the hippocampus of rats. The Nrf2-antioxidant response element (ARE) signaling pathway may be involved in the neuroprotective effects of KFL against HPM-induced oxidative stress. In summary, the dietary supplement KFL is a promising natural complex, which ameliorates oxidative stress, with neuroprotective effects against HPM.

(E) İkinci A, Mercantepe T, Unal D, Erol HS, Şahin A, Aslan A, Baş O, Erdem H, Sönmez OF, Kaya H, Odacı E. Morphological and antioxidant impairments in the spinal cord of male offspring rats following exposure to a continuous 900-MHz electromagnetic field during early and mid-adolescence. J Chem Neuroanat. 2015 Dec 17. pii: S0891-0618(15)00096-4. doi: 10.1016/j.jchemneu.2015.11.006. [Epub ahead of print]

The effects on human health of devices emitting electromagnetic field (EMF) have become the subject of intense research among scientists due to the rapid increase in their use. Children and adolescents are particularly attracted to the use of devices emitting EMF, such as mobile phones. The aim of this study was therefore to investigate changes in the spinal cords of male rat pups exposed to the effect of 900 megahertz (MHz) EMF. The study began with 24 Sprague Dawley male rats aged 3 weeks. Three groups containing equal numbers of rats were established - control group (CG), sham group (SG) and EMF group (EMFG). EMFG rats were placed inside an EMF cage every day between postnatal days (PD) 21 and 46 and exposed to the effect of 900MHz EMF for 1hour. SG rats were kept in the EMF cage for 1hour without being exposed to the effect of EMF. At the end of the study, the spinal cords in the upper thoracic region of all rats were removed. Tissues were collected for biochemistry, light microscopy (LM) and transmission electron microscopic (TEM) examination. Biochemistry results revealed significantly increased malondialdehyde and glutathione levels in EMFG compared to CG and SG, while SG and EMFG catalase and superoxide dismutase levels were significantly higher than those in CG. In EMFG, LM revealed atrophy in the spinal cord, vacuolization, myelin thickening and irregularities in the perikarya. TEM revealed marked loss of myelin sheath integrity and invagination into the axon and broad vacuoles in axoplasm. The study results show that biochemical alterations and pathological changes may occur in the spinal cords of male rats following exposure to 900MHz EMF for 1hour a day on PD 21-46.

(E) Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M, Akyol O, Ozen S. Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain. Clin Chim Acta. 340(1-2): 153-162, 2004.

BACKGROUND: The widespread use of mobile phones (MP) in recent years has raised the research activities in many countries to determine the consequences of exposure to the low-intensity electromagnetic radiation (EMR) of mobile phones. Since several experimental studies suggest a role of reactive oxygen species (ROS) in EMR-induced oxidative damage in tissues, in this study, we investigated the effect of Ginkgo biloba (Gb) on MP-induced oxidative damage in brain tissue of rats. METHODS: Rats (EMR+) were exposed to 900 MHz EMR from MP for 7 days (1 h/day). In the EMR+Gb groups, rats were exposed to EMR and pretreated with Gb. Control and Gb-administrated groups were produced by turning off the mobile phone while the animals were in the same exposure conditions. Subsequently, oxidative stress markers and pathological changes in brain tissue were examined for each groups. RESULTS: Oxidative damage was evident by the: (i) increase in malondialdehyde (MDA) and nitric oxide (NO) levels in brain tissue, (ii) decrease in brain superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and (iii) increase in brain xanthine oxidase (XO) and adenosine deaminase (ADA) activities. These alterations were prevented by Gb treatment. Furthermore, Gb prevented the MP-induced cellular injury in brain tissue histopathologically. CONCLUSION: Reactive oxygen species may play a role in the mechanism that has been proposed to explain the biological side effects of MP, and Gb prevents the MP-induced oxidative stress to preserve antioxidant enzymes activity in brain tissue.

(E) Imge EB, Kiliçoğlu B, Devrim E, Cetin R, Durak I. Effects of mobile phone use on brain tissue from the rat and a possible protective role of vitamin C - a preliminary study. Int J Radiat Biol. 86(12):1044-1049, 2010.

Purpose: To evaluate effects of mobile phone use on brain tissue and a possible protective role of vitamin C. Materials and methods: Forty female rats were divided into four groups randomly (Control, mobile phone, mobile phone plus vitamin C and, vitamin C alone). The mobile phone group was exposed to a mobile phone signal (900 MHz), the mobile phone plus vitamin C group was exposed to a mobile phone signal (900 MHz) and treated with vitamin C administered orally (per os). The vitamin C group was also treated with vitamin C per os for four weeks. Then, the animals were sacrificed and brain tissues were dissected to be used in the analyses of malondialdehyde (MDA), antioxidant potential (AOP), superoxide dismutase, catalase (CAT), glutathione peroxidase (GSH-Px), xanthine oxidase, adenosine deaminase (ADA) and 5'nucleotidase (5'-NT). Results: Mobile phone use caused an inhibition in 5'-NT and CAT activities as compared to the control group. GSH-Px activity and the MDA level were also found to be reduced in the mobile phone group but not significantly. Vitamin C caused a significant increase in the activity of GSH-Px and non-significant increase in the activities of 5'-NT, ADA and CAT enzymes. Conclusion: Our results suggest that vitamin C may play a protective role against detrimental effects of mobile phone radiation in brain tissue.

(E) Irmak MK, Fadillioglu E, Gulec M, Erdogan H, Yagmurca M, Akyol O. Effects of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. Cell Biochem Funct. 20(4):279-283, 2002.

The number of reports on the effects induced by electromagnetic radiation (EMR) in various cellular systems is still increasing. Until now no satisfactory mechanism has been proposed to explain the biological effects of this radiation. Oxygen free radicals may play a role in mechanisms of adverse effects of EMR. This study was undertaken to investigate the influence of electromagnetic radiation of a digital GSM mobile telephone (900 MHz) on oxidant and antioxidant levels in rabbits. Adenosine deaminase, xanthine oxidase, catalase, myeloperoxidase, superoxide dismutase (SOD) and glutathione peroxidase activities as well as nitric oxide (NO) and malondialdehyde levels were measured in sera and brains of EMR-exposed and sham-exposed rabbits. Serum SOD activity increased, and serum NO levels decreased in EMR-exposed animals compared to the sham group. Other parameters were not changed in either group. This finding may indicate the possible role of increased oxidative stress in the pathophysiology of adverse effect of EMR. Decreased NO levels may also suggest a probable role of NO in the adverse effect.

(E) **Jelodar G, Akbari A, Nazifi S. The prophylactic effect of vitamin C on oxidative stress indexes in rat eyes following exposure to radiofrequency wave generated by a BTS antenna model. Int J Radiat Biol. 89(2):128-131, 2013.

Purpose: This study was conducted to evaluate the effect of radiofrequency wave (RFW)-induced oxidative stress in the eye and the prophylactic effect of vitamin C on this organ by measuring the antioxidant enzymes activity including: glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), and malondialdehyde (MDA). Materials and methods: Thirty-two adult male Sprague-Dawley rats were randomly divided into four experimental groups and treated daily for 45 days as follows: Control, vitamin C (L-ascorbic acid 200 mg/kg of body weight/day by gavage), test (exposed to 900 MHz RFW) and the treated group (received vitamin C in addition to exposure to RFW). At the end of the experiment all animals were sacrificed, their eyes were removed and were used for measurement of antioxidant enzymes and MDA activity. Results: The results indicate that exposure to RFW in the test group decreased antioxidant enzymes activity and increased MDA compared with the control groups ($P < 0.05$). In the treated group vitamin C improved antioxidant enzymes activity and reduced MDA compared to the test group ($P < 0.05$). Conclusions: It can be concluded that RFW causes oxidative stress in the eyes and vitamin C improves the antioxidant enzymes activity and decreases MDA.

(E) *Jelodar G, Nazifi S, Akbari A. The prophylactic effect of vitamin C on induced oxidative stress in rat testis following exposure to 900 MHz radio frequency wave generated by a BTS antenna model. Electromagn Biol Med. 32(3):409-416, 2013.

Radio frequency wave (RFW) generated by base transceiver station (BTS) has been reported to make deleterious effects on reproduction, possibly through oxidative stress. This study was conducted to evaluate the effect of RFW generated by BTS on oxidative stress in testis and the prophylactic effect of vitamin C by measuring the antioxidant enzymes activity, including glutathione peroxidase, superoxide dismutase (SOD) and catalase, and malondialdehyde

(MDA). Thirty-two adult male Sprague-Dawley rats were randomly divided into four experimental groups and treated daily for 45 days as follows: sham, sham+vitamin C (l-ascorbic acid 200 mg/kg of body weight/day by gavage), RFW (exposed to 900 MHz RFW) 'sham' and 'RFW' animals were given the vehicle, i.e., distilled water and the RFW+vitamin C group (received vitamin C in addition to exposure to RFW). At the end of the experiment, all the rats were sacrificed and their testes were removed and used for measurement of antioxidant enzymes and MDA activity. The results indicate that exposure to RFW in the test group decreased antioxidant enzymes activity and increased MDA compared with the control groups ($p < 0.05$). In the treated group, vitamin C improved antioxidant enzymes activity and reduced MDA compared with the test group ($p < 0.05$). It can be concluded that RFW causes oxidative stress in testis and vitamin C improves the antioxidant enzymes activity and decreases MDA.

(NE) Jeong YJ, Son Y , Han NK, Choi HD, Pack JK, Kim N, Lee YS, Lee HJ. Impact of Long-Term RF-EMF on Oxidative Stress and Neuroinflammation in Aging Brains of C57BL/6 Mice. Int J Mol Sci. 2018 Jul 19;19(7). pii: E2103.

The expansion of mobile phone use has raised questions regarding the possible biological effects of radiofrequency electromagnetic field (RF-EMF) exposure on oxidative stress and brain inflammation. Despite accumulative exposure of humans to radiofrequency electromagnetic fields (RF-EMFs) from mobile phones, their long-term effects on oxidative stress and neuroinflammation in the aging brain have not been studied. In the present study, middle-aged C57BL/6 mice (aged 14 months) were exposed to 1950 MHz electromagnetic fields for 8 months (specific absorption rate (SAR) 5 W/kg, 2 h/day, 5 d/week). Compared with those in the young group, levels of protein (3-nitro-tyrosine) and lipid (4-hydroxy-2-nonenal) oxidative damage markers were significantly increased in the brains of aged mice. In addition, levels of markers for DNA damage (8-hydroxy-2'-deoxyguanosine, p53, p21, γH2AX, and Bax), apoptosis (cleaved caspase-3 and cleaved poly(ADP-ribose) polymerase 1 (PARP-1)), astrocyte (GFAP), and microglia (Iba-1) were significantly elevated in the brains of aged mice. However, long-term RF-EMF exposure did not change the levels of oxidative stress, DNA damage, apoptosis, astrocyte, or microglia markers in the aged mouse brains. Moreover, long-term RF-EMF exposure did not alter locomotor activity in aged mice. Therefore, these findings indicate that long-term exposure to RF-EMF did not influence age-induced oxidative stress or neuroinflammation in C57BL/6 mice.

(cancer) (E) Kahya MC, Nazıroğlu M, Cığ B. Selenium Reduces Mobile Phone (900 MHz)-Induced Oxidative Stress, Mitochondrial Function, and Apoptosis in Breast Cancer Cells. Biol Trace Elem Res. 160: 285-293, 2014.

Exposure to mobile phone-induced electromagnetic radiation (EMR) may affect biological systems by increasing free oxygen radicals, apoptosis, and mitochondrial depolarization levels although selenium may modulate the values in cancer. The present study was designed to

investigate the effects of 900 MHz radiation on the antioxidant redox system, apoptosis, and mitochondrial depolarization levels in MDA-MB-231 breast cancer cell line. Cultures of the cancer cells were divided into four main groups as controls, selenium, EMR, and EMR + selenium. In EMR groups, the cells were exposed to 900 MHz EMR for 1 h (SAR value of the EMR was 0.36 ± 0.02 W/kg). In selenium groups, the cells were also incubated with sodium selenite for 1 h before EMR exposure. Then, the following values were analyzed: (a) cell viability, (b) intracellular ROS production, (c) mitochondrial membrane depolarization, (d) cell apoptosis, and (e) caspase-3 and caspase-9 values. Selenium suppressed EMR-induced oxidative cell damage and cell viability (MTT) through a reduction of oxidative stress and restoring mitochondrial membrane potential. Additionally, selenium indicated anti-apoptotic effects, as demonstrated by plate reader analyses of apoptosis levels and caspase-3 and caspase-9 values. In conclusion, 900 MHz EMR appears to induce apoptosis effects through oxidative stress and mitochondrial depolarization although incubation of selenium seems to counteract the effects on apoptosis and oxidative stress.

(E) Kamali K, Taravati A, Sayyadi S, Gharib FZ, Maftoon H. Evidence of oxidative stress after continuous exposure to Wi-Fi radiation in rat model. Environ Sci Pollut Res Int. 25(35):35396-35403, 2018.

Exposure to electromagnetic radiation (EMR) is rapidly increasing in everyday environment, consequently conferring potential health effects. Oxidative stress is emerging as a mechanism implicated in pathophysiology and progression of various diseases. To our knowledge, no report has been made on the status of antioxidant redox systems after continuous exposure to radiofrequency radiation emitted from a Wi-Fi access point in animal model so far. Therefore, we aimed to continuously subject rats in the experimental group to radiofrequency (RF) radiation emitted from a commercially available Wi-Fi device. Male Wister rats were exposed to 2.45 GHz RF radiation emitted from a Wi-Fi for 24 h/day for 10 consecutive weeks. In order to assess the change in antioxidant redox system of plasma after continuous exposure to a Wi-Fi device, the total antioxidant capacity of plasma, level of thiobarbituric acid reactive substances, concentration of reduced glutathione (GSH), and activity of different enzymatic antioxidants, e.g., superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GSH-Px], and glutathione S-transferase [GST], were measured. In the Wi-Fi exposed group, a significant decrease was detected in total antioxidant capacity of plasma and the activities of several antioxidant enzymes, including CAT, GSH-Px, and SOD ($P < 0.05$). Meanwhile, the GST activity was significantly increased in this group ($P < 0.05$). However, no significant changes were found in GSH and TBARS levels following exposure to RF radiation. According to the results, oxidative defense system in rats exposed to Wi-Fi signal was significantly affected compared to the control group. Further studies are needed to better understand the possible biological mechanisms of EMR emitted from Wi-Fi device and relevant outcomes

(NE) *Kang KA, Lee HC, Lee JJ, Hong MN, Park MJ, Lee YS, Choi HD, Kim N, Ko YK, Lee JS. Effects of combined radiofrequency radiation exposure on levels of reactive

oxygen species in neuronal cells. J Radiat Res. 2013 Oct 8. [Epub ahead of print]

The objective of this study was to investigate the effects of the combined RF radiation (837 MHz CDMA plus 1950 MHz WCDMA) signal on levels of intracellular reactive oxygen species (ROS) in neuronal cells. Exposure of the combined RF signal was conducted at specific absorption rate values of 2 W/kg of CDMA plus 2 W/kg of WCDMA for 2 h. Co-exposure to combined RF radiation with either H2O2 or menadione was also performed. The experimental exposure groups were incubator control, sham-exposed, combined RF radiation-exposed with or without either H2O2 or menadione groups. The intracellular ROS level was measured by flow cytometry using the fluorescent probe dichlorofluorescein diacetate. Intracellular ROS levels were not consistently affected by combined RF radiation exposure alone in a time-dependent manner in U87, PC12 or SH-SY5Y cells. In neuronal cells exposed to combined RF radiation with either H2O2 or menadione, intracellular ROS levels showed no statically significant alteration compared with exposure to menadione or H2O2 alone. These findings indicate that neither combined RF radiation alone nor combined RF radiation with menadione or H2O2 influences the intracellular ROS level in neuronal cells such as U87, PC12 or SH-SY5Y.

(E) Kazemi E, Mortazavi SM, Ali-Ghanbari A, Sharifzadeh S, Ranjbaran R, Mostafavi-Pour Z, Zal F, Haghani M. Effect of 900 MHz Electromagnetic Radiation on the Induction of ROS in Human Peripheral Blood Mononuclear Cells. J Biomed Phys Eng. **5(3):105-114, 2015.**

BACKGROUND: Despite numerous studies over a decade, it still remains controversial about the biological effects of RF EMF emitted by mobile phone telephony. OBJECTIVE: Here we investigated the effect of 900 MHz GSM on the induction of oxidative stress and the level of intracellular reactive oxygen species (ROS) in human mononuclear cells, monocytes and lymphocytes as defence system cells. METHOD: 6 ml Peripheral Blood samples were obtained from 13 healthy volunteers (21-30 year-old). Each sample was devided into 2 groups: one was exposed RF radiation emitted from a mobile phone simulator for 2 hour and the other used as control group which was not exposed to any fields. After that, mononuclear cells were isolated from peripheral blood by density gradient centrifugation in Ficoll-Paque. The intracellular ROS content in monocytes and lymphocytes was measured by the CM-H2DCFDA fluorescence probe using flowcytometry technique. RESULTS: Our results showed significant increase in ROS production after exposure in population rich in monocytes. This effect was not significant in population rich in lymphocytes in comparison with non exposed cells. CONCLUSION: The results obtained in this study clearly showed the oxidative stress induction capability of RF electromagnetic field in the portion of PBMCs mostly in monocytes, like the case of exposure to micro organisms, although the advantages or disadvantages of this effect should be evaluated.

(E) Kerimoğlu G, Aslan A, Baş O, Çolakoğlu S, Odacı E. Adverse effects in lumbar spinal cord morphology and tissue biochemistry in Sprague Dawley male rats following exposure to a continuous 1-h a day 900-MHz electromagnetic field throughout adolescence. J Chem Neuroanat. 78:125-130, 2016.

Cell phones, an indispensable element of daily life, are today used at almost addictive levels by adolescents. Adolescents are therefore becoming increasingly exposed to the effect of the electromagnetic field (EMF) emitted by cell phones. The purpose of this study was to investigate the effect of exposure to a 900-MHz EMF throughout adolescence on the lumbar spinal cord using histopathological, immunohistochemical and biochemical techniques. Twenty-four Sprague Dawley (28.3-43.9g) aged 21days were included in the study. These were divided equally into three groups - control (CG), sham (SG) and electromagnetic (ELMAG). No procedure was performed on the CG rats until the end of the study. SG and ELMAG rats were kept inside an EMF cage (EMFC) for 1h a day every day at the same time between postnatal days 22 and 60. During this time, ELMAG rats were exposed to the effect of a 900-MHz EMF, while the SG rats were kept in the EMFC without being exposed to EMF. At the end of the study, the lumbar regions of the spinal cords of all rats in all groups were extracted. Half of each extracted tissue was stored at -80°C for biochemical analysis, while the other half was used for histopathological and immunohistochemical analyses. In terms of histopathology, a lumbar spinal cord with normal morphology was observed in the other groups, while morphological irregularity in gray matter, increased vacuolization and infiltration of white matter into gray matter were pronounced in the ELMAG rats. The cytoplasm of some neurons in the gray matter was shrunken and stained dark, and vacuoles were observed in the cytoplasms. The apoptotic index of glia cells and neurons were significantly higher in ELMAG compared to the other groups. Biochemical analysis revealed a significantly increased MDA value in ELMAG compared to CG, while SOD and GSH levels decreased significantly. In conclusion, our study results suggest that continuous exposure to a 900-MHz EMF for 1h a day through all stages of adolescence can result in impairments at both morphological and biochemical levels in the lumbar region spinal cords of Sprague Dawley rats.

(E) Kerimoğlu G, Hancı H, Baş O, Aslan A, Erol HS, Turgut A, Kaya H, Çankaya S, Sönmez OF, Odacı E. Pernicious effects of long-term, continuous 900-MHz electromagnetic field throughout adolescence on hippocampus morphology, biochemistry and pyramidal neuron numbers in 60-day-old Sprague Dawley male rats. J Chem Neuroanat. 77:169-175, 2016.

The central nervous system (CNS) begins developing in the intrauterine period, a process that continues until adulthood. Contact with chemical substances, drugs or environmental agents such as electromagnetic field (EMF) during adolescence therefore has the potential to disturb the development of the morphological architecture of components of the CNS (such as the hippocampus). The hippocampus is essential to such diverse functions as memory acquisition and integration and spatial maneuvering. EMF can result in severe damage to both the morphology of the hippocampus and its principal functions during adolescence. Although children and adolescents undergo greater exposure to EMF than adults, the information currently available regarding the effects of exposure to EMF during this period is as yet insufficient. This study investigated the 60-day-old male rat hippocampus following exposure to 900 megahertz (MHz) EMF throughout the adolescent period using stereological, histopathological and biochemical analysis techniques. Eighteen male Sprague Dawley rats aged 21days were assigned into control, sham and EMF groups on a random basis. No procedure was performed on the control group rats. The EMF group (EMFGr) was exposed to

a 900-MHz EMF for 1h daily from beginning to end of adolescence. The sham group rats were held in the EMF cage but were not exposed to EMF. All rats were sacrificed at 60days of age. Their brains were extracted and halved. The left hemispheres were set aside for biochemical analyses and the right hemispheres were subjected to stereological and histopathological evaluation. Histopathological examination revealed increased numbers of pyknotic neurons with black or dark blue cytoplasm on EMFGr slides stained with cresyl violet. Stereological analyses revealed fewer pyramidal neurons in EMFGr than in the other two groups. Biochemical analyses showed an increase in malondialdehyde and glutathione levels, but a decrease in catalase levels in EMFGr. Our results indicate that oxidative stress-related morphological damage and pyramidal neuron loss may be observed in the rat hippocampus following exposure to 900-MHz EMF throughout the adolescent period.

(E) Kerimoğlu G, Mercantepe T, Erol HS, Turgut A, Kaya H, Çolakoğlu S, Odacı E. Effects of long-term exposure to 900 megahertz electromagnetic field on heart morphology and biochemistry of male adolescent rats. Biotech Histochem. 2016 Aug 11:1-10. [Epub ahead of print]

The pathological effects of exposure to an electromagnetic field (EMF) during adolescence may be greater than those in adulthood. We investigated the effects of exposure to 900 MHz EMF during adolescence on male adult rats. Twenty-four 21-day-old male rats were divided into three equal groups: control (Cont-Gr), sham (Shm-Gr) and EMF-exposed (EMF-Gr). EMF-Gr rats were placed in an EMF exposure cage (Plexiglas cage) for 1 h/day between postnatal days 21 and 59 and exposed to 900 MHz EMF. Shm-Gr rats were placed inside the Plexiglas cage under the same conditions and for the same duration, but were not exposed to EMF. All animals were sacrificed on postnatal day 60 and the hearts were extracted for microscopic and biochemical analyses. Biochemical analysis showed increased levels of malondialdehyde and superoxide dismutase, and reduced glutathione and catalase levels in EMF-Gr compared to Cont-Gr animals. Hematoxylin and eosin stained sections from EMF-Gr animals exhibited structural changes and capillary congestion in the myocardium. The percentage of apoptotic myocardial cells in EMF-Gr was higher than in either Shm-Gr or Cont-Gr animals. Transmission electron microscopy of myocardial cells of EMF-Gr animals showed altered structure of Z bands, decreased myofilaments and pronounced vacuolization. We found that exposure of male rats to 900 MHz EMF for 1 h/day during adolescence caused oxidative stress, which caused structural alteration of male adolescent rat heart tissue.

(E) Kerimoğlu G, Güney C, Ersöz Ş, Odacı E.A histopathological and biochemical evaluation of oxidative injury in the sciatic nerves of male rats exposed to a continuous 900-megahertz electromagnetic field throughout all periods of adolescence. J Chem Neuroanat. 91:1-7, 2018.

The effects on human health of the electromagnetic field (EMF) emitted by mobile phones, used by approximately 7 billion people worldwide, have become an important subject for scientific research. Studies have suggested that the EMF emitted by mobile phones can cause oxidative stress in different tissues and age groups. Young people in adolescence, a time period when risky behaviors and dependences increase, use mobile phones more than adults. The EMF emitted by mobile phones, which are generally carried in the pocket or in bags when not in use, will very probably affect the sciatic

nerve. No previous study has investigated the effect of mobile phone use in adolescence on peripheral nerve. This study was planned accordingly. Twenty-four male Sprague Dawley rats aged 21 days were divided equally into control (CGr), Sham (SGr) and EMF (EMFGr) groups. No procedure was performed on CGr rats. EMFGr were exposed to the effect of a 900-megahertz (MHz) EMF for 1 h at the same time every day between postnatal days 21-59 (the entire adolescent period) inside a cage in the EMF apparatus. SGr rats were placed inside the cage for 1 h every day without being exposed to EMF. All rats were sacrificed at the end of the study period, and 1 cm sections of sciatic nerve were extracted. Malondialdehyde (MDA), glutathione, catalase (CAT) superoxide dismutase (SOD) values were investigated biochemically in half of the right sciatic nerve tissues. The other halves of the nerve tissues were subjected to routine histopathological tissue procedures, sectioned and stained with hematoxylin and eosin (H&E) and Masson's trichrome. Histopathological evaluation of slides stained with Masson's trichrome and H&E revealed a normal appearance in Schwann cells and axons in all groups. However, there was marked thickening in the epineurium of sciatic nerves from EMFGr rats. MDA, SOD and CAT levels were higher in EMFGr than in CGr and SGr at biochemical analyses. Apoptotıc index (AI) analysis revealed a significant increase in the number of TUNEL (+) cells when EMFGr was compared with CGr and SGr. In conclusion, our study results suggest that continuous exposure to a 900-MHz EMF for 1 h throughout adolescence can cause oxidative injury and thickening in the epineurium in the sciatic nerve in male rats.

(E) *Kesari KK, Behari J. Microwave exposure affecting reproductive system in male rats. Appl Biochem Biotechnol 31:495-498, 2010. (LI)

The object of present study is to investigate the effects of 50 GHz microwave frequency electromagnetic fields on reproductive system of male rats. Male rats of Wistar strain were used in the study. Animals 60 days old were divided into two groups-group I sham exposed and group II experimental (microwave exposed). During exposure, rats were confined in Plexiglas cages with drilled ventilation holes for 2 h a day for 45 days continuously at a specified specific absorption rate of 8.0 x 10(-4) W/kg. After the last exposure, the rats were sacrificed immediately and sperms were collected. Antioxidant enzyme (superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase), histone kinase, apoptosis, and cell cycle were analyzed in sperm cells. Result shows a significant decrease in the level of sperm GPx and SOD activity ($p </= 0.05$), whereas catalase shows significant increase in exposed group of sperm samples as compared with control ($p < 0.02$). We observed a statistically significant decrease in mean activity of histone kinase as compared to the control ($p < 0.016$). The percentage of cells dividing in a spermatogenesis was estimated by analyzing DNA per cell by flow cytometry. The percentage of apoptosis in electromagnetic field exposed group shows increased ratio as compared to sham exposed ($p < 0.004$). There were no significant differences in the G(0)/G phase; however, a significant decrease ($p < 0.026$) in S phase was obtained. Results also indicate a decrease in percentage of G/M transition phase of cell cycle in exposed group as compared to sham exposed ($p < 0.019$). We conclude that these radiations may have a significant effect on reproductive system of male rats, which may be an indication of male infertility.

(E) Kesari KK, Behari J. Evidence for mobile phone radiation exposure effects on reproductive pattern of male rats: role of ROS. Electromagn Biol Med. 31(3):213-22, 2012.

The relationship between radiofrequency electromagnetic fields emitted from mobile phone and infertility is a matter of continuing debate. It is postulated that these radiations may affect the reproduction pattern spell by targeting biochemistry of sperm. In an attempt to expedite the issue, 70 days old Wistar rats (n = 6) were exposed to mobile phone radiofrequency (RF) radiation for 2 h per day for 45 days and data compared with sham exposed (n = 6) group. A significant decrease ($P < 0.05$) in the level of testosterone and an increase in caspase-3 activity were found in the RF-exposed animals. Distortions in sperm head and mid piece of sperm mitochondrial sheath were also observed as captured by Transmission Electron Microscope (TEM). In addition, progeny from RF-exposed rats showed significant decreases in number and weight as compared with that of sham-exposed animals. A reduction in testosterone, an increase in caspase-3, and distortion in spermatozoa could be caused by overproduction of reactive oxygen species (ROS) in animals under mobile phone radiation exposure. Our findings on these biomarkers are clear indications of possible health implications of repeated exposure to mobile phone radiation.

(E) Kesari KK, Kumar S, Behari J. Mobile phone usage and male infertility in Wistar rats. Indian J Exp Biol. 48(10):987-992, 2010.

A significant decrease in protein kinase C and total sperm count along with increased apoptosis were observed in male Wistar rats exposed to mobile phone frequencies (2 h/day x 35 days at 0.9 W/kg specific absorption rate). The results suggest that a reduction in protein kinase activity may be related to overproduction of reactive oxygen species (ROS) under microwave field exposure. Decrease in sperm count and an increase in apoptosis may be causative factor due to mobile radiation exposure leading to infertility.

(E) Kesari KK, Kumar S, Behari J. Effects of Radiofrequency Electromagnetic Wave Exposure from Cellular Phones on the Reproductive Pattern in Male Wistar Rats. Appl Biochem Biotechnol. 164(4):546-559, 2011.

The present study investigates the effect of free radical formation due to mobile phone exposure and effect on fertility pattern in 70-day-old male Wistar rats (sham exposed and exposed). Exposure took place in Plexiglas cages for 2 h a day for 35 days to mobile phone frequency. The specific absorption rate was estimated to be 0.9 W/kg. An analysis of antioxidant enzymes glutathione peroxidase ($P < 0.001$) and superoxide dismutase ($P < 0.007$) showed a decrease, while an increase in catalase ($P < 0.005$) was observed. Malondialdehyde ($P < 0.003$) showed an increase and histone kinase ($P = 0.006$) showed a significant decrease in the exposed group. Micronuclei also show a significant decrease ($P < 0.002$) in the exposed group. A significant change in sperm cell cycle of G(0)-G(1) ($P = 0.042$) and G(2)/M ($P = 0.022$) were recorded. Generation of free radicals was recorded to be significantly increased ($P = 0.035$). Our findings on antioxidant, malondialdehyde, histone kinase, micronuclei, and sperm cell cycle are clear indications

of an infertility pattern, initiated due to an overproduction of reactive oxygen species. It is concluded that radiofrequency electromagnetic wave from commercially available cell phones might affect the fertilizing potential of spermatozoa.

(E) *Kesari KK, Kumar S, Behari J. 900-MHz microwave radiation promotes oxidation in rat brain. Electromagn Biol Med. 30(4):219-234, 2011.

Recently, there have been several reports referring to detrimental effects due to radio frequency electromagnetic fields (RF-EMF) exposure. Special attention was given to investigate the effect of mobile phone exposure on the rat brain. Since the integrative mechanism of the entire body lies in the brain, it is suggestive to analyze its biochemical aspects. For this, 35-day old Wistar rats were exposed to a mobile phone for 2 h per day for a duration of 45 days where specific absorption rate (SAR) was 0.9 W/Kg. Animals were divided in two groups: sham exposed (n = 6) and exposed group (n = 6). Our observations indicate a significant decrease ($P < 0.05$) in the level of glutathione peroxidase, superoxide dismutase, and an increase in catalase activity. Moreover, protein kinase shows a significant decrease in exposed group ($P < 0.05$) of hippocampus and whole brain. Also, a significant decrease ($P < 0.05$) in the level of pineal melatonin and a significant increase ($P < 0.05$) in creatine kinase and caspase 3 was observed in exposed group of whole brain as compared with sham exposed. Finally, a significant increase in the level of ROS (reactive oxygen species) ($P < 0.05$) was also recorded. The study concludes that a reduction or an increase in antioxidative enzyme activities, protein kinase C, melatonin, caspase 3, and creatine kinase are related to overproduction of reactive oxygen species (ROS) in animals under mobile phone radiation exposure. Our findings on these biomarkers are clear indications of possible health implications.

(E) *Khalil AM, Gagaa MH, Alshamali AM. 8-Oxo-7, 8-dihydro-2'-deoxyguanosine as a biomarker of DNA damage by mobile phone radiation. Hum Exp Toxicol. 31(7):734-740, 2012.

We examined the effect of exposure to mobile phone 1800 MHz radio frequency radiation (RFR) upon the urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG), one major form of oxidative DNA damage, in adult male Sprague-Dawley rats. Twenty-four rats were used in three independent experiments (RFR exposed and control, 12 rats, each). The animals were exposed to RFR for 2 h from Global System for Mobile Communications (GSM) signal generator with whole-body-specific absorption rate of 1.0 W/kg. Urine samples were collected from the rat while housed in a metabolic cage during the exposure period over a 4-h period at 0.5, 1.0, 2.0 and 4.0 h from the beginning of exposure. In the control group, the signal generator was left in the turn-off position. The creatinine-standardized concentrations of 8-oxodG were measured. With the exception of the urine collected in the last half an hour of exposure, significant elevations were noticed in the levels of 8-oxodG in urine samples from rats exposed to RFR when compared to control animals. Significant differences were seen overall across time points of urine collection with a maximum at 1 h after exposure, suggesting repair of the DNA lesions leading to 8-oxodG formation.

(NE) *Khalil AM, Abu Khadra KM, Aljaberi AM, Gagaa MH, Issa HS. Assessment of oxidant/antioxidant status in saliva of cell phone users. Electromagn Biol Med. 33(2):92-97, 2014.

Abstract Hazardous health effects resulting from exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from cell phones have been reported in the literature. However, the cellular and molecular targets of RF-EMR are still controversial. The aim of this study was to examine the oxidant/antioxidant status in saliva of cell phone users. Saliva samples collected before using a cell phone as well as at the end of 15 and 30 min calls were tested for two commonly used oxidative stress biomarkers: malondialdehyde (MDA) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-Oxo-dG). The 8-oxo-dG levels were determined by enzyme-linked immunosorbent (ELISA) competitive assay, while the MDA levels were measured using the OxiSelect MDA adduct ELISA Kit. The antioxidant capacity of the saliva was evaluated using the oxygen radical absorption capacity (ORAC) and the hydroxyl radical averting capacity (HORAC) assays according to the manufacture instructions. The mean 8-oxo-dG and the Bradford protein concentrations (ng/ml and mg/ml, respectively) peaked at 15 min. The levels of HORAC, ORAC and MDA progressively increased with time and reached maximum at 30 min. However, there was no significant effect of talking time on the levels of 8-OxodG and MDA. Similarly, there was no statistically significant effect of talking time on the oxygen and hydroxyl radicals averting capacities, (ORAC) and (HORAC), respectively. These findings suggest that there is no relationship between exposure to radio frequency radiation (RFR) and changes in the salivary oxidant/antioxidant profile.

(E) Kim JY, Kim HJ, Kim N, Kwon JH, Park MJ. Effects of radiofrequency field exposure on glutamate-induced oxidative stress in mouse hippocampal HT22 cells. Int J Radiat Biol. 93(2):249-256, 2017.

PURPOSE: To define the impact of radiofrequency (RF) under in vitro experimental Alzheimer's disease conditions, we investigated the effect of RF radiation on glutamate-induced oxidative stress in mouse hippocampal neuronal HT22 cells. MATERIALS AND METHODS: Cell survival rate was measured by MTT and trypan blue exclusion assays. Cell cycle distribution, cell death, and ROS production were analyzed using flow cytometry. Expression of proteins was analyzed by Western blot. RESULTS: RF exposure alone had a marginal impact on cell proliferation, however significantly enhanced glutamate-induced cytotoxicity in HT22 cells. Glutamate augmented the subG1 fraction of cell cycle, annexin/propidium iodide positive cell population, and expression of cleaved poly (ADP ribose) polymerase, which were further increased by RF exposure. Glutamate induced reactive oxygen species (ROS) generation and RF exposure further upregulated it. N-acetylcysteine (NAC) treatment completely abrogated glutamate- and RF-induced ROS production followed by cell death and restored cell proliferation in HT22 cells. Finally, glutamate phosphorylated c-Jun N-terminal kinase (JNK) and RF increased this event further. Treatment with NAC and inhibitor of JNK decreased JNK phosphorylation and restored cell proliferation, respectively. CONCLUSIONS: Our results demonstrate that RF exposure enhanced glutamate-induced cytotoxicity by further increase of ROS production in HT22 cells.

(E) 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 **green tea** catechin on oxidative damage in microwave-exposed rats. The microwave-exposed rats received one of three diets: catechin-free (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 **green tea** 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.

(NE) *Kismali G, Ozgur E, Guler G, Akcay A, Sel T, Seyhan N. The influence of 1800 MHz GSM-like signals on blood chemistry and oxidative stress in non-pregnant and pregnant rabbits. Int J Radiat Biol. 88(5):414-419, 2012.

PURPOSE: Environmental electromagnetic fields originate from man-made sources, such as mobile phones and base stations, and have led to increasing public concern about their possible adverse health effects. We aimed to investigate the possible effects of radiofrequency radiation (RFR) generated from these devices on oversensitive animals, such as pregnant rabbits. **MATERIALS AND METHODS:** In the present study, the effects of whole body 1800 MHz Global System for Mobile Communications (GSM)-like RFR exposure for 15 min/day for seven days on blood chemistry and lipid peroxidation levels in both non-pregnant and pregnant New Zealand White rabbits were investigated. Thirteen-month-old rabbits were studied in the following four groups: Non-pregnant control, non-pregnant RFR-exposed, pregnant control and pregnant RFR-exposed. **RESULTS:** Lipid peroxidation, namely malondialdehyde (MDA) levels, did not change after RFR exposure. However, blood chemistry parameters, such as cholesterol (CHO), total protein (TP), albumin (ALB), uric acid, creatinin and creatine kinase (CK) and creatine kinase-myocardial band isoenzyme (CK-MB) changed due to both pregnancy and RFR exposure. **CONCLUSION:** Our investigations have been shown that no indication for oxidative stress was detected in the blood of pregnant rabbits upon RF exposure at specific conditions employed in the present study. Minor changes in some blood chemistry parameters were detected but CK-MB and CK

increases were found remarkable. Studies on RFR exposure during pregnancy will help establish international standards for the protection of pregnant women from environmental RFR.

(E) Koylu H, Mollaoglu H, Ozguner F, Nazyroglu M, Delibab N. Melatonin modulates 900 Mhz microwave-induced lipid peroxidation changes in rat brain. Toxicol Ind Health. 22(5):211-216, 2006.

Microwaves (MW) from cellular phones may affect biological systems by increasing free radicals, which may enhance lipid peroxidation levels of the brain, thus leading to oxidative damage. Melatonin is synthesized in and secreted by the pineal gland at night and exhibits anti-oxidant properties. Several studies suggest that supplementation with anti-oxidant can influence MW-induced brain damage. The present study was designed to determine the effects of MW on the brain lipid peroxidation system, and the possible protective effects of melatonin on brain degeneration induced by MW. Twenty-eight Sprague-Dawley male rats were randomly divided into three groups as follows: (1) sham-operated control group (N = 8); (2) study 900-MHz MW-exposed group (N = 8); and (3) 900-MHz MW-exposed+melatonin (100 microg/kg sc before daily MW exposure treated group) (N = 10). Cortex brain and hippocampus tissues were removed to study the levels of lipid peroxidation as malonyl dialdehyde. The levels of lipid peroxidation in the brain cortex and hippocampus increased in the MW group compared with the control group, although the levels in the hippocampus were decreased by MW+melatonin administration. The brain cortex lipid peroxidation levels were unaffected by melatonin treatment. We conclude that melatonin may prevent MW-induced oxidative changes in the hippocampus by strengthening the anti-oxidant defense system, by reducing oxidative stress products.

(E) Kulaber A, Kerimoğlu G, Ersöz Ş, Çolakoğlu S, Odacı E. Alterations of thymic morphology and antioxidant biomarkers in 60-day-old male rats following exposure to a continuous 900 MHz electromagnetic field during adolescence. Biotech Histochem. 92(5):331-337, 2017.

We investigated changes in thymic tissue of male rats exposed to a 900 megahertz (MHz) electromagnetic field (EMF) on postnatal days 22-59. Three groups of six 21-day-old male Sprague-Dawley rats were allocated as: control (CG), sham (SG) and EMF (EMFG) groups. No procedure was performed on the CG rats. SG rats were placed in a Plexiglas cage for 1 h every day between postnatal days 22 and 59 without exposure to EMF. EMFG rats were placed in the same cage for the same periods as the SG rats and were exposed to 900 MHz EMF. Rats were sacrificed on postnatal day 60. Sections of thymus were stained for histological assessment. Oxidant/antioxidant parameters were investigated biochemically. Malondialdehyde (MDA) levels in EMFG increased compared to the other groups. Extravascular erythrocytes were observed in the medullary/corticomedullary regions in EMFG sections. We found that 900 MHz EMF applied for 1 h/day on postnatal days 22-59 can increase tissue MDA and histopathological changes in male rat thymic tissue.

(E) Kumar S, Nirala JP, Behari J, Paulraj R. Effect of electromagnetic irradiation produced by 3G mobile phone on male rat reproductive system in a simulated scenario. Indian J Exp Biol. 52(9):890-897, 2014.

Reports of declining male fertility have renewed interest in assessing the role of electromagnetic fields (EMFs). Testicular function is particularly susceptible to the radiation emitted by EMFs. Significant decrease in sperm count, increase in the lipid peroxidation damage in sperm cells, reduction in seminiferous tubules and testicular weight and DNA damage were observed following exposure to EMF in male albino rats. The results suggest that mobile phone exposure adversely affects male fertility.

(E) Kuzay D, Ozer C, Sirav B, Canseven AG, Seyhan N. Oxidative effects of extremely low frequency magnetic field and radio frequency radiation on testes tissues of diabetic and healthy rats. Bratisl Lek Listy. 118(5):278-282, 2017.

With the development of technology, people are increasingly under the exposure of electromagnetic fields. Individuals with chronic diseases such as diabetes are now long-term exposed to Radio Frequency-RF radiation and extremely low frequency (ELF) magnetic fields (MFs). The purpose of this present study is to investigate oxidative effects and antioxidant parameters of ELF MFs and RF radiation on testis tissue in diabetic and healthy rats. Wistar male rats were divided into 10 groups. Intraperitoneal single dose STZ (65 mg/kg) dissolved in citrate buffer (0.1M (pH 4.5)) was injected to diabetes groups. ELF MFs and RF radiation were used as an electromagnetic exposure for 20 min/day, 5 days/week for one month. Testis tissue oxidant malondialdehyde (MDA), and antioxidants glutathione (GSH), and total nitric oxide (NOx) levels were determined. The results of ANOVA and Mann-Whitney tests were compared; $p < 0.05$ was considered significant. ELF and RF radiation resulted in an increase in testicular tissue MDA and NOX levels ($p < 0.05$), and caused a decrease in GSH levels ($p < 0.05$) in both healthy and diabetic rats, yet more distinctively in diabetic rats. The most pronounced effect was recorded in D-RF + ELF group ($p < 0.005$). Both radiation practices increased the oxidative stress in testis tissue while causing a decrease in antioxidant level which was more distinctive in diabetic rats (Tab. 1, Fig. 3, Ref. 30).

(E) 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/cm2, 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-phenylnitrone (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.

(NE) Lantow M, Schuderer J, Hartwig C, Simko M. Free Radical Release and HSP70 Expression in Two Human Immune-Relevant Cell Lines after Exposure to 1800 MHz Radiofrequency Radiation. Radiat Res. 165(1):88-94, 2006.

The goal of this study was to investigate whether radiofrequency (RF) electromagnetic-field (EMF) exposure at 1800 MHz causes production of free radicals and/or expression of heat-shock proteins (HSP70) in human immune-relevant cell systems. Human Mono Mac 6 and K562 cells were used to examine free radical release after exposure to incubator control, sham, RF EMFs, PMA, LPS, heat (40 degrees C) or co-exposure conditions. Several signals were used: continuous-wave, several typical modulations of the Global System for Mobile Communications (GSM): GSM-non DTX (speaking only), GSM-DTX (hearing only), GSM-Talk (34% speaking and 66% hearing) at specific absorption rates (SARs) of 0.5, 1.0, 1.5 and 2.0 W/kg. Heat and PMA treatment induced a significant increase in superoxide radical anions and in ROS production in the Mono Mac 6 cells when compared to sham and/ or incubator conditions. No significant differences in free radical production were detected after RF EMF exposure or in the respective controls, and no additional effects on superoxide radical anion production were detected after co-exposure to RF EMFs+PMA or RF EMFs+LPS. The GSM-DTX signal at 2 W/kg produced a significant difference in free radical production when the data were compared to sham because of the decreasing sham value. This difference disappeared when data were compared to the incubator controls. To determine the involvement of heat-shock proteins as a possible inhibitor of free radical production, we investigated the HSP70 expression level after different RF EMF exposures; no significant effects were detected.

(NE) Lantow M, Lupke M, Frahm J, Mattsson MO, Kuster N, Simko M. ROS release and Hsp70 expression after exposure to 1,800 MHz radiofrequency electromagnetic fields in primary human monocytes and lymphocytes. Radiat Environ Biophys. 45(1):55-62, 2006.

The aim of this study is to investigate if 1,800 MHz radiofrequency electromagnetic fields (RF-EMF) can induce reactive oxygen species (ROS) release and/or changes in heat shock protein 70 (Hsp70) expression in human blood cells, using different exposure and co-exposure conditions. Human umbilical cord blood-derived monocytes and lymphocytes were used to examine ROS release after exposure to continuous wave or different GSM signals (GSM-DTX and GSM-Talk) at 2 W/kg for 30 or 45 min of continuous or intermittent (5 min ON/5 min OFF) exposure. The cells were exposed to incubator conditions, to sham, to RF-EMF, or to chemicals in parallel. Cell stimulation with the phorbol ester phorbol-12-myristate-13-acetate (PMA; 1 muM) was used as positive control for ROS release. To investigate the effects on Hsp70 expression, the human monocytes were exposed to the GSM-DTX signal at 2 W/kg for 45 min, or to heat treatment (42 degrees C) as positive control. ROS production and Hsp70 expression were determined by flow cytometric analysis. The data were compared

to sham and/or to control values and the statistical analysis was performed by the Student's t-test ($P<0.05$). The PMA treatment induced a significant increase in ROS production in human monocytes and lymphocytes when the data were compared to sham or to incubator controls. After continuous or intermittent GSM-DTX signal exposure (2 W/kg), a significantly different ROS production was detected in human monocytes if the data were compared to sham. However, this significant difference appeared due to the lowered value of ROS release during sham exposure. In human lymphocytes, no differences could be detected if data were compared either to sham or to incubator control. The Hsp70 expression level after 0, 1, and 2 h post-exposure to GSM-DTX signal at 2 W/kg for 1 h did not show any differences compared to the incubator or to sham control.

(E) Lewicka M, Henrykowska GA, Pacholski K, Szczęsny A, Dziedziczak-Buczyńska M, Buczyński A. The impact of electromagnetic radiation of different parameters on platelet oxygen metabolism - in vitro studies. Adv Clin Exp Med. 24(1):31-35, 2015.

BACKGROUND: Electromagnetic radiation emitted by a variety of devices, e.g. cell phones, computers and microwaves, interacts with the human body in many ways. Research studies carried out in the last few decades have not yet resolved the issue of the effect of this factor on the human body and many questions are left without an unequivocal answer. Various biological and health-related effects have not been fully recognized. Thus further studies in this area are justified. OBJECTIVES: A comparison of changes within catalase enzymatic activity and malondialdehyde concentration arising under the influence of the electromagnetic radiation emitted by car electronics, equipment used in physiotherapy and LCD monitors. MATERIAL AND METHODS: The suspension of human blood platelets at a concentration of 1 × 109/0.001 dm 3, obtained from whole blood by manual apheresis, was the study material. Blood platelets were exposed to an electromagnetic field for 30 min in a laboratory stand designed for the reconstruction of the electromagnetic radiation generated by car electronics, physiotherapy equipment and LCD monitors. The changes in catalase activity and malondialdehyde concentration were investigated after the exposure and compared to the control values (unexposed material). RESULTS: An increase in catalase activity and malondialdehyde concentration was observed after 30 min exposure of platelets to EMF regardless of the radiation source. The most significant changes determining the degree of oxidative stress were observed after exposure to the EMF generated by car electronics. CONCLUSIONS: The low frequency electromagnetic fields generated by car electronics, physiotherapy equipment and LCD monitors may be a cause of oxidative stress in the human body and may lead to free radical diseases.

(E) Li R, Ma M, Li L, Zhao L, Zhang T, Gao X, Zhang D, Zhu Y, Peng Q, Luo X, Wang M. The Protective Effect of Autophagy on DNA Damage in Mouse Spermatocyte-Derived Cells Exposed to 1800 MHz Radiofrequency Electromagnetic Fields. Cell Physiol Biochem. 48(1):29-41, 2018.

BACKGROUND/AIMS: The effects of exposure to radiofrequency electromagnetic fields (RF-EMFs) on the male reproductive system have raised public concern and studies have shown that exposure to RF-EMFs can induce DNA damage and autophagy. However, there are no related reports on the role of autophagy in DNA

damage in spermatocytes, especially after exposure to RF-EMFs. The aim of the present study was to determine the mechanism and role of autophagy induced by RF-EMFs in spermatozoa cells. METHODS: Mouse spermatocyte-derived cells (GC-2) were exposed to RF-EMFs 4 W/kg for 24 h. The level of reactive oxygen species (ROS) was determined by ROS assay kit. Comet assay was utilized to detect DNA damage. Autophagy was detected by three indicators: LC3II/LC3I, autophagic vacuoles, and GFP-LC3 dots, which were measured by western blot, transmission electron microscopy, and transfection with GFP-LC3, respectively. The expression of the molecular signaling pathway AMP-activated protein kinase (AMPK)/mTOR was determined by western blot. RESULTS: The results showed that RF-EMFs induced autophagy and DNA damage in GC-2 cells via ROS generation, and the autophagy signaling pathway AMPK/mTOR was activated by ROS generation. Furthermore, following inhibition of autophagy by knockdown of AMPKα, increased DNA damage was observed in GC-2 cells following RF-EMFs exposure, and overexpression of AMPKα promoted autophagy and attenuated DNA damage. CONCLUSIONS: These findings demonstrated that the autophagy which was induced by RF-EMFs via the AMPK/mTOR signaling pathway could prevent DNA damage in spermatozoa cells**.**

(E) *Liu C, Duan W, Xu S, Chen C, He M, Zhang L, Yu Z, Zhou Z. Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line. Toxicol Lett 218(1): 2-9, 2013.

Whether exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from mobile phones can induce DNA damage in male germ cells remains unclear. In this study, we conducted a 24 h intermittent exposure (5 min on and 10 min off) of a mouse spermatocyte-derived GC-2 cell line to 1800 MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at specific absorption rates (SAR) of 1 W/kg, 2 W/kg or 4 W/kg. Subsequently, through the use of formamidopyrimidine DNA glycosylase (FPG) in a modified comet assay, we determined that the extent of DNA migration was significantly increased at a SAR of 4 W/kg. Flow cytometry analysis demonstrated that levels of the DNA adduct 8-oxoguanine (8-oxoG) were also increased at a SAR of 4 W/kg. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS); these phenomena were mitigated by co-treatment with the antioxidant α-tocopherol. However, no detectable DNA strand breakage was observed by the alkaline comet assay. Taking together, these findings may imply the novel possibility that RF-EMR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells.

(E) Liu K, Zhang G, Wang Z, Liu Y, Dong J, Dong X, Liu J, Cao J, Ao L, Zhang S. The protective effect of autophagy on mouse spermatocyte derived cells exposure to 1800MHz radiofrequency electromagnetic radiation. Toxicol Lett. 2014 May 8. pii: S0378-4274(14)00195-7. doi: 10.1016/j.toxlet.2014.05.004. [Epub ahead of print]

The increasing exposure to radiofrequency (RF) radiation emitted from mobile phone use has raised public concern regarding the biological effects of RF exposure on the male reproductive system. Autophagy contributes to maintaining intracellular homeostasis under environmental stress. To clarify whether RF exposure could induce autophagy in the spermatocyte, mouse spermatocyte-derived cells (GC-2) were exposed to 1800MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at specific absorption rate (SAR) values of 1 w/kg, 2w/kg or 4w/kg for 24h, respectively. The results indicated that the expression of LC3-II increased in a dose- and time-dependent manner with RF exposure, and showed a significant change at the SAR value of 4w/kg. The autophagosome formation and the occurrence of autophagy were further confirmed by GFP-LC3 transient transfection assay and transmission electron microscopy (TEM) analysis. Furthermore, the conversion of LC3-I to LC3-II was enhanced by co-treatment with Chloroqrine (CQ), indicating autophagic flux could be enhanced by RF exposure. Intracellular ROS levels significantly increased in a dose- and time-dependent manner after cells were exposed to RF. Pretreatment with anti-oxidative NAC obviously decreased the conversion of LC3-I to LC3-II and attenuated the degradation of p62 induced by RF exposure. Meanwhile, phosphorylated extracellular-signal-regulated kinase (ERK) significantly increased after RF exposure at the SAR value of 2w/kg and 4w/kg. Moreover, we observed that RF exposure did not increase the percentage of apoptotic cells, but inhibition of autophagy could increase the percentage of apoptotic cells. These findings suggested that autophagy flux could be enhanced by 1800MHz GSM exposure (4w/kg), which is mediated by ROS generation. Autophagy may play an important role in preventing cells from apoptotic cell death under RF exposure stress.

(E) Liu Q, Si T, Xu X, Liang F, Wang L, Pan S. Electromagnetic radiation at 900 MHz induces sperm apoptosis through bcl-2, bax and caspase-3 signaling pathways in rats. Reprod Health. 12:65, 2015.

BACKGROUND: The decreased reproductive capacity of men is an important factor contributing to infertility. Accumulating evidence has shown that Electromagnetic radiation potentially has negative effects on human health. However, whether radio frequency electromagnetic radiation (RF-EMR) affects the human reproductive system still requires further investigation. Therefore, The present study investigates whether RF-EMR at a frequency of 900 MHz can trigger sperm cell apoptosis and affect semen morphology, concentration, and microstructure. METHODS: Twenty four rats were exposed to 900 MHz electromagnetic radiation with a special absorption rate of 0.66 ± 0.01 W/kg for 2 h/d. After 50d, the sperm count, morphology, apoptosis, reactive oxygen species (ROS), and total antioxidant capacity (TAC), representing the sum of enzymatic and nonenzymatic antioxidants, were investigated. Western blotting and reverse transcriptase PCR were used to determine the expression levels of apoptosis-related proteins and genes, including bcl-2, bax, cytochrome c, and capase-3.

RESULTS: In the present study, the percentage of apoptotic sperm cells in the exposure group was significantly increased by 91.42% compared with the control group. Moreover, the ROS concentration in exposure group was increased by 46.21%, while the TAC was decreased by 28.01%. Radiation also dramatically decreased the protein and mRNA expression of bcl-2 and increased that of bax, cytochrome c, and capase-3. CONCLUSION: RF-EMR increases the ROS level and decreases TAC in rat sperm. Excessive oxidative stress alters the expression levels of apoptosis-related genes and triggers sperm apoptosis through bcl-2, bax, cytochrome c and caspase-3 signaling pathways.

(E) López-Furelos A, Salas-Sánchez AA, Ares-Pena FJ, Leiro-Vidal JM, López-Martín E. Exposure to radiation from single or combined radio frequencies provokes macrophage dysfunction in the RAW 264.7 cell line. Int J Radiat Biol. (6):607-618, 2018.

PURPOSE: The aim of this study was to determine whether exposure to radiation from single or multiple radio-frequency (RF) signals at 900 and 2450 MHz would induce effects in the RAW 264.7 cell line. MATERIALS AND METHODS: Cell cultures were exposed to single or combined RF for 4, 24, 48, or 72 h in a GTEM electromagnetic test chamber. At the end of the radiation exposure time, viability and cell growth were analyzed by flow cytometry, nitric oxide (NO) production was measured by colorimetry, the expression of HSP70 and TNF-α was ascertained by qPCR, and the phagocytic activity was observed by microscopy. RESULTS: NO production increased after 48 h exposure at 2450 MHz, compared with controls. The group subjected to the combined interaction of two RFs showed an increase of HSP70 after 48 h exposure and a significant increase of NO and TNF-α after 72 h. The phagocytic activity of macrophages decreased in all groups as exposure time increased. CONCLUSIONS: Our results indicated a decrease in phagocytic activity and an increase in inflammatory, cytoprotective, and cytotoxic responses in macrophages after continuous and combined exposure of multiple RF signals. Multiple RF interact in everyday life, the immune response in humans is unknown.

(E) Lu YS, Huang BT, Huang YX. Reactive Oxygen Species Formation and Apoptosis in Human Peripheral Blood Mononuclear Cell Induced by 900 MHz Mobile Phone Radiation. Oxid Med Cell Longev. 2012:740280, 2012.

We demonstrate that reactive oxygen species (ROS) plays an important role in the process of apoptosis in human peripheral blood mononuclear cell (PBMC) which is induced by the radiation of 900 MHz radiofrequency electromagnetic field (RFEMF) at a specific absorption rate (SAR) of ~0.4 W/kg when the exposure lasts longer than two hours. The apoptosis is induced through the mitochondrial pathway and mediated by activating ROS and caspase-3, and decreasing the mitochondrial potential. The activation of ROS is triggered by the conformation disturbance of lipids, protein, and DNA induced by the exposure of GSM RFEMF. Although human PBMC was found to have a self-

protection mechanism of releasing carotenoid in response to oxidative stress to lessen the further increase of ROS, the imbalance between the antioxidant defenses and ROS formation still results in an increase of cell death with the exposure time and can cause about 37% human PBMC death in eight hours.

(E) Luo YP, Ma HR, Chen JW, Li JJ, Li CX. [Effect of American Ginseng Capsule on the liver oxidative injury and the Nrf2 protein expression in rats exposed by electromagnetic radiation of frequency of cell phone.] [Article in Chinese]. Zhongguo Zhong Xi Yi Jie He Za Zhi. 34(5):575-580, 2014. (In Chinese)

OBJECTIVE: To observe the effect of American Ginseng Capsule (AGC) on the liver oxidative injury and the Nrf2 protein expression in the liver tissue of rats exposed by 900 MHz cell phone electromagnetic radiation. METHODS: Totally 40 male SD rats were randomly divided into the normal control group, the model group, the Shuifei Jibin Capsule (SJC) group, and the AGC group,10 in each group. Rats in the normal control group were not irradiated. Rats in the rest three groups were exposed by imitated 900 MHz cellular phone for 4 h in 12 consecutive days. Meanwhile, rats in the SJC group and the AGC group were intragastrically administrated with suspension of SJC and AGC (1 mL/200 g body weight) respectively. Normal saline was administered to rats in the normal control group and the model group. The histolomorphological changes of the liver tissue were observed by HE staining. Contents of malonic dialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GSH-PX)were detected by colorimetry. The Nrf2 protein expression of hepatocytes was detected by immunohistochemical assay and Western blot. RESULTS: Compared with the normal control group, hepatocyte nucleus was atrophied or partially disappeared, the contents of liver MDA and Nrf2 protein obviously increased (P <0. 05, P <0. 01); contents of liver SOD and GSH decreased (P <0. 05) in the model group. Compared with the model group, karyopyknosis was obviously attenuated and approached to the normal level in the SJC group and the AGC group. The contents of liver MDA and Nrf2 protein expression decreased (P <0. 05), and the contents of liver SOD, GSH, and GSH-PX obviously increased (P < 0.05) in the SJC group. The contents of liver MDA and the Nrf2 protein expression decreased (P < 0.05), and contents of SOD and GSH obviously increased in the AGC group (P <0.01, P <0.05). CONCLUSIONS: The electromagnetic radiation induced by 900 MHz cell phone could affect the expression of Nrf2 protein, induce oxidative injury, and induce abnormal morphology of liver cells. SJC and AGC could promote the morphological recovery of the liver cells. Its mechanism might be related to affecting the expression of Nrf2 protein and attenuating oxidative damage of liver cells.

(cancer) (E) Luukkonen J, Hakulinen P, Mäki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872MHz radiofrequency radiation. Mutat Res. 662(1-2):54-58, 2009.

The objective of the study was to investigate effects of 872MHz radiofrequency (RF) radiation on intracellular reactive oxygen species (ROS) production and DNA damage at a relatively high SAR value (5W/kg). The experiments also involved combined exposure to RF radiation

and menadione, a chemical inducing intracellular ROS production and DNA damage. The production of ROS was measured using the fluorescent probe dichlorofluorescein and DNA damage was evaluated by the Comet assay. Human SH-SY5Y neuroblastoma cells were exposed to RF radiation for 1h with or without menadione. Control cultures were sham exposed. Both continuous waves (CW) and a pulsed signal similar to that used in global system for mobile communications (GSM) mobile phones were used. Exposure to the CW RF radiation increased DNA breakage ($p<0.01$) in comparison to the cells exposed only to menadione. Comparison of the same groups also showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60min after the end of exposure ($p<0.05$ and $p<0.01$, respectively). No effects of the GSM signal were seen on either ROS production or DNA damage. The results of the present study suggest that 872MHz CW RF radiation at 5W/kg might enhance chemically induced ROS production and thus cause secondary DNA damage. However, there is no known mechanism that would explain such effects from CW RF radiation but not from GSM modulated RF radiation at identical SAR.

(NE) Luukkonen J, Juutilainen J, Naarala J. Combined effects of 872 MHz radiofrequency radiation and ferrous chloride on reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells. Bioelectromagnetics. 31(6):417-424, 2010.

The aim of the present study was to investigate possible cooperative effects of radiofrequency (RF) radiation and ferrous chloride (FeCl(2)) on reactive oxygen species (ROS) production and DNA damage. In order to test intracellular ROS production as a possible underlying mechanism of DNA damage, we applied the fluorescent probe DCFH-DA. Integrity of DNA was quantified by alkaline comet assay. The exposures to 872 MHz RF radiation were conducted at a specific absorption rate (SAR) of 5 W/kg using continuous waves (CW) or a modulated signal similar to that used in Global System for Mobile Communications (GSM) phones. Four groups were included: (1) Sham exposure (control), (2) RF radiation, (3) Chemical treatment, (4) Chemical treatment, and RF radiation. In the ROS production experiments, human neuroblastoma (SH-SY5Y) cells were exposed to RF radiation and 10 microg/ml FeCl(2) for 1 h. In the comet assay experiments, the exposure time was 3 h and an additional chemical (0.015% diethyl maleate) was used to make DNA damage level observable. The chemical treatments resulted in statistically significant responses, but no effects from either CW or modulated RF radiation were observed on ROS production, DNA damage or cell viability.

(E) Ma HR, Ma ZH, Wang GY, Song CM, Ma XL, Cao XH, Zhang GH. Impacts of exposure to 900 MHz **mobile phone radiation on liver function in rats. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 31(6):567-571, 2015.**

OBJECTIVE: To study the impacts of exposure to electromagnetic radiation (EMR) on liver function in rats. METHODS: Twenty adult male Sprague-Dawley rats were randomly divided into normal group and radiated group. The rats in normal group were not radiated, those in radiated group were exposed to EMR 4 h/ d for 18 consecutive days. Rats were sacrificed immediately after the end of the experiment. The serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and those of malondialdehyde (MDA) and

glutathione (GSH) in liver tissue were evaluated by colorimetric method. The liver histopathological changes were observed by hematoxylin and eosin staining and the protein expression of bax and bcl- 2 in liver tissue were detected by immunohistochemical method. Terminal-deoxynucleotidyl transferase mediated nick and labelling (TUNEL) method was used for analysis of apoptosis in liver. RESULTS: Compared with the normal rats, the serum levels of ALT and AST in the radiated group had no obvious changes (P>0.05), while the contents of MDA increased (P < 0.01) and those of GSH decreased (P < 0.01) in liver tissues. The histopathology examination showed diffuse hepatocyte swelling and vacuolation, small pieces and focal necrosis. The immunohistochemical results displayed that the expression of the bax protein was higher and that of bcl-2 protein was lower in radiated group. The hepatocyte apoptosis rates in radiated group was higher than that in normal group (all P < 0.01). CONCLUSION: The exposure to **900 MHz** mobile phone 4 h/d for 18 days could induce the liver histological changes, which may be partly due to the apoptosis and oxidative stress induced in liver tissue by electromagnetic radiation.

(E) *Maaroufi K, Save E, Poucet B, Sakly M, Abdelmelek H, Had-Aissouni L. Oxidative stress and prevention of the adaptive response to chronic iron overload in the brain of young adult rats exposed to a 150 kilohertz electromagnetic field. Neuroscience. 186:39-47, 2011.

Iron surcharge may induce an oxidative stress-based decline in several neurological functions. In addition, electromagnetic fields (EMF) of frequencies up to about 100 kHz, emitted by electric/electronic devices, have been suggested to enhance free radical production through an iron dependent pathway. The purpose of this study was therefore to determine a possible relationship between iron status, exposure to EMF, and brain oxidative stress in young adult rats. Samples were micro-dissected from prefrontal cortex, hippocampus, striatum, and cerebellum after chronic saline or iron overload (IO) as well as after chronic sham exposure or exposure to a 150 kHz EMF or after combining EMF exposure with IO. The brain samples were used to monitor oxidative stress-induced lipid peroxidation and activity of the antioxidant enzymes superoxide dismutase and catalase. While IO did not induce any oxidative stress in young adult rats, it stimulated antioxidant defenses in the cerebellum and prefrontal cortex in particular. On the contrary, EMF exposure stimulated lipid peroxidation mainly in the cerebellum, without affecting antioxidant defenses. When EMF was coapplied with IO, lipid peroxidation was further increased as compared to EMF alone while the increase in antioxidant defenses triggered by the sole IO was abolished. These data suggest that EMF exposure may be harmful in young adults by impairing the antioxidant defenses directed at preventing iron-induced oxidative stress.

(E) Manta AK, Papadopoulou D, Polyzos AP, Fragopoulou AF, Skouroliakou AS, Thanos D, Stravopodis DJ, Margaritis LH. Mobile-phone Radiation-induced Perturbation of Gene-expression Profiling, Redox Equilibrium and Sporadic-apoptosis Control in the Ovary of Drosophila melanogaster. Fly (Austin). 11(2):75-95, 2017.

BACKGROUND: The daily use by people of wireless communication devices has increased exponentially in the last decade, begetting concerns regarding its potential health hazards.

METHODS: Drosophila melanogaster four days-old adult female flies were exposed for 30 min to radiation emitted by a commercial mobile phone at a SAR of 0.15 W/kg and a SAE of 270 J/kg. ROS levels and apoptotic follicles were assayed in parallel with a genome-wide microarrays analysis. RESULTS: ROS cellular contents were found to increase by 1.6 fold (x), immediately after the end of exposure, in follicles of pre-choriogenic stages (germarium - stage 10), while sporadically generated apoptotic follicles (germarium 2b and stages 7-9) presented with an averaged 2x upregulation in their sub-population mass, 4 h after fly's irradiation with mobile device. Microarray analysis revealed 168 genes being differentially expressed, 2 h post-exposure, in response to radiofrequency (RF) electromagnetic field-radiation exposure (≥1.25x, $P<0.05$) and associated with multiple and critical biological processes, such as basic metabolism and cellular subroutines related to stress response and apoptotic death. CONCLUSION: Exposure of adult flies to mobile-phone radiation for 30 min has an immediate impact on ROS production in animal's ovary, which seems to cause a global, systemic and non-targeted transcriptional reprogramming of gene expression, 2 h post-exposure, being finally followed by induction of apoptosis 4 h after the end of exposure. Conclusively, this unique type of pulsed radiation, mainly being derived from daily used mobile phones, seems capable of mobilizing critical cytopathic mechanisms, and altering fundamental genetic programs and networks in D. melanogaster.

(E) Mailankot M, Kunnath AP, Jayalekshmi H, Koduru B, Valsalan R. Radio frequency electromagnetic radiation (RF-EMR) from GSM (0.9/1.8GHz) mobile phones induces oxidative stress and reduces sperm motility in rats. Clinics (Sao Paulo). 64(6):561-565, 2009.

INTRODUCTION: Mobile phones have become indispensable in the daily lives of men and women around the globe. As cell phone use has become more widespread, concerns have mounted regarding the potentially harmful effects of RF-EMR from these devices. OBJECTIVE: The present study was designed to evaluate the effects of RF-EMR from mobile phones on free radical metabolism and sperm quality. MATERIALS AND METHODS: Male albino Wistar rats (10-12 weeks old) were exposed to RF-EMR from an active GSM (0.9/1.8 GHz) mobile phone for 1 hour continuously per day for 28 days. Controls were exposed to a mobile phone without a battery for the same period. The phone was kept in a cage with a wooden bottom in order to address concerns that the effects of exposure to the phone could be due to heat emitted by the phone rather than to RF-EMR alone. Animals were sacrificed 24 hours after the last exposure and tissues of interest were harvested. RESULTS: One hour of exposure to the phone did not significantly change facial temperature in either group of rats. No significant difference was observed in total sperm count between controls and RF-EMR exposed groups. However, rats exposed to RF-EMR exhibited a significantly reduced percentage of motile sperm. Moreover, RF-EMR exposure resulted in a significant increase in lipid peroxidation and low GSH content in the testis and epididymis. CONCLUSION: Given the results of the present study, we speculate that RF-EMR from mobile phones negatively affects semen quality and may impair male fertility.

(E) *Manta AK, Stravopodis DJ, Papassideri IS, Margaritis LH. Reactive oxygen species elevation and recovery in Drosophila bodies and ovaries following short-term

and long-term exposure to DECT base EMF. Electromagn Biol Med. 33(2):118-131, 2014. (LI)

Abstract The objective of this study was to approach the basic mechanism(s) underlying reported ovarian apoptotic cell death and fecundity decrease induced by nonionizing radiation (NIR) in Drosophila melanogaster. ROS (Reactive Oxygen Species) levels were measured in the bodies and the ovaries of (sexually mature) 4-day-old flies, following exposure for 0.5, 1, 6, 24 and 96 h to a wireless DECT (Digital Enhanced Cordless Telephone) base radiation (1.88-1.90 GHz). Electrical field intensity was 2.7 V/m, measured within the fly vials and calculated SAR (Specific Absorption Rate) value = 0.009 W/Kg. Male and female bodies showed twofold increase in ROS levels ($p < 0.001$) after 6 h of exposure, slightly increasing with more irradiation (24 and 96 h). Ovaries of exposed females had a quick response in ROS increase after 0.5 h (1.5-fold, $p < 0.001$), reaching 2.5-fold after 1 h with no elevation thereafter at 6, 24 and 96 h. ROS levels returned to normal, in the male and the female bodies 24 h after 6 h of exposure of the flies ($p < 0.05$) and in the ovaries 4 h after 1 h exposure of the females ($p < 0.05$). It is postulated that the pulsed (at 100 Hz rate and 0.08 ms duration) idle state of the DECT base radiation is capable of inducing free radical formation albeit the very low SAR, leading rapidly to accumulation of ROS in a level-saturation manner under continuous exposure, or in a recovery manner after interruption of radiation, possibly due to activation of the antioxidant machinery of the organism.

(E) Marconi A, Tasteyre A, de Seze R, Fogel P, Simoneau G, Conti M, Sarbach C, Young SS, Gilbert J-E, Thomas Y. Journal Scientific Exploration. 29 (3): 449-465, 2015.

A multidisciplinary project was conducted to study the possible biological impact of mobile phone emissions. As part of that project, we conducted a pilot study on 18 human volunteers, with the treatment being GSM mobile phone exposure. The volunteers were randomized and the study was a double-blind, crossover design. Two categories of oxidative stress biomarkers were followed and measured in blood and exhaled air: those assessing oxidative attacks of cell membrane lipids (malondialdehyde, exhaled alkanes, aldehydes, and isoprene) and those accounting for the organism's antioxidant defense systems (superoxyde dismutase, glutathion Peroxydase, and exhaled halogenated alkanes).The overall entropy of the system with and without GSM exposure was then calculated for each volunteer, using a statistical approach based on the global entropie difference of raw data. A significant modulation of organization of the biomarkers after 30 minutes of mobile phone exposure was found, as evidenced by a decreased entropy of the dataset associated to the emitting mobile phone condition. While these results illustrate neither deleterious effects nor the innocuity of mobile phone use, they nonetheless constitute evidence of actual interactions of these wavelengths with complex biological systems. These results will need to be confirmed in larger, future studies.

(E) Marjanovic AM, Pavicic I, Trosic I, Cell oxidation–reduction imbalance after modulated radiofrequency radiation. Electromagn Biol Med. 34(4):381-386, 2015.

Aim of this study was to evaluate an influence of modulated radiofrequency field (RF) of 1800 MHz, strength of 30 V/m on oxidation–reduction processes within the cell. The assigned

RF field was generated within Gigahertz Transversal Electromagnetic Mode cell equipped by signal generator, modulator, and amplifier. Cell line V79, was irradiated for 10, 30, and 60 min, specific absorption rate was calculated to be 1.6 W/kg. Cell metabolic activity and viability was determined by MTT assay. In order to define total protein content, colorimetric method was used. Concentration of oxidised proteins was evaluated by enzyme-linked immunosorbent assay. Reactive oxygen species (ROS) marked with fluorescent probe 2′,7′-dichlorofluorescin diacetate were measured by means of plate reader device. In comparison with control cell samples, metabolic activity and total protein content in exposed cells did not differ significantly. Concentrations of carbonyl derivates, a product of protein oxidation, insignificantly but continuously increase with duration of exposure. In exposed samples, ROS level significantly ($p < 0.05$) increased after 10 min of exposure. Decrease in ROS level was observed after 30-min treatment indicating antioxidant defence mechanism activation. In conclusion, under the given laboratory conditions, modulated RF radiation might cause impairment in cell oxidation–reduction equilibrium within the growing cells.

(E) Marjanovic Cermak AM, Pavicic I, Tariba Lovakovic B, Pizent A, Trosic I. In vitro non-thermal oxidative stress response after 1800 MHz radiofrequency radiation. Gen Physiol Biophys. 36(4): 407-414, 2017.

In this study possible connection between radiofrequency exposure (RF) and development of oxidative stress was investigated by measuring impairment in cellular oxidation-reduction balance immediately after RF exposure. Fibroblast cells V79 were exposed for 10, 30 and 60 minutes to 1800 MHz RF radiation. Electric field strength was 30 V/m and specific absorption rate (SAR) was calculated to be 1.6 W/kg. Electromagnetic field was generated within Gigahertz Transversal Electromagnetic Mode cell (GTEM) equipped by signal generator, amplifier and modulator. Cell viability was determined by CCK-8 colorimetric assay and level of reactive oxygen species (ROS) was detected by dihydroethidium staining. Reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) were used to assess cell antioxidant activity while lipid oxidative damage was evaluated measuring concentration of malondialdehyde. Viability of V79 cells remained within normal physiological values regardless of exposure time. Increased level of superoxide radicals was detected after 60-min exposure. Significantly higher GSH level was observed immediately after 10-min exposure with higher but insignificant activity of GSH-Px. Lipid oxidative damage in exposed cell samples was not observed. Short-term RF exposure revealed transient oxidation-reduction imbalance in fibroblast cells following adaptation to applied experimental conditions.

(cancer) (E) Marjanovic Cermak AM, Pavicic I, Trosic I. Oxidative stress response in SH-SY5Y cells exposed to short-term 1800 MHz radiofrequency radiation. J Environ Sci Health A Tox Hazard Subst Environ Eng. 53(2):132-138, 2018.

The exact mechanism that could explain the effects of radiofrequency (RF) radiation exposure at non-thermal level is still unknown. Increasing evidence suggests a possible involvement of reactive oxygen species (ROS) and development of oxidative stress. To test the proposed hypothesis, human neuroblastoma cells (SH-SY5Y) were exposed to 1800 MHz short-term RF exposure for 10, 30 and 60 minutes. Electric field strength within Gigahertz Transverse

Electromagnetic cell (GTEM) was 30 V m-1 and specific absorption rate (SAR) was calculated to be 1.6 W kg-1. Cellular viability was measured by MTT assay and level of ROS was determined by fluorescent probe 2',7'-dichlorofluorescin diacetate. Concentrations of malondialdehyde and protein carbonyls were used to assess lipid and protein oxidative damage and antioxidant activity was evaluated by measuring concentrations of total glutathione (GSH). After radiation exposure, viability of irradiated cells remained within normal physiological values. Significantly higher ROS level was observed for every radiation exposure time. After 60 min of exposure, the applied radiation caused significant lipid and protein damage. The highest GSH concentration was detected after 10 minute-exposure. The results of our study showed enhanced susceptibility of SH-SY5Y cells for development of oxidative stress even after short-term RF exposure.

(E) Masoumi A, Karbalaei N, Mortazavi SMJ, Shabani M. Radiofrequency radiation emitted from Wi-Fi (2.4 GHz) causes impaired insulin secretion and increased oxidative stress in rat pancreatic islets. Int J Radiat Biol. 94(9):850-857, 2018.

PURPOSE: There is a great concern regarding the possible adverse effects of electromagnetic radiation (EMR). This study investigated the effects of EMR induced by Wi-Fi (2.45 GHz) on insulin secretion and antioxidant redox systems in the rat pancreas. MATERIALS AND METHODS: Adult male Sprague-Dawley rats in the weight range of 230-260 g were divided into control, sham, Wi-Fi exposed groups. After long-term exposure (4 h/day for 45 days) to Wi-Fi EMR, plasma levels of glucose and insulin during intraperitoneal glucose tolerance test were measured. Islet insulin secretion and content, lipid peroxidation, and antioxidant status in pancreas of rats were determined. RESULTS: Our data showed that the weight gain in the WI-FI exposed group was significantly lower than the control group ($p < .05$). Wi-Fi (2.45 GHz)-exposed group showed hyperglycemia. Plasma insulin level and glucose-stimulated insulin secretion from pancreatic islet were significantly reduced in the Wi-Fi-exposed group. EMR emitted from Wi-Fi caused a significant increase in lipid peroxidation and a significant decrease in GSH level, SOD, and GPx activities of the pancreas. CONCLUSIONS: These data showed that EMR of Wi-Fi leads to hyperglycemia, increased oxidative stress, and impaired insulin secretion in the rat pancreatic islets.

(E) Marzook EA, Abd El Moneim AE, Elhadary AA. Prootective role of seame oil against mobile phone base station-induced oxidative stress. J Rad Res Appl Sci 7(1):1-6, 2014.

The present study was undertaken to shed the light on the environmental threats associated with the wireless revolution and the health hazards associated with exposure to mobile base station (MBS). Besides, studying the possible protective role of sesame oil (SO) as an antioxidant against oxidative stress. Therefore, the present work was designed to study the effect of chronic exposure to electromagnetic radiations (EMR), produced by a cellular tower for mobile phone and the possible protective role of sesame oil on glutathione reductase

(GSH-Rx), superoxide dismutase (SOD), catalase (CAT), total testosterone and lipid profile (total cholesterol (Tch), triglycerides (TG), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c) in male albino rats. Rats were arranged into four groups: the control unexposed, the exposed untreated and the exposed treated groups (1.5 and 3 ml oil). Exposed groups were subjected to electromagnetic field at frequency of 900 MHz, for 24 h/day for 8 weeks, at the same time both treated groups were supplied with oral injection of sesame oil three times per week. At the end of the experiment, blood samples were obtained for determination of the above mentioned variables in serum. The results obtained revealed that TG and testosterone were raised significantly over control in all groups and the significant increase in oil groups occurred in dose dependent manner. SOD and CAT activities were reduced significantly in exposed rats than control and increased significantly in sesame oil groups as the dose of oil increased. Total cholesterol only showed remarkable reduction in the group treated with 3 ml sesame oil. Also, in this latter group, significant elevation of GSH-Rx was recorded. Changes in serum HDL-c and LDL-c followed an opposite trend in exposed and sesame oil groups reflecting their affectation by EMR or sesame oil. In conclusion, all results of the current study proved that sesame oil can be used as an edible oil to attenuate the oxidative stress which could be yielded as a result of chronic exposure to EMR.

(E) *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. 33(2):81-91, 2014.

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^2) 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.

(E) *Megha K, Deshmukh PS, Banerjee BD, Tripathi AK, Abegaonkar MP. Microwave radiation induced oxidative stress, cognitive impairment and inflammation in brain of Fischer rats. Indian J Exp Biol. 50(12):889-896, 2012. (LI)

Public concerns over possible adverse effects of microwave radiation emitted by mobile phones on health are increasing. To evaluate the intensity of oxidative stress, cognitive impairment and inflammation in brain of Fischer rats exposed to microwave radiation, male Fischer-344 rats were exposed to 900 MHz microwave radiation (SAR = 5.953 x 10(-4) W/kg) and 1800 MHz microwave radiation (SAR = 5.835 x 10(-4) W/kg) for 30 days (2 h/day). Significant impairment in cognitive function and induction of oxidative stress in brain tissues of microwave exposed rats were observed in comparison with sham exposed groups. Further, significant increase in level of cytokines (IL-6 and TNF-alpha) was also observed following microwave exposure. Results of the present study indicated that increased oxidative stress due to microwave exposure may contribute to cognitive impairment and inflammation in brain.

(E) Meral I, Mert H, Mert N, Deger Y, Yoruk I, Yetkin A, Keskin S. Effects of 900-MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels of guinea pigs. Brain Res. 1169:120-124, 2007.

This study was designed to demonstrate the effects of 900-MHz electromagnetic field (EMF) emitted from cellular phone on brain tissue and also blood malondialdehyde (MDA), glutathione (GSH), retinol (vitamin A), vitamin D(3) and tocopherol (vitamin E) levels, and catalase (CAT) enzyme activity of guinea pigs. Fourteen male guinea pigs, weighing 500-800 g were randomly divided into one of two experimental groups: control and treatment (EMF-exposed), each containing seven animals. Animals in treatment group were exposed to 890- to 915-MHz EMF (217-Hz pulse rate, 2-W maximum peak power, SAR 0.95 w/kg) of a cellular phone for 12 h/day (11-h 45-min stand-by and 15-min spiking mode) for 30 days. Control guinea pigs were housed in a separate room without exposing EMF of a cellular phone. Blood samples were collected through a cardiac puncture and brains were removed after decapitation for the biochemical analysis at the end of the 30 days of experimental period. It was found that the MDA level increased ($P<0.05$), GSH level and CAT enzyme activity decreased ($P<0.05$), and vitamins A, E and D(3) levels did not change ($P>0.05$) in the brain tissues of EMF-exposed guinea pigs. In addition, MDA, vitamins A, D(3) and E levels, and CAT enzyme activity increased ($P<0.05$), and GSH level decreased ($P<0.05$) in the blood of EMF-exposed guinea pigs. It was concluded that electromagnetic field emitted from cellular phone might produce oxidative stress in brain tissue of guinea pigs. However, more studies are needed to demonstrate whether these effects are harmful or/and affect the neural functions.

(E) Monselise EB, Levkovitz A, Gottlieb HE, Kost D. Bioassay for assessing cell stress in the vicinity of radio-frequency irradiating antennas. J Environ Monit. 13(7):1890-1896, 2011.

The 24 h exposure of water plants (etiolated duckweed) to RF-EMF between 7.8 V m(-1) and 1.8 V m(-1), generated by AM 1.287 MHz transmitting antennas, resulted in alanine accumulation in the plant cells, a phenomenon we have previously shown to be a universal stress signal. The magnitude of the effect corresponds qualitatively to the level of RF-EMF exposure. In the presence of 10 mM vitamin C, alanine accumulation is

completely suppressed, suggesting the involvement of free radicals in the process. A unique biological connection has thus been made between exposure to RF-EMF and cell stress, in the vicinity of RF transmitting antennas. This simple test, which lasts only 24 h, constitutes a useful bioassay for the quick detection of biological cell stress caused in the vicinity of RF irradiating antennas.

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

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

(E) Mortazavi SMJ, Mostafavi-Pour Z, Daneshmand M, Zal F, Zare R, Mosleh-Shirazi MA. Adaptive Response Induced by Pre-Exposure to 915 MHz Radiofrequency: A Possible Role for Antioxidant Enzyme Activity. J Biomed Phys Eng. 7(2):137-142, 2017.

BACKGROUND: Over the past few years, the rapid use of high frequency electromagnetic fields like **mobile phones** has raised global concerns about the negative health effects of its use. Adaptive response is the ability of a **cell** or tissue to better resist stress damage by prior exposure to a lesser amount of stress. This study aimed to assess whether radiofrequency radiation can induce adaptive response by changing the antioxidant balance.MATERIALS AND METHODS: In order to assess RF-induced adaptive response in tissues, we evaluated the level of GSH and the activity of GR in liver. 50 rats were divided into 5 groups. Three groups were pre-exposed to 915 MHz RF radiation, 4 hours per day for one week at different powers, as low, medium and high. 24 hours after the last exposure to radiation, they were exposed to 4 Gy sublethal dose of gamma radiation and then sacrificed after 5 hours. Their livers were removed, washed and were kept at -80o C until used.RESULTS: Our finding showed that pre-exposure to 915 MHz radiofrequency radiation with specific power could induce adaptive response in liver by inducing changes in the activity and level of antioxidant enzymes.CONCLUSION: It can be concluded that pre-exposure to microwave radiation could increase the level of GSH and the activity of GR enzyme, although these increases were seen just in low power group, and the GR activity was indicated in medium power group. This increase protects tissue from oxidative damage induced by sublethal dose of gamma radiation.

(E) Motawi TK, Darwish HA, Moustafa YM, Labib MM. Biochemical Modifications and Neuronal Damage in Brain of Young and Adult Rats After Long-Term Exposure to Mobile Phone Radiations. Cell Biochem Biophys. 2014 May 7. [Epub ahead of print]

This study investigated the effect of exposure to mobile phone radiations on oxidative stress and apoptosis in brain of rats. Rats were allocated into six groups (three young and three adult). Groups 1 and 4 were not subjected to the radiation source and served as control groups. In groups 2 and 5, the mobile phones were only connected to the global system for mobile communication, while in groups 3 and 6, the option of calling was in use. Microwaves were generated by a mobile test phone (SAR = 1.13 W/kg) during 60 days (2 h/day). Significant increments in conjugated dienes, protein carbonyls, total oxidant status, and oxidative stress index along with a significant reduction of total antioxidant capacity levels were evident after exposure. Bax/Bcl-2 ratio, caspase-3 activity, and tumor necrosis factor-alpha level were enhanced, whereas no DNA fragmentation was detected. The relative brain weight of young rats was greatly affected, and histopathological examination reinforced the neuronal damage. The study highlights the detrimental effects of mobile phone radiations on brain during young and adult ages. The interaction of these radiations with brain is via dissipating its antioxidant status and/or triggering apoptotic cell death.

(E) Moustafa YM, Moustafa RM, Belacy A, Abou-El-Ela SH, Ali FM. Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. J Pharm Biomed Anal 26(4):605-608, 2001.

Radiofrequency fields of cellular phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation, and by changing the antioxidase activities of human blood thus leading to oxidative stress. To test this, we have investigated the effect of acute exposure to radiofrequency fields of commercially

available cellular phones on some parameters indicative of oxidative stress in 12 healthy adult male volunteers. Each volunteer put the phone in his pocket in standby position with the keypad facing the body. The parameters measured were lipid peroxide and the activities of superoxide dismutase (SOD), total glutathione peroxidase (GSH-Px) and catalase. The results obtained showed that the plasma level of lipid peroxide was significantly increased after 1, 2 and 4 h of exposure to radiofrequency fields of the cellular phone in standby position. Moreover, the activities of SOD and GSH-Px in human erythrocytes showed significant reduction while the activity of catalase in human erythrocytes did not decrease significantly. These results indicate that acute exposure to radiofrequency fields of commercially available cellular phones may modulate the oxidative stress of free radicals by enhancing lipid peroxidation and reducing the activation of SOD and GSH-Px, which are free radical scavengers. Therefore, these results support the interaction of radiofrequency fields of cellular phones with biological systems.

(NE) Nakatani-Enomoto S, Okutsu M, Suzuki S, Suganuma R, Groiss SJ, Kadowaki S, Enomoto H, Fujimori K, Ugawa Y. Effects of 1950 MHz W-CDMA-like signal on human spermatozoa. Bioelectromagnetics. 2016 Jun 11. doi: 10.1002/bem.21985. [Epub ahead of print]

There are growing concerns about how electromagnetic waves (EMW) emitted from mobile phones affect human spermatozoa. Several experiments have suggested harmful effects of EMW on human sperm quality, motility, velocity, or the deoxyribonucleic acid (DNA) of spermatozoa. In this study, we analyzed the effects on human spermatozoa (sperm motility and kinetic variables) induced by 1 h of exposure to 1950 MHz Wideband Code Division Multiple Access (W-CDMA)-like EMW with specific absorption rates of either 2.0 or 6.0 W/kg, using a computer-assisted sperm analyzer system. We also measured the percentage of 8-hydroxy-2'-deoxyguanosine (8-OHdG) positive spermatozoa with flow cytometry to evaluate damage to DNA. No significant differences were observed between the EMW exposure and the sham exposure in sperm motility, kinetic variables, or 8-OHdG levels. We conclude that W-CDMA-like exposure for 1 h under temperature-controlled conditions has no detectable effect on normal human spermatozoa. Differences in exposure conditions, humidity, temperature control, baseline sperm characteristics, and age of donors may explain inconsistency of our results with several previous studies.

(E) Narayanan SN, Kumar RS, Kedage V, Nalini K, Nayak S, Bhat PG. Evaluation of oxidant stress and antioxidant defense in discrete brain regions of rats exposed to 900 MHz radiation. Bratisl Lek Listy. 115(5):260-266, 2014.

AIM: In the current study, the effects of 900 MHz radio-frequency electromagnetic radiation (RF-EMR) on levels of thiobarbituric acid-reactive substances (TBARS), total antioxidants (TA), and glutathione S-transferase (GST) activity in discrete brain regions were studied in adolescent rats. MATERIALS AND METHODS: Thirty-six male Wistar rats (6-8 weeks old) were allotted into three groups (n = 12 in each group). Control group (1) remained undisturbed in their home cage; sham group (2) was exposed to mobile phone in switch off mode for four weeks; RF-EMR-exposed group (3) was exposed to

900 MHz of RF-EMR (1 hr/day with peak power density of 146.60 µW/cm2) from an activated Global System for Mobile communication (GSM) mobile phone (kept in silent mode; no ring tone and no vibration) for four weeks. On 29th day, behavioral analysis was done. Followed by this, six animals from each group were sacrificed and biochemical parameters were studied in amygdala, hippocampus, frontal cortex, and cerebellum. RESULTS: Altered behavioral performances were found in RF-EMR-exposed rats. Additionally, elevated TBARS level was found with all brain regions studied. RF-EMR exposure significantly decreased TA in the amygdala and cerebellum but its level was not significantly changed in other brain regions. GST activity was significantly decreased in the hippocampus but, its activity was unaltered in other brain regions studied. CONCLUSION: RF-EMR exposure for a month induced oxidative stress in rat brain, but its magnitude was different in different regions studied. RF-EMR-induced oxidative stress could be one of the underlying causes for the behavioral deficits seen in rats after RF-EMR exposure (Fig. 5, Ref. 37).

(E) 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.

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

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^{2+} and cell numbers were higher in 2.45 GHz groups than in the controls. The increase of cytosolic free Ca^{2+} 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^{2+} 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^{2+} influx although blocking of transient receptor potential melastatin 2 channels by 2-aminoethyl diphenylborinate seems to counteract the effects on Ca^{2+} ions influx.

(E) *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, 2012b.

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.

(NE) Nazıroğlu M, Özkan FF, Hapil SR, Ghazizadeh V, Çiğ B. Epilepsy but not mobile phone frequency (900 MHz) induces apoptosis and calcium entry in hippocampus of epileptic rat: involvement of TRPV1 channels. J Membr Biol. 248(1):83-91, 2015.

Electromagnetic radiation (EMR) and epilepsy are reported to mediate the regulation of apoptosis and oxidative stress through Ca(2+) influx. Results of recent reports indicated that EMR can increase temperature and oxidative stress of body cells, and TRPV1 channel is activated by noxious heat, oxidative stress, and capsaicin (CAP). We investigated the effects of mobile phone (900 MHz) EMR exposure on Ca(2+) influx, apoptosis, oxidative stress, and TRPV1 channel activations in the hippocampus of pentylenetetrazol (PTZ)-induced epileptic rats. Freshly isolated hippocampal neurons of twenty-one rats were used in study within three groups namely control, PTZ, and PTZ + EMR. The neurons in the three groups were

stimulated by CAP. Epilepsy was induced by PTZ administration. The neurons in PTZ + EMR group were exposed to the 900 MHz EMR for 1 h. The apoptosis, mitochondrial membrane depolarization, intracellular reactive oxygen species (ROS), and caspase-3 and caspase-9 values were higher in PTZ and PTZ + EMR groups than in control. However, EMR did not add additional increase effects on the values in the hippocampal neurons. Intracellular-free Ca(2+) concentrations in fura-2 analyses were also higher in PTZ + CAP group than in control although their concentrations were decreased by TRPV1 channel blocker, capsazepine. However, there were no statistical changes on the Ca(2+) concentrations between epilepsy and EMR groups. In conclusion, apoptosis, mitochondrial, ROS, and Ca(2+) influx via TRPV1 channel were increased in the hippocampal neurons by epilepsy induction although the mobile phone did not change the values. The results indicated that TRPV1 channels in hippocampus may possibly be a novel target for effective target of epilepsy.

(cancer) (Review) **Naziroğlu M, Tokat S, Demirci S. Role of melatonin on electromagnetic radiation-induced oxidative stress and Ca2+ signaling molecular pathways in breast cancer. J Recept Signal Transduct Res. 32(6):290-297, 2012c.**

AIMS: Exposure to electromagnetic radiation (EMR) may increase breast cancer risk by inducing oxidative stress and suppressing the production of melatonin. Aim of the present review is to discuss the mechanisms and risk factors of EMR and oxidative stress-induced breast cancer, to summarize the controlled studies evaluating measures for prevention, and to conclude with evidence-based strategies for prevention. MATERIALS: Review of the relevant literature and results from our recent basic studies, as well as critical analyses of published systematic reviews were obtained from the Pubmed and the Science Citation Index. RESULTS: It has been proposed that chronic exposure to EMR may increase the risk of breast cancer by suppressing the production of melatonin; this suppression may affect the development of breast cancer either by increasing levels of circulation of estrogen or through over production of free oxygen radicals. Most epidemiological studies have also indicated overall effect of EMR exposure in premenopausal women, particularly for estrogen receptor positive breast tumors. Enhanced voltage-dependent Ca(2+) current and impaired inhibitory G-protein function, and derangement of intracellular organelles with a Ca(2+) buffering effect, such as endoplasmic reticulum and mitochondria have been also shown to contribute to disturbed Ca(2+) signaling in breast cancer. CONCLUSION: Melatonin may modulate breast cancer through modulation of enhanced oxidative stress and Ca(2+) influx in cell lines. However, there is not enough evidence on increased risk of breast cancer related to EMR exposure.

(Review) **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.

(E) Nirwane A, Sridhar V, Majumdar A. Neurobehavioural Changes and Brain Oxidative Stress Induced by Acute Exposure to GSM900 Mobile Phone Radiations in Zebrafish (Danio rerio). Toxicol Res. 2016 Apr;32(2):123-32. doi: 10.5487/TR.2016.32.2.123. Epub 2016 Apr 30.

The impact of mobile phone (MP) radiation on the brain is of specific interest to the scientific community and warrants investigations, as MP is held close to the head. Studies on humans and rodents revealed hazards MP radiation associated such as brain tumors, impairment in cognition, hearing etc. Melatonin (MT) is an important modulator of CNS functioning and is a neural antioxidant hormone. Zebrafish has emerged as a popular model organism for CNS studies. Herein, we evaluated the impact of GSM900MP (GSM900MP) radiation exposure daily for 1 hr for 14 days with the SAR of 1.34W/Kg on neurobehavioral and oxidative stress parameters in zebrafish. Our study revealed that, GSM900MP radiation exposure, significantly decreased time spent near social stimulus zone and increased total distance travelled, in social interaction test. In the novel tank dive test, the GSM900MP radiation exposure elicited anxiety as revealed by significantly increased time spent in bottom half; freezing bouts and duration and decreased distance travelled, average velocity, and number of entries to upper half of the tank. Exposed zebrafish spent less time in the novel arm of the Y-Maze, corroborating significant impairment in learning as compared to the control group. Exposure decreased superoxide dismutase (SOD), catalase (CAT) activities whereas, increased levels of reduced glutathione (GSH) and lipid peroxidation (LPO) was encountered showing compromised antioxidant defense. Treatment with MT significantly reversed the above neurobehavioral and oxidative derangements induced by GSM900MP radiation exposure. This study traced GSM900MP radiation exposure induced neurobehavioral aberrations and alterations in brain oxidative status. Furthermore, MT proved to be a promising therapeutic candidate in ameliorating such outcomes in zebrafish.

(E) Odacı E, Unal D, Mercantepe T, Topal Z, Hancı H, Türedi S, Erol H, Mungan S, Kaya H, Colakoğlu S. Pathological effects of prenatal exposure to a 900 MHz electromagnetic field on the 21-day-old male rat kidney. Biotech Histochem. 2014 Aug 27:1-9. [Epub ahead of print]

We investigated the effects on kidney tissue of 900 megahertz (MHz) EMF applied during the prenatal period. Pregnant rats were exposed to 900 MHz EMF, 1 h/day, on days 13-21 of

pregnancy; no procedure was performed on control group pregnant rats or on mothers or newborns after birth. On postnatal day 21, kidney tissues of male rat pups from both groups were examined by light and electron microscopy. Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione levels also were investigated. Light microscopy revealed some degenerative changes in the tubule epithelium, small cystic formations in the primitive tubules and large cysts in the cortico-medullary or medullary regions in the experimental group. Electron microscopy revealed a loss of peritubular capillaries and atypical parietal layer epithelial cells in the experimental group. Biochemical analysis showed significantly increased MDA levels in the experimental group and decreased SOD and CAT levels. EMF applied during the prenatal period can caused pathological changes in kidney tissue in 21-day-old male rats owing to oxidative stress and decreased antioxidant enzyme levels.

(E) Odacı E, Özyılmaz C. Exposure to a 900 MHz electromagnetic field for one hour a day over 30 days does change the histopathology and biochemistry of the rat testis. Int J Radiat Biol. 2015 Mar 19:1-20. [Epub ahead of print]

PURPOSE: This study investigated the effect of exposure to a 900-megahertz (MHz) electromagnetic field (EMF) on the rat testicle. MATERIALS AND METHODS: Twenty-four adult male rats were divided into control, sham and EMF groups. The EMF group rats were exposed to 900-MHz EMF (1 h / 30 day), and testicles were extracted at the end of the experiment. Malondialdehyde, superoxide dismutase, catalase and glutathione levels and apoptotic index and histopathological damage scores were compared. RESULTS: Histopathologically, EMF group rats exhibited vacuoles in seminiferous tubules basal membrane and edema in the intertubular space. Seminiferous tubule diameters and germinal epithelium thickness were both smaller, and apoptotic index was higher, in the EMF group than in the other groups. Malondialdehyde, superoxide dismutase, catalase and glutathione values in the EMF group decreased significantly compared to those of the control group. CONCLUSIONS: The results show that exposure to 900-MHz EMF causes alterations in adult rat testicular morphology and biochemistry.

(E) *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(-1) 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.

(E) Oktem F, Ozguner F, Mollaoglu H, Koyu A, Uz E. Oxidative Damage in the Kidney Induced by 900-MHz-Emitted Mobile Phone: Protection by Melatonin. Arch Med Res. 36(4):350-355, 2005.

BACKGROUND: The mobile phones emitting 900-MHz electromagnetic radiation (EMR) may be mainly absorbed by kidneys because they are often carried in belts. Melatonin, the chief secretory product of the pineal gland, was recently found to be a potent free radical scavenger and antioxidant. The aim of this study was to examine 900-MHz mobile phone-induced oxidative stress that promotes production of reactive oxygen species (ROS) on renal tubular damage and the role of melatonin on kidney tissue against possible oxidative damage in rats. METHODS: The animals were randomly grouped as follows: 1) sham-operated control group and 2) study groups: i) 900-MHz EMR exposed (30 min/day for 10 days) group and ii) 900-MHz EMR exposed+melatonin (100 mug kg(-1) s.c. before the daily EMR exposure) treated group. Malondialdehyde (MDA), an index of lipid peroxidation), and urine N-acetyl-beta-d-glucosaminidase (NAG), a marker of renal tubular damage were used as markers of oxidative stress-induced renal impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status. RESULTS: In the EMR-exposed group, while tissue MDA and urine NAG levels increased, SOD, CAT, and GSH-Px activities were reduced. Melatonin treatment reversed these effects as well. In this study, the increase in MDA levels of renal tissue and in urine NAG and also the decrease in renal SOD, CAT, GSH-Px activities demonstrated the role of oxidative mechanism induced by 900-MHz mobile phone exposure, and melatonin, via its free radical scavenging and antioxidant properties, ameliorated oxidative tissue injury in rat kidney. CONCLUSIONS: These results show that melatonin may exhibit a protective effect on mobile phone-induced renal impairment in rats.

(E) Olgar Y, Hidisoglu E, Celen MC, Yamasan BE, Yargicoglu P, Ozdemir S. 2.1 GHz electromagnetic field does not change contractility and intracellular Ca2+ transients but decreases β-adrenergic responsiveness through nitric oxide signaling in rat ventricular myocytes. Int J Radiat Biol. 2015 Jul 1:1-23. [Epub ahead of print]

PURPOSE: Due to the increasing use of wireless technology in developing countries, particularly mobile phones, the influence of electromagnetic fields (EMF) on biologic systems has become the subject of an intense debate. Therefore, in this study we investigated the effect of 2.1 GHz EMF on contractility and beta-adrenergic (β-AR) responsiveness of ventricular myocytes. MATERIALS AND METHODS: Rats were randomized to the following groups: sham rats (SHAM) and rats exposed to 2.1 GHz EMF for 2 hours/day for 10 weeks (EM-10). Sarcomere shortening and Ca^{2+} transients were recorded in isolated myocytes loaded with Fura2-AM and electrically stimulated at 1 Hz, while L-type Ca^{2+} currents (I_{CaL}) were measured using whole-cell patch clamping at 36±1°C. Cardiac nitric oxide (NO) levels were

measured in tissue samples using a colorimetric assay kit. RESULTS: Fractional shortening and amplitude of the matched Ca^{2+} transients were not changed in EM-10 rats. Although the isoproterenol-induced (10^{-6} M) I_{CaL} response was reduced in rats exposed to EMF, basal I_{CaL} density in myocytes was similar between the two groups ($p<0.01$). Moreover, EMF exposure led to a significant increase in nitric oxide levels in rat heart ($p<0.02$). CONCLUSIONS: Long-term exposure to 2.1 GHz EMF decreases β-AR responsiveness of ventricular myocytes through NO signaling.

(E) Oral B, Guney M, Ozguner F, Karahan N, Mungan T, Comlekci S, Cesur G. Endometrial Apoptosis Induced by a 900-MHz Mobile Phone: Preventive Effects of Vitamins E and C. Adv Ther. 23(6):957-973, 2006.

Numerous reports have described the effects induced by an electromagnetic field (EMF) in various cellular systems. The purposes of this study were to examine oxidative stress that promotes production of reactive oxygen species induced by a 900-megahertz (MHz) mobile phone and the possible ameliorating effects of vitamins E and C on endometrial tissue against EMF-induced endometrial impairment and apoptosis in rats. Animals were randomly grouped as follows: (1) sham-operated control group (n=8), (2) 900 MHz EMF-exposed group (n=8; 30 min/d for 30 d), and (3) 900 MHz EMF-exposed group, treated with vitamins E and C (n=8; 50 mg/kg intramuscularly and 20 mg/kg body weight intraperitoneally before daily EMF exposure). Malondialdehyde (an index of lipid peroxidation) was used as a marker of oxidative stress-induced endometrial impairment; Bcl-2, Bax, caspase-3, and caspase-8 were assessed immunohistochemically. In this study, increased malondialdehyde levels in endometrial tissue and apoptosis illustrated the role of the oxidative mechanism induced by exposure to a 900-MHz mobile phone-like device and vitamins E and C; via free radical scavenging and antioxidant properties, oxidative tissue injury and apoptosis were ameliorated in rat endometrium. In conclusion, exposure to 900-MHz radiation emitted by mobile phones may cause endometrial apoptosis and oxidative stress, but treatment with vitamins E and C can diminish these changes and may have a beneficial effect in preventing endometrial changes in rats.

(cancer) (E) **Osera C, Amadio M, Falone S, Fassina L, Magenes G, Amicarelli F, Ricevuti G, Govoni S, Pascale A. Pre-exposure of neuroblastoma cell line to pulsed electromagnetic field prevents H2 O2 -induced ROS production by increasing mnSOD activity. Bioelectromagnetics. 2015 Feb 23. doi: 10.1002/bem.21900. [Epub ahead of print]**

Electromagnetic fields (EMFs) have been linked to increased risk of cancers and neurodegenerative diseases; however, EMFs can also elicit positive effects on biological systems, and redox status seems crucially involved in EMF biological effects. This study aimed to assess whether a short and repeated pulsed EMF (PEMF) could trigger adaptive responses against an oxidative insult in a neuronal cellular model. We found that a 40 min overall (four times a week, 10 min each) pre-exposure to PEMF did not affect major physiological parameters and led to a significant increase of Mn-dependent superoxide dismutase activity in the human neuroblastoma SH-SY5Y cell line. In addition, we found PEMF-pre-exposed cells exhibited decreased reactive oxygen species production following a

30 min H_2 O_2 challenge, with respect to non pre-exposed cells. Our findings might provide new insights on the role played by short and repeated PEMF stimulations in the enhancement of cellular defenses against oxidative insults. Although studies in normal neuronal cells would be useful to further confirm our hypothesis, we suggest that specific PEMF treatments may have potential biological repercussions in diseases where oxidative stress is implicated.

(E) Othman H, Ammari M, Sakly M, Abdelmelek H. Effects of prenatal exposure to WIFI signal (2.45GHz) on postnatal development and behavior in rat: Influence of maternal restraint. Behav Brain Res. 326:291-302, 2017.

The present study was carried out to investigate the potential combined influence of maternal restraint stress and 2.45GHz WiFi signal exposure on postnatal development and behavior in the offspring of exposed rats. 24 pregnant albino Wistar rats were randomly assigned to four groups: Control, WiFi-exposed, restrained and both WiFi-exposed and restrained groups. Each of WiFi exposure and restraint occurred 2h/day along gestation till parturition. The pups were evaluated for physical development and neuromotor maturation. Moreover, elevated plus maze test, open field activity and stationary beam test were also determined on postnatal days 28, 30 and 31, respectively. After behavioral tests, the rats were anesthetized and their brains were removed for biochemical analysis. Our main findings showed no detrimental effects on gestation progress and outcomes at delivery in all groups. Subsequently, WiFi and restraint, per se and mainly in concert altered physical development of pups with slight differences between genders. Behaviorally, the gestational WiFi irradiation, restraint and especially the associated treatment affected the neuromotor maturation mainly in male progeny. At adult age, we noticed anxiety, motor deficit and exploratory behavior impairment in male offspring co-exposed to WiFi radiation and restraint, and in female progeny subjected to three treatments. The biochemical investigation showed that, all three treatments produced global oxidative stress in brain of both sexes. As for serum biochemistry, phosphorus, magnesium, glucose, triglycerides and calcium levels were disrupted. Taken together, prenatal WiFi radiation and restraint, alone and combined, provoked several behavioral and biochemical impairments at both juvenile and adult age of the offspring.

(E) Othman H, Ammari M, Rtibi K, Bensaid N, Sakly M, Abdelmelek H. Postnatal development and behavior effects of in-utero exposure of rats to radiofrequency waves emitted from conventional WiFi devices. Environ Toxicol Pharmacol. 52:239-247, 2017.

The present work investigated the effects of prenatal exposure to radiofrequency waves of conventional WiFi devices on postnatal development and behavior of rat offspring. Ten Wistar albino pregnant rats were randomly assigned to two groups (n=5). The experimental group was exposed to a 2.45GHz WiFi signal for 2h a day throughout gestation period. Control females were subjected to the same conditions as treated group without applying WiFi radiations. After delivery, the offspring was tested for physical and neurodevelopment during its 17 postnatal days (PND), then for anxiety (PND 28) and motricity (PND 40-43), as well as for cerebral oxidative stress response and cholinesterase activity in brain and serum (PND 28 and 43). Our main results showed that the in-utero WiFi exposure impaired offspring

neurodevelopment during the first seventeen postnatal days without altering emotional and motor behavior at adult age. Besides, prenatal WiFi exposure induced cerebral oxidative stress imbalance (increase in malondialdehyde level (MDA) and hydrogen peroxide (H_2O_2) levels and decrease in catalase (CAT) and superoxide dismutase (SOD) activities) at 28 but not 43days old, also the exposure affected acethylcolinesterase activity at both cerebral and seric levels. Thus, the current study revealed that maternal exposure to WiFi radiofrequencies led to various adverse neurological effects in the offspring by affecting neurodevelopment, cerebral stress equilibrium and cholinesterase activity.

(E) Othman H, Ammari M , Sakly M, Abdelmelek H. Effects of repeated restraint stress and WiFi signal exposure on behavior and oxidative stress in rats. Metab Brain Dis. 32(5):1459-1469, 2017.

Today, due to technology development and aversive events of daily life, Human exposure to both radiofrequency and stress is unavoidable. This study investigated the co-exposure to repeated restraint stress and WiFi signal on cognitive function and oxidative stress in brain of male rats. Animals were divided into four groups: Control, WiFi-exposed, restrained and both WiFi-exposed and restrained groups. Each of WiFi exposure and restraint stress occurred 2 h (h)/day during 20 days. Subsequently, various tests were carried out for each group, such as anxiety in elevated plus maze, spatial learning abilities in the water maze, cerebral oxidative stress response and cholinesterase activity in brain and serum. Results showed that WiFi exposure and restraint stress, alone and especially if combined, induced an anxiety-like behavior without impairing spatial learning and memory abilities in rats. At cerebral level, we found an oxidative stress response triggered by WiFi and restraint, per se and especially when combined as well as WiFi-induced increase in acetylcholinesterase activity. Our results reveal that there is an impact of WiFi signal and restraint stress on the brain and cognitive processes especially in elevated plus maze task. In contrast, there are no synergistic effects between WiFi signal and restraint stress on the brain.

(E) Oyewopo AO, Olaniyi SK, Oyewopo CI, Jimoh AT.Radiofrequency electromagnetic radiation from cell phone causes defective testicular function in male Wistar rats. Andrologia. 2017 Dec;49(10). doi: 10.1111/and.12772. Epub 2017 Mar 6

Cell phones have become an integral part of everyday life. As cell phone usage has become more widespread, concerns have increased regarding the harmful effects of radiofrequency electromagnetic radiation from these devices. The current study was undertaken to investigate the effects of the emitted radiation by cell phones on testicular histomorphometry and biochemical analyses. Adult male Wistar rats weighing 180-200 g were randomly allotted to control, group A (switched off mode exposure), group B (1-hr exposure), group C (2-hr exposure) and group D (3-hr exposure). The animals were exposed to radiofrequency electromagnetic radiation of cell phone for a period of 28 days. Histomorphometry, biochemical and histological investigations were carried out. The histomorphometric parameters showed no significant change ($p < .05$) in the levels of germinal epithelial diameter in all the experimental groups compared with the control

group. There was no significant change ($p < .05$) in cross-sectional diameter of all the experimental groups compared with the control group. Group D rats showed a significant decrease ($p < .05$) in lumen diameter compared with group B rats. There was an uneven distribution of germinal epithelial cells in groups B, C and D. However, there was degeneration of the epithelia cells in group D when compared to the control and group B rats. Sera levels of malondialdehyde (MDA) and superoxide dismutase (SOD), which are markers of reactive oxygen species, significantly increased (MDA) and decreased (SOD), respectively, in all the experimental groups compared with the control group. Also sera levels of gonadotropic hormones (FSH, LH and testosterone) significantly decreased ($p < .05$) in groups C and D compared with the control group. The study demonstrates that chronic exposure to radiofrequency electromagnetic radiation of cell phone leads to defective testicular function that is associated with increased oxidative stress and decreased gonadotropic hormonal profile.

(E) Ozguner F, Aydin G, Mollaoglu H, Gokalp O, Koyu A, Cesur G. Prevention of mobile phone induced skin tissue changes by melatonin in rat: an experimental study. Toxicol Ind Health. 20(6-10):133-139, 2004.

Most of the mobile phones in Turkey emit 900 MHz radiation which is mainly absorbed by the skin and, to a lesser extent, muscle. The aim of this study was to investigate the effects the 900 MHz electromagnetic irradiation emitted by these devices on the induction of histopathologic changes in skin and the effect of melatonin (Mel) on any of these changes. Thirty male Wistar-Albino rats were used in the study. The experimental groups were composed of: a nontreated control group, an irradiated group (IR) without Mel and an irradiated with Mel treatment group (IR + Mel). 900 MHz radiation was applied to IR group for 10 days (30 min/day). The IR + Mel group received 10 mg/kg per day melatonin in tap water for 10 days before irradiation. At the end of the tenth day, the skin graft was excized from the thoraco-abdominal area. Histopathologic changes in skin were analyzed. In the IR group, increased thickness of stratum corneum, atrophy of epidermis, papillamatosis, basal cell proliferation, increased granular cell layer (hypergranulosis) in epidermis and capillary proliferation, impairment in collagen tissue distribution and separation of collagen bundles in dermis were all observed compared to the control group. Most of these changes, except hypergranulosis, were prevented with melatonin treatment. In conclusion, exposure to 900 MHz radiation emitted by mobile phones caused mild skin changes. Furthermore, melatonin treatment can reduce these changes and may have a beneficial effect to prevent 900 MHz mobile phone-induced rat skin changes.

(E) Ozguner F, Oktem F, Ayata A, Koyu A, Yilmaz HR. A novel antioxidant agent caffeic acid phenethyl ester prevents long-term mobile phone exposure-induced renal impairment in rat. Prognostic value of malondialdehyde, N-acetyl-beta-D-glucosaminidase and nitric oxide determination. Mol Cell Biochem. 277(1-2):73-80, 2005.

Caffeic acid phenethyl ester (CAPE), a flavonoid like compound, is one of the major components of honeybee propolis. It has been used in folk medicine for many years in Middle East countries. It was found to be a potent free radical scavenger and antioxidant recently. The

aim of this study was to examine long-term applied 900 MHz emitting mobile phone-induced oxidative stress that promotes production of reactive oxygen species (ROS) and, was to investigate the role of CAPE on kidney tissue against the possible electromagnetic radiation (EMR)-induced renal impairment in rats. In particular, the ROS such as superoxide and nitric oxide (NO) may contribute to the pathophysiology of EMR-induced renal impairment. Malondialdehyde (MDA, an index of lipid peroxidation) levels, urinary N-acetyl-beta-D: -glucosaminidase (NAG, a marker of renal tubular injury) and nitric oxide (NO, an oxidant product) levels were used as markers of oxidative stress-induced renal impairment and the success of CAPE treatment. The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in renal tissue were determined to evaluate the changes of antioxidant status. The rats used in the study were randomly grouped (10 each) as follows: i) Control group (without stress and EMR), ii) Sham-operated rats stayed without exposure to EMR (exposure device off), iii) Rats exposed to 900 MHz EMR (EMR group), and iv) A 900 MHz EMR exposed + CAPE treated group (EMR + CAPE group). In the EMR exposed group, while tissue MDA, NO levels and urinary NAG levels increased ($p < 0.0001$), the activities of SOD, CAT, and GSH-Px in renal tissue were reduced ($p < 0.001$). CAPE treatment reversed these effects as well ($p < 0.0001$, $p < 0.001$ respectively). In conclusion, the increase in NO and MDA levels of renal tissue, and in urinary NAG with the decrease in renal SOD, CAT, GSH-Px activities demonstrate the role of oxidative mechanisms in 900 MHz mobile phone-induced renal tissue damage, and CAPE, via its free radical scavenging and antioxidant properties, ameliorates oxidative renal damage. These results strongly suggest that CAPE exhibits a protective effect on mobile phone-induced and free radical mediated oxidative renal impairment in rats.

(E) Ozguner F, Oktem F, Armagan A, Yilmaz R, Koyu A, Demirel R, Vural H, Uz E. Comparative analysis of the protective effects of melatonin and caffeic acid phenethyl ester (CAPE) on mobile phone-induced renal impairment in rat. Mol Cell Biochem. 276(1-2):31-37, 2005.

Melatonin and caffeic acid phenethyl ester (CAPE), a component of honeybee propolis, were recently found to be potent free radical scavengers and antioxidants. There are a number of reports on the effects induced by electromagnetic radiation (EMR) in various cellular systems. Mechanisms of adverse effects of EMR indicate that reactive oxygen species may play a role in the biological effects of this radiation. The present study was carried out to compare the protective effects of melatonin and CAPE against 900 MHz EMR emitted mobile phone-induced renal tubular injury. Melatonin was administered whereas CAPE was given for 10 days before the exposure. Urinary N-acetyl-beta-D-glucosaminidase (NAG, a marker of renal tubular injury) and malondialdehyde (MDA, an index of lipid peroxidation), were used as markers of oxidative stress-induced renal impairment in rats exposed to EMR. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status in renal tissue. Urinary NAG and renal MDA were increased in EMR exposed rats while both melatonin and CAPE caused a significant reduction in the levels of these parameters. Likewise, renal SOD and GSH-Px activities were decreased in EMR exposed animals while melatonin caused a significant increase in the activities of these antioxidant enzymes but CAPE did not. Melatonin caused a significant decrease in urinary NAG activity and MDA levels which were increased because

of EMR exposure. CAPE also reduced elevated MDA levels in EMR exposed renal tissue, but the effect of melatonin was more potent than that of CAPE. Furthermore, treatment of EMR exposed rats with melatonin increased activities of SOD and GSH-Px to higher levels than those of control rats. In conclusion, melatonin and CAPE prevent renal tubular injury by reducing oxidative stress and protect the kidney from oxidative damage induced by 900 MHz mobile phone. Nevertheless, melatonin seems to be a more potent antioxidant compared with CAPE in kidney.

(E) Ozguner F, Altinbas A, Ozaydin M, Dogan A, Vural H, Kisioglu AN, Cesur G, Yildirim NG. Mobile phone-induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. Toxicol Ind Health. 21(9):223-230, 2005.

Electromagnetic radiation (EMR) or radiofrequency fields of cellular mobile phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation, and by changing the antioxidant defense systems of human tissues, thus leading to oxidative stress. Mobile phones are used in close proximity to the heart, therefore 900 MHz EMR emitting mobile phones may be absorbed by the heart. Caffeic acid phenethyl ester (CAPE), one of the major components of honeybee propolis, was recently found to be a potent free radical scavenger and antioxidant, and is used in folk medicine. The aim of this study was to examine 900 MHz mobile phone-induced oxidative stress that promotes production of reactive oxygen species (ROS) and the role of CAPE on myocardial tissue against possible oxidative damage in rats. Thirty rats were used in the study. Animals were randomly grouped as follows: sham-operated control group (N: 10) and experimental groups: (a) group II: 900 MHz EMR exposed group (N: 10); and (b) group III: 900 MHz EMR exposed+CAPE-treated group (N: 10). A 900 MHz EMR radiation was applied to groups II and III 30 min/day, for 10 days using an experimental exposure device. Malondialdehyde (MDA, an index of lipid peroxidation), and nitric oxide (NO, a marker of oxidative stress) were used as markers of oxidative stress-induced heart impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status. In the EMR exposed group, while tissue MDA and NO levels increased, SOD, CAT and GSH-Px activities were reduced. CAPE treatment in group III reversed these effects. In this study, the increased levels of MDA and NO and the decreased levels of myocardial SOD, CAT and GSH-Px activities demonstrate the role of oxidative mechanisms in 900 MHz mobile phone-induced heart tissue damage, and CAPE, via its free radical scavenging and antioxidant properties, ameliorates oxidative heart injury. These results show that CAPE exhibits a protective effect on mobile phone-induced and free radical mediated oxidative heart impairment in rats.

(E) Ozguner F, Bardak Y, Comlekci S. Protective effects of melatonin and caffeic acid phenethyl ester against retinal oxidative stress in long-term use of mobile phone: A comparative study. Mol Cell Biochem. 282(1-2):83-88, 2006.

There are numerous reports on the effects of electromagnetic radiation (EMR) in various cellular systems. Melatonin and caffeic acid phenethyl ester (CAPE), a component of honeybee propolis, were recently found to be potent free radical scavengers and antioxidants. Mechanisms of adverse effects of EMR indicate that reactive oxygen species may play a role

in the biological effects of this radiation. The present study was carried out to compare the efficacy of the protective effects of melatonin and CAPE against retinal oxidative stress due to long-term exposure to 900 MHz EMR emitting mobile phones. Melatonin and CAPE were administered daily for 60 days to the rats prior to their EMR exposure during our study. Nitric oxide (NO, an oxidant product) levels and malondialdehyde (MDA, an index of lipid peroxidation), were used as markers of retinal oxidative stress in rats following to use of EMR. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status in retinal tissue. Retinal levels of NO and MDA increased in EMR exposed rats while both melatonin and CAPE caused a significant reduction in the levels of NO and MDA. Likewise, retinal SOD, GSH-Px and CAT activities decreased in EMR exposed animals while melatonin and CAPE caused a significant increase in the activities of these antioxidant enzymes. Treatment of EMR exposed rats with melatonin or CAPE increased the activities of SOD, GSH-Px and CAT to higher levels than those of control rats. In conclusion, melatonin and CAPE reduce retinal oxidative stress after long-term exposure to 900 MHz emitting mobile phone. Nevertheless, there was no statistically significant difference between the efficacies of these two antioxidants against to EMR induced oxidative stress in rat retina. The difference was in only GSH-Px activity in rat retina. Melatonin stimulated the retinal GSH-Px activity more efficiently than CAPE did.

(E) Ozgur E, Güler G, Seyhan N. Mobile phone radiation-induced free radical damage in the liver is inhibited by the antioxidants n-acetyl cysteine and epigallocatechin-gallate. Int J Radiat Biol. 86(1):935-945, 2010.

Purpose: To investigate oxidative damage and antioxidant enzyme status in the liver of guinea pigs exposed to mobile phone-like radiofrequency radiation (RFR) and the potential protective effects of N-acetyl cysteine (NAC) and epigallocatechin-gallate (EGCG) on the oxidative damage. Materials and methods: Nine groups of guinea pigs were used to study the effects of exposure to an 1800-MHz Global System for Mobile Communications (GSM)-modulated signal (average whole body Specific Absorption Rate (SAR) of 0.38 W/kg, 10 or 20 min per day for seven days) and treatment with antioxidants. Results: Significant increases in malondialdehyde (MDA) and total nitric oxide (NO(x)) levels and decreases in activities of superoxide dismutase (SOD), myeloperoxidase (MPO) and glutathione peroxidase (GSH-Px) were observed in the liver of guinea pigs after RFR exposure. Only NAC treatment induces increase in hepatic GSH-Px activities, whereas EGCG treatment alone attenuated MDA level. Extent of oxidative damage was found to be proportional to the duration of exposure ($P < 0.05$). Conclusion: Mobile phone-like radiation induces oxidative damage and changes the activities of antioxidant enzymes in the liver. The adverse effect of RFR may be related to the duration of mobile phone use. NAC and EGCG protect the liver tissue against the RFR-induced oxidative damage and enhance antioxidant enzyme activities.

(E) *Ozgur E, Kismali G, Guler G, Akcay A, Ozkurt G, Sel T, Seyhan N. Effects of Prenatal and Postnatal Exposure to GSM-Like Radiofrequency on Blood Chemistry and Oxidative Stress in Infant Rabbits, an Experimental Study. Cell Biochem Biophys. 67(2):743-751, 2013.

We aimed to investigate the potential hazardous effects of prenatal and/or postnatal exposure to 1800 MHz GSM-like radiofrequency radiation (RFR) on the blood chemistry and lipid peroxidation levels of infant rabbits. A total of 72 New Zealand female and male white rabbits aged 1-month were used. Thirty-six female and 36 male were divided into four groups which were composed of nine infants: (i) Group 1 were the sham exposure (control), (ii) Group 2 were exposed to RFR, 15 min daily for 7 days in the prenatal period (between 15th and 22nd days of the gestational period) (prenatal exposure group). (iii) Group 3 were exposed to RFR 15 min/day (14 days for male, whereas 7 days for female) after they reached 1-month of age (postnatal exposure group). (iv) Group 4 were exposed to RFR for 15 min daily during 7 days in the prenatal period (between 15th and 22nd days of the gestational period) and 15 min/day (14 days for male, whereas 7 days for female) after they reached 1-month of age (prenatal and postnatal exposure group). Results showed that serum lipid peroxidation level in both female and male rabbits changed due to the RFR exposure. However, different parameters of the blood biochemistry were affected by exposure in male and female infants. Consequently, the whole-body 1800 MHz GSM-like RFR exposure may lead to oxidative stress and changes on some blood chemistry parameters. Studies on RFR exposure during prenatal and postnatal periods will help to establish international standards for the protection of pregnants and newborns from environmental RFR.

(E) Ozgur E, Sahin D, Tomruk A, Guler G, Sepici-Dinçel A, Altan N, Seyhan N. The Effects of N-acetyl-L-cysteine and Epigallocatechin-3-gallate on Liver Tissue Protein Oxidation and Antioxidant Enzyme Levels After the Exposure to Radio Frequency Radiation. Int J Radiat Biol. 2014 Sep 24:1-19. [Epub ahead of print]

PURPOSE: The widespread and sustained use of mobile and cordless phones causes unprecedented increase of radiofrequency radiation (RFR). The aim of this experimental study was to investigate the effect of 900 MHz Global System for Mobile Communications (GSM) modulated RFR (average whole body Specific Absorption Rate (SAR) of 0,4 W/kg, 10 or 20 min daily for consecutive 7 days) to the liver tissue of guinea pigs and the protective effects of antioxidant treatments. MATERIALS and METHODS: Adult male guinea pigs were randomly divided into nine groups as; Group I (Sham/saline), Group II (Sham/EGCG), Group III (Sham/NAC), Group IV (10-min RF-exposure/saline), Group V (20-min RF-exposure/saline), Group VI (10-min RF-exposure/EGCG), Group VII (20-min RF-exposure/EGCG), Group VIII (10-min RF-exposure/NAC), Group IX (20-min RF-exposure/NAC). Protein oxidation (PCO), advanced oxidation protein products (AOPP) and antioxidant enzyme activities of superoxide dismutase (SOD) were evaluated after the exposure and the treatments with N-acetylcysteine (NAC) and (-)-epigallocatechin-3-gallate (EGCG). RESULTS and CONCLUSIONS: Significant decreases in the activities of SOD were observed in the liver of guinea pigs after RFR exposure. Protein damage did not change due to RFR exposure. On the other hand, only NAC treatment induces increase PCO levels, whereas EGCG treatment alone elevated the level of AOPP. Due to antioxidants have pro-oxidant behavior, the well decided doses and treatment time tables of NAC and ECGC is needed.

(E) *Ozorak A, Nazıroğlu M, Celik O, Yüksel M, Ozçelik D, Ozkaya MO, Cetin H, Kahya MC, Kose SA. Wi-Fi (2.45 GHz)- and Mobile Phone (900 and 1800 MHz)-

Induced Risks on Oxidative Stress and Elements in Kidney and Testis of Rats During Pregnancy and the Development of Offspring. Biol Trace Elem Res. 156(1-3):221-229, 2013.

The present study was designed to determine the effects of both Wi-Fi (2.45 GHz)- and mobile phone (900 and 1800 MHz)-induced electromagnetic radiation (EMR) on oxidative stress and trace element levels in the kidney and testis of growing rats from pregnancy to 6 weeks of age. Thirty-two rats and their 96 newborn offspring were equally divided into four different groups, namely, control, 2.45 GHz, 900 MHz, and 1800 MHz groups. The 2.45 GHz, 900 MHz, and 1,800 MHz groups were exposed to EMR for 60 min/day during pregnancy and growth. During the fourth, fifth, and sixth weeks of the experiment, kidney and testis samples were taken from decapitated rats. Results from the fourth week showed that the level of lipid peroxidation in the kidney and testis and the copper, zinc, reduced glutathione (GSH), glutathione peroxidase (GSH-Px), and total antioxidant status (TAS) values in the kidney decreased in the EMR groups, while iron concentrations in the kidney as well as vitamin A and vitamin E concentrations in the testis increased in the EMR groups. Results for fifth-week samples showed that iron, vitamin A, and β-carotene concentrations in the kidney increased in the EMR groups, while the GSH and TAS levels decreased. The sixth week results showed that iron concentrations in the kidney and the extent of lipid peroxidation in the kidney and testis increased in the EMR groups, while copper, TAS, and GSH concentrations decreased. There were no statistically significant differences in kidney chromium, magnesium, and manganese concentrations among the four groups. In conclusion, Wi-Fi- and mobile phone-induced EMR caused oxidative damage by increasing the extent of lipid peroxidation and the iron level, while decreasing total antioxidant status, copper, and GSH values. Wi-Fi- and mobile phone-induced EMR may cause precocious puberty and oxidative kidney and testis injury in growing rats.

(E) **Pandey N, Giri S, Das S, Upadhaya P. Radiofrequency radiation (900 MHz)-induced DNA damage and cell cycle arrest in testicular germ cells in swiss albino mice. Toxicol Ind Health. 33(4):373-384, 2017.**

Even though there are contradictory reports regarding the cellular and molecular changes induced by mobile phone emitted radiofrequency radiation (RFR), the possibility of any biological effect cannot be ruled out. In view of a widespread and extensive use of mobile phones, this study evaluates alterations in male germ cell transformation kinetics following RFR exposure and after recovery. Swiss albino mice were exposed to RFR (900 MHz) for 4 h and 8 h duration per day for 35 days. One group of animals was terminated after the exposure period, while others were kept for an additional 35 days post-exposure. RFR exposure caused depolarization of mitochondrial membranes resulting in destabilized cellular redox homeostasis. Statistically significant increases in the damage index in germ cells and sperm head defects were noted in RFR-exposed animals. Flow cytometric estimation of germ cell subtypes in mice testis revealed 2.5-fold increases in spermatogonial populations with significant decreases in spermatids. Almost fourfold reduction in spermatogonia to spermatid turnover (1C:2C) and three times reduction in primary spermatocyte to spermatid turnover (1C:4C) was found indicating arrest in the premeiotic stage of spermatogenesis, which resulted in loss of post-meiotic germ cells apparent from testis histology and low sperm count

in RFR-exposed animals. Histological alterations such as sloughing of immature germ cells into the seminiferous tubule lumen, epithelium depletion and maturation arrest were also observed. However, all these changes showed recovery to varied degrees following the post-exposure period indicating that the adverse effects of RFR on mice germ cells are detrimental but reversible. To conclude, RFR exposure-induced oxidative stress causes DNA damage in germ cells, which alters cell cycle progression leading to low sperm count in mice.

(E) Pandey N, Giri S. Melatonin attenuates radiofrequency radiation (900 MHz)-induced oxidative stress, DNA damage and cell cycle arrest in germ cells of male Swiss albino mice. Toxicol Ind Health. 34(5):315-327, 2018.

Increasing male infertility of unknown aetiology can be associated with environmental factors. Extensive use of mobile phones has exposed the general population to unprecedented levels of radiofrequency radiations (RFRs) that may adversely affect male reproductive health. Therefore, the present study investigated the effect of RFR Global System for Mobile communication (GSM) type, 900 MHz and melatonin supplementation on germ cell development during spermatogenesis. Swiss albino mice were divided into four groups. One group received RFR exposure for 3 h twice/day for 35 days and the other group received the same exposure but with melatonin (N-acetyl-5-methoxytryptamine) (MEL; 5 mg/kg bw/day). Two other groups received only MEL or remain unexposed. Sperm head abnormality, total sperm count, biochemical assay for lipid peroxides, reduced glutathione, superoxide dismutase activity and testis histology were evaluated. Additionally, flow cytometric evaluation of germ cell subtypes and comet assay were performed in testis. Extensive DNA damage in germ cells of RFR-exposed animals along with arrest in pre-meiotic stages of spermatogenesis eventually leading to low sperm count and sperm head abnormalities were observed. Furthermore, biochemical assays revealed excess free radical generation resulting in histological and morphological changes in testis and germ cells morphology, respectively. However, these effects were either diminished or absent in RFR-exposed animals supplemented with melatonin. Hence, it can be concluded that melatonin inhibits pre-meiotic spermatogenesis arrest in male germ cells through its anti-oxidative potential and ability to improve DNA reparative pathways, leading to normal sperm count and sperm morphology in RFR-exposed animals.

(E) Paredi P, Kharitonov SA, Hanazawa T, Barnes PJ, Local vasodilator response to mobile phones. Laryngoscope 111(1):159-162, 2001.

OBJECTIVES: The use of mobile phones with the resulting generation of potentially harmful electromagnetic fields (EMF) is the focus of public interest. Heat generation and the activation of the inducible form of nitric oxide (NO) synthase may be possible causes of the biological effects of EMF exposure. We investigated if a mobile telephone conversation can modify skin temperature, NO, and nasal resistance. METHODS: We studied the effect of an EMF (900 MHz) generated by a commercially available cellular phone during a 30-minute telephone conversation on skin temperature, nasal NO measured by chemiluminescence, and nasal minimal cross-sectional area (MCA) measured by rhinometry. Eleven normal subjects (mean

age +/- standard error of mean [SEM], 32 +/- 5 y; 10 male) were studied. RESULTS: There was a similar and significant increase in skin temperature of the nostril and occipital area on the same side as the telephone (maximal increase 2.3 +/- 0.2 degrees C at 6 min) as well as a tendency for higher nasal NO levels (maximal increase 12.9 +/- 4.9% at 10 min), whereas the MCA was significantly reduced (maximal decrease -27 +/- 6% at 15 min). Such changes were not recorded when an earpiece was used to avoid the direct exposure to the electromagnetic field. There were no changes in the skin temperature and nasal NO measured on the opposite side to the mobile phone, whereas the MCA was significantly increased (38 +/- 10%). CONCLUSIONS: Exposure to EMF produced by a mobile phone produces biological effects that can be easily measured. Microwaves may increase skin temperature and therefore cause vasodilation and reduce MCA. Further studies are needed to study the long-term effects of mobile phone use and the relation among NO production, vasodilation, and temperature.

(E) Payez A, Ghanati F, Behmanesh M, Abdolmaleki P, Hajnorouzi A, Rajabbeigi E. Increase of seed germination, growth and membrane integrity of wheat seedlings by exposure to static and a 10-KHz electromagnetic field. Electromagn Biol Med. 32(4):417-429, 2013.

There is a large body of experimental data demonstrating various effects of magnetic field (MF) on plants growth and development. Although the mechanism(s) of perception of MF by plants is not yet elucidated, there is a possibility that like other stimuli, MF exerts its effects on plants by changing membrane integrity and conductance of its water channels, thereby influencing growth characteristics. In this study, the seeds of wheat (Triticum aestivum L. cv. Kavir) were imbibed in water overnight and then treated with or without a 30-mT static magnetic field (SMF) and a 10-kHz electromagnetic field (EMF) for 4 days, each 5 h. Water uptake of seeds reduced 5 h of the treatment with EMF but did not show changes in SMF treatment. Exposure to both magnetic fields did not affect germination percent of the seeds but increased the speed of germination, compared to the control group. Treatment with EMF significantly reduced seedling length and subsequently vigor index I, while SMF had no effects on these parameters. Both treatments significantly increased vigor index II, compared to the control group. These treatments also remarkably increased catalase activity and proline contents of seedlings but reduced the activity of peroxidase, the rate of lipid peroxidation and electrolyte leakages of membranes. The results suggest promotional effects of EMFs on membrane integrity and growth characteristics of wheat seedlings.

(E) Piccinetti CC, De Leo A, Cosoli G, Scalise L, Randazzo B, Cerri G, Olivotto I. Measurement of the 100 MHz EMF radiation in vivo effects on zebrafish D. rerio embryonic development: A multidisciplinary study. Ecotoxicol Environ Saf. 154:268-279, 2018.

The augmented exposure of both environment and human being to electromagnetic waves and the concomitant lack of an unequivocal knowledge about biological consequences of these radiations, raised public interest on electromagnetic pollution. In this context, the present study aims to evaluate the biological effects on zebrafish (ZF) embryos of 100 MHz radiofrequency electromagnetic field (RF-EMF) exposure

through a multidisciplinary protocol. Because of the shared synteny between human and ZF genomes that validated its use in biomedical research, toxicology and developmental biology studies, ZF was here selected as experimental model and a measurement protocol and biological analyses have been set up to clearly discriminate between RF-EMF biological and thermal effects. The results showed that a 100 MHz EMF was able to affect ZF embryonic development, from 24 to 72 h post fertilization (hpf) in all the analyzed pathways. Particularly, at the 48 hpf stage, a reduced growth, an increased transcription of oxidative stress genes, the onset of apoptotic/autophagic processes and a modification in cholesterol metabolism were detected. ZF embryos faced stress induced by EMF radiation by triggering detoxification mechanisms and at 72 hpf they partially recovered from stress reaching the hatching time in a comparable way respect to the control group. Data here obtained showed unequivocally the in vivo effects of RF-EMF on an animal model, excluding thermal outcomes and thus represents the starting point for more comprehensive studies on dose response effects of electromagnetic fields radiations consequences.

(E) Pilla AA. Electromagnetic fields instantaneously modulate nitric oxide signaling in challenged biological systems. Biochem Biophys Res Commun. 426(3):330-333, 2012.

This study shows that a non-thermal pulse-modulated RF signal (PRF), configured to modulate calmodulin (CaM) activation via acceleration of Ca(2+) binding kinetics, produced an immediate nearly 3-fold increase in nitric oxide (NO) from dopaminergic MN9D cultures ($P<0.001$). NO was measured electrochemically in real-time using a NO selective membrane electrode, which showed the PRF effect occurred within the first seconds after lipopolysaccharide (LPS) challenge. Further support that the site of action of PRF involves CaM is provided in human fibroblast cultures challenged with low serum and exposed for 15min to the identical PRF signal. In this case a CaM antagonist W-7 could be added to the culture 3h prior to PRF exposure. Those results showed the PRF signal produced nearly a two-fold increase in NO, which could be blocked by W-7 ($P<0.001$). To the authors' knowledge this is the first report of a real-time effect of non-thermal electromagnetic fields (EMF) on NO release from challenged cells. The results provide mechanistic support for the many reported bioeffects of EMF in which NO plays a role. Thus, in a typical clinical application for acute post operative pain, or chronic pain from, e.g., osteoarthritis, EMF therapy could be employed to modulate the dynamics of NO via Ca/CaM-dependent constitutive nitric oxide synthase (cNOS) in the target tissue. This, in turn, would modulate the dynamics of the signaling pathways the body uses in response to the various phases of healing after physical or chemical insult or injury.

(NE) Poulletier de Gannes F, Haro E, Hurtier A, Taxile M, Ruffié G, Billaudel B, Veyret B, Lagroye I. Effect of exposure to the edge signal on oxidative stress in brain cell models. Radiat Res. 175(2):225-230, 2011.

In this study we investigated the effect of the Enhanced Data rate for GSM Evolution (EDGE) signal on cells of three human brain cell lines, SH-SY5Y, U87 and CHME5, used as models of neurons, astrocytes and microglia, respectively, as well as on primary

cortical neuron cultures. SXC-1800 waveguides (IT'IS-Foundation, Zürich, Switzerland) were modified for in vitro exposure to the EDGE signal radiofrequency (RF) radiation at 1800 MHz. Four exposure conditions were tested: 2 and 10 W/kg for 1 and 24 h. The production of reactive oxygen species (ROS) was measured by flow cytometry using the dichlorofluorescein diacetate (DCFH-DA) probe at the end of the 24-h exposure or 24 h after the 1-h exposure. Rotenone treatment was used as a positive control. All cells tested responded to rotenone treatment by increasing ROS production. These findings indicate that exposure to the EDGE signal does not induce oxidative stress under these test conditions, including 10 W/kg. Our results are in agreement with earlier findings that RF radiation alone does not increase ROS production.

(E) Qin F, Yuan H, Nie J, Cao Y, Tong J. [Effects of nano-selenium on cognition performance of mice exposed in 1800 MHz radiofrequency fields]. Wei Sheng Yan Jiu. 43(1):16-21, 2014. [Article in Chinese]

OBJECTIVE: To study the effects of nano-selenium (NSe) on cognition performance of mice exposed to 1800 MHz radiofrequency fields (RF).METHODS: Male mice were randomly divided into four groups, control and nano-Se low, middle and high dose groups (L, M, H). Each group was sub-divided into three groups, RF 0 min, RF 30 min and RF 120 min. Nano-se solution (2, 4 and 8 microg/ml) were administered to mice of L, M, H groups by intra-gastric injection respectively, 0.5 ml/d for 50 days, the conctral group were administered with distilled water. At the 21st day, the mice in RF subgroup were exposed to 208 microW/cm2 1800 MHz radiofrequency fields (0, 30 and 120 min/d respectively) for 30 days. The cognitive ability of the mice were tested with Y-maze. Further, the levels of MDA, GABA, Glu, Ach and the activities of CAT and GSH-Px in cerebra were measured. RESULTS: Significant impairments in learning and memory ($P < 0.05$) were observed in the RF 120 min group, and with reduction of the Ach level and the activities of CAT and GSH-Px and increase of the content of GABA, Glu and MDA in cerebrum. NSe enhanced cognitive performance of RF mice, decreased GABA, Glu and MDA levels, increased Ach levels, GSH-Px and CAT activities. CONCLUSION: NSe could improve cognitive impairments of mice exposed to RF, the mechanism of which might involve the increasing antioxidation, decreasing free radical content and the changes of cerebra neurotransmitters.

(E) Ragy MM. Effect of exposure and withdrawal of 900-MHz-electromagnetic waves on brain, kidney and liver oxidative stress and some biochemical parameters in male rats. Electromagn Biol Med. 2014 Apr 8. [Epub ahead of print]

Increasing use of mobile phones in daily life with increasing adverse effects of electromagnetic radiation (EMR), emitted from mobile on some physiological processes, cause many concerns about their effects on human health. Therefore, this work was designed to study the effects of exposure to mobile phone emits 900-MHz EMR on the brain, liver and kidney of male albino rats. Thirty male adult rats were randomly divided into four groups (10 each) as follows: control group (rats without exposure to EMR), exposure group (exposed to 900-MHz EMR for 1 h/d for 60 d) and withdrawal group (exposed to 900-MHz electromagnetic wave for 1 h/d for 60 d then left for 30 d without exposure). EMR emitted from mobile phone led to a significant increase in malondialdehyde (MDA) levels and significant decrease total antioxidant capacity (TAC) levels in brain, liver and kidneys tissues.

The sera activity of alanine transaminase (ALT), aspartate aminotransferase (AST), urea, creatinine and corticosterone were significantly increased ($p < 0.05$), while serum catecholamines were insignificantly higher in the exposed rats. These alterations were corrected by withdrawal. In conclusion, electromagnetic field emitting from mobile phone might produce impairments in some biochemicals changes and oxidative stress in brain, liver and renal tissue of albino rats.

(E) Şahin D, Özgür E, Güler G, Tomruk A, Ünlü İ, Sepici-Dinçel A, Seyhan N. The 2100MHz radiofrequency radiation of a 3G-mobile phone and the DNA oxidative damage in brain. J Chem Neuroanat. 2016 Jan 8. pii: S0891-0618(16)00004-1. doi: 10.1016/j.jchemneu.2016.01.002. [Epub ahead of print]

We aimed to evaluate the effect of 2100MHz radiofrequency radiation emitted by a generator, simulating a 3G-mobile phone on the brain of rats during 10 and 40 days of exposure. The female rats were randomly divided into four groups. Group I; exposed to 3G modulated 2100MHz RFR signal for 6h/day, 5 consecutive days/wk for 2 weeks, Group II; control 10 days, were kept in an inactive exposure set-up for 6h/day, 5 consecutive days/wk for 2 weeks, Group III; exposed to 3G modulated 2100MHz RFR signal for 6h/day, 5 consecutive days/wk for 8 weeks and Group IV; control 40 days, were kept in an inactive exposure set-up for 6h/day, 5 consecutive days/wk for 8 weeks. After the genomic DNA content of brain was extracted, oxidative DNA damage (8-hydroxy-2'deoxyguanosine, pg/mL) and malondialdehyde (MDA, nmoL/g tissue) levels were determined. Our main finding was the increased oxidative DNA damage to brain after 10 days of exposure with the decreased oxidative DNA damage following 40 days of exposure compared to their control groups. Besides decreased lipid peroxidation end product, MDA, was observed after 40 days of exposure. The measured decreased quantities of damage during the 40 days of exposure could be the means of adapted and increased DNA repair mechanisms.

(E) Saikhedkar N, Bhatnagar M, Jain A, Sukhwal P, Sharma C, Jaiswal N. Effects of mobile phone radiation (900 MHz radiofrequency) on structure and functions of rat brain. Neurol Res. 2014 May 26:1743132814Y0000000392. [Epub ahead of print]

Objectives: The goals of this study were: (1) to obtain basic information about the effects of long-term use of mobile phone on cytological makeup of the hippocampus in rat brain (2) to evaluate the effects on antioxidant status, and (3) to evaluate the effects on cognitive behavior particularly on learning and memory. Methods: Rats (age 30 days, 120 ± 5 g) were exposed to 900 MHz radio waves by means of a mobile hand set for 4 hours per day for 15 days. Effects on anxiety, spatial learning, and memory were studied using open field test, elevated plus maze, Morris water maze (MWM), and classic maze test. Effects on brain antioxidant status were also studied. Cresyl violet staining was done to access the neuronal damage. Result: A significant change in behavior, i.e., more anxiety and poor learning was shown by test animals as compared to controls and sham group. A significant change in level of antioxidant enzymes and non-enzymatic antioxidants, and increase in lipid peroxidation were observed in test rats. Histological examination showed neurodegenerative cells in hippocampal sub regions and cerebral cortex. Discussion: Thus our findings indicate extensive neurodegeneration on exposure to radio waves. Increased production of reactive oxygen species due to exhaustion of

enzymatic and non-enzymatic antioxidants and increased lipid peroxidation are indicating extensive neurodegeneration in selective areas of CA1, CA3, DG, and cerebral cortex. This extensive neuronal damage results in alterations in behavior related to memory and learning.

(E) Salah MB, Abdelmelek H, Abderraba M. Effects of olive leave 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 leave 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.

(E) Saygin M, Asci H, Ozmen O, Cankara FN, Dincoglu D, Ilhan I. Impact of 2.45 GHz microwave radiation on the testicular inflammatory pathway biomarkers in young rats: The role of gallic acid. Environ Toxicol. 2015 Aug 13. doi: 10.1002/tox.22179. [Epub ahead of print]

The aim of this study was to investigate electromagnetic radiation (EMR) transmitted by wireless devices (2.45 GHz), which may cause physiopathological or ultrastructural changes, in the testes of rats. We addressed if the supplemental gallic acid (GA) may reduce these adverse effects. Six-week-old male Sprague Dawley rats were used in this study. Forty eight rats were equally divided into four groups, which were named: Sham, EMR only (EMR, 3 h day^{-1} for 30 days), EMR + GA (30 mg/kg/daily), and GA (30 mg/kg/daily) groups. Malondialdehyde (MDA) and total oxidant status (TOS) levels increased ($p = 0.001$ for both) in EMR only group. TOS and oxidative stress index (OSI) levels decreased in GA treated group significantly ($p = 0.001$ and $p = 0.045$, respectively). Total antioxidant status (TAS) activities decreased in EMR only group and increased in GA treatment group ($p = 0.001$ and $p = 0.029$, respectively). Testosterone and vascular endothelial growth factor (VEGF) levels decreased in EMR only group, but this was not statistically significant. Testosterone and VEGF levels increased in EMR+GA group, compared with EMR only group ($p = 0.002$), and also increased in GA group compared with the control and EMR only group ($p = 0.044$ and $p = 0.032$, respectively). Prostaglandin E_2 (PGE_2) and calcitonin gene releated peptide (CGRP) staining increased in tubules of the testes in EMR only group ($p < 0.001$ for both) and decreased in tubules of the testes in EMR+GA group ($p < 0.001$ for all parameters). In EMR only group, most

of the tubules contained less spermatozoa, and the spermatozoon counts decreased in tubules of the testes. All these findings and the regenerative reaction, characterized by mitotic activity, increased in seminiferous tubules cells of the testes in EMR+GA group ($p < 0.001$). Long term EMR exposure resulted in testicular physiopathology via oxidative damage and inflammation. GA may have ameliorative effects on the prepubertal rat testes physiopathology.

(E) Sefidbakht Y, Moosavi-Movahedi AA, Hosseinkhani S, Khodagholi F, Torkzadeh-Mahani M, Foolad F, Faraji-Dana R. Effects of 940 MHz EMF on bioluminescence and oxidative response of stable luciferase producing HEK cells. Photochem Photobiol Sci. 2014 Jun 2. [Epub ahead of print]

The effects of mobile phone frequency electromagnetic field (RF-EMF, 940 MHz) on a stable cell line (HEK293T) harbouring the firefly luciferase gene were evaluated. A waveguide exposure system with 1 W input power provided the mean specific absorption rate of ≈0.09 W kg^{-1} in 35 mm Petri dishes. The effects of exposure duration (15, 30, 45, 60 and 90 min) on luciferase activity and oxidative response elements were investigated. Endogenous luciferase activity was reduced after 30 and 45 min of continuous exposure, while after 60 min, the exposed cell lysate showed higher luciferase activity compared with the non-exposed control. Reactive oxygen species (ROS) generation was highest in the 30 min exposed cells as studied by 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescence. The observed boost in ROS was then followed by a sharp rise in catalase (CAT) and superoxide dismutase (SOD) activity and elevation of glutathione (GSH) during the 45 min exposure. Decrease in lipid peroxidation (malondialdehyde, MDA) was meaningful for the 45 and 60 min exposed cells. Therefore, it appears that an increase in the activity of luciferase after 60 min of continuous exposure could be associated with a decrease in ROS level caused by activation of the oxidative response. This ability in cells to overcome oxidative stress and compensate the luciferase activity could also be responsible for the adaptive response mechanism detected in ionizing radiation studies with RF-EMF pre-treatments.

(E) Sepehrimanesh M, Kazemipour N, Saeb M, Nazifi S. Analysis of rat testicular proteome following 30-days exposure to 900 MHz electromagnetic field radiation. Electrophoresis. 2014 Aug 21. doi: 10.1002/elps.201400273. [Epub ahead of print]

The use of electromagnetic field (EMF) generating apparatuses such as cell phones is increasing, and has caused an interest in the investigations of its effects on human health. We analyzed proteome in preparations from the whole testis in adult male Sprague-Dawley rats exposed for 1, 2 or 4 h/d for 30 consecutive days to 900 MHz EMF radiation, simulating a range of possible human cell phone use. Subjects were sacrificed immediately after the end of the experiment and testes fractions were solubilized and separated via high resolution 2-dimensional electrophoresis, and gel patterns were scanned, digitized and processed. Thirteen of the proteins which found only in sham or in exposure groups were identified by MALDI-TOF/TOF-MS. Among them, heat shock proteins, superoxide dismutase, peroxiredoxin-1 and other proteins related to misfolding of proteins and/or stress were identified. These results demonstrate significant effects of radio-frequency modulated electromagnetic fields (RF-EMF) exposure on proteome, particularly in protein species in the rodent testis, and suggest

that a 30 d exposure to EMF radiation induces non-thermal stress in testicular tissue. The functional implication of the identified proteins was discussed.

(E) Shahin S, Mishra V, Singh SP, Chaturvedi CM. 2.45-GHz microwave irradiation adversely affects reproductive function in male mouse, Mus musculus by inducing oxidative and nitrosative stress. Free Radic Res. 48(5):511-525, 2014.

Electromagnetic radiations are reported to produce long-term and short-term biological effects, which are of great concern to human health due to increasing use of devices emitting EMR especially microwave (MW) radiation in our daily life. In view of the unavoidable use of MW emitting devices (microwaves oven, mobile phones, Wi-Fi, etc.) and their harmful effects on biological system, it was thought worthwhile to investigate the long-term effects of low-level MW irradiation on the reproductive function of male Swiss strain mice and its mechanism of action. Twelve-week-old mice were exposed to non-thermal low-level 2.45-GHz MW radiation (CW for 2 h/day for 30 days, power density = 0.029812 mW/cm(2) and SAR = 0.018 W/Kg). Sperm count and sperm viability test were done as well as vital organs were processed to study different stress parameters. Plasma was used for testosterone and testis for 3β HSD assay. Immunohistochemistry of 3β HSD and nitric oxide synthase (i-NOS) was also performed in testis. We observed that MW irradiation induced a significant decrease in sperm count and sperm viability along with the decrease in seminiferous tubule diameter and degeneration of seminiferous tubules. Reduction in testicular 3β HSD activity and plasma testosterone levels was also noted in the exposed group of mice. Increased expression of testicular i-NOS was observed in the MW-irradiated group of mice. Further, these adverse reproductive effects suggest that chronic exposure to nonionizing MW radiation may lead to infertility via free radical species-mediated pathway.

(E) Shahin S, Singh VP, Shukla RK, Dhawan A, Gangwar RK, Singh SP, Chaturvedi CM. 2.45 GHz microwave irradiation-induced oxidative stress affects implantation or pregnancy in mice, Mus musculus. Appl Biochem Biotechnol. 169(5):1727-1751, 2013.

The present experiment was designed to study the 2.45 GHz low-level microwave (MW) irradiation-induced stress response and its effect on implantation or pregnancy in female mice. Twelve-week-old mice were exposed to MW radiation (continuous wave for 2 h/day for 45 days, frequency 2.45 GHz, power density=0.033549 mW/cm(2), and specific absorption rate=0.023023 W/kg). At the end of a total of 45 days of exposure, mice were sacrificed, implantation sites were monitored, blood was processed to study stress parameters (hemoglobin, RBC and WBC count, and neutrophil/lymphocyte (N/L) ratio), the brain was processed for comet assay, and plasma was used for nitric oxide (NO), progesterone and estradiol estimation. Reactive oxygen species (ROS) and the activities of ROS-scavenging enzymes- superoxide dismutase, catalase, and glutathione peroxidase-were determined in the liver, kidney and ovary. We observed that implantation sites were affected significantly in MW-irradiated mice as compared to control. Further, in addition to a significant increase in ROS, hemoglobin ($p<0.001$), RBC and WBC counts ($p<0.001$), N/L ratio ($p<0.01$), DNA damage ($p<0.001$) in brain cells, and plasma estradiol concentration ($p<0.05$), a significant decrease was observed in NO level ($p<0.05$) and antioxidant enzyme activities of MW-exposed mice. Our findings led us to conclude that a low level of MW irradiation-induced

oxidative stress not only suppresses implantation, but it may also lead to deformity of the embryo in case pregnancy continues. We also suggest that MW radiation-induced oxidative stress by increasing ROS production in the body may lead to DNA strand breakage in the brain cells and implantation failure/resorption or abnormal pregnancy in mice.

(E) Shahin S, Singh SP, Chaturvedi CM. Mobile phone (1800MHz) radiation impairs female reproduction in mice, Mus musculus, through stress induced inhibition of ovarian and uterine activity. Reprod Toxicol. 73:41-60, 2017.

Present study investigated the long-term effects of mobile phone (1800MHz) radiation in stand-by, dialing and receiving modes on the female reproductive function (ovarian and uterine histo-architecture, and steroidogenesis) and stress responses (oxidative and nitrosative stress). We observed that mobile phone radiation induces significant elevation in ROS, NO, lipid peroxidation, total carbonyl content and serum corticosterone coupled with significant decrease in antioxidant enzymes in hypothalamus, ovary and uterus of mice. Compared to control group, exposed mice exhibited reduced number of developing and mature follicles as well as corpus lutea. Significantly decreased serum levels of pituitary gonadotrophins (LH, FSH), sex steroids (E2 and P4) and expression of SF-1, StAR, P-450scc, 3β-HSD, 17β-HSD, cytochrome P-450 aromatase, ER-α and ER-β were observed in all the exposed groups of mice, compared to control. These findings suggest that mobile phone radiation induces oxidative and nitrosative stress, which affects the reproductive performance of female mice.

(E) Sharma VP, Singh HP, Kohli RK, Batish DR. Mobile phone radiation inhibits Vigna radiata (mung bean) root growth by inducing oxidative stress. Sci Total Environ. 407(21):5543-5547, 2009.

During the last couple of decades, there has been a tremendous increase in the use of cell phones. It has significantly added to the rapidly increasing EMF smog, an unprecedented type of pollution consisting of radiation in the environment, thereby prompting the scientists to study the effects on humans. However, not many studies have been conducted to explore the effects of cell **phone** EMFr on growth and biochemical changes in plants. We investigated whether EMFr from cell phones inhibit growth of Vigna radiata (mung bean) through induction of conventional stress responses. Effects of cell **phone** EMFr (power density: 8.55 microW cm(-2); 900 MHz band width; for 1/2, 1, 2, and 4 h) were determined by measuring the generation of reactive oxygen species (ROS) in terms of malondialdehyde and hydrogen peroxide (H(2)O(2)) content, root oxidizability and changes in levels of antioxidant enzymes. Our results showed that cell **phone** EMFr significantly inhibited the germination (at > or =2 h), and radicle and plumule growths (> or =1 h) in mung bean in a time-dependent manner. Further, cell **phone** EMFr enhanced MDA content (indicating lipid peroxidation), and increased H(2)O(2) accumulation and root oxidizability in mung bean roots, thereby inducing oxidative stress and **cellular** damage. In response to EMFr, there was a significant upregulation in the activities of scavenging enzymes, such as superoxide dismutases, ascorbate peroxidases, guaiacol peroxidases, catalases and glutathione reductases, in mung bean roots. The study concluded that cell **phone** EMFr inhibit root growth of mung

bean by inducing ROS-generated oxidative stress despite increased activities of antioxidant enzymes.

(E) Sharma VP, Singh HP, Batish DR, Kohli RK. Cell phone radiations affect early growth of Vigna radiata (mung bean) through biochemical alterations. Z Naturforsch C. 65(1-2):66-72, 2010.

The indiscriminate use of wireless technologies, particularly of cell phones, has increased the health risks among living organisms including plants. We investigated the impact of cell phone electromagentic field (EMF) radiations (power density, 8.55 microW cm(-2)) on germination, early growth, proteins and carbohydrate contents, and activities of some enzymes in Vigna radiata. Cell phone EMF radiations significantly reduced the seedling length and dry weight of V radiata after exposure for 0.5, 1, 2, and 4 h. Furthermore, the contents of proteins and carbohydrates were reduced in EMF-exposed plants. However, the activities of proteases, alpha-amylases, beta-amylases, polyphenol oxidases, and peroxidases were enhanced in EMF-exposed radicles indicating their role in providing protection against EMF-induced stress. The study concludes that cell phone EMFs impair early growth of V radiata seedlings by inducing biochemical changes.

(E) Sharma A, Kesari KK, Saxena VK, Sisodia R. Ten gigahertz microwave radiation impairs spatial memory, enzymes activity, and histopathology of developing mice brain. Mol Cell Biochem. 2017 May 3. doi: 10.1007/s11010-017-3051-8. [Epub ahead of print]

For decades, there has been an increasing concern about the potential hazards of non-ionizing electromagnetic fields that are present in the environment and alarming as a major pollutant or electro-pollutant for health risk and neuronal diseases. Therefore, the objective of the present study was to explore the effects of 10 GHz microwave radiation on developing mice brain. Two weeks old mice were selected and divided into two groups (i) sham-exposed and (ii) microwave-exposed groups. Animals were exposed for 2 h/day for 15 consecutive days. After the completion of exposure, within an hour, half of the animals were autopsied immediately and others were allowed to attain 6 weeks of age for the follow-up study. Thereafter results were recorded in terms of various biochemical, behavioral, and histopathological parameters. Body weight result showed significant changes immediately after treatment, whereas non-significant changes were observed in mice attaining 6 weeks of age. Several other endpoints like brain weight, lipid peroxidation, glutathione, protein, catalase, and superoxide dismutase were also found significantly ($p < 0.05$) altered in mice whole brain. These significant differences were found immediately after exposure and also in follow-up on attaining 6 weeks of age in microwave exposure group. Moreover, statistically significant ($p < 0.001$) effect was investigated in spatial memory of the animals, in learning to locate the position of platform in Morris water maze test. Although in probe trial test, sham-exposed animals spent more time in searching for platform into the target quadrant than in opposite or other quadrants. Significant alteration in histopathological parameters (qualitative and quantitative) was also observed in CA1 region of the hippocampus, cerebral cortex, and ansiform lobule of cerebellum. Results from the present study concludes that the brain of 2 weeks aged mice was very sensitive to microwave exposure as observed immediately after exposure and during follow-up study at 6 weeks of age.

(E) Shehu A, Mohammed A, Magaji RA, Muhammad MS. Exposure to mobile phone electromagnetic field radiation, ringtone and vibration affects anxiety-like behaviour and oxidative stress biomarkers in albino wistar rats. Metab Brain Dis. 2015 Nov 7. [Epub ahead of print]

Research on the effects of Mobile phone radio frequency emissions on biological systems has been focused on noise and vibrations as auditory stressors. This study investigated the potential effects of exposure to mobile phone electromagnetic field radiation, ringtone and vibration on anxiety-like behaviour and oxidative stress biomarkers in albino wistar rats. Twenty five male wistar rats were randomly divided into five groups of 5 animals each: group I: exposed to mobile phone in switched off mode (control), group II: exposed to mobile phone in silent mode, group III: exposed to mobile phone in vibration mode, group IV: exposed to mobile phone in ringtone mode, group V: exposed to mobile phone in vibration and ringtone mode. The animals in group II to V were exposed to 10 min call (30 missed calls for 20 s each) per day for 4 weeks. Neurobehavioural studies for assessing anxiety were carried out 24 h after the last exposure and the animals were sacrificed. Brain samples were collected for biochemical evaluation immediately. Results obtained showed a significant decrease ($P < 0.05$) in open arm duration in all the experimental groups when compared to the control. A significant decrease ($P < 0.05$) was also observed in catalase activity in group IV and V when compared to the control. In conclusion, the results of the present study indicates that 4 weeks exposure to electromagnetic radiation, vibration, ringtone or both produced a significant effect on anxiety-like behavior and oxidative stress in young wistar rats.

(E) Shivashankara AR, Joy J, Sunitha V, Rai MP, Rao S, Nambranathayil S, Baliga MS. Effect of cell phone use on salivary total protein, enzymes and oxidative stress markers in young adults: a pilot study. J Clin Diagn Res. 9(2):BC19-22, 2015.

INTRODUCTION: The present study aimed to assess the levels of salivary enzymes, protein and oxidant-antioxidant system in young college-going cell phone users. MATERIALS AND METHODS: The cell users (students) were categorized in to two groups - less mobile users and high mobile users, based on the duration and frequency of cell use. Unstimulated whole saliva samples of the volunteers were analysed for amylase, lactate dehydrogenase (LDH), malondialdehdye (MDA) and glutathione (GSH). RESULTS: High mobile users had significantly higher levels of amylase ($p = 0.001$), LDH ($p = 0.002$) and MDA ($p = 0.002$) in saliva, when compared to less mobile users. The marginal decrease in salivary total proteins, GSH and flow rate were statistically not significant ($p > 0.05$). CONCLUSION: Significant changes in salivary enzymes and MDA suggest adverse effect of high use of cell phones on cell health.

(NE) Silva V, Hilly O, Strenov Y, Tzabari C, Hauptman Y, Feinmesser R. Effect of cell phone-like electromagnetic radiation on primary human thyroid cells. Int J Radiat Biol. 2015 Dec 21:1-9. [Epub ahead of print]

Purpose To evaluate the potential carcinogenic effects of radiofrequency energy (RFE) emitted by cell phones on human thyroid primary cells. Materials and methods Primary thyroid cell culture was prepared from normal thyroid tissue obtained from patients who underwent surgery at our department. Subconfluent thyroid cells were irradiated under different conditions inside a cell incubator using a device that simulates cell phone-RFE. Proliferation of control and irradiated cells was assessed by the immunohistochemical staining of antigen Kiel clone-67 (Ki-67) and tumor suppressor p53 (p53) expression. DNA ploidy and the stress biomarkers heat shock protein 70 (HSP70) and reactive oxygen species (ROS) was evaluated by fluorescence-activated cell sorting (FACS). Results Our cells highly expressed thyroglobulin (Tg) and sodium-iodide symporter (NIS) confirming the origin of the tissue. None of the irradiation conditions evaluated here had an effect neither on the proliferation marker Ki-67 nor on p53 expression. DNA ploidy was also not affected by RFE, as well as the expression of the biomarkers HSP70 and ROS. Conclusion Our conditions of RFE exposure seem to have no potential carcinogenic effect on human thyroid cells. Moreover, common biomarkers usually associated to environmental stress also remained unchanged. We failed to find an association between cell phone-RFE and thyroid cancer. Additional studies are recommended.

(NE) **Simkó M, Hartwig C, Lantow M, Lupke M, Mattsson MO, Rahman Q, Rollwitz J. Hsp70 expression and free radical release after exposure to non-thermal radio-frequency electromagnetic fields and ultrafine particles in human Mono Mac 6 cells. Toxicol Lett. (1):73-82, 2006.**

The contemporary urban environment has become increasingly complex in its composition, leading to discussions regarding possible novel health effects. Two factors that recently have received considerable attention are ultrafine particles (UFP; <0.1 microm) produced by combustion processes and emissions from wireless communication devices like mobile phones that emit in the radio-frequency (RF) part of the spectrum. Several studies have shown biological effects of both these exposures in various cell systems. Here we investigate if exposure to UFP (12-14 nm, 100 microg/ml) and RF-electromagnetic fields (EMF; 2 W/kg specific absorption rate (SAR); continuous wave (CW) or modulated (217Hz or GSM-nonDTX)), alone or in combination influences levels of the superoxide radical anion or the stress protein heat-shock protein (Hsp70) in the human monocyte cell line Mono Mac 6. Heat treatment (42-43 degrees C, 1h) was used as positive control for both stress reaction and for heat development in the RF exposure setup. Our results clearly show that Mono Mac 6 cells are capable to internalise UFP, and that this phagocytic activity is connected to an increased release of free radicals. This increase (40-45% above negative control) is stronger than the effect of heat treatment. On the other hand, none of the employed RF exposures showed any effects on free radical levels. Co-exposure of RF and UFP did not potentiate the UFP effect either. Our investigations showed a significantly increased Hsp70 expression level by heat treatment in a time-dependent manner, whereas UFP, RF, or UFP+RF were without any effect. Therefore, we conclude that in the investigated Mono Mac 6 cells, RF exposure alone or in combination with UFP cannot influence stress-related responses.

(E) Singh HP, Sharma VP, Batish DR, Kohli RK. Cell phone electromagnetic field radiations affect rhizogenesis through impairment of biochemical processes. Environ Monit Assess. 184(4):1813-1821, 2012.

Indiscriminate adoption and use of cell phone technology has tremendously increased the levels of electromagnetic field radiations (EMFr) in the natural environment. It has raised the concerns among the scientists regarding the possible risks of EMFr to living organisms. However, not much has been done to assess the damage caused to plants that are continuously exposed to EMFr present in the environment. The present study investigated the biochemical mechanism of interference of 900 MHz cell phone EMFr with root formation in mung bean (Vigna radiata syn. Phaseolus aureus) hypocotyls, a model system to study rhizogenesis in plants. Cell phone EMFr enhanced the activities of proteases (by 1.52 to 2.33 times), polyphenol oxidases (by 1.5 to 4.3 times), and peroxidases (by 1.5 to 2.0 times) in mung bean hypocotyls over control. Further, EMFr enhanced malondialdehyde (an indicator of lipid peroxidation), hydrogen peroxide, and proline content, indicating a reactive oxygen species-mediated oxidative damage in hypocotyls. It was confirmed by the upregulation in the activities of antioxidant enzymes (superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, catalase, and glutathione reductase) suggesting their possible role in providing protection against EMFr-induced oxidative damage. The study concluded that cell phone radiations affect the process of rhizogenesis through biochemical alterations that manifest as oxidative damage resulting in root impairment.

(E) Sokolovic D, Djindjic B, Nikolic J, Bjelakovic G, Pavlovic D, Kocic G, Krstic D, Cvetkovic T, Pavlovic V. Melatonin reduces oxidative stress induced by chronic exposure of microwave radiation from mobile phones in rat brain. J Radiat Res (Tokyo). 49(6):579-586, 2008.

PURPOSE: The aim of the study was to evaluate the intensity of oxidative stress in the brain of animals chronically exposed to mobile phones and potential protective effects of melatonin in reducing oxidative stress and brain injury. MATERIALS AND METHODS: Experiments were performed on Wistar rats exposed to microwave radiation during 20, 40 and 60 days. Four groups were formed: I group (control)- animals treated by saline, intraperitoneally (i.p.) applied daily during follow up, II group (Mel)- rats treated daily with melatonin (2 mg kg(-1) body weight i.p.), III group (MWs)- microwave exposed rats, IV group (MWs + Mel)- MWs exposed rats treated with melatonin (2 mg kg(-1) body weight i.p.). The microwave radiation was produced by a mobile test phone (SAR = 0.043-0.135 W/kg). RESULTS: A significant increase in the brain tissue malondialdehyde (MDA) and carbonyl group concentration was registered during exposure. Decreased activity of catalase (CAT) and increased activity of xanthine oxidase (XO) remained after 40 and 60 days of exposure to mobile phones. Melatonin treatment significantly prevented the increase in the MDA content and XO activity in the brain tissue after 40 days of exposure while it was unable to prevent the decrease of CAT activity and increase of carbonyl group contents. CONCLUSION: We demonstrated two important findings; that mobile phones caused oxidative damage biochemically by increasing the levels of MDA, carbonyl groups, XO activity and decreasing CAT activity;

and that treatment with the melatonin significantly prevented oxidative damage in the brain.

(E) Sokolovic D, Djordjevic B, Kocic G, Stoimenov TJ, Stanojkovic Z, Sokolovic DM, et al. The Effects of Melatonin on Oxidative Stress Parameters and DNA Fragmentation in Testicular Tissue of Rats Exposed to Microwave Radiation. Adv Clin Exp Med. 24(3):429-436, 2015.

BACKGROUND: Microwaves from mobile phones are one of the environmental toxicants that are capable of compromising male fertility by inducing oxidative stress and apoptosis in the testes. Melatonin is a lipophilic tryptophan indole amine and a potent antioxidant. OBJECTIVES: The aim of the study was to evaluate the effect of melatonin treatment on oxidative stress parameters and DNA fragmentation in the testicular tissue of rats exposed to microwave radiation (4 h/day). MATERIAL AND METHODS: Adult Wistar rats were divided in 4 groups: I - treated with saline; II - treated with melatonin; III - exposed to microwaves; IV - exposed to microwaves and treated with melatonin. The melatonin (2 mg/kg ip) was administered daily. The animals were sacrificed after 20, 40 and 60 days. RESULTS: Melatonin treatment prevented previously registered increases in malondialdehyde after only 20 days. Furthermore, it reversed the effects of microwave exposure on xanthine oxidase (after 40 days) and acid-DNase activity (after 20 days). However, neither protein carbonyl content nor catalase and alkaline Dnase activity were changed due to melatonin treatment. CONCLUSIONS: Melatonin exerts potent antioxidant effects in the testes of rats exposed to microwaves by decreasing the intensity of oxidative stress; it also reduces DNA fragmentation.

(cancer) (E) Sun Y, Zong L, Gao Z, Zhu S, Tong J, Cao Y. Mitochondrial DNA damage and oxidative damage in HL-60 cells exposed to 900MHz radiofrequency fields. Mutat Res. 797-799:7-14, 2017.

HL-60 cells, derived from human promyelocytic leukemia, were exposed to continuous wave 900MHz radiofrequency fields (RF) at 120μW/cm2 power intensity for 4h/day for 5 consecutive days to examine whether such exposure is capable damaging the mitochondrial DNA (mtDNA) mediated through the production of reactive oxygen species (ROS). In addition, the effect of RF exposure was examined on 8-hydroxy-2'-dexoyguanosine (8-OHdG) which is a biomarker for oxidative damage and on the mitochondrial synthesis of adenosine triphosphate (ATP) which is the energy required for cellular functions. The results indicated a significant increase in ROS and significant decreases in mitochondrial transcription factor A, mtDNA polymerase gamma, mtDNA transcripts and mtDNA copy number in RF-exposed cells compared with those in sham-exposed control cells. In addition, there was a significant increase in 8-OHdG and a significant decrease in ATP in RF-exposed cells. The response in positive control cells exposed to gamma radiation (GR, which is also known to induce ROS) was similar to those in RF-exposed cells. Thus, the overall data indicated that RF exposure was capable of inducing mtDNA damage mediated through ROS pathway which also induced oxidative damage.

Prior-treatment of RF- and GR-exposed the cells with melatonin, a well-known free radical scavenger, reversed the effects observed in RF-exposed cells.

(E) Tkalec M, Malaric K, Pevalek-Kozlina B. Influence of 400, 900, and 1900 MHz electromagnetic fields on Lemna minor growth and peroxidase activity. Bioelectromagnetics. 26(3):185-193, 2005.

Increased use of radio and microwave frequencies requires investigations of their effects on living organisms. Duckweed (Lemna minor L.) has been commonly used as a model plant for environmental monitoring. In the present study, duckweed growth and peroxidase activity was evaluated after exposure in a Gigahertz Transversal Electromagnetic (GTEM) cell to electric fields of frequencies 400, 900, and 1900 MHz. The growth of plants exposed for 2 h to the 23 V/m electric field of 900 MHz significantly decreased in comparison with the control, while an electric field of the same strength but at 400 MHz did not have such effect. A modulated field at 900 MHz strongly inhibited the growth, while at 400 MHz modulation did not influence the growth significantly. At both frequencies a longer exposure mostly decreased the growth and the highest electric field (390 V/m) strongly inhibited the growth. Exposure of plants to lower field strength (10 V/m) for 14 h caused significant decrease at 400 and 1900 MHz while 900 MHz did not influence the growth. Peroxidase activity in exposed plants varied, depending on the exposure characteristics. Observed changes were mostly small, except in plants exposed for 2 h to 41 V/m at 900 MHz where a significant increase (41%) was found. Our results suggest that investigated electromagnetic fields (EMFs) might influence plant growth and, to some extent, peroxidase activity. However, the effects of EMFs strongly depended on the characteristics of the field exposure.

(E) Tkalec M, Malarić K, Pevalek-Kozlina B. Exposure to radiofrequency radiation induces oxidative stress in duckweed Lemna minor L. Sci Total Environ. 388(1-3):78-89, 2007.

Widespread use of radiofrequency radiation emitting devices increased the exposure to electromagnetic fields (EMFs) from 300 MHz to 300 GHz. Various biological effects of exposure to these fields have been documented so far, but very little work has been carried out on plants. The aim of the present work was to investigate the physiological responses of the plant Lemna minor after exposure to radiofrequency EMFs, and in particular, to clarify the possible role of oxidative stress in the observed effects. Duckweed was exposed for 2 h to EMFs of 400 and 900 MHz at field strengths of 10, 23, 41 and 120 V m(-1). The effect of a longer exposure time (4 h) and modulation was also investigated. After exposure, parameters of oxidative stress, such as lipid peroxidation, H(2)O(2) content, activities and isoenzyme pattern of antioxidative enzymes as well as HSP70 expression were evaluated. At 400 MHz, lipid peroxidation and H(2)O(2) content were significantly enhanced in duckweed exposed to EMFs of 23 and 120 V m(-1) while other exposure treatments did not have an effect. Compared to the controls, the activities of antioxidative enzymes showed different behaviour: catalase (CAT) activity increased after most exposure treatments while pyrogallol (PPX) and ascorbate peroxidase (APX) activities were not changed. Exceptions were reduced PPX and APX activity after longer exposure at 23 V m(-1) and increased PPX activity after exposures at 10 and 120 V m(-

1). By contrast, at 900 MHz almost all exposure treatments significantly increased level of lipid peroxidation and H(2)O(2) content but mostly decreased PPX activity and did not affect CAT activity. Exceptions were exposures to a modulated field and to the field of 120 V m(-1) which increased PPX and CAT activity. At this frequency APX activity was significantly decreased after exposure at 10 V m(-1) and longer exposure at 23 V m(-1) but it increased after a shorter exposure at 23 V m(-1). At both frequencies no differences in isoenzyme patterns of antioxidative enzymes or HSP70 level were found between control and exposed plants. Our results showed that non-thermal exposure to investigated radiofrequency fields induced oxidative stress in duckweed as well as unspecific stress responses, especially of antioxidative enzymes. However, the observed effects markedly depended on the field frequencies applied as well as on other exposure parameters (strength, modulation and exposure time). Enhanced lipid peroxidation and H(2)O(2) content accompanied by diminished antioxidative enzymes activity caused by exposure to investigated EMFs, especially at 900 MHz, indicate that oxidative stress could partly be due to changed activities of antioxidative enzymes.

(E) *Tkalec M, Stambuk A, Srut M, Malarić K, Klobučar GI. Oxidative and genotoxic effects of 900 MHz electromagnetic fields in the earthworm Eisenia fetida. Ecotoxicol Environ Saf. 90:7-12, 2013. (WS) (LI)

Accumulating evidence suggests that exposure to radiofrequency electromagnetic field (RF-EMF) can have various biological effects. In this study the oxidative and genotoxic effects were investigated in earthworms Eisenia fetida exposed in vivo to RF-EMF at the mobile phone frequency (900 MHz). Earthworms were exposed to the homogeneous RF-EMF at field levels of 10, 23, 41 and 120 V m(-1) for a period of 2h using a Gigahertz Transversal Electromagnetic (GTEM) cell. At the field level of 23 V m(-1) the effect of longer exposure (4h) and field modulation (80% AM 1 kHz sinusoidal) was investigated as well. All exposure treatments induced significant genotoxic effect in earthworms coelomocytes detected by the Comet assay, demonstrating DNA damaging capacity of 900 MHz electromagnetic radiation. Field modulation additionally increased the genotoxic effect. Moreover, our results indicated the induction of antioxidant stress response in terms of enhanced catalase and glutathione reductase activity as a result of the RF-EMF exposure, and demonstrated the generation of lipid and protein oxidative damage. Antioxidant responses and the potential of RF-EMF to induce damage to lipids, proteins and DNA differed depending on the field level applied, modulation of the field and duration of E. fetida exposure to 900 MHz electromagnetic radiation. Nature of detected DNA lesions and oxidative stress as the mechanism of action for the induction of DNA damage are discussed.

(E) *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.

(E) Tomruk A, Guler G, Dincel AS. The influence of 1800 MHz GSM-like signals on hepatic oxidative DNA and lipid damage in nonpregnant, pregnant, and newly born rabbits. Cell Biochem Biophys. 56(1):39-47, 2010.

The aim of our study is to evaluate the possible biological effects of whole-body 1800 MHz GSM-like radiofrequency (RF) radiation exposure on liver oxidative DNA damage and lipid peroxidation levels in nonpregnant, pregnant New Zealand White rabbits, and in their newly borns. Eighteen nonpregnant and pregnant rabbits were used and randomly divided into four groups which were composed of nine rabbits: (i) Group I (nonpregnant control), (ii) Group II (nonpregnant-RF exposed), (iii) Group III (pregnant control), (iv) Group IV (pregnant-RF exposed). Newborns of the pregnant rabbits were also divided into two groups: (v) Group V (newborns of Group III) and (vi) Group VI (newborns of Group III). 1800 MHz GSM-like RF radiation whole-body exposure (15 min/day for a week) was applied to Group II and Group IV. No significant differences were found in liver 8 OHdG/10(6) dG levels of exposure groups (Group II and Group IV) compared to controls (Group I and Group III). However, in Group II and Group IV malondialdehyde (MDA) and ferrous oxidation in xylenol orange (FOX) levels were increased compared to Group I ($P < 0.05$, Mann-Whitney). No significant differences were found in liver tissue of 8 OHdG/10(6) dG and MDA levels between Group VI and Group V ($P > 0.05$, Mann-Whitney) while liver FOX levels were found significantly increased in Group VI with respect to Group V ($P < 0.05$, Mann-Whitney). Consequently, the whole-body 1800 MHz GSM-like RF radiation exposure may lead to oxidative destruction as being indicators of subsequent reactions that occur to form oxygen toxicity in tissues.

(E) Topal Z, Hanci H, Mercantepe T, Erol HS, Keleş ON, Kaya H, Mungan S, Odaci E. The effects of prenatal long-duration exposure to 900-MHz electromagnetic field on the 21-day-old newborn male rat liver. Turk J Med Sci. 45(2):291-297, 2015.

BACKGROUND/AIM: To determine what effect a 900-MHz electromagnetic field (EMF) applied in the prenatal period would have on the liver in the postnatal period. MATERIALS AND METHODS: At the start of the study, adult pregnant rats were divided into two groups, control and experimental. The experimental group was exposed to a 900-MHz EMF for 1 h

daily during days 13-21 of pregnancy. After birth, no procedure was performed on either mothers or pups. Male rat pups (n = 6) from the control group mothers (CGMR) and male rat pups (n = 6) from the experimental group mothers (EGMR) were sacrificed on postnatal day 21. RESULTS: Biochemical analyses showed that malondialdehyde and superoxide dismutase values increased and glutathione levels decreased in the EGMR pups. Marked hydropic degeneration in the parenchyma, particularly in pericentral regions, was observed in light microscopic examination of EGMR sections stained with hematoxylin and eosin. Examinations under transmission electron microscope revealed vacuolization in the mitochondria, expansion in the endoplasmic reticulum, and necrotic hepatocytes. CONCLUSION: The study results show that a 900-MHz EMF applied in the prenatal period caused oxidative stress and pathological alterations in the liver in the postnatal period.

(E) Tsoy A, Saliev T, Abzhanova E, Turgambayeva A, Kaiyrlykyzy A, Akishev M, Saparbayev S, Umbayev B, Askarova S. The Effects of Mobile Phone Radiofrequency Electromagnetic Fields on β-Amyloid-Induced Oxidative Stress in Human and Rat Primary Astrocytes. Neuroscience. 408:46-57, 2019.

Amyloid beta peptide (Aβ) is implicated in the development of pathological reactions associated with Alzheimer's disease (AD), such as oxidative stress, neuro-inflammation and death of brain cells. Current pharmacological approaches to treat AD are not able to control the deposition of Aβ and suppression of Aβ-induced cellular response. There is a growing body of evidence that exposure to radiofrequency electromagnetic field (RF-EMF) causes a decrease of beta-amyloid deposition in the brains and provides cognitive benefits to Alzheimer's Tg mice. Herein, we investigated the effects of mobile phone radiofrequency EMF of 918 MHz on reactive oxygen species (ROS) formation, mitochondrial membrane potential (MMP), activity of NADPH-oxidase, and phosphorylation of p38MAPK and ERK1/2 kinases in human and rat primary astrocytes in the presence of $A\beta_{42}$ and H_2O_2. Our data demonstrate that EMF is able to reduce $A\beta_{42}$- and H_2O_2-induced cellular ROS, abrogate $A\beta_{42}$-induced production of mitochondrial ROS and the co-localization between the cytosolic (p47-phox) and membrane (gp91-phox) subunits of NADPH oxidase, while increasing MMP, and inhibiting H_2O_2-induced phosphorylation of p38MAPK and ERK1/2 in primary astrocytes. Yet, EMF was not able to modulate alterations in the phosphorylation state of the MAPKs triggered by $A\beta_{42}$. Our findings provide an insight into the mechanisms of cellular and molecular responses of astrocytes on RF-EMF exposure and indicate the therapeutic potential of RF-EMF for the treatment of Alzheimer's disease.

(E) Türedi S, Hancı H, Topal Z, Unal D, Mercantepe T, Bozkurt I, Kaya H, Odacı E. The effects of prenatal exposure to a 900-MHz electromagnetic field on the 21-day-old male rat heart. Electromagn Biol Med. 2014 Aug 28:1-8. [Epub ahead of print]

Abstract The growing spread of mobile phone use is raising concerns about the effect on human health of the electromagnetic field (EMF) these devices emit. The purpose of this study was to investigate the effects on rat pup heart tissue of prenatal exposure to a 900 megahertz (MHz) EMF. For this purpose, pregnant rats were divided into experimental and control

groups. Experimental group rats were exposed to a 900 MHz EMF (1 h/d) on days 13-21 of pregnancy. Measurements were performed with rats inside the exposure box in order to determine the distribution of EMF intensity. Our measurements showed that pregnant experimental group rats were exposed to a mean electrical field intensity of 13.77 V/m inside the box (0.50 W/m^2). This study continued with male rat pups obtained from both groups. Pups were sacrificed on postnatal day 21, and the heart tissues were extracted. Malondialdehyde, superoxide dismutase and catalase values were significantly higher in the experimental group rats, while glutathione values were lower. Light microscopy revealed irregularities in heart muscle fibers and apoptotic changes in the experimental group. Electron microscopy revealed crista loss and swelling in the mitochondria, degeneration in myofibrils and structural impairments in Z bands. Our study results suggest that exposure to EMF in the prenatal period causes oxidative stress and histopathological changes in male rat pup heart tissue.

(E)Türedi S, Kerimoğlu G, Mercantepe T, Odacı E. Biochemical and pathological changes in the male rat kidney and bladder following exposure to continuous 900-MHz electromagnetic field on postnatal days 22-59. Int J Radiat Biol. 93(9):990-999, 2017.

PURPOSE: To investigate the effect on male rat kidney and bladder tissues of exposure to 900-megahertz (MHz) electromagnetic field (EMF) applied on postnatal days 22-59, inclusive. **MATERIALS AND METHODS:** Twenty-four male Sprague Dawley rats, aged 21 days, were used. These were divided equally into one of three groups, control (CG), sham (SG) or EMF (EMFG). CG was not exposed to any procedure. SG rats were kept inside a cage, without being exposed to the effect of EMF, for 1 h a day on postnatal days 22-59, inclusive. EMFG rats were exposed to continuous 900-MHz EMF for 1 h a day under the same conditions as those for the SG rats. Rats were sacrificed on postnatal day 60, and the kidney and bladder tissues were removed. Tissues were stained with hematoxylin and eosin (H&E) and Masson trichrome for histomorphological evaluation. The TUNEL method was used to assess apoptosis. Transmission electron microscopy (TEM) was also used for the kidney tissue. Oxidant/antioxidant parameters were studied in terms of biochemical values. **RESULTS:** The findings showed that tissue malondialdehyde increased in EMFG compared to CG and SG in both kidney ($p = 0.004$ and $p = 0.004$, respectively) and bladder tissue ($p = 0.004$, $p = 0.006$, respectively), while catalase and glutathione levels decreased compared to CG ($p = 0.004$; $p = 0.004$, respectively) and SG ($p = 0.004$; $p = 0.004$, respectively). In the EMF group, pathologies such as dilatation and vacuolization in the distal and proximal tubules, degeneration in glomeruli and an increase in cells tending to apoptosis were observed in kidney tissue. In bladder tissue, degeneration in the transitional epithelium and stromal irregularity and an increase in cells tending to apoptosis were observed in EMFG. Additionally, EMFG samples exhibited glomerular capillary degeneration with capillary basement membranes under TEM. **CONCLUSIONS:** We conclude that continuous exposure to the effect of 900-MHz EMF for 1 h a day on postnatal days 22-59, inclusive, causes an increase in

oxidative stress and various pathological changes in male rat kidney and bladder tissues.

(E) 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 β-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.

(E) Ulubay M, Yahyazadeh A, Deniz OG, Kıvrak EG, Altunkaynak BZ, Erdem G, Kaplan S. Effects of prenatal 900 MHz electromagnetic field exposures on the histology of rat kidney. Int J Radiat Biol. 91(1):35-41, 2015.

Purpose: To research the harmful effects of prenatal exposure of 900 megahertz (MHz) electromagnetic field (EMF) on kidneys of four-week-old male rats and to determine protective effects of melatonin (MEL) and omega-3 (ω-3). Materials and methods: Twenty-one Wistar albino rats were randomly placed into seven groups as follows: control (Cont), Sham, MEL, ω-3, EMF, EMF+MEL and EMF+ω-3. After mating, three groups (EMF, EMF+MEL, EMF+ ω-3) were exposed to an EMF. In the fourth week subsequent to parturition, six rats were randomly chosen from each group. Mean volume of kidneys and renal cortices, the total number of glomeruli and basic histological structure of kidney were evaluated by stereological and light microscopical methods, respectively. Results: Stereological results determined the mean volume of the kidneys and cortices were significantly increased in EMF-exposed groups compared to the Cont group. However, EMF-unexposed groups were not significantly modified compared to the Cont group. Additionally, the total number of glomeruli was significantly higher in EMF-unexposed groups compared to the Cont group. Alternatively, the number of glomeruli in EMF-exposed groups was decreased compared to the Cont group. Conclusions: Prenatal exposure of rat kidneys to 900 MHz EMF resulted in increased total kidney volume and decreased the numbers of glomeruli. Moreover, MEL and ω-3 prevented adverse effects of EMF on the kidneys.

(E) Varghese R, Majumdar A, Kumar G, Shukla A. Rats exposed to 2.45GHz of non-ionizing radiation exhibit behavioral changes with increased brain expression of apoptotic caspase 3. Pathophysiology. 2017 Nov 14. pii: S0928-4680(17)30052-4. doi: 10.1016/j.pathophys.2017.11.001.

In recent years there has been a tremendous increase in use of Wi-Fi devices along with mobile phones, globally. Wi-Fi devices make use of 2.4 GHz frequency. The present study evaluated the impact of 2.45 GHz radiation exposure for 4h/day for 45 days on behavioral and oxidative stress parameters in female Sprague Dawley rats. Behavioral tests of anxiety, learning and memory were started from day 38. Oxidative stress parameters were estimated in brain homogenates after sacrificing the rats on day 45. In morris water maze, elevated plus maze and light dark box test, the 2.45 GHz radiation exposed rats elicited memory decline and anxiety behavior. Exposure decreased activities of super oxide dismutase, catalase and reduced glutathione levels whereas increased levels of brain lipid peroxidation was encountered in the radiation exposed rats, showing compromised anti-oxidant defense. Expression of caspase 3 gene in brain samples were quantified which unraveled notable increase in the apoptotic marker caspase 3 in 2.45 GHz radiation exposed group as compared to sham exposed group. No significant changes were observed in histopathological examinations and brain levels of TNF-α. Analysis of dendritic arborization of neurons showcased reduction in number of dendritic branching and intersections which corresponds to alteration in dendritic structure of neurons, affecting neuronal signaling. The study clearly indicates that exposure of rats to microwave radiation of 2.45GHz leads to detrimental changes in brain leading to lowering of learning and memory and expression of anxiety behavior in rats along with fall in brain antioxidant enzyme systems.

(E) Wang X, Liu C, Ma Q, Feng W, Yang L, Lu Y, Zhou Z, Yu Z, Li W, Zhang L. 8-oxoG DNA Glycosylase-1 Inhibition Sensitizes Neuro-2a Cells to Oxidative DNA Base Damage Induced by 900 MHz Radiofrequency Electromagnetic Radiation. Cell Physiol Biochem. 37(3):1075-1088, 2015.

BACKGROUND/AIMS: The purpose of this study was to explore the in vitro putative genotoxicity during exposure of Neuro-2a cells to radiofrequency electromagnetic fields (RF-EMFs) with or without silencing of 8-oxoG DNA glycosylase-1 (OGG1). METHODS: Neuro-2a cells treated with or without OGG1 siRNA were exposed to 900 MHz Global System for Mobile Communication (GSM) Talk signals continuously at a specific absorption rate (SAR) of 0, 0.5, 1 or 2 W/kg for 24 h. DNA strand breakage and DNA base damage were measured by the alkaline comet assay and a modified comet assay using formamidopyrimidine DNA glycosylase (FPG), respectively. Reactive oxygen species (ROS) levels and cell viability were monitored using the non-fluorescent probe 2, 7-dichlorofluorescein diacetate (DCFH-DA) and CCK-8 assay. RESULTS: Exposure to 900 MHz RF-EMFs with insufficient energy could induce oxidative DNA base damage in Neuro-2a cells. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS). Without OGG1 siRNA, 2 W/kg RF-EMFs induced oxidative DNA base damage in Neuro-2a cells. Interestingly, with OGG1 siRNA, RF-EMFs could cause DNA base damage in Neuro-2a cells as low as 1 W/kg. However, neither DNA strand breakage nor altered cell viability was observed. CONCLUSION: Even if further studies

remain conducted we support the hypothesis that OGG1 is involved in the process of DNA base repair and may play a pivotal role in protecting DNA bases from RF-EMF induced oxidative damage.

(E) Wu W, Yao K, Wang KJ, Lu DQ, He JL, Xu LH, Sun WJ. [Blocking 1800 MHz mobile phone radiation-induced reactive oxygen species production and DNA damage in lens epithelial cells by noise magnetic fields]. [Article in Chinese] Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):34-38, 2008.

OBJECTIVE: To investigate whether the exposure to the electromagnetic noise can block reactive oxygen species (ROS) production and DNA damage of lens epithelial cells induced by 1800 MHz mobile phone radiation. METHODS: The DCFH-DA method and comet assay were used respectively to detect the intracellular ROS and DNA damage of cultured human lens epithelial cells induced by 4 W/kg 1800 MHz mobile phone radiation or/and 2 muT electromagnetic noise for 24 h intermittently. RESULT: 1800 MHz mobile phone radiation at 4 W/kg for 24 h increased intracellular ROS and DNA damage significantly ($P<0.05$). However, the ROS level and DNA damage of mobile phone radiation plus noise group were not significant enhanced ($P>0.05$) as compared to sham exposure group. CONCLUSION: Electromagnetic noise can block intracellular ROS production and DNA damage of human lens epithelial cells induced by 1800 MHz mobile phone radiation.

(E) Xu S, Zhou Z, Zhang L, Yu Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Zhong M. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res. 1311:189-196, 2010.

Increasing evidence indicates that oxidative stress may be involved in the adverse effects of radiofrequency (RF) radiation on the brain. Because mitochondrial DNA (mtDNA) defects are closely associated with various nervous system diseases and mtDNA is highly susceptible to oxidative stress, the purpose of this study was to determine whether radiofrequency radiation can cause oxidative damage to mtDNA. In this study, we exposed primary cultured cortical neurons to pulsed RF electromagnetic fields at a frequency of 1800 MHz modulated by 217 Hz at an average special absorption rate (SAR) of 2 W/kg. At 24h after exposure, we found that RF radiation induced a significant increase in the levels of 8-hydroxyguanine (8-OHdG), a common biomarker of DNA oxidative damage, in the mitochondria of neurons. Consistent with this finding, the copy number of mtDNA and the levels of mitochondrial RNA (mtRNA) transcripts showed an obvious reduction after RF exposure. Each of these mtDNA disturbances could be reversed by pretreatment with melatonin, which is known to be an efficient in the brain. Together, these results suggested that 1800 MHz RF radiation could cause oxidative damage to mtDNA in primary cultured neurons. Oxidative damage to mtDNA may account for the neurotoxicity of RF radiation in the brain.

(Review) Yakymenko I, Tsybulin O, Sidorik E, Henshel D, Kyrylenko O, Kyrylenko S. Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. Electromagn Biol Med. 35(2):186-202, 2016.

This review aims to cover experimental data on oxidative effects of low-intensity radiofrequency radiation (RFR) in living cells. Analysis of the currently available peer-reviewed scientific literature reveals molecular effects induced by low-intensity RFR in living cells; this includes significant activation of key pathways generating reactive oxygen species (ROS), activation of peroxidation, oxidative damage of DNA and changes in the activity of antioxidant enzymes. It indicates that among 100 currently available peer-reviewed studies dealing with oxidative effects of low-intensity RFR, in general, 93 confirmed that RFR induces oxidative effects in biological systems. A wide pathogenic potential of the induced ROS and their involvement in cell signaling pathways explains a range of biological/health effects of low-intensity RFR, which include both cancer and non-cancer pathologies. In conclusion, our analysis demonstrates that low-intensity RFR is an expressive oxidative agent for living cells with a high pathogenic potential and that the oxidative stress induced by RFR exposure should be recognized as one of the primary mechanisms of the biological activity of this kind of radiation.

(E) Yakymenko I, Burlaka A, Tsybulin I, Brieieva I, Buchynska L, Tsehmistrenko I, Chekhun F. Oxidative and mutagenic effects of low intensity GSM 1800 MHz microwave radiation. Exp Oncol. 40(4):282-287, 2018.

AIM: Despite a significant number of epidemiological studies on potential carcinogenicity of microwave radiation (MWR) from wireless devices and a bulk of experimental studies on oxidative and mutagenic effects of low intensity MWR, the discussion on potential carcinogenicity of low intensity MWR is going on. This study aims to assess oxidative and mutagenic effects of low intensity MWR from a typical commercial model of a modern smartphone. MATERIALS AND METHODS: The model of developing quail embryos has been used for the assessment of oxidative and mutagenic effects of Global System for Mobile communication (GSM) 1800 MHz MWR from a commercial model of smartphone. The embryos were exposed in ovo to 0.32 μW/cm2, discontinuously - 48 s - On, 12 s - Off, during 5 days before and 14 days through the incubation period. RESULTS: The exposure of quail embryos before and during the incubation period to low intensity GSM 1800 MHz has resulted in expressive statistically significant oxidative effects in embryonic cells, including a 2-fold increase in superoxide generation rate and 85% increase in nitrogen oxide generation rate, damages of DNA integrity and oxidative damages of DNA (up to twice increased levels of 8-oxo-dG in cells of 1-day old chicks from the exposed embryos). Finally, the exposure resulted in a significant, almost twice, increase of embryo mortality. CONCLUSION: The exposure of model biological system to low intensity GSM 1800 MHz MWR resulted in significant oxidative and mutagenic effects in exposed cells, and thus should be recognized as a significant risk factor for living cells.

(E) Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, Ye J, Sun L. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol Vis. 14:964-969, 2008.

PURPOSE: The goal of this study was to investigate whether superposing of electromagnetic noise could block or attenuate DNA damage and intracellular reactive oxygen species (ROS) increase of cultured human lens epithelial cells (HLECs) induced by acute exposure to 1.8

GHz radiofrequency field (RF) of the Global System for Mobile Communications (GSM). METHODS: An sXc-1800 RF exposure system was used to produce a GSM signal at 1.8 GHz (217 Hz amplitude-modulated) with the specific absorption rate (SAR) of 1, 2, 3, and 4 W/kg. After 2 h of intermittent exposure, the ROS level was assessed by the fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DNA damage to HLECs was examined by alkaline comet assay and the phosphorylated form of histone variant H2AX (gammaH2AX) foci formation assay. RESULTS: After exposure to 1.8 GHz RF for 2 h, HLECs exhibited significant intracellular ROS increase in the 2, 3, and 4 W/kg groups. RF radiation at the SAR of 3 W/kg and 4 W/kg could induce significant DNA damage, examined by alkaline comet assay, which was used to detect mainly single strand breaks (SSBs), while no statistical difference in double strand breaks (DSBs), evaluated by gammaH2AX foci, was found between RF exposure (SAR: 3 and 4 W/kg) and sham exposure groups. When RF was superposed with 2 muT electromagnetic noise could block RF-induced ROS increase and DNA damage. CONCLUSIONS: DNA damage induced by 1.8 GHz radiofrequency field for 2 h, which was mainly SSBs, may be associated with the increased ROS production. Electromagnetic noise could block RF-induced ROS formation and DNA damage.

(E) Yariktas M, Doner F, Ozguner F, Gokalp O, Dogru H, Delibas N. Nitric oxide level in the nasal and sinus mucosa after exposure to electromagnetic field. Otolaryngol Head Neck Surg. 132(5):713-716, 2005.

OBJECTIVE: The purpose of this study was to examine the changes in nitric oxide (NO) level in the nasal and paranasal sinus mucosa after exposure radiofrequency electromagnetic fields (EMF). STUDY DESIGN AND SETTING: Thirty male Sprague-Dawley rats were randomly grouped as follows: EMF group (group I; n, 10), EMF group in which melatonin received (group II; n, 10) and the control (sham operated) group (group III; n, 10). Groups I and II were exposed to a 900 MHz. Oral melatonin was given in group II. Control rats (group III) were also placed in the tube as the exposure groups, but without exposure to EMF. At the end of 2 weeks, the rats were sacrificed, and the nasal and paranasal sinus mucosa dissected. NO was measured in nasal and paranasal mucosa. RESULTS: The nasal and paranasal sinus mucosa NO levels of group I were significantly higher than those of the control group (group III) ($P < 0.05$). However, there was no statistically significant difference between group II and the control group (group III) regarding NO output ($P > 0.05$). CONCLUSION: Exposure to EMF released by mobile phones (900 MHz) increase NO levels in the sinus and nasal mucosa. SIGNIFICANCE: Increased NO levels may act as a defense mechanism and presumably related to tissue damage. In addition, melatonin may have beneficial effect to prevent these changes in the mucosa.

(E) Yüksel M, Nazıroğlu M, Özkaya MO. Long-term exposure to electromagnetic radiation from mobile phones and Wi-Fi devices decreases plasma prolactin, progesterone, and estrogen levels but increases uterine oxidative stress in pregnant rats and their offspring. Endocrine. 2015 Nov 14. [Epub ahead of print]

We investigated the effects of mobile phone (900 and 1800 MHz)- and Wi-Fi (2450 MHz)-induced electromagnetic radiation (EMR) exposure on uterine oxidative stress and plasma hormone levels in pregnant rats and their offspring. Thirty-two rats and their forty newborn

offspring were divided into the following four groups according to the type of EMR exposure they were subjected to: the control, 900, 1800, and 2450 MHz groups. Each experimental group was exposed to EMR for 60 min/day during the pregnancy and growth periods. The pregnant rats were allowed to stand for four generations (total 52 weeks) before, plasma and uterine samples were obtained. During the 4th, 5th, and 6th weeks of the experiment, plasma and uterine samples were also obtained from the developing rats. Although uterine lipid peroxidation increased in the EMR groups, uterine glutathione peroxidase activity (4th and 5th weeks) and plasma prolactin levels (6th week) in developing rats decreased in these groups. In the maternal rats, the plasma prolactin, estrogen, and progesterone levels decreased in the EMR groups, while the plasma total oxidant status, and body temperatures increased. There were no changes in the levels of reduced glutathione, total antioxidants, or vitamins A, C, and E in the uterine and plasma samples of maternal rats. In conclusion, although EMR exposure decreased the prolactin, estrogen, and progesterone levels in the plasma of maternal rats and their offspring, EMR-induced oxidative stress in the uteri of maternal rats increased during the development of offspring. Mobile phone- and Wi-Fi-induced EMR may be one cause of increased oxidative uterine injury in growing rats and decreased hormone levels in maternal rats. TRPV1 cation channels are the possible molecular pathways responsible for changes in the hormone, oxidative stress, and body temperature levels in the uterus of maternal rats following a year-long exposure to electromagnetic radiation exposure from mobile phones and Wi-Fi devices. It is likely that TRPV1-mediated Ca^{2+} entry in the uterus of pregnant rats involves accumulation of oxidative stress and opening of mitochondrial membrane pores that consequently leads to mitochondrial dysfunction, substantial swelling of the mitochondria with rupture of the outer membrane and release of oxidants such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). The superoxide radical is converted to H_2O_2 by superoxide dismutase (SOD) enzyme. Glutathione peroxidase (GSH-Px) is an important antioxidant enzyme for removing lipid hydroperoxides and hydrogen peroxide and it catalyzes the reduction of H_2O_2 to water.

(E) Yurekli AI, Ozkan M, Kalkan T, Saybasili H, Tuncel H, Atukeren P, Gumustas K, Seker S. GSM Base Station Electromagnetic Radiation and Oxidative Stress in Rats. Electromagn Biol Med. 2006;25(3):177-188, 2006. (LI)

The ever increasing use of cellular phones and the increasing number of associated base stations are becoming a widespread source of nonionizing electromagnetic radiation. Some biological effects are likely to occur even at low-level EM fields. In this study, a gigahertz transverse electromagnetic (GTEM) cell was used as an exposure environment for plane wave conditions of far-field free space EM field propagation at the GSM base transceiver station (BTS) frequency of 945 MHz, and effects on oxidative stress in rats were investigated. When EM fields at a power density of 3.67 W/m2 (specific absorption rate = 11.3 mW/kg), which is well below current exposure limits, were applied, MDA (malondialdehyde) level was found to increase and GSH (reduced glutathione) concentration was found to decrease significantly ($p < 0.0001$). Additionally, there was a less significant ($p = 0.0190$) increase in SOD (superoxide dismutase) activity under EM exposure.

(E) Zeni, O., Di Pietro, R., d'Ambrosio, G., Massa, R., Capri, M., Naarala, J., Juutilainen, J. and Scarfi, M. R. Formation of Reactive Oxygen Species in L929 Cells after Exposure to 900 MHz RF Radiation with and without Co-exposure to 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone. Radiat. Res. 167, 306-311, 2007.

The aim of this study was to investigate the induction of reactive oxygen species in murine L929 fibrosarcoma cells exposed to radiofrequency (RF) radiation at 900 MHz, with or without co-exposure to 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a potent environmental carcinogen produced during chlorination of drinking water. Both continuous-wave and GSM mobile phone signals were applied for 10 or 30 min at specific absorption rates of 0.3 and 1 W/kg. Simultaneous sham exposures were performed for each exposure condition. MX treatment was performed at a subtoxic level of 500 muM, and the RF-field exposure was carried out during the first 10 or 30 min of the chemical treatment. The formation of reactive oxygen species was followed soon after the exposure and at different harvesting times until 1 h after RF-field treatment. The studied provided no indication that 900 MHz RF-field exposure, either alone or in combination with MX, induced formation of reactive oxygen species under any of the experimental conditions investigated. In contrast, exposure to MX resulted in a statistically significant increase in the formation of reactive oxygen species for all the treatment durations investigated, confirming that MX is an inductor of oxidative stress in L929 cells.

(E) Zhang X, Gao Y, Dong J, Wang S, Yao B, et al. (2014) The Compound Chinese Medicine "Kang Fu Ling" Protects against High Power Microwave-Induced Myocardial Injury. PLoS ONE 9(7): e101532. doi:10.1371/journal.pone.0101532.

Background. The prevention and treatment of Microwave-caused cardiovascular injury remains elusive. This study investigated the cardiovascular protective effects of compound Chinese medicine "Kang Fu Ling" (KFL) against high power microwave (HPM)-induced myocardial injury and the role of the mitochondrial permeability transition pore (mPTP) opening in KFL protection. Methods. Male Wistar rats (100) were divided into 5 equal groups: no treatment, radiation only, or radiation followed by treatment with KFL at 0.75, 1.5, or 3 g/kg/day. Electrocardiography was used to Electrophysiological examination. Histological and ultrastructural changes in heart tissue and isolated mitochondria were observed by light microscope and electron microscopy. mPTP opening and mitochondrial membrane potential were detected by confocal laser scanning microscopy and fluorescence analysis. Connexin-43 (Cx-43) and endothelial nitric oxide synthase (eNOS) were detected by immunohistochemistry. The expression of voltage-dependent anion channel (VDAC) was detected by western blotting. Results. At 7 days after radiation, rats without KFL treatment showed a significantly lower heart rate ($P<0.01$) than untreated controls and a J point shift. Myocyte swelling and rearrangement were evident. Mitochondria exhibited rupture, and decreased fluorescence intensity, suggesting opening of mPTP and a consequent reduction in mitochondrial membrane potential. After treatment with 1.5 g/kg/day KFL for 7 d, the heart rate increased significantly ($P<0.01$), and the J point shift was reduced flavorfully ($P<0.05$) compared to untreated, irradiated rats; myocytes and mitochondria were of normal

morphology. The fluorescence intensities of dye-treated mitochondria were also increased, suggesting inhibition of mPTP opening and preservation of the mitochondrial membrane potential. The microwave-induced decrease of Cx-43 and VDAC protein expression was significantly reversed. Conclusion. Microwave radiation can cause electrophysiological, histological and ultrastructural changes in the heart. KFL at 1.5 g/kg/day had the greatest protective effect on these cardiovascular events. mPTP plays an important role in the protective effects of KFL against microwave-radiation-induced myocardial injury. (See Hu et al., 2014).

(E) Zhu W, Cui Y, Feng X, Li Y, Zhang W, Xu J, Wang H, Lv S. The apoptotic effect and the plausible mechanism of microwave radiation on rat myocardial cells. Can J Physiol Pharmacol. 94(8):849-857, 2016.

.Microwaves may exert adverse biological effects on the cardiovascular system at the integrated system and cellular levels. However, the mechanism underlying such effects remains poorly understood. Here, we report a previously uncharacterized mechanism through which microwaves damage myocardial cells. Rats were treated with 2450 MHz microwave radiation at 50, 100, 150, or 200 mW/cm(2) for 6 min. Microwave treatment significantly enhanced the levels of various enzymes in serum. In addition, it increased the malondialdehyde content while decreasing the levels of antioxidative stress enzymes, activities of enzyme complexes I-IV, and ATP in myocardial tissues. Notably, irradiated myocardial cells exhibited structural damage and underwent apoptosis. Furthermore, Western blot analysis revealed significant changes in expression levels of proteins involved in oxidative stress regulation and apoptotic signaling pathways, indicating that microwave irradiation could induce myocardial cell apoptosis by interfering with oxidative stress and cardiac energy metabolism. Our findings provide useful insights into the mechanism of microwave-induced damage to the cardiovascular system.

(E) Zmyslony M, Politanski P, Rajkowska E, Szymczak W, Jajte J. Acute exposure to 930 MHz CW electromagnetic radiation in vitro affects reactive oxygen species level in rat lymphocytes treated by iron ions. Bioelectromagnetics. 25(5):324-328, 2004.

The aim of this study was to test the hypothesis that the 930 MHz continuous wave (CW) electromagnetic field, which is the carrier of signals emitted by cellular phones, affects the reactive oxygen species (ROS) level in living cells. Rat lymphocytes were used in the experiments. A portion of the lymphocytes was treated with iron ions to induce oxidative processes. Exposures to electromagnetic radiation (power density 5 W/m2, theoretical calculated SAR = 1.5 W/kg) were performed within a GTEM cell. Intracellular ROS were measured by the fluorescent probe dichlorofluorescin diacetate (DCF-DA). The results show that acute (5 and 15 min) exposure does not affect the number of produced ROS. If, however, FeCl2 with final concentration 10 microg/ml was added to the lymphocyte suspensions to stimulate ROS production, after both durations of exposure, the magnitude of fluorescence (ROS level during the experiment) was significantly greater in the exposed lymphocytes. The character of the changes in the number of free radicals observed in our experiments was qualitatively compatible with the theoretical prediction from the model of electromagnetic radiation effect on radical pairs.

(E) Zong C, Ji Y, He Q, Zhu S, Qin F, Tong J, Cao Y. Adaptive Response in Mice Exposed to 900 MHz Radiofrequency Fields: Bleomycin-induced DNA and Oxidative Damage/Repair. Int J Radiat Biol. 2014 Oct 27:1-21. [Epub ahead of print]

Purpose: To determine whether mice exposed to radiofrequency fields (RF) and then injected with a radiomimetic drug, bleomycin (BLM), exhibit adaptive response and provide some mechanistic evidence for such response. Materials and methods: Adult mice were exposed to 900 MHz RF at 120 $\mu W/cm^2$ power density for 4 hours/day for 7 days. Immediately after the last exposure, some mice were sacrificed while the others were injected with BLM 4 hours later. In each animal: (i) the primary DNA damage and BLM-induced damage as well as its repair kinetics were determined in blood leukocytes; (ii) the oxidative damage was determined from malondialdehyde (MDA) levels and the antioxidant status was assessed from superoxide dismutase (SOD) levels in plasma, liver and lung tissues. Results: There were no indications for increased DNA and oxidative damages in mice exposed to RF alone in contrast to those treated with BLM alone. Mice exposed to RF+BLM showed significantly: (a) reduced BLM-induced DNA damage and that is remaining after each 30, 60, 90, 120 and 150 minutes repair time, (c) decreased levels of MDA in plasma and liver, and increased SOD level in the lung. Conclusions: The overall data suggested that RF exposure was capable of inducing adaptive response and mitigated BLM-induced DNA and oxidative damages by activating certain cellular processes.

(E) Zothansiama, Zosangzuali M, Lalramdinpuii M, Jagetia GC. Impact of radiofrequency radiation on DNA damage and antioxidants in peripheral blood lymphocytes of humans residing in the vicinity of mobile phone base stations. Electromagn Biol Med. 36(3):295-305, 2017

Radiofrequency radiations (RFRs) emitted by mobile phone base stations have raised concerns on its adverse impact on humans residing in the vicinity of mobile phone base stations. Therefore, the present study was envisaged to evaluate the effect of RFR on the DNA damage and antioxidant status in cultured human peripheral blood lymphocytes (HPBLs) of individuals residing in the vicinity of mobile phone base stations and comparing it with healthy controls. The study groups matched for various demographic data including age, gender, dietary pattern, smoking habit, alcohol consumption, duration of mobile phone use and average daily mobile phone use. The RF power density of the exposed individuals was significantly higher ($p < 0.0001$) when compared to the control group. The HPBLs were cultured and the DNA damage was assessed by cytokinesis blocked micronucleus (MN) assay in the binucleate lymphocytes. The analyses of data from the exposed group ($n = 40$), residing within a perimeter of 80 m of mobile base stations, showed significantly ($p < 0.0001$) higher frequency of micronuclei when compared to the control group, residing 300 m away from the mobile base station/s. The analysis of various antioxidants in the plasma of exposed individuals revealed a significant attrition in glutathione (GSH) concentration ($p < 0.01$), activities of catalase (CAT) ($p < 0.001$) and superoxide dismutase (SOD) ($p < 0.001$) and rise in lipid peroxidation (LOO) when compared to controls. Multiple linear regression analyses revealed a

significant association among reduced GSH concentration ($p < 0.05$), CAT ($p < 0.001$) and SOD ($p < 0.001$) activities and elevated MN frequency ($p < 0.001$) and LOO ($p < 0.001$) with increasing RF power density.

(E) Zuo WQ, Hu YJ, Yang Y, Zhao XY, Zhang YY, Kong W, Kong WJ. Sensitivity of spiral ganglion neurons to damage caused by mobile phone electromagnetic radiation will increase in lipopolysaccharide-induced inflammation in vitro model. J Neuroinflammation. 2015 May 29;12(1):105. [Epub ahead of print]

BACKGROUND: With the increasing popularity of mobile phones, the potential hazards of radiofrequency electromagnetic radiation (RF-EMR) on the auditory system remain unclear. Apart from RF-EMR, humans are also exposed to various physical and chemical factors. We established a lipopolysaccharide (LPS)-induced inflammation in vitro model to investigate whether the possible sensitivity of spiral ganglion neurons to damage caused by mobile phone electromagnetic radiation (at specific absorption rates: 2, 4 W/kg) will increase. METHODS: Spiral ganglion neurons (SGN) were obtained from neonatal (1- to 3-day-old) Sprague Dawley® (SD) rats. After the SGN were treated with different concentrations (0, 20, 40, 50, 100, 200, and 400 μg/ml) of LPS, the Cell Counting Kit-8 (CCK-8) and alkaline comet assay were used to quantify cellular activity and DNA damage, respectively. The SGN were treated with the moderate LPS concentrations before RF-EMR exposure. After 24 h intermittent exposure at an absorption rate of 2 and 4 W/kg, DNA damage was examined by alkaline comet assay, ultrastructure changes were detected by transmission electron microscopy, and expression of the autophagy markers LC3-II and Beclin1 were examined by immunofluorescence and confocal laser scanning microscopy. Reactive oxygen species (ROS) production was quantified by the dichlorofluorescin-diacetate assay. RESULTS: LPS (100 μg/ml) induced DNA damage and suppressed cellular activity ($P < 0.05$). LPS (40 μg/ml) did not exhibit cellular activity changes or DNA damage ($P > 0.05$); therefore, 40 μg/ml was used to pretreat the concentration before exposure to RF-EMR. RF-EMR could not directly induce DNA damage. However, the 4 W/kg combined with LPS (40 μg/ml) group showed mitochondria vacuoles, karyopyknosis, presence of lysosomes and autophagosome, and increasing expression of LC3-II and Beclin1. The ROS values significantly increased in the 4 W/kg exposure, 4 W/kg combined with LPS (40 μg/ml) exposure, and H_2O_2 groups ($P < 0.05$, 0.01). CONCLUSIONS: Short-term exposure to radiofrequency electromagnetic radiation could not directly induce DNA damage in normal spiral ganglion neurons, but it could cause the changes of cellular ultrastructure at special SAR 4.0 W/kg when cells are in fragile or micro-damaged condition. It seems that the sensitivity of SGN to damage caused by mobile phone electromagnetic radiation will increase in a lipopolysaccharide-induced inflammation in vitro model.

BioInitiative Working Group; Preliminary Opinion on Potential Health Effects of Exposure to Electromagnetic Fields (EMF); 2014

16 April 2014

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Re: ***Preliminary Opinion on Potential Health Effects of Exposure to Electromagnetic Fields (EMF)***

Gentlemen,

The BioInitiative Working Group has reviewed the *Preliminary Opinion on Potential Health Effects of Exposure to Electromagnetic Fields (EMF*) dated November 29, 2013. We submit the following comments and suggested revisions. Thank you for providing this opportunity for comment. We hope these suggested revisions will be incorporated in the Final Opinion.

OVERALL COMMENTS

1. This Preliminary Opinion is an inadequate basis for updating the 2009 EU opinion on '*Health Effects of Electromagnetic Fields (EMF)*' and should be sent back for major revisions. The conclusions drawn from the data presented are unreliable for judging possible health risks.

2. The Committee has not answered the question it was appointed to investigate. There is no conclusion in the Executive Summary on whether the Committee determined that possible health effects of EMF are established for childhood leukemia and exist for genotoxicity, for neurological effects, for brain tumors, male fertility, fetal and neonatal effects or other key areas of research. The title of the Opinion is 'Preliminary Opinion on **Possible Effects** of Electromagnetic Fields (EMF) on Human Health" (emphasis added). The Committee has given an answer to a different question, limiting its conclusions to whether certainty or causal effect is established. This was also the central failing of the SCENIHR 2009 Opinion on EMF. This Opinion is better titled "Preliminary Opinion on Scientific Certainty of Health Harm from Electromagnetic Fields (EMF).

3. The Opinion should be revised to clearly state whether the evidence supports a finding of **possible risk** for each type of evidence considered (each section). This report is not useful for the purpose intended due to the ambiguous basis for judging the sufficiency of the scientific evidence, which will eventually form a basis for concluding whether changes in the ICNIRP standards are warranted. The lack of a clear statement about the basis for judging what constitutes sufficient evidence of "Possible Effects", and the embedded up-shifting language to instead require a demonstration of 'conclusive or unequivocal evidence' (Exhibit A).

4. Sections on brain tumors are flawed. The report consistently ignores or dismisses published scientific studies that report positive findings at exposure levels below ICNIRP standards (Exhibit B-Hardell). The SCENIHR conclusion that evidence for glioma is weaker now than in 2009 is unjustified, and can only be reached by excluding key scientific studies that reach the opposite conclusion. *There is a consistent pattern of increased risk for glioma (a malignant brain tumor) and acoustic neuroma with use of mobile and cordless phones* according to studies from Orebro University, Sweden released in 2012 and 2013.

5. Further, the Opinion misreads evidence of effects of some studies it does present when drawing conclusions (Exhibit C: Misreading Evidence - De Iuliis). In one example, statistically significant damage to sperm DNA and sperm motility and vitality was reported at cell phone radiation exposure of only 1 W/kg. The preliminary Opinion on page 77 wrongly characterizes the evidence to show that only very high SARs cause this effect. It says *"(T)he authors claimed that their results clearly demonstrated that RF exposure can damage sperm function via mechanisms involving the leakage of electrons from the mitochondria and the induction of oxidative stress but* ***the employed SAR values are very high and not relevant to cell phone users."*** (emphasis added). Finally, the entire body of new evidence for risks to fertility and reproduction is dismissed in the Executive Summary with "*The previous SCENIHR opinion concluded that there were no adverse effects on reproduction and development from RF fields at exposure levels below existing limits. The inclusion of more recent human and animal data does not change that assessment"* and in Section 3.13.4 "*(T)herefore, it is concluded that there is strong overall weight of evidence against an effect of low level RF fields on reproduction or development."* These conclusions are possible only by omitting key data, ignoring the conclusions of the authors, and dismantling the significance of the De Iuliis et al results by misreporting it. Critical evidence is misquoted, and then relied on by SCENIHR to dismiss the essential point.

6. Evidence for neurological effects (Exhibit D) should be incorporated into the analysis and conclusions of the Final Opinion. The involvement of oxidative stress on neurological/behavioral effects of ELF EMF and RFR were dismissed as "*not firmly identified*" in the Executive Summary. Exhibit D documents a significant number of overlooked studies of extremely-low frequency radiation that are reported to cause nervous system effects in 90% of the 105 studies available from 2007 to 2014. New neurological RFR studies report effects in 68% of studies on radiofrequency radiation (or 144 of 211 studies) in 2014. This has increased from 63% in 2012 (93 of 150 studies) in 2012. These studies should be included in the Final Opinion. They will likely change the Preliminary Opinion that now avoids making a judgment about whether neurological effects are sufficiently established as a cause of possible health effects.

7. Genetic effects (damage to DNA) from radiofrequency radiation are reported in 65% (or 74 of 114 studies); and 83% (or 49 of 59 studies) of extremely-low frequency studies (Exhibit E). These studies span the 2006/2007 to 2014 time period and many are overlooked. They should be included in the Final Opinion. They will likely change the conclusion of the Preliminary Opinion that skirt the issue of whether genotoxicity is sufficiently established as a cause of possible health effects (Sections 3.5.2.5, 3.7.2.5, and 3.11.3).

8. Evidence for Impacts of Physical and Biological Variables on Study Results (Exhibit F)
The main flaw of the preliminary Opinion is in neglecting the mechanistic data on non-thermal (NT) effects of microwaves (MW). As reported in multiple studies in Exhibit F , these effects depend on a variety of biological and physical parameters including polarization, frequency and environmental EMF. *In vitro* and *in vivo* negative studies have covered a negligible minority of

real cell phone signals, so the studies cannot provide evidence that the vast majority of other real cell phone signals are safe. Thus, the results of negative studies profiled in the Opinion cannot be extrapolated to the issue of the safety or lack of safety of cell phones in use today. Well-conducted positive studies cannot be negated by poorly conducted negative studies. The claim of "inconsistency" in *in vitro* and *in vivo* data and "conflicting results" has at least one simple explanation. The studies were performed under different conditions. Thus, results cannot be directly compared. The SCENIHR report on inconsistency and conflicting results may rather reflect the level of superficial analysis of these studies. Another fundamental flaw is in neglecting many studies showing dependence of the NT MW effects on exposure duration or dose (defined in radiation physics as multiplication of SAR on exposure duration), see for review (Belyaev 2010 in Exhibit F). In addition to laboratory studies, when brain cancer risk was epidemiologically examined as a function of dose received in different time windows before diagnosis, increasing trend was observed with increasing RF dose (for exposures 7 years or more in the past) (Cardis, Armstrong et al. 2011). This study provided straightforward evidence for one of the most important Bradford Hill criteria which is dependence on dose.

Good epidemiological evidence for brain tumors from many other studies has been excluded (see Section 1 and Exhibits B and F). The SCENIHR preliminary Opinion is heavily biased in favor of the Danish subscriber cohort study of mobile phone subscribers. This study has major flaws that have been substantially documented since it's publication. It is not informative even according to the requirement of SCENIHR which says *"(T)he minimum requirement for exposure assessment for an epidemiological study to be informative is to include reasonably accurate individual exposure characterization over a relevant period of time capturing all major sources of exposure for the pertinent part of the body*" (page 10).

None of the sections adequately address the literature on mitochondrial function and ELF-EMF and RFR. The studies in Table 7 are largely negative studies, and do not begin to address the central questions. This section needs to be revised to more comprehensively document existing literature as shown in Exhibit G.

Mitochondria are commonly discussed in terms of the biochemical pathways and cascades of events by which they metabolize glucose and generate energy. But in parallel with this level of function there also appears to be a dimension of electromagnetic radiation that is part of the activity of these organelles. For example, electromagnetic radiation can be propagated through the mitochondrial reticulum, which along with the mitochondria has a higher refractive index than the surrounding cell and can serve to propagate electromagnetic radiation within the network (Exhibit G). These electromagnetic aspects of mitochondrial physiology and pathophysiology could very well be impacted by ELF-EMF and RFR (i.e. a possible health effect that should be documented in the Final Opinion).

Electrophysiology: None of the sections adequately address the literature on changes in electrophysiology with exposure to ELF-EMF and RFR. This is a major area of importance and many papers are available for review. This section needs to be revised to more comprehensively document existing literature, especially in the context of blood-brain barrier changes and the propensity for seizures with disrupted electrophysiology (Exhibit G).

Epileptic seizures can be both caused by and cause oxidative stress and mitochondrial dysfunction. Seizures can cause extravasation of plasma into brain parenchyma which can trigger a vicious circle of tissue damage from albumin and greater irritability, as discussed above. Evidence suggests that if the blood-brain barrier (BBB) is already disrupted, there will be greater sensitivity to EMF/RFR exposure than if the BBB were intact suggesting that such exposures can

further exacerbate vicious circles already underway. The combination of pathophysiological and electrophysiological vulnerabilities has been explored in relation to the impact of EMF/RFR on people with epilepsy. EMF/RFR exposures from mobile phone emissions have been shown to modulate brain excitability and to increase interhemispheric functional coupling. In a rat model the combination of picrotoxin and microwave exposure at mobile phone-like intensities led to a progressive increase in neuronal activation and glial reactivity, with regional variability in the fall-off of these responses three days after picrotoxin treatment, suggesting a potential for interaction between a hyperexcitable brain and EMF/RFR exposure.

All of these comments and criticisms argue most strongly for a conclusion in the SCENIHR Final Opinion on EMF that health effects are possible, and in some cases such effects are established.

EXHIBITS AND SPECIFIC FINDINGS AND CONCLUSIONS of The BioInitiative Working Group

Here the BioInitiative Working Group provides specific comments keyed to sections of the preliminary Opinion.

1. Evidence for Brain Tumors

The report consistently ignores or dismisses published scientific studies that report positive findings at exposure levels below ICNIRP standards (Exhibit A-Hardell). The SCENIHR conclusion that evidence for glioma is weaker now than in 2009 is unjustified, and can only be reached by excluding key scientific studies that reach the opposite conclusion. *There is a consistent pattern of increased risk for glioma (a malignant brain tumor) and acoustic neuroma with use of mobile and cordless phones* according to studies from Orebro University, Sweden released in 2012 and 2013.

Had the preliminary Opinion not excluded key papers by Hardell et al, there would be more evidence about the higher risks to adults (and children) of glioma with cell phone use starting early in life. It is another compelling reason to include these Hardell et al studies that have been ignored. Inclusion of the Hardell et al studies provides valuable evidence of possible risks to children from cell phone use. Excluding these key papers has allowed the SCENIHR Committee to avoid making the necessary judgment that evidence already exists that children were reported in at least one study to have higher rates of glioma with mobile phone use than adults. It would lead to the conclusion that "brain tumors are a possible health effect of use of a mobile phone in children, and that risk appears to be far higher than for adults".

Because key studies are omitted, and because the standard for judging possible health effects has morphed into "unequivocal evidence" or "causal evidence", then the Preliminary Opinion wrongly concludes that no risks are established. These sections highlight the problems (yellow highlight).

Health effects from Radiofrequency (RF) fields, Page 4

Epidemiological studies on RF EMF exposure do not unequivocally indicate an increased risk of brain tumours, and do not indicate an increased risk for other cancers of the head and neck region, or other

malignant diseases including childhood cancer. Earlier studies raised open questions regarding an increased risk of glioma and acoustic neuroma in heavy users of mobile phones. Based on the most recent cohort and incidence time trend studies, it appears that the evidence for an increased risk of glioma became weaker while the possibility of an association of RF EMF exposure with acoustic neuroma remains open.

Health effects from RF fields, Page 12

Epidemiological studies on RF exposure do not unequivocally indicate an increased risk of brain tumours, and do not indicate an increased risk for other cancers of the head and neck region, or other malignant diseases including childhood cancer. Earlier studies raised open questions regarding an increased risk of glioma and acoustic neuroma in heavy users of mobile phones. Based on the most recent cohort and incidence time trend studies, it appears that the evidence for glioma became weaker while the possibility of an association with acoustic neuroma remains open.

Discussion of brain tumours and other tumours of the head and neck area, Pages 65-66

Overall, there is little evidence that moderate mobile phone use is associated with any cancer in the head and neck region. This is supported by large-scale epidemiological studies of three different designs. Only one case-control study shows risk increases at moderate usage levels, but the results are incompatible with observed time trends in incidence rates in reality checks and can therefore not be used for hazard assessment. Evidence is more controversial for heavy users of mobile phones; "heavy use" is a qualitative characterisation and difficult to quantify as the users with the highest life-long use are compared to those with lesser use (combining years of use and amount of daily use), with various definistons and cut-points. For instance, in Interphone, "heavy users"were approximately 10% of life-long heaviest regular users (or about 5% of all study subjects). It corresponds to, for example, half an hour of daily use over 10 years or more (in the communication of the outcome of the IARC Monograph (IARC 2013)), but this figure must not be interpreted as any suggestion of a safety limit. For the segment of the heaviest users, the largest case-control study in particular observed about 40% increased risks for glioma and for acoustic neuroma. It cannot be concluded from the available studies whether this reflects a causal association. Limitations of the case-control studies, including selection bias and reporting bias, raise concern that the observed association in small subgroups could be attributable to methodological shortcomings. Time trend analysis in incidence rates and the two cohort studies show no evidence of any risk, but would not detect small risk increases after longer latencies in heavy users only.

RF Epidemiological Studies: Conclusions on epidemiology of neoplastic diseases, Page 67

Epidemiological studies do not unequivocally indicate an increased risk of brain tumors, other cancers of the head and neck region, or other malignant diseases including childhood cancer.

Page 172.
Further studies of the effects of RF fields associated with mobile phone use and brain tumours in children are recommended as a high priority [R19]. These should include children of a younger age than those that have been studied to date, and be of sufficient duration to include assessments of cancer risk later in life.

Inclusion of the Hardell et al studies provides valuable evidence of possible risks to children from cell phone use. Excluding it has allowed the SCENIHR Committee to avoid making the necessary judgment that evidence already exists that children have higher rates of glioma with mobile phone use. It would lead to the conclusion that 'brain tumors are a possible health effect of use of a mobile phone in children, and that risk appears to be far higher than for adults'.

2. Misreading Evidence - Evidence for Effects on Fertility and Reproduction

The section must be rewritten based on the following peer-reviewed studies, and their conclusions, but particularly because of the mishandling of the De Iuliis et al (2009) study. This conclusion is also contradicted by a large number of new studies of RFR on sperm quality, motility and other male fertility parameters with very low-intensity cell phone radiation exposures; on pathological changes in the testes, and other serious health impacts that are reported by multiple laboratories around the world (see Exhibit B for references).

The De Iuliis study reports that very LOW SARs of 1.0 W/kg (which are well below today's safety limits) significantly reduced sperm quality parameters, and not just the higher SARs of 27 W/kg and higher which were also reported to decrease motility.

De Iuliis et al conclude that "(*H)igh quality spermatozoa selected in discontinuous Percoll gradients displayed a decline in both vitality and motility after exposure to RF-EMR in a dose-dependent manner. The control populations maintained an average vitality of 89%; however, significant reductions in vitality were observed at exposure levels as low as 1.0 W/kg (p,0.01) (Figure 2A). Similarly, the control populations maintained motilities at an average of 86% over the incubation period, however after exposure to RF-EMR at levels of 1.0 W/kg, motility was observed to significantly decrease to 68% (p,0.05) and decreased still further at higher SAR exposures (Figure 2B).*"

Further, "*The research described in this article suggests that one of the key environmental factors involved in the stimulation of sperm mitochondria to produce high levels of ROS, might be excess exposure to RF-EMR from sources such as mobile phones*."

DeIullis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa *in vitro*. PLos One 2009;4(7):e6446.

The SCENIHR preliminary Opinion mischaracterizes the fundamental exposure results the De Iullis et al, 2009 study and should be corrected. The preliminary Opinion on page 77 wrongly concludes that only very high SARs that are not relevant for cell phone users resulted in sperm damage. In fact, SAR levels as low as 1 W/kg can be common in men who keep a cell phone in their pants pocket, or use them near the genitals while sitting may experience such exposures.

Executive Summary, Page 13: Page 120: Conclusions on Reproduction and Developmental Effects: Page 172 – 173: and Page 177-179 Literature Identified but Not Cited.

It is quite stunning that the preliminary Opinion simply does not evaluate many key papers on RFR impacts to sperm and male fertility that it clearly knows to exist, because it lists them in Section 7 as "Literature Identified but Not Cited", and still has the temerity to conclude that the evidence for potential effects of RF fields on male fertility is weak. It would not be weak if these papers were properly included in the review (see Exhibit B: Reference List for Important Fertility and Reproduction Papers).

3.13.4. RF fields

"*The evidence suggesting that RF fields affect male fertility is weak and the existing ex vivo studies reporting positive effects have methodological problems. Cohort studies are recommended only if a study design is available that can overcome potential confounding and recall bias regarding phone use and the study has appropriate exposure assessment.*"

"*The previous SCENIHR opinion concluded that there were no adverse effects on reproduction and development from RF fields at non-thermal exposure levels. The inclusion of more recent human and animal data does not change this assessment. Therefore, it is concluded that there is strong overall weight of evidence against an effect of low level RF fields on reproduction or development.*"

"*De Iuliis et al, after 16 h exposure at 1800 MHz, SAR from 0.4 up to 27.5 W/kg also found an increase in ROS generation by the whole cell and mitochondria in a SAR-dependent manner, together with oxidative DNA damage (8-OHdG) and DNA fragmentation. Such effects translated to reduction in sperms motility and vitality. The authors claimed that their results clearly demonstrated that RF exposure can damage sperm function via mechanisms involving the leakage of electrons from the mitochondria and the induction of oxidative stress, but the employed SAR values are very high and not relevant to cell phone users.*"

This kind of reporting misquotes the statistics, and thus wrongly dismisses the significance of the De Iuliis et al results by not pointing out that a) these important adverse effects occur at as low an SAR as 1.0 W/kg which is half of the ICNIRP safety limit of 2 W/kg (the FCC/IEEE safety limit is 1.6 W/kg). The reporting also does not differentiate between very high SAR exposures of up to 27.5 W/kg and lower SARs where DNA damage is reported as well. De Iuliis et al point directly to a threat from cell phone use but the preliminary Opinion misquotes the authors, saying the levels where such effects were seen are 'not relevant to cell phone users.' It directly misrepresents both data and conclusions of this important paper.

Human sperm are are reported to be damaged by cell phone radiation at very low intensities in other studies, some reporting damage at exposure levels as low as 0.00034 – 0.07 μW/cm2 (Exhibit B). There is a veritable flood of new studies reporting sperm damage in humans and animals, leading to substantial concerns for fertility, reproduction and health of the offspring (unrepaired de novo mutations in sperm). Exposure levels are similar to those resulting from wearing a cell phone on the belt, or in the pants pocket, or using a wireless laptop computer on the lap. Sperm lack the ability to repair DNA damage (Exhibit C and Chart)

Several international laboratories have replicated studies showing adverse effects on sperm quality, motility and pathology in men who use and particularly those who wear a cell phone, PDA or pager on their belt or in a pocket (See Section 18 for references - Agarwal et al, 2008; Agarwal et al, 2009; Wdowiak et al, 2007; De Iuliis et al, 2009; Fejes et al, 2005; Aitken et al, 2005; Kumar, 2012). Other studies conclude that usage of cell phones, exposure to cell phone radiation, or storage of a mobile phone close to the testes of human males affect sperm counts, motility, viability and structure (Aitken et al, 2004; Agarwal et al, 2007; Erogul et al, 2006). Animal studies have demonstrated oxidative and DNA damage, pathological changes in the testes of animals, decreased sperm mobility and viability, and other measures of deleterious damage to the male germ line (Dasdag et al, 1999; Yan et al, 2007; Otitoloju et al, 2010; Salama et al, 2008; Behari et al, 2006; Kumar et al, 2012). There are fewer animal studies that have studied effects of cell phone radiation on female fertility parameters. Panagopoulous et al (2012) report decreased ovarian development and size of ovaries, and premature cell death of ovarian follicles and nurse cells in Drosophila melanogaster. Gul et al (2009) reported rats exposed to stand-by level RFR (phones on but not transmitting calls) had a decrease in the number of ovarian follicles in pups born to these exposed dams. Magras and Xenos (1997) reported irreversible infertility in mice after five (5) generations of exposure to RFR at cell phone tower exposure levels of less than one microwatt per centimeter squared (μW/cm2). See Exhibit C for references.

Though causal evidence of one or more mechanism(s) are not yet fully refined, it is generally accepted that oxidative stress and free radical action may be responsible for the recorded genotoxic effects of EMFs which may lead to impairments in fertility and reproduction. Free radical action and/or hydrolytic enzymes like DNAase induced by exposure to EMFs may

constitute the biochemical actions leading to adverse changes in hormones essential in males and female reproduction, DNA damage, which in turn causes damage to sperm motility, viability, and sperm morphology. Such exposures are now common in men who use and who wear wireless devices on their body, or use wireless-mode laptop computers. It may also account for damage to ovarian cells and female fertility, and miscarriage in women (ELF-EMF at 16 mG intermittent exposure). Section 18: Fertility and Reproduction, BioInitiative 2012 Report at www.bioinitiative.org

3. Evidence for Neurological and Behavioral Effects (Effects on the Nervous System)
Executive Summary, Page 14, Section 3.5.2.5

Evidence for neurological effects from a more comprehensive review of relevant papers should be incorporated into the analysis and conclusions of the Final Opinion (Exhibit D). The involvement of oxidative stress on neurological/behavioral effects of ELF EMF and RFR were dismissed as "*not firmly identified*" in the Executive Summary on page 14, but clearly the evidence supports a finding of 'possible health effect' if not 'probable effect'.

New neurological RFR studies to 2014 report effects in 68% of studies on radiofrequency radiation (or 144 of 211 studies) in 2014. This has increased from 63% in 2012 (93 of 150 studies) in 2012 (Exhibit D).

Studies of extremely-low frequency radiation are reported to cause nervous system effects in 90% of the 105 studies available in 2014.

These studies should be included in the Final Opinion. They will likely change the Preliminary Opinion that now avoids making a judgment about whether neurological effects are sufficiently established as a cause of possible health effects.

The Preliminary Opinion unnecessarily omits relevant studies on neurological effects (Exhibit D). Were they properly included, the Committee's conclusions would be different, i.e., a finding of possible health effect would have to be the clear conclusion.

There are studies on the interaction of cell phone radiation on EEG during sleep. Changes in sleep EEG have been reported by Hung et al. (2007), Regel et al. (2007), Lowden et al (2011), Schmid et al. (2012), Loughran et al. (2012), Mohammed et al. (2013), and Pelletier et al. (2012), whereas no significant effect was reported by Fritzer et al (2007), Mohler et al. (2010, 2012) and Nakatani-Enomoto et al. (2013). Loughran et al. (2012) provided an interesting conclusion in their paper: "(T)hese results confirm previous findings of mobile phone-like emissions affecting the EEG during non-REM sleep. Importantly, this low-level effect was also shown to be sensitive to individual variability. Furthermore, this indicates that "previous negative results are not strong evidence for a lack of an effect…"

Considering the effects of neurological/behavioral effects of radiofrequency radiation published since 2007, there are 30 human study papers of which 11 showed effects. The effects studied included behavioral arousal, memory effects to cognitive functions. There are 34 animal studies, of which 32 showed effects. Effects studies included motor hyperactivity to cognitive behaviors. A difference between the humans and animal studies is that most of the animal studies deal with chronic/repeated exposure, whereas the human studies are mostly acute (one time) exposure. Effects of chronic/repeated exposure studies should play more weight in considering the risk effect. It must be pointed out that neurophysiological and behavioral changes have been reported

in both animals and humans after acute (one time) exposure to RFR, and most of the EEG studies are acute exposure experiments.

Behavioral effects of ELF EMF have been further substantiated in research since 2007. These included: changes in locomotor activity (9 studies), learning and memory functions (10 studies), anxiety (5 studies); depression-like behavior (2 studies), perception (1 study), cognitive dysfunction (1 study), emotional state (1 study), sleep onset (1 study), and comb building in hornets (1 study. Since different behavioral effects have been observed in different exposure conditions, species of animals, and testing paradigms, they provide the strongest evidence that exposure to ELF EMF can affect the nervous system.

The involvement of oxidative stress on neurological/behavioral effects of ELF EMF was not carefully considered. Oxidative changes (free radicals) seens to play a critical role (Akdag et al., 2010, 2013; Akpinar et al., 2013; Cho et al., 2012; Chu et al., 2011; Ciejka et al., 2011; Deng et al., 2013; Coskun et al., 2009; Cui et al., 2012; Cui et al., 2012; Di Loreto et al., 2009; Duan et al., 2013; Falone et al., 2008; Manikonda et al., 2013; Martinez-Samano et al., 2012; Rauš Balind et al., 2014; Selaković et al., 2013; Tassel et al., 2012a, Turkozer et al., 2008). Other physiological factors, e.g., sex, age, stress, etc, that can affect the effects of ELF EMF should be considered. A paper by Falone et al. (2008) reported the brain of young rats showed an increase in anti-oxidative enzymes and defense against oxidative damage, whereas that of old rat showed a decrease. Janac et al. (2012) reported age-dependent effects of ELF EMF on locomotor activity in the Gerbils. Reyes-Guerrero et al. (2010) found that the fields affected olfactory bulb estrogen receptors in female but not in male rats. Sun et al. (2010) reported that, after in ovo (in the egg) exposure to ELF EMF, chicks showed memory deficit only when they were under stress.

Effects have been reported after exposure to low (environmental) levels of ELF EMF. For example, Ross et al (2008) showed 'perception' alternation in human subjects exposed to magnetic field at 10 nT (0.00001 mT); a study by Fournier et al (2012) on effect of brain development in the rat at 30 nT (0.00003 mT), and Stevens (2007) indicated changes in emotional states in humans exposed to 8-12 Hz magnetic field at 5 mT (0.005 mT).

Executive Summary, Page 14, Section 3.7.2.5.

A summary of the research literature on the neurological effects of ELF EMF published in 2007-2014 allows the SCENIHR Committee to survey the relevant literature more comprehensively. (In most studies, even only magnetic field was mentioned; there was no explicit statement that electric fields had been eliminated. In most ELF EMF exposure systems used in laboratory system, electric fields were also generated unless grounding was done. Thus, cells or animals were actually exposed to both magnetic and electric fields.)

- Neurotransmitters are chemicals that carry (transmit) signals from one nerve cell to another. Neurotransmitters are released from one nerve cell and react with molecules called receptors on another nerve cell. The reaction alters the activity of the second nerve cell. Activities in nerve cell could also change the properties of these receptors (mainly by changing the concentration or the affinity of the receptors to neurotransmitters). In the updated EMF literature, all the studies are on the effects of ELF EMF exposure on neurotransmitter receptors. Manikonda et al. (2007) reported effects of chronic ELF EMF exposure on NMDA receptors in the hippocampus of the rat. Salunke et al. (2013) reported that ELF EMF-induced anxiety in the rat involved NMDA receptors in the brain. There is a report on effects of magnetic field serotonin and dopamine receptors in the brain of the rat (Janac et al., 2009). Changes in subtypes of serotonin receptors 5HT(2A)

in the prefrontal cortex was reported. However, Masuda et al. (2011) reported that another type of serotonin receptor 5HT (1B) was not significantly affected after magnetic field exposure in an *in vitro* experiment. The researchers were trying to replicate two experiments carried out previously showing magnetic field exposure affected 5HT(1B) receptor. Some of the co-authors of the Musuda study were actually co-authors of one of these earlier studies. However, the 5HT(2A) receptors, particularly in the frontal cortex, are believed to be related to the psychiatric syndromes of depression in humans. Kitaoka et al. (2013) and Szemerszky et al. (2010) did report depression-like behavior in mice and rats, respectively, after chronic exposure to magnetic fields. There are two reports on dopamine receptors. Shin et al. (2007, 2011) reported an increase in D-1 dopamine receptors and activity in the striatum of the rat after magnetic field exposure. Dopamine in the striatum is involved in Parkinson's disease. Wang et al. (2008) reported that ELF magnetic fields potentiated morphine-induced decrease in D-2 dopamine receptors. The implication of these data is not readily clear. Both D-1 and D-2 dopamine receptors in the brain are involved in depression and drug addiction. There is one study on the cholinergic system. Ravera et al. (2010) reported changes in the enzyme acetylcholinesterase in cell membrane isolated from the cerebellum after magnetic field exposure. Interesting, these researchers also reported 'frequency window' effects in their experiment. Window effects, i.e., effects are observed at a certain range(s) of EMF frequency or intensity, were first reported by Ross Adey and Susan Bawin and Carl Blackman in the 1980s. A recently study by Fournier et al. (2012) reported an 'intensity window' effect of ELF magnetic field on neurodevelopment in the rat. The cholinergic systems in the brain play a major role in learning and memory functions.

• Behavioral effects of ELF EMF have been further substantiated in recent research. These included: changes in locomotor activity (Balassa et al., 2009; Dimitrijevic et al., 2014; Janac et al., 2012; Legros et al., 2012; Raus et al., 2012b; Shin et al., 2007, 2011; Todorovic et al., 2012), learning and memory functions (Che et al., 2007; Corbacio et al., 2011; Cui et al., 2012; Duan et al., 2013; Fournier et al., 2012; Fu et al., 2008; Harakawa et al., 2008; He et al., 2011; Liu et al., 2008b; Sun et al., 2010), anxiety (Balassa et al., 2009; He et al., 2011; Korpinar et al., 2012; Liu et al., 2008a; Salunke et al., 2013); depression-like behavior (Kitaoka et al., 2013; Szemerszky et al., 2011), perception (Ross et al., 2008), cognitive dysfunction (Davanipour et al., 2014), emotional state (Stevens, 2007), sleep onset (Hung et al., 2007), and comb building in hornets (Ishay et al., 2007). Since different behavioral effects have been observed in different exposure conditions, species of animals, and testing paradigms, they provide the strongest evidence that exposure to ELF EMF can affect the nervous system.

• In some of these observed neurological effects, oxidative changes (free radicals) again seemed to play a role (Akdag et al., 2010, 2013; Akpinar et al., 2013; Cho et al., 2012; Chu et al., 2011; Ciejka et al., 2011; Deng et al., 2013; Coskun et al., 2009; Cui et al., 2012; Cui et al., 2012; Di Loreto et al., 2009; Duan et al., 2013; Falone et al., 2008; Manikonda et al., 2013; Martinez-Samano et al., 2012; Rauš Balind et al., 2014; Selaković et al., 2013; Tassel et al., 2012a, Turkozer et al., 2008). Increase in free radicals causes cellular damages. Most of these effects are changes in enzymes involved in maintenance of oxidative balance in cells. A paper by Falone et al. (2008) reported an interesting finding. The researchers observed that, after magnetic field exposure, the brain of young rats showed an increase in anti-oxidative enzymes and defense against oxidative damage, whereas that of old rat showed a decrease. Thus, aging may make an individual more susceptible to the detrimental effects of ELF EMF. There are other factors that could affect an animal's response to ELF EMF. Janac et al. (2012) reported

age-dependent effects of ELF EMF on locomotor activity in the Gerbils. Reyes-Guerrero et al. (2010) found that the fields affected olfactory bulb estrogen receptors in female but not in male rats. Sun et al. (2010) reported that, after in ovo exposure to ELF EMF, chicks showed memory deficit only when they were under stress. Indeed, Lahijani et al. (2011) reported histological changes in the brain of chicks exposed to ELF EMF in ovo.

• The possible medical applications of ELF EMF should be given more attention. Several studies indicate that ELF EMF could enhance recovery of functions after nervous system damage and have protective effects against development of neurodegenerative diseases. Cuccurazzu et al. (2010) reported an ELF EMF-induced neurogenesis and repair of the nervous system after damage. Kumar et al. (2010) and Das et al. (2012) showed an enhanced restoration of functions after spinal injury in the rat. Kumar et al. (2013) further showed that ELF EMF exposure restored spinal cord injury-induced tonic pain and changes in neurotransmitter concentrations in the brain of the rat. Maestú et al. (2013) reported improvement in pain sensation in fibromyalgia patients after magnetic field stimulation. A possible beneficial effect on cerebral ischemia has been reported by Rauš Balind et al. (2014). Piacentini et al. (2008) reported a promotion of neural differentiation by ELF EMF. Kim et al. (2013) and Bai et al. (2013) reported stimulation by ELF EMF on neural differentiation of stem cells. Effects on stem cells and hippocampal neurogenesis also have been reported by Podda et al. (2013) and Leone et al. (2014). Protective effects of ELF EMF have been reported by Raus et al (2012a, b) after cerebral ischemia, Tassel et al. (2012a, b) on the development of Huntington's Disease, and Manjhi et al. (2013) on spinal cord injury induced osteoporosis. Furthermore, Cvetkovic et al. (2009) reported alteration of EEG by application of certain frequencies of magnetic fields. This may be useful in the treatment of certain neurological disorders such as sleep and psychiatric disorders. Static magnetic field has been shown by Wang et al. (2010) to act like an anti-Parkinson drug. Static magnetic field also has been shown to have anti-angiogenesis properties (Wang Z, Yang P, Xu H, Qian A, Hu L, Shang P. Inhibitory effects of a gradient static magnetic field on normal angiogenesis are reported in Bioelectromagnetics (6):446-453, 2009), which can be translated into an anticancer activity. Use of ELF EMF for cancer treatment has been extensively investigated. There is a study showing that pulsed electromagnetic fields turned on adenosine receptors in brain cancer cells that inhibit cancer growth (Vincenzi F, Targa M, Corciulo C, Gessi S, Merighi S, Setti S, Cadossi R, Borea PA, Varani K. The anti-tumor effect of A_3 adenosine receptors is potentiated by pulsed electromagnetic fields in cultured neural cancer cells is reported in PLoS One 7(6):e39317, 2012). Interesting, this effect was not observed when normal brain cells were exposed to magnetic field. The waveform of the fields may play an important role in the effect produced. There are several studies on pulsed (instead of sinusoidal) magnetic fields (Aldinucci et al., 2009; Capone et al., 2009; Cook et al. 2009; Glover et al., 2009) and complex fields (Ross et al., 2008). It has been speculated that intermittent EMF or fields that have a transient nature could be more biologically potent than constant fields. The conditions and parameters of the fields that could produce either detrimental or beneficial effects need further investigation. Furthermore, it is still not clear whether acute (one time) exposure would elicit effects different from chronic/repeated exposure. In the 2007-2014 literature, there are many studies reporting effects of chronic/repeated exposure. The study by Liu et al. (2008a) indicates that duration of exposure could be an important factor.

• The majority of the studies used magnetic fields above 0.1 mT (1 gauss; the highest was 8 mT). The intensities are much higher than those in the public environment. Thus,

caution should be taken in extrapolating the high-intensity cell and animal studies to environmental human exposure situation. Exposure to magnetic fields of 0.4 mT (0.0004 mT) has been implication in an increased risk of childhood leukemia. And, the recent report by Li et al. (Li DK, Ferber JR, Odouli R, Quesenberry CP Jr. A Prospective Study of In-utero Exposure to Magnetic Fields and the Risk of Childhood Obesity. Sci Rep. 2:540, 2012) on an increased risk of obesity of humans exposed prenatally to magnetic field at 0.25 mT (0.00025 mT). There is also a report of a blood pressure lowering effect in humans with mild-to-moderate hypertension after exposure to magnetic fields at 1 μT (0.001mT) (Nishimura T, Tada H, Guo X, Murayama T, Teramukai S, Okano H, Yamada J, Mohri K, Fukushima M. A 1-μT extremely low-frequency electromagnetic field vs. sham control for mild-to-moderate hypertension: a double-blind, randomized study. Hypertens Res. 34(3):372-377, 2011.) Apparently, humans are sensitive to magnetic field at level less than 1 mT. There is a study by Ross et al (2008) showing 'perception' alteration in human subjects exposed to magnetic field at 10 nT (0.00001 mT), a study by Fournier et al (2012) on effect of brain development in the rat at 30 nT (0.00003 mT), and a study by Stevens (2007) indicating changes in emotional states in humans exposed to 8-12 Hz magnetic field at 5 mT (0.005 mT). These data do suggest magnetic fields at very low intensities could cause neurological effects in humans. In the 1990s, there were a series of more than 20 studies published by Reuven Sandyk showing that pulsed magnetic fields at pT (1 pT = 0.000000001 mT) levels could have therapeutic effects on Parkinson's disease and multiple sclerosis (see e.g., Sandyk R. Reversal of cognitive impairment in an elderly Parkinsonian patient by transcranial application of picotesla electromagnetic fields. Int J Neurosci. 91(1-2):57-68, 1997, or, search for 'Sandyk R' in the PubMed.) However, Sandyk's findings have never been independently confirmed.

- In summary, ELF EMF affects neurological functions and behavior in animals and humans. There is no definite data showing that these effects are detrimental to human health. However, since effects have been observed, it is advisable that one should limit one's exposure to EMF.

Exhibit D is a summary of the research literature on the neurological effects of ELF EMF published in 2007-2014.

4. Evidence for Genotoxicity (Genetic Damage to DNA)

There are many more publications on genotoxicity of ELF-EMF and RFR since 2007 than the SCENIHR Working Group considered.

Genetic effects (damage to DNA) from radiofrequency radiation are reported in 65% (or 74 of 114 studies) (Exhibit E).

For ELF-EMF, genetic effects are reported to occur in 83% (or 49 of 59 studies) of extremely-low frequency studies (Exhibit E).

These studies should be included in the Final Opinion. They will likely change the conclusion of the Preliminary Opinion that skirt the issue of whether genotoxicity is sufficiently established as a cause of possible health effects (Sections 3.5.2.5, and 3.11.3).

Effects of EMF on oxidative status, a change of which disturbs all physiological functions is poorly analyzed because many relevant peer-reviewed papers are missing from the assessment.

• The effects of both RF and ELF fields are very similar. This is surprising because the energies carried by these EMFs are billions of folds different. An explanation for similar genetic effects has been provided by a recent paper by Blank and Goodman (Blank M, Goodman R. DNA is a fractal antenna in electromagnetic fields. Int. J. Radiat. Biol. 87(4):409-415, 2011) in which they stated that '…the wide frequency range of interaction with EMF is the functional characteristic of a fractal antenna, and DNA appears to possess the two structural characteristics of fractal antennas, electronic conduction and self symmetry.' However, similarities in effects between ELF and RF fields have also been reported in studies of other physiological processes, e.g., neurochemical and behavioral effects (Cf. Lai, H., Carino, M.A., Horita, A. and Guy, A.W. Opioid receptor subtypes that mediate a microwave-induced decrease in central cholinergic activity in the rat. Bioelectromagnetics 13:237-246, 1992; Lai, H. and Carino, M.A.

Intracerebroventricular injections of mu and delta-opiate receptor antagonists block 60-Hz magnetic field-induced decreases in cholinergic activity in the frontal cortex and hippocampus of the rat. Bioelectromagnetics 19:433-437, 1998; Lai, H., Carino, M.A. and Ushijima, I. Acute exposure to a 60 Hz magnetic field affects rats' performance in the water maze. Bioelectromagnetics 19:117-122, 1998; Wang, B.M. and Lai, H. Acute exposure to pulsed 2450-MHz microwaves affects water maze learning in the rat. Bioelectromagnetics 21:52-56, 2000.) Thus, there is a basic interaction mechanism of biological tissues with electromagnetic fields that is independent of frequency.
Many studies have implicated the involvement of free radical processes in the genetic effects of EMF: ELF-EMF (Butdak et al., 2012; Jouni et al., 2012; Luukkonen et al., 2014; Tiwari et al.,2014); RFR (Agarwal et al., 2009; Atasoy et al., 2012; Burlaka et al., 2013; Campisi et al., 2010; De Iuliis et al., 2009; Esmekaya et al., 2011; Ferreira et al., 2006; Gajski and Garaj-Vrhovac, 2009; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Khalil et al., 2012; Kumar et al., 2010; Liu et al., 2013a,b; Luukkonan et al., 2009; Tomruk et al., 2010; Tkalec et al., 2013; Wu et al., 2008; Xu et al., 2010; Yao et al., 2003). Increase in free radical activity and changes in enzymes involved in cellular oxidative processes are the most consistent effects observed in cells and animals after EMF exposure. However, they are reports indicating that EMF could induce genetic effects without the involvement of free radicals (ELF- Alcaraz et al., 2013; RFR- Ferreira et al., 2006; Furtado-Filho et al., 2013) and increase in free radical after EMF exposure did not lead to genetic effects (Frahm et al., 2006). There are at least a couple of hundred published papers on the effects of EMF exposure on cellular oxidative processes. Many biological effects of EMF can be explained by intracellular changes in oxidative status, including the genetic effects reported in this review.

• An important observation of the studies is that EMF can interact with other entities and synergistically cause genetic effects. These entities include: ELF-EMF- cisplastin (Buldak et al., 2012; El-Bialy et al., 2013), bleomycin (Cho et al., 2007), gadolinium (Cho et al., 2014); hydrogen peroxide and methyl methane sulfonate (Koyama et al., 2008), menadione (Luukkonan et al., 2011, 2014; Markkanen et al., 2008), ionizing radiation (Mairs et al., 2007; Journi et al., 2012 Yoon et al., 2014); RFR- chemical mutagens (Baohong et al., 2005), clastogens (Kim et al., 2008), x-rays (Manti et al., 2008), ultraviolet ray (Baohong et al., 2007), aphidicolin (Tiwari et al., 2008), picrotoxin (López-Martín et al., 2009), doxorubicin (Zhijian et al., 2010), and incoherent

electromagnetic noise (Wu et al., 2008; Yao et al., 2008). Most of the compounds that interact with EMF are mutagens. This is important because in real life situations, a person is usually exposed to many different environmental factors simultaneously. Synergism of these factors with EMF should be considered more seriously.

• Several long term/repeated exposure papers are included in this update: ELF-EMF (Borhani et al., 2011; Cuccurazzu et al., 2010; Erdal et al., 2007; Fedrowitz and Loscher, 2012; Mariucci et al., 2010; Panagopoulous et al., 2013; Udroiu et al., 2006), and RFR (Asasoy et al., 2012; Atli Serkeroglu et al., 2013; Burlaka et al., 2013; Chavdoula et al., 2010; Deshmukh et al., 2013; Ferreira et al., 2006; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Lakshmi et al., 2010; Paulraj and Behari, 2006; Tomruk et al., 2010; Yan et al., 2008). These data are important in the understanding of the biological effects of EMF exposure in real life situation, since human environmental EMF exposure is both chronic and intermittent. Within these long-term exposure studies, there are several that investigated the effect of EMF exposure on developing animals (ELF-EMF: Borhani et al., 2011; Cuccurazzu et al., 2010; Panagopoulous et al., 2013; Udroiu et al., 2006, RFR: Burlaka et al., 2013; Ferreira et al., 2006; Guler et al., 2010, 2012; Serkeroglu et al., 2013; Tomruk et al., 2010; Zalata et al., In press). Data of effects of EMF exposure on growth and development of young animals are urgently needed. There are several studies indicating that RFR may affect reproduction, particularly with effects on sperm physiology and DNA (Agarwal et al., 2009; Atasoy et al., 2012; Avendano et al., 2012; Chavdoula et al., 2010; de Iuliis et al., 2009; Liu et al., 2013b; Panagopoulous et al., 2007). Similar effects of ELF-EMF on sperm have also been reported, e.g., Hong R, Zhang Y, Liu Y, Weng EQ. Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 23(6):414-417, 2005; Iorio R, Scrimaglio R, Rantucci E, Delle Monache S, Di Gaetano A, Finetti N, Francavilla F, Santucci R, Tettamanti E, Colonna R. A preliminary study of oscillating electromagnetic field effects on human spermatozoon motility. Bioelectromagnetics. 28(1):72-75, 2007; Iorio R, Delle Monache S, Bennato F, Di Bartolomeo C, Scrimaglio R, Cinque B, Colonna RC. Involvement of mitochondrial activity in mediating ELF-EMF stimulatory effect on human sperm motility. Bioelectromagnetics. 32(1):15-27, 2011.

• Another area that needs more research is the biological effects of low-intensity exposure. This is particularly true for ELF-EMF, since intensities of ELF-EMF in the environment are in microtesla (mT) levels. There are many studies on biological effects of low-intensity RFR (see Table 1 in Levitt, B.B. and Lai, H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays. Environ. Rev. 18:369-395, 2010.) However, most cell and animal studies in ELF-EMF used fields in the millitesla (mT) level. Exceptions are the study of Sarimov et al. (2011) listed below in the reference section and the study of de Bruyn and de Jager (2010) (**de Bruyn L** and **de Jager L**. Effect of long-term exposure to a randomly varied 50 Hz power frequency **magnetic field** on the fertility of the mouse. **Electromag. Biol. Med.** 29(1-2):52-61, 2010).

• Two other important findings of these recent studies are that the effects of EMF are shown to be waveform specific and cell-type specific. Regarding waveform specificity, Campisi et al. (2010) reported increases in free radical activity and DNA fragmentation in brain cells after acute exposure to a 50-Hz amplitude-modulated 900-MHz RFR, whereas

a continuous-wave 9000-MHz field produced no effect. Franzellitti et al. (2010) showed increased DNA strand breaks in trophoblasts after exposure to a 217-Hz modulated 1.8 GHz-RFR, but a continuous-wave field of the same carrier frequency was without effect. Tkalec et al (2013) reported that AM-modulated (1 KHz sinusoidal) 900-MHz RFR is more potent than non-modulated field in causing DNA damage in coelomocytes of exposed earthworms. Luukkonen et al. (2009) reported a continuous-wave 872-MHz RFR increased chemically-induced DNA strand breaks and free radicals in human neuroblastoma cells, whereas a GSM-modulated 872-MHz field had no significant effect. Zhang et al. (2008) found that gene expression in rat neurons is more sensitive to intermittent than continuous exposure to a 1.8 GHz-RFR. López-Martín et al. (2009) found that GSM and unmodulated RFR caused different effects on c-Fos gene expression in the rat brain. Regarding cell-type specificity, Nylund and Leszczynski (2006) and Remondini et al. (2006) reported different patterns of gene expression in different types of cells after exposure to RFR. Zhao et al. (2007) found than neurons are more sensitive to a 1.9 GHz cell phone radiation than astrocytes. Schwarz et al. (2008) reported DNA strand breaks and micronucleus formation in human fibroblasts, but not in lymphocytes, after exposure to a 1950-MHz UMTS field. Furthermore, Xu et al (2013) found DNA damages in some cell types and not in others after exposure to 1800-MHz RFR. Valbonesi et at. (2014) reported that HSP70 expression and MAPK signaling pathways in PC12 cells were affected by GSM-217 Hz signal and not by CW or GSM-talk signals. In ELF-EM research, Giorgi et al. (2011) found that DNA transposition in E. coli was *decreased* after exposure to a sinusoidal magnetic field and *increased* after exposure to a pulsed magnetic field. Kim et al. (2012) described DNA strand breaks in human fibroblasts after exposure to ELF magnetic field. They found that the pattern of changes depended on the eddy current and Lorentz force in the field. Nahab et al. (2007) reported that a square-continuous ELF magnetic field was more effective than sinusoidal-continuous or pulsed field in inducing sister chromatid exchange in human lymphocytes. These findings underscore the complexity of interaction of EMF with biological tissues and may partially explain why effects were observed in some studies and not others. It is essential to understand why and how certain wave-characteristics of an EMF are more effective than other characteristics in causing biological effects, and why certain types of cells are more susceptible to the effect of EMF? That there are different biological effects elicited by different EMF wave characteristics is critical proof for the existence of nonthermal effects.

• Many biological/health effects have been reported in cells and animals after exposure to EMFs in both the ELF and RF ranges. (Sixty-five percent of the RFR papers and 82% of the ELF-EMF papers in the publication list below reported effects.) It is highly dishonest for a scientist to summarily deny the existence of biological effects of EMF. A biological effect of EMF can be detrimental to health, but can also be turned into a beneficial means for the treatment of human diseases. Denying any effects hampers the development of electromagnetic treatments for diseases. Examples of possible clinical uses of EMF are: Alzheimer's disease (Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C. Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. J Alzheimers Dis. 19(1):191-210, 2010); Parkinson's disease (Wang Z, Che PL, Du J, Ha B, Yarema KJ. Static magnetic field exposure reproduces cellular effects of the Parkinson's disease drug candidate ZM241385. PLoS One. 5(11):e13883, 2010); bone regeneration (Lee HM, Kwon UH, Kim H, Kim HJ, Kim B, Park JO, Moon ES, Moon SH. Pulsed electromagnetic field stimulates cellular proliferation in human intervertebral disc cells. Yonsei Med. J. 51(6):954-959, 2010);

cancer treatment (Costa FP, de Oliveira AC, Meirelles R, Machado MC, Zanesco T, Surjan R, Chammas MC, de Souza Rocha M, Morgan D, Cantor A, Zimmerman J, Brezovich I, Kuster N, Barbault A, Pasche B. Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields. Br. J. Cancer. 105(5):640-648, 2011), and tissue regeneration (Gaetani R, Ledda M, Barile L, Chimenti I, De Carlo F, Forte E, Ionta V, Giuliani L, D'Emilia E, Frati G, Miraldi F, Pozzi D, Messina E, Grimaldi S, Giacomello A, Lisi A. Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic fields. Cardiovasc. Res. 82(3):411-420, 2009).

- It must be pointed out that, consistent with previous research, not very much of the cellular and animal genetic research data directly indicate that EMF (both RF and ELF EMF) is a carcinogen. However, the data show that EMF can possibly alter genetic functions and thus it is advisable that one should limit one's exposure to EMF.

The genotoxicity assessment flaws lead to dismissal of the fertility implications of oxidative damage on sperm. Both genotoxicity (DNA damage to genes) in general and the consequence that genotoxicity from mechanisms related to free-radicals (oxidative damage to DNA) to sperm from cell phone radiation (RFR) mean that two promising lines of scientific evidence in SCENIHR's Opinion are compromised.

5. Evidence for Fetal and Neonatal Effects

Effects on the developing fetus from in-utero exposure to cell phone radiation have been observed in both human and animal studies since 2006. Sources of fetal and neonatal exposures of concern include cell phone radiation (both paternal use of wireless devices worn on the body and maternal use of wireless phones during pregnancy). Sources include exposure to whole-body RFR from base stations and WI-FI, use of wireless laptops, use of incubators for newborns with excessively high ELF-EMF levels resulting in altered heart rate variability and reduced melatonin levels in newborns, fetal exposures to MRI of the pregnant mother, and greater susceptibility to leukemia and asthma in the child where there have been maternal exposures to ELF-EMF. Divan et al (2008) found that children born to mothers who used cell phones during pregnancy develop more behavioral problems by the time they have reached school age than children whose mothers did not use cell phones during pregnancy. Children whose mothers used cell phones during pregnancy had 25% more emotional problems, 35% more hyperactivity, 49% more conduct problems and 34% more peer problems (Divan et al, 2008). Aldad et al (2012) showed that cell phone radiation significantly altered fetal brain development and produced ADHD-like behavior in the offspring of pregnant mice. Exposed mice had a dose-dependent impaired glutamatergic synaptic transmission onto Layer V pyramidal neurons of the prefrontal cortex. The authors conclude the behavioral changes were the result of altered neuronal developmental programming in utero. Offspring mice were hyperactive and had impaired memory function and behavior problems, much like the human children in Divan et al (2008). Fetal (in-utero) and early childhood exposures to cell phone radiation and wireless technologies in general may be a risk factor for hyperactivity, learning disorders and behavioral problems in school.

See Herbert and Sage, Section 19: Fetal and Neonatal Effects of EMF and Section 20: Findings in Autism (ASC) Consistent with Electromagnetic Fields (EMF) and Radiofrequency Radiation (RFR) Exposure in the BioInitiative 2012 Report at www.bioinitiative.org for references and as published in Pathophysiology, Volume 20, Issue 3.

[Herbert M, Sage C (2013) Autism and EMF/RFR? Plausibility of a Pathophysiological Link-Part I. Pathophysiology Volume 20, Issue 3, 191-209, June 2013]

Fragopoulou et al (2012) reports that brain astrocyte development followed by proteomic studies is adversely affected by DECT (cordless phone radiation) and mobile phone radiation (Fragopoulou and Margaritis, Section 5: EMF Transcriptomics and Proteomics Research 2007-2012, BioInitiative 2012 Report at www.bioinitiative.org)

Common sense measures to limit both ELF-EMF and RF EMF in these populations is needed, especially with respect to avoidable exposures like incubators that can be modified; and where education of the pregnant mother with respect to laptop computers, mobile phones and other sources of ELF-EMF and RF EMF are easily instituted. A precautionary approach may provide the frame for decision-making where remediation actions have to be realized to prevent high exposures of children and pregnant woman.

(Bellieni and Pinto, 2012 – Section 19, Fetal and Neonatal Effects, BioInitiative 2012 Report at www.bioinitiative.org)

6. Evidence for Heat Shock Protein Effects

3.5.1.4 Conclusions on neoplastic diseases from RF Exposure and
3.7.1.4 Conclusions on neoplastic diseases from ELF Exposure

SCENIHR emphasizes epidemiology studies of health effects such as cancers that generally affect a relatively small percentage of those exposed and take many years to develop. It does not include studies of the natural protective mechanisms in virtually all cells that protect against the immediate changes that lead to the long term health effects. Living cells synthesize stress proteins when exposed to potentially harmful stimuli that include electromagnetic fields (EMF) across a wide range of non-ionizing frequencies. Stress protein synthesis and oxidative damage to DNA stimulated by EMF are considered likely to lead to cancer and other diseases. Like the DNA damage, these effects occur at exposures well below levels that are now considered safe. Stress proteins can also be protective when induced prior to surgery, as in reducing oxidative damage following heart bypass surgery. Given the goals of SCENIHR, analysis of cell biology studies is essential. An EMF safety standard, based on the far more sensitive natural biological response, would not only be more realistic than the thermal criterion, but more protective as well.

7. Evidence for Impacts of Physical and Biological Variables on Study Results

The main flaw of the preliminary Opinion is in neglecting the mechanistic data on non-thermal (NT) effects of microwaves (MW). As reported in multiple studies in Exhibit F , these effects depend on variety of biological and physical parameters including polarization, frequency and environmental EMF. *In vitro* and *in vivo* negative studies have covered a negligible minority of real cell phone signals, so the studies cannot provide evidence that the vast majority of other real cell phone signals are safe. Thus, the results of negative studies profiled in the Opinion cannot be extrapolated to the issue of the safety or lack of safety of cell phones in use today. Well conducted positive studies cannot be negated by poorly conducted negative studies. The claimed of "inconsistency" in *in vitro* and *in vivo* data and "conflicting results" has at least one simple explanation. The studies were performed under different conditions. Thus, results cannot be directly compared. The SCENIHR report on inconsistency and conflicting results may rather reflect the level of superficial analysis of these studies. Another fundamental flaw is in

neglecting many studies showing dependence of the NT MW effects on exposure duration or dose (defined in radiation physics as multiplication of SAR on exposure duration), see for review (Belyaev 2010 in Exhibit F). In addition to laboratory studies, when brain cancer risk was epidemiologically examined as a function of dose received in different time windows before diagnosis, increasing trend was observed with increasing RF dose (for exposures 7 years or more in the past) (Cardis, Armstrong et al. 2011). This study provided straightforward evidence for one of most important Bradford Hill criteria which is dependence on dose.

Good epidemiological evidence for brain tumors from many other studies has been excluded (see Section 1 and Exhibits B and F). The SCENIHR preliminary Opinion is heavily biased in favor of the Danish subscriber cohort study of mobile phone subscribers. This study has major flaws that have been substantially documented since it's publication. It is not informative even according to the requirement of SCENIHR which says *"(T)he minimum requirement for exposure assessment for an epidemiological study to be informative is to include reasonably accurate individual exposure characterization over a relevant period of time capturing all major sources of exposure for the pertinent part of the body*" (page 10).

ELF Carcinogenicity: Page 131 of the SCENIHR provides misleading and flawed conclusions on ELF and neoplastic diseases. As a matter of fact, the increased risk of childhood leukemia with daily average exposure above 0.3 to 0.4 μT is as strong as never before. All available studies from Europe, America and Asia consistently show such correlation. It has been further supported by recent meta-analysis by Zhao et al. (Zhao, Liu et al. 2014). The statement of lack of mechanisms for ELF effects is wrong. Recent studies provided more evidence for such mechanisms even if they have not been comprehensively studied, see below. Considerations of ELF carcinogenicity in the SCENIHR report did not use standard methods such as the Bradford Hill criteria which do not require complete knowledge of mechanisms in case when epidemiological evidence is overwhelming as in case of childhood leukemia (Zhao, Liu et al. 2014).

ELF affects cell proliferation: In line with many previous studies, new studies unmentioned in the SCENIHR report provide further evidence that ELF can affect cell proliferation under specific conditions of exposure (Segatore, Setacci et al. 2012; Bae, Do et al. 2013; Jadidi, Safari et al. 2013). Bai et al. investigated ELF effects on proliferation of epidermal stem cells (ESC) (Bai, Zhang et al. 2012). See additional comments in Exhibit F.

ELF induced ROS and genomic instability: Induction ROS and is generally considered as a candidate mechanism for carcinogenicity for EMF (IARC 2013). Several recent studies unmentioned in the SCENIHR report provided further evidence for this mechanism in case of ELF exposure (Duan, Wang et al. 2013; Khaki, Khaki et al. 2013)**.** See additional comments in Exhibit F.

Mechanisms for effects of weak ELF: While all mechanisms of ELF effects are not known with certainty, new important data emerged about these mechanisms which were neglected by the SCENIHR report. For ELF fields, these mechanisms involve magnetoreception of fields in the μT-range which is observed in many studied animals including lizards (Nishimura, Okano et al. 2010). It should be stressed that the lack of precise knowledge for this mechanism (radical pairs and magnetite are mainly considered) does not preclude general acceptance of these phenomena. In analogy, and in accordance to the Bradford Hill criteria, lack of precise knowledge on mechanism for leukemogenesis of weak ELF ≥0.3 μT, which was consistently shown in children

in multiple studies (Zhao, Liu et al. 2014) should not preclude classification of µT-range ELF as an IARC carcinogen group 1. The SCENIHR report completely neglects variety of mechanisms based on ELF effects on ions (Halgamuge and Abeyrathne 2011; Foletti, Grimaldi et al. 2013). See additional comments in Exhibit F.

ELF section omits significant number of ELF positive studies: Except for aforementioned studies, ELF section of the SCENIHR report omits significant number of other ELF positive studies. These include but not limited to (Mariucci, Villarini et al. 2010; Nishimura, Okano et al. 2010; Ravera, Bianco et al. 2010; Severini, Bosco et al. 2010; Ulku, Akdag et al. 2011; Bai, Zhang et al. 2012; Ince, Akdag et al. 2012; Martirosyan 2012; Portelli, Madapatha et al. 2012; Balassa, Varro et al. 2013; Gang, Parker et al. 2013; Iorio, Bennato et al. 2013; Kang, Hong et al. 2013; Khaki, Khaki et al. 2013; Li, Zhang et al. 2013; Martirosyan, Baghdasaryan et al. 2013; Panagopoulos, Karabarbounis et al. 2013; Shams Lahijani, Tehrani et al. 2013; Villarini, Ambrosini et al. 2013) See additional comments in Exhibit F.

8. Literature Identified but Not Cited (pages 217-219).

Entire bodies of relevant evidence are ignored, or key papers are not quoted (but they appear in the reference list as "literature identified but not cited"). This is not explained, and functionally disables scientific review of highly relevant emerging scientific studies. An explanation is needed. Further, revisions should be made to include many or most of them in the Final Opinion to include these and other relevant papers. These papers are included as 'literature identified but not cited' – as examples of the problem.

Blood-Brain Barrier Evidence
Nittby H, Brun A, Strömblad S, Moghadam MK, Sun W, Malmgren L, Eberhardt J, Persson BR, Salford LG (2011). Nonthermal GSM RF and ELF EMFELF MF effects upon rat BBB permeability. Environmentalist, 31(2), 140-8

Heat Shock Protein (Stress Protein) Evidence
Calabrò E, Condello S, Currò M, Ferlazzo N, Caccamo D, Magazù S, Ientile R. Modulation of heat shock protein response in SH-SY5Y by mobile phone microwaves. World J Biol Chem. 3 (2):34-40, 2012. (3.5)

Perez FP, Zhou X, Morisaki J, Jurivich D. Electromagnetic field therapy delays cellular senescence and death by enhancement of the heat shock response. Exp Gerontol. 2008 Apr;43(4):307-16. Epub 2008 Jan 29. (3.5 & 3.10)

Two other highly relevant papers on stress proteins that were ignored and should be incorporated. They are:

Blank M, Goodman R (2009) Electromagnetic Fields Stress Living Cells. Pathophysiology 16:71-78.

Blank M (2012) Evidence for Stress Response (Stress Proteins). In BioInitiative Report (2012) A Scientific Perspective on Health Risk of Electromagnetic Fields. Section 7, pp. 1-39. Published Online December 31, 2012
http://www.bioinitiative.org/report/index.htm

9. Mitochondrial Function and Disruptions in Electrophysiology

None of the sections adequately address the literature on mitochondrial function and ELF-EMF and RFR. The studies in Table 7 are largely negative studies, and do not begin to address the central questions. This section needs to be revised to more comprehensively document existing literature as shown in Exhibit G.

Mitochondria are broadly vulnerable, in part because the integrity of their membranes is vital to their optimal functioning – including channels and electrical gradients, and their membranes can be damaged by free radicals which can be generated in myriad ways including ELF-EMF and RFR exposure at environmental levels. Moreover, just about every step in their metabolic pathways can be targeted by environmental agents, including toxicants and drugs, as well as mutations.

Mitochondria are commonly discussed in terms of the biochemical pathways and cascades of events by which they metabolize glucose and generate energy. But in parallel with this level of function there also appears to be a dimension of electromagnetic radiation that is part of the activity of these organelles. For example, electromagnetic radiation can be propagated through the mitochondrial reticulum, which along with the mitochondria has a higher refractive index than the surrounding cell and can serve to propagate electromagnetic radiation within the network (Exhibit G). These electromagnetic aspects of mitochondrial physiology and pathophysiology could very well be impacted by ELF-EMF and RFR (i.e. a possible health effect that should be documented in the Final Opinion).

Other types of mitochondrial damage have been reported in at least some of the studies that have examined the impacts of EMF/RFR upon mitochondria. These include reduced or absent mitochondrial crista, mitochondrial DNA damage, swelling and crystallization, alterations and decreases in various lipids suggesting an increase in their use in cellular energetics, damage to mitochondrial DNA, and altered mobility and lipid peroxidation after exposures. Also noted has been enhancement of brain mitochondrial function in Alzheimer's transgenic mice and normal mice. The existence of positive as well as negative effects gives an indication of the high context dependence of exposure impacts, including physical factors such as frequency, duration, and tissue characteristics (Exhibit G).

Secondary mitochondrial dysfunction (i.e. environmentally triggered rather than rooted directly in genetic mutations) could result from EMF/RFR to damage channels, membranes and mitochondria themselves as well as from toxicant exposures and immune challenges. In a meta-analysis of studies of children with mitochondrial disorder and autism, the spectrum of severity varied, and 79% of the cases were identified by laboratory findings without associated genetic abnormalities.

Electrophysiology: None of the sections adequately address the literature on changes in electrophysiology with exposure to ELF-EMF and RFR. This is a major area of importance and many papers are available for review. This section needs to be revised to more comprehensively document existing literature, especially in the context of blood-brain barrier changes and the propensity for seizures with disrupted electrophysiology (Exhibit G).

Nervous system electrophysiology when disrupted by ELF-EMF and RFR can produce alterations in molecular, cellular and systems physiological function. It occurs in the brain as well as in the body, and impacts the transduction into the electrical signaling activities of the brain and nervous

system. If the cells responsible for generating synapses and oscillatory signaling are laboring under cellular and oxidative stress, lipid peroxidation, impaired calcium and other signaling system abnormalities, then mitochondrial metabolism will fall short, all the more so because of the challenges from the immune system which in turn can be triggered to a major extent by environment. How well will synaptic signals be generated? How well will immune-activated and thereby distracted glial cells be able to modulate synaptic and network activity? Microglial activation can impact excitatory neurotransmission mediated by astrocytes. Cortical innate immune response increases local neuronal excitability and can lead to seizures. Inflammation can play an important role in epilepsy.

Epileptic seizures can be both caused by and cause oxidative stress and mitochondrial dysfunction. Seizures can cause extravasation of plasma into brain parenchyma which can trigger a vicious circle of tissue damage from albumin and greater irritability, as discussed above. Evidence suggests that if the blood-brain barrier (BBB) is already disrupted, there will be greater sensitivity to EMF/RFR exposure than if the BBB were intact suggesting that such exposures can further exacerbate vicious circles already underway. The combination of pathophysiological and electrophysiological vulnerabilities has been explored in relation to the impact of EMF/RFR on people with epilepsy. EMF/RFR exposures from mobile phone emissions have been shown to modulate brain excitability and to increase interhemispheric functional coupling. In a rat model the combination of picrotoxin and microwave exposure at mobile phone-like intensities led to a progressive increase in neuronal activation and glial reactivity, with regional variability in the fall-off of these responses three days after picrotoxin treatment, suggesting a potential for interaction between a hyperexcitable brain and EMF/RFR exposure.

One critical issue here is nonlinearity and context and parameter sensitivity of impact. In one study, rat brain slices exposed to EMF/RFR showed reduced synaptic activity and diminution of amplitude of evoked potentials, while whole body exposure to rats led to synaptic facilitation and increased seizure susceptibility in the subsequent analysis of neocortical slices. Another study unexpectedly identified enhanced rat pup post-seizure mortality after perinatal exposure to a specific frequency and intensity of exposure, and concluded that apparently innocuous exposures during early development might lead to vulnerability to stimuli presented later in development.

10. ELF Studies Support a Finding of "Probable' or 'Known' Carcinogen

Overall, the ELF MF epidemiological evidence points consistently to an increased risk for childhood leukemia. In such circumstances, considering that no other interpretation (chance, bias, or confounding) could be substantiated in the past decade, the association became more credible and even in the absence of a mechanistic interpretation ELF MF should be upgraded to a 2A or even a group 1 carcinogen.

Many epidemiological studies of ELF MF and childhood leukemia were of high quality and there are no shortcomings that may prevent a causal interpretation. The WHO IARC panel was of the opinion that the studies allowed a causal interpretation; otherwise no classification into group 2B would have been possible. Only bias and confounding could not be ruled out with sufficient scientific certainty. This assessment was also supported by the lack of consistent support by *in vitro* and animal evidence.

3.7. Health effects from ELF fields (Page 123)

3.7.1. Neoplastic diseases

3.7.1.1. Epidemiological studies

Page 123, lines 24-31:

In summarizing the previous SCENIHR statement that endorsed the IARC classification of ELF magnetic fields as possibly carcinogenic to humans "due to consistently observed increased childhood leukemia risk in epidemiological studies" SCENIHR claimed that shortcomings in these studies prevented a causal interpretation.

In fact, the IARC panel was of the opinion that the studies allowed a causal interpretation; otherwise no classification into group 2B would have been possible. Only bias and confounding could not be ruled out with sufficient scientific certainty. This assessment was also supported by the lack of consistent support by *in vitro* and animal evidence.

Many epidemiological studies of ELF MF and childhood leukemia were of high quality and there are no shortcomings that may prevent a causal interpretation. Of course, the retrospective nature of most studies and the inevitable misclassification if measurements are done years after the assumed initiation of the disease introduce problems of interpretation. Considering the potential sources of bias IARC noted that although selection bias could have led to higher risk estimates, not the whole effect can be attributed to bias. This is corroborated by the fact that studies that relied on distance from power lines or wire-codes only and did not contact participants found the same effects. Misclassification bias, on the other hand, if non-differential would lead to reduced risk estimates. The same is true for the most likely scenarios of differential misclassification.

Overall, the epidemiological evidence points consistently to an increased risk. In such circumstances, considering that no other interpretation (chance, bias, or confounding) could be substantiated in the past decade, the association became more credible and even in the absence of a mechanistic interpretation ELF MF should be upgraded to a 2A or even a group 1 carcinogen.

In a recent study of distance from power lines and childhood cancer in Britain covering the period from 1962 through 2008 (Bunch et al. 2014) elevated childhood leukemia risks were reported for distances below 200 or 600 m from high-voltage power lines (400/275 kV) from the 1960s to the 1980s but no significant increases in more recent years. Authors interpreted this result as more likely due to changing population characteristics among those living near power lines than to physical factors. Indeed, the reported data speak for a change in population distribution around power lines. This study raises important questions about the importance of stability of residences for epidemiological studies of localized exposures, but overall speaks in favor of a relationship between ELF MF and childhood leukemia.

Page124-125:

SCENIHR mentioned in their previous report (2009) two studies that addressed the issue of survival from childhood leukemia and exposure to ELF MF. These studies reported poorer survival at increased levels of exposure (above 0.2/0.3 μT). In 2012 Schüz et al. reported results of a pooled study including data from 6 countries. This pooled analysis reported somewhat increased hazard ratios at moderately increased average exposure levels up to 0.3 μT but no increased hazard ratios above 0.3 μT. This study has been mentioned in the new SCENIHR report but without further discussion of its implications. Although the study included more than 3000 cases the small number of children at elevated exposure levels and the lack of follow-up data on post diagnostic exposure for most of the cases prohibit far reaching conclusions.

Page 126-131:

SCENIHR provides a brief overview of *in vitro* and *in vivo* animal studies of ELF MF exposure and endpoints relevant for the issue of potential mechanisms of a relationship between ELF-MF and neoplastic diseases. While the presentation encompasses all publications of relevance since the last report it again lacks a discussion of the difficulties of such studies and the very small likelihood to detect an effect of exposure due to the lack of a profound biophysical mechanism as a starting point.

In conclusion, the preliminary opinion of SCENIHR concerning ELF MF covers the relevant literature and no essential omission has been detected. However, it is recommended to not separate the findings from previous reports but to assess the evidence as a whole. Furthermore, it appears that SCENIHR does not sufficiently challenge the validity especially of studies that did not find an effect of exposure.

Contributed by Prof. Michael Kundi, PhD med habil Institute of Environmental Health, Medical University of Vienna, Vienna, Austria

11. RFR Studies Support a Finding of 'Probable' or 'Known' Human Carcinogen

A recent publication by Hardell and Carlberg reports that "(*F)urther research has thus strengthened the evidence in support of an increased risk of malignant brain tumours and acoustic neuroma associated with use of mobile phones. Based on the latest findings and using the so called Hill viewpoints from the 1960's exposure to RF-EMF from mobile phones may now be classified as a human cancer causing agent, Group 1, according to the definitions used by IARC*."

Hardell L, Carlberg M. Using the Hill viewpoints from 1965 for evaluating strengths of evidence of the risk for brain tumors associated with use of mobile and cordless phones. *Rev Environ Health* 2013;38:97-106. doi: 10.1515/reveh-2013-0006**.**

There is credible scientific evidence that RF exposures cause changes in cell membrane function, metabolism and cellular signal communication, as well as activation of proto-oncogenes and triggering of the production of stress proteins at exposure levels thousands of times below current regulatory limits. There is also generation of reactive oxygen species, which cause single- and double-strand DNA damage, chromosomal aberrations and nerve cell death. A number of different effects on the central nervous system have also been documented, including activation of the endogenous opioid systems, changes in brain function including memory loss, slowed learning, motor dysfunction and performance impairment in children, and increased frequency of headaches, fatigue and sleep disorders. Melatonin secretion is reduced, resulting in altered circadian rhythms and disruption of several physiological functions. See Chapters 1, 5–12 of the 2007 BioInitiative Report [1], [2-6] and Chapters 1, 5-24 of the 2012 BioInitiative Report [7]. These effects can reasonably be presumed to result in adverse health effects and disease with chronic and uncontrolled exposures, and children may be particularly vulnerable [1,19] . The young are also largely unable to remove themselves from such environments. Second-hand non-ionizing radiation, like second-hand smoke may be considered a public health concern based on the evidence at hand.

Exposure to electromagnetic fields (both extremely low-frequency ELF-EMF from power frequency sources like power lines and appliances; and radiofrequency radiation or RFR) has

been linked to a variety of adverse health outcomes that may have significant public health consequences. The most serious health endpoints that have been reported to be associated with extremely low frequency (ELF) and/or radiofrequency radiation (RFR) include childhood and adult leukemia, childhood and adult brain tumors, and increased risk of the neurodegenerative diseases, Alzheimer's and amyotrophic lateral sclerosis (ALS). In addition, there are reports of increased risk of breast cancer in both men and women, genotoxic effects (DNA damage, chromatin condensation, micronucleation, impaired repair of DNA damage in human stem cells), pathological leakage of the blood–brain barrier, altered immune function including increased allergic and inflammatory responses, miscarriage and some cardiovascular effects. Insomnia (sleep disruption) is reported in studies of people living in very low-intensity RF environments with WI-FI and cell tower-level exposures. Short-term effects on cognition, memory and learning, behavior, reaction time, attention and concentration, and altered brainwave activity (altered EEG) are also reported in the scientific literature. Biophysical mechanisms that may account for such effects can be found in various articles and reviews. [2-7]

The BioInitiative Working Group concluded in 2007 that existing public safety limits were inadequate to protect public health, and agreed that new, biologically-based public safety limits were needed more than five years ago. The 2007 BioInitiative Report was been prepared by more than a dozen world-recognized experts in science and public health policy; and outside reviewers also contributed valuable content and perspective.

From a public health standpoint, experts reasoned that it was not in the public interest to wait. In 2007, the evidence at hand coupled with the enormous populations placed at possible risk was argued as sufficient to warrant strong precautionary measures for RFR, and lowered safety limits for ELF-EMF. The ELF recommendations were biologically-based and reflected the ELF levels consistently associated with increased risk of childhood cancer, and further incorporated a safety factor that is proportionate to others used in similar circumstances. The public health cost of doing nothing was judged to be unacceptable in 2007.

12. Plausible Biological Mechanisms are Known

Oxidative stress through the action of free radical damage to DNA is a plausible biological mechanism for cancer and diseases that involve damage from ELF to the central nervous system.

Plausible biological mechanisms are already identified that can reasonably account for most biological effects reported for exposure to RF and ELF at low-intensity levels (oxidative stress and DNA damage from free radicals leading to genotoxicity; molecular mechanisms at very low energies are plausible links to disease, e.g., effect on electron transfer rates linked to oxidative damage, DNA activation linked to abnormal biosynthesis and mutation). It is also important to remember that traditional public health and epidemiological determinations do not require a proven mechanism before inferring a causal link between EMFs exposure and disease. Many times, proof of mechanism is not known before wise public health responses are implemented.

"Obviously, melatonin's ability to protect DNA from oxidative damage has implications for many types of cancer, including leukemia, considering that DNA damage due to free radicals is believed to be the initial oncostatic event in a majority of human cancers [Cerutti et al., 1994]. In addition to cancer, free radical damage to the central nervous system is a significant component of a variety of neurodegenerative diseases of the aged including Alzheimer's disease and Parkinsonism. In experimental animal models of both of these conditions, melatonin has proven highly effective in forestalling their onset, and reducing their severity." [9]

The De Iuliis et al study which is quoted by the SCENIHR committee with respect to both genotoxicity and oxidative stress, and to sperm motility damage discusses that oxidative damage is a plausible mechanism for these effects.

"Oxidative stress has been known for some time to limit the fertilizing potential of human spermatozoa through the induction of peroxidative damage to the sperm plasma membrane [13,20]. Oxidative stress is also known to be associated with DNA damage in human spermatozoa [21]. Furthermore, the source of the free radicals responsible for generating such stress appears to be the mitochondria [15]. However, the factors responsible for inducing the mitochondria to leak electrons and propagate the production of ROS have not been elucidated. The research described in this article suggests that one of the key environmental factors involved in the stimulation of sperm mitochondria to produce high levels of ROS, might be excess exposure to RF-EMR from sources such as mobile phones."

See also Exhibit C: Reference List for Important Fertility and Reproduction Papers

13. Consistent Failure to Identify the Potential for Health Effects (Opinion-wide)

The evaluative language quoted below indicates the disparity between what was asked of the authors (to identify Possible Effects of EMF) and what they eventually chose to use as a basis for their analysis process that no change in the ICNIRP standards is warranted at this time (see Exhibit A).

> SIXTEEN (16) instances of "no causal evidence" or "prevents a causal interpretation" or "is not causally linked" or "not informative for causal linkage".
>
> THREE (3) instances of "does not provide convincing evidence".
>
> THREE (3) instances of "not definitive".
>
> SEVEN (7) instances of "do not unequivocally indicate".

These criteria are inconsistent with a review that is titled "Possible Effects". Further, the approach in judging the emerging evidence is inconsistent with the charter of the Scientific Committee* to give advice needed for "*consumer safety, public health and the environment on new or emerging problems.*" Some statements acknowledge important new evidence of effect; yet then shift the burden of proof to a higher level requiring that adverse health effect, a known mechanism, a causal level of evidence be conclusively demonstrated, or physical evidence of harm be demonstrated. There is nothing in the report that says the authors were directed to provide proof of effect (or consistent indications, or consistent demonstration of effect; or consistent support for, or certainty of effects) at levels below ICNIRP limits. With the same flawed approach in drawing conclusions from emerging science as demonstrated by the SCENIHR, hardly any environmental or occupational condition would be qualified as an emerging or newly identified health risk*.

*Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention

to the new or emerging problems which may pose an actual or potential threat. They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the **Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)** and are made up of external experts.

Preparation of this review has been completed with generous donations of the time and resources of authors of the BioInitiative Working Group.

Qualifications of the BioInitiative 2012 Working Group

The 2012 BioInitiative Report was prepared by 29 authors from ten countries, ten holding medical degrees (MDs), 21 PhDs, and three MsC, MA or MPHs. Among the authors are three former Presidents of the Bioelectromagnetics Society and five full members of BEMS. One distinguished author is the Chair of the Russian National Committee on Non-Ionizing Radiation. Three were members of the 2011 IARC Working Group that established RFR as a Group 2B Possible Human Carcinogen (Hardell, Belyaev and Blackman). Another was until recently a Senior Advisor on Science, Policy, Emerging Issues, Integrated Environmental Assessment to the European Environmental Agency. Full titles and affiliations of authors is in Section 25 of the BioInitiative Report at www.bioinitiative.org. See specific conclusions and findings of the BioInitiative 2012 Report at www.bioinitiative.org. It is incorporated by reference in this comment.

In twenty-four technical chapters, the BioInitiative Working Group authors discuss the content and implications of about 1800 new studies since 2007. Overall, these new studies report abnormal gene transcription (Section 5); genotoxicity and single-and double-strand DNA damage (Section 6); stress proteins because of the fractal RF-antenna like nature of DNA (Section 7); chromatin condensation and loss of DNA repair capacity in human stem cells (Sections 6 and 15); reduction in free-radical scavengers - particularly melatonin (Sections 5, 9, 13, 14, 15, 16 and 17); neurotoxicity in humans and animals (Section 9); carcinogenicity in humans (Sections 11, 12, 13, 14, 15, 16 and 17); serious impacts on human and animal sperm morphology and function (Section 18); effects on the fetus, neonate and offspring (Section 18 and 19); effects on brain and cranial bone development in the offspring of animals that are exposed to cell phone radiation during pregnancy (Sections 5 and 18); and findings in autism spectrum disorders consistent with EMF/RFR exposure effects. Global precautionary actions that have been taken in countries around the world, and recommended by medical and research experts are documented in Section 22. Use of the Precautionary Principal and it's relevance are presented in Section 23. Key scientific evidence and public health policy recommendations are in Section 24.

Respectfully submitted on behalf of the BioInitiative Working Group by:

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BioInitiative Working Group; Consistent Failure to Identify the Potential for Health Effects (Exhibit A); 2014

Exhibit A: Consistent Failure to Identify the Potential for Health Effects (Opinion-wide)

The evaluative language quoted below indicates the disparity between what was asked of the authors (to identify Possible Effects of EMF) and what they eventually chose to use as a basis for their analysis process that no change in the ICNIRP standards is warranted at this time.

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All of the areas highlighted in yellow in the preliminary Opinion indicate problems (omissions, mischaracterizations of exposure data leading to erroneous conclusions about possible public health risks, misreading of original study results, dismissal of important findings, need for a known mechanism, and failure to use proper criteria for judging potential for health effects as opposed to causal effects).

There is a serious consequence which comes from dismissing effects linking EMF/RFR exposures reported in scientific studies to an 'all or none' finding by using embedded criteria that demand 'causal' or 'conclusive' or 'definitive' or 'consistent demonstration of effect'. It is clear that such erasing possible impacts of great global health consequence will chill public health responses that would otherwise occur if the correct standards for judging the evidence were used in this Opinion. Public health activities hinge on not causality but sufficiency of evidence to warrant a proportionate preventative action in line with established precautionary principles. This draft Opinion provides no guidance in this area.

Since the charge to the Scientific Committee is to evaluate the possible health effects (not to prove beyond a shadow of a scientific doubt the causality of such exposures to health harm), the Opinion needs complete re-working. It may be also that the Committee needs new membership capable of a different, and more appropriate approach to the important assessment that SCENIHR is charged to prepare. Page and line numbers are included to key to the Opinion sections.

Page 5: Health effects from Extremely Low Frequency (ELF) fields
12 The new epidemiological studies are consistent with earlier findings of an increased risk
13 of childhood leukemia with long-term average exposure to magnetic fields above 0.3 to
14 0.4 μT. However, as stated in the previous opinions, no mechanisms have been identified
15 that could explain these findings. The lack of experimental support and shortcomings
16 identified for the epidemiological studies prevent a causal interpretation.

Page 12-13: Health effects from RF fields
42 Epidemiological studies on RF exposure do not unequivocally indicate an increased risk of
43 brain tumours

20-22 However, research conducted since the previous SCENIHR opinion adds weight to the conclusion that RF exposure is not causally linked to these symptoms,

Page 13 Health effects from ELF fields
49 The new epidemiological studies are consistent with earlier findings of an increased risk
50 of childhood leukemia with daily average exposure above 0.3 to 0.4 μT. As stated in the
51 previous SCENIHR opinions, no mechanisms have been identified in experimental studies
52 that could explain these findings. Due to lack of support from experimental data and shortcomings in the epidemiological studies, evidence remains weak that the observed association reflects a causal effect.

For symptoms associated with longer-term exposures (measured in days to months), the
30 evidence from observational studies against a causative association with RF exposure is
31 broadly consistent but has gaps, most notably in terms of the objective monitoring of
32 exposure.

Page 58
24-28 They reported higher incidence rates of brain cancers in countries with the most frequent mobile phone subscriptions. The study is not informative for causal inference, as popular use of mobile phones can also reflect standard of living, which is also associated with, for example, availability of diagnostic services.

Page 65-66 Discussion of brain tumours and other tumours of the head and neck area
5-7 For the segment of the heaviest users, the largest case-control study in particular observed about 40% increased risks for glioma and for acoustic neuroma. It cannot be concluded from the available studies whether this reflects a causal association.

Here, the conclusion that there might legitimately be causal evidence for increased risk for brain tumors with cell phone use but it no longer matters, because, indeed, technologies might change in the future. This is a preposterous statement. It has the impact of trivializing the issue, minimizing identified risks and leaping to an irrational conclusion that negates any need for the Scientific Committee to advise caution.

19-25 Therefore, the increased risks seen in heavy users in the case-control studies, mainly driven by technologies not in operation anymore or operating more efficiently today, could perhaps not be due to methodological shortcomings but indeed reflect a causal association. This finding might be irrelevant for any future cancer prevention activities since those relevant cumulative RF exposure levels

are not reached anymore, not even among those using mobile phones for longer duration or much more often than the users of the 1980s or 1990s.

Pages 114-115 *Provocation Studies*
The fact that these effects disappear once blinding is used and the participant is therefore unaware of the exposure suggests first, that no casual (causal) *effect of RF exposure exists and second, that believing RF 48 to be present is sufficient to induce symptoms via a nocebo effect. While further work using this paradigm would be beneficial, at present these studies suggest there is no causal link between exposure and symptoms.*

Page 123 *3.7. Health effects from ELF fields*
22 3.7.1. Neoplastic diseases
23 3.7.1.1. Epidemiological studies
24 What was already known on this subject?
25 The previous SCENIHR statement endorsed the IARC assessment of classifying ELF
26 magnetic fields as possibly carcinogenic to humans due to consistently observed
27 increased childhood leukaemia risk in epidemiological studies (SCENIHR, 2009); the
28 latter stems mainly from two pooled analyses based on studies completed before the
29 year 2000, showing a two-fold risk increase with ELF magnetic fields above 0.3-0.4 μT
30 (time-weighted average) but raising concerns about shortcomings of those studies
31 preventing a causal interpretation (Ahlbom et al., 2000; Greenland et al., 2000).

Page 125 *Discussion on epidemiological studies*
27 Pooled analyses of the more recent studies on ELF magnetic fields and childhood
28 leukaemia confirm those of earlier studies, however, the new generation of studies shows
29 little methodological advancement compared to the ones conducted before 2000.
30 Therefore it remains difficult to judge whether the apparently quite robust empirical
31 association is likely to be causal or a result of methodological shortcomings of the
32 studies.

Page 125 *Conclusions on epidemiological studies*
42 The previous assessment of the 2009 SCENIHR statement of a possible association
43 between long term exposure to ELF magnetic fields and an increased risk of childhood
44 leukaemia remains valid. From an epidemiological point of view, the association appears
45 to be robust, having been observed in multiple studies in different settings at different
46 points in time. Unfortunately, little progress has been made in explaining the finding,
47 both in terms of finding a plausible mechanism for a causal association or in identifying
48 alternative explanations.

Page 131 *3.7.1.4. Conclusions on neoplastic diseases*
18 The new epidemiological studies are consistent with earlier findings of an increased risk
19 of childhood leukemia with daily average exposure above 0.3 to 0.4 μT. As stated in the
20 previous opinions, no mechanisms have been identified in experimental studies that
21 could explain these findings. Lack of support from experimental studies and shortcomings
22 of the epidemiological studies prevent a causal interpretation.

Page 141 *3.7.3.1 Conclusions on Symptoms*
The 2009 opinion concluded that no consistent relationship had been demonstrated between ELF exposure and symptoms, neither in the general public nor in people with IEI-EMF.

Page *142* *Conclusions on symptoms 3.7.3 Other Health Effects*
49 The studies published since the 2009 opinion show discordant results. However,
50 observational studies suffered from weaknesses and do not provide convincing evidence
51 of an effect of ELF exposure on symptoms in the general population and most
52 experimental evidence also points to the absence of any causal effect.

Page 144-145 *Neoplastic diseases*
48 The new epidemiological studies are consistent with earlier findings of an increased risk
49 of childhood leukemia with daily average exposures above 0.3 to 0.4 μT. As stated in the
50 previous opinions, no mechanisms have been identified in experimental studies that
1 could explain these findings. Lack of support from experimental studies and shortcomings
2 of the epidemiological studies prevent a causal interpretation.

Page 170 *3.13. Research recommendations*
44 Research to date has not been able to identify with any certainty any adverse health
45 effect resulting from exposure to EMFs at any frequency or intensity typically found in the
46 workplace or everyday environment. Epidemiological studies have reported associations
47 between EMF exposure and certain diseases, most notably for an increased risk of
48 childhood leukaemia with exposure to low frequency magnetic fields, but none of these
49 associations can be considered causal

Page 176. *3.14. Guidance on research methods*
Indeed, organ-specific dosimetry is considered necessary to help to establish causality.

Page 177 - 178
To give particular attention to issues affected by important gaps in knowledge in the previous opinions, especially:

35 2a. the potential adverse effects of EMF on the nervous system, including neurobehavioural disorders and on the risk of neo-plastic diseases;

37 RF fields
38 Previous studies suggesting that RF exposure may affect brain activities as reflected by
39 changes in the EEG during wake and sleep are further substantiated by the results of
40 more recent studies. However, given the variety of applied fields, duration of exposure,
41 number of considered leads, and statistical methods it is difficult to derive firm
42 conclusions. For event-related potentials and slow brain oscillations results are
43 inconsistent. Likewise, studies on cognitive functions in humans lack consistency. The
44 biological relevance of reported small physiological EEG changes remains unclear, and
45 mechanistic explanation is still lacking.
46 A reasonable body of experimental evidence now suggests that exposure to RF does not
47 trigger symptoms, at least in the short-term. While additional observational studies are
48 required to assess whether longer-term exposure could be associated with symptoms,
49 the evidence to date weighs against a causal effect.

2 Studies on neurological diseases and symptoms show no clear effect, but the evidence is
3 limited. Human studies on child development and behavioural problems provide only
4 weak evidence because of conflicting results and methodological limitations. Direct
5 effects of exposure from mother's mobile phone use during pregnancy are not plausible
6 owing to extremely low fetal exposure to mobile phone EMF.
7 Epidemiological studies on RF exposure do not unequivocally indicate an increased risk of
8 brain tumours, and do not indicate an increased risk for other cancers of the head and
9 neck region, or other malignant diseases including childhood cancer. Earlier studies
10 raised open questions regarding an increased risk of glioma and acoustic neuroma in
11 heavy long-term users of mobile phones. Based on the most recent cohort and incidence
12 time trend studies, the evidence for glioma became weaker while the possibility of an
13 association with acoustic neuroma remains open.
14 A considerable number of well-performed in vivo studies using a wide variety of animal
15 models have been mostly negative in outcome. These studies are considered to provide
16 evidence for the absence of a carcinogenic effect.
17 A large number of in vitro studies pertaining to genotoxic as well as non-genotoxic end
18 points have been published since the last opinion. In most of the studies, no effects of

19 exposure at levels below exposure limits were recorded, although in some cases DNA
20 strand breaks and spindle disturbances were observed.

Page 178 ELF fields
The new epidemiological studies are consistent with earlier findings of an increased risk
41 of childhood leukemia with long-term daily average exposures above 0.3 to 0.4 μT. As
42 stated in the previous opinions, no mechanisms have been identified and no support is
43 existing from experimental studies that could explain these findings, which, together
44 with shortcomings of the epidemiological studies prevent a causal interpretation.

BioInitiative Working Group; Reference List for Important Fertility and Reproduction Papers (Exhibit C); 2014

Exhibit C: Reference List for Important Fertility and Reproduction Papers

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Exhibit B-1
RF Color Charts for Fertility and Reproduction Studies with Exposures

PUBLISHED STUDIES RELEVANT TO SCENIHR REVIEW OF EMF – FERTILITY AND REPRODUCTION
C. SAGE, BIOINITIATIVE WORKING GROUP, APRIL 2014

Agarwal A, Deepinder F, Sharma RK, Ranga G, Li J. 2008. Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. Fertil Steril. 89(1):124-8.

1.0 W/Kg	Motility, sperm count, sperm morphology, and viability reduced in active cell phone users (human r manner.

Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R. 2009. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. Fertil Steril. 92(4) 1318-1325.

1.0 W/Kg	Motility, sperm count, sperm morphology, and viability reduced in active cell phone users (human r manner.

Aitken RJ, Bennetts LE, Sawyer D, Wiklendt AM, King BV. 2005 Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline 28:171-179.

0.09 W/Kg	900 MHz study of mice for 7 days, 12-hr per day (whole-body) resulted in significant effect on mito

Aldad TS Gan G Gao XB Taylor HS. 2012. Fetal radiofrequency radiation exposure from 800-1900 MHz rated cellular telephones affects neurodevelopment and behavior in mice. Sci. Rep. 2, 312. DOI: 10.1038/srep00312

0.0003 - 0.06 W/Kg	Neurobehavioral disorders in offspring of pregnant mice exposed in utero to cell phones - dose-respo synaptic transmission onto layer V pyramidal neurons of the prefrontal cortex. Hyperactivity and im offspring. Altered brain development.

Atasoy HI, Gunal MY, Atasoy P, Elgun S, Bugdayci G. 2012 Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices. J Pediatr Urol. [Epub ahead of print]

0.091 W/Kg	Wireless internet 2400 MHz, 24-hrs per day/20 weeks increased DNA damage and reduced DNA re Authors say "findings raise questions about safety of radiofrequency exposure from Wi-Fi internet acc organisms of reproductive age, with a potential effect on fertility and integrity of germ cells" (male g cells=sperm)

Avendano C, Mata A, Sanchez Sarmiento CA, Doncei GF. 2012. Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. Fertility and Sterility. American Society for Reproductive Medicine, Published by Elsevier Inc. doi:10.1016/j.fertnstert.2011.10.012.

0.5 - 1.0 uW/cm2	Wi-FI level laptop exposure for 4-hr resulted in decrease in sperm viability, DNA fragmentation wit dishes under a laptop connected via WI-FI to the internet.

Behari J, Kesari KK 2006. Effects of microwave radiations on reproductive system of male rats. Embryo Talk 1 (Suppl.1):81-5.

0.00034 uW/cm2	Chronic exposure to mobile phone pulsed RF significantly reduced sperm count,

Dasdag, S et al, 1999. Whole-body microwave exposure emitted by cellular phones and testicular function of rats. Urological Research 27(3):219-223.

0.141 W/Kg	Structural changes in testes - smaller diameter of seminiferous

De Iuliis GN, Newey RJ, King BV, Aitken RJ. 2009. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4(7):e6446.

0.4 - 1.0 W/Kg	One 6-hr exposure to 1800 MHz cell phone radiation in human sperm cells caused a significant dos motility and viability; reactive oxygen species levels were significantly increased after exposure to 1. detrimental effects of RF/MW to human sperm. The authors conclude "(T)hese findings have clear im extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and their offspring."

De Iuliis GN, Newey RJ, King BV, Aitken RJ. 2009. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4(7):e6446.

1.0 W/Kg	Human semen degraded by exposure to cell phone frequency RF increased free-radical damage.

Forgács Z, Somosy Z, Kubinyi G, Bakos J, Hudák A, Surján A, Thuróczy G. Effect of whole-body 1800 MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice. Reprod Toxicol. 2006; Jul;22(1):111-7.

45 uW/cm2	Pulsed RFR affected serum testosterone levels in mice

A.F. Fragopoulou, et al., Cranial and postcranial skeletal variations induced in mouse embryos by mobile phone radiation, Pathophysiology (2009), doi:10.1016/j.pathophys.2009.10.002

0.6 - 0.9 W/Kg	Mouse embryos develop fragile cranial bones from in utero 900 MHz The authors say "(O)ur results modest exposure (e.g., 6 min daily for 21 days" is sufficient to interfere with the normal mouse deve

Gul A, Celebi H, Ugras S. The effects of microwaves emitted by cellular phones on ovarian follicles in rats. Archives of Gynecology

< 1.0 W/Kg	Rats exposed to mobile phone radiation on STANDBY ONLY for 11-hr 45-min plus 15-min TRANSMI 21 days showed decreased number of ovarian follicles in pups born to these pregnant rats. The auth number of follicles in pups exposed to mobile phone microwaves suggest that intrauterine exposure h

Kumar S Behari J Sisodia R. 2012. Impact of Microwave at X-Band in the aetiology of

male infertility. Electromagnetic Biology and Medicine, 31(3): 223–232. online DOI: 10.3109/15368378.2012.700293.

0.014 W/Kg	Sperm damage from oxidative stress and lowered melatonin levels resulted from 2-hr per day/45 d

Magras, IN & Zenos, TD, 1997. RF Radiation-induced changes in the prenatal development of mice. Bioelectromagnetics 18:455-461.

0.168 - 1.053 uW/cm2	Irreversible infertility in mice after 5 generations of exposure to RFR from an 'antenna park'

Navakatikian, MA & Tomashevskaya, LA, 1994 Phasic behavioral and endocrine effects of microwaves of nonthermal intensity. In: Biological Effects of Electric and Magnetic Fields, Volume 1, Carpenter, DO, (Ed.) Academic Press, Inc., San Diego, CA., pp. 333-342.

100 uW/cm2	A 24.3% drop in testosterone after 6 hours of CW RFR exposure

Otitoloju AA, Obe IA, Adewale OA, Otubanjo OA, Osunkalu VO. 2010. Preliminary study on the induction of sperm head abnormalities in mice, Mus musculus, exposed to radiofrequency radiations from global system for mobile communication base stations. Bulletin of Environmental Contamination and Toxicology 84(1):51-4.

0.07 - 0.1 uW/cm2	Sperm head abnormalities in mice exposed for 6-months to base station level RF/MW. Sperm head to 46% exposed mice (only 2% in controls) abnormalities was also found to be dose dependent. The banana-shaped sperm head. The occurrence of sperm head observed increase occurrence of sperm reproductive health of humans living in close proximity to GSM base stations were discussed."

Panagopoulos DJ. 2012. Effect of microwave exposure on the ovarian development of Drosophila melanogaster. Cell Biochem Biophys. 63(2):121-132.

0.795 W/Kg	GSM 900 MHz, 217 Hz significantly decreases ovarian development and size of ovaries, due to DNA death of nurse cells and follicles in ovaries (that nourish egg cells)

Salama N, Kishimoto T, Kanayama HO. Effects of exposure to a mobile phone on testicular function and structure in adult rabbit. International Journal of Andrology 2010;33(1):88-94.

0.43 W/Kg	Significant decrease in sperm mobility; drop in sperm concentration; and decrease in seminiferous 12 weeks, with mobile phone radiation level on STANDBY ONLY (in rabbits)

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

60 uW/cm2	RFR caused structural changes in cells of mouse embryos

Chart References

Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R (2009). Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. Fertil Steril, 92(4), 1318-25.

Al-Damegh MA (2012). Rat testicular impairment induced by electromagnetic radiation from a conventional cellular telephone and the protective effects of the antioxidants vitamins C and E. Clinics (Sao Paulo), 67(7), 785-92.

Kesari KK, Behari J (2102). Evidence for mobile phone radiation exposure effects on reproductive pattern of male rats: role of ROS. Electromagn Biol Med, 31(3), 213-22.
Kesari KK, Kumar S, Behari J (2010). Mobile phone usage and male infertility in Wistar rats. Indian J Exp Biol, 48 (10), 987-92.

Kesari KK, Kumar S, Behari J (2011). Effects of radiofrequency electromagnetic wave exposure from cellular phones on the reproductive pattern in male Wistar rats. Appl Biochem Biotechnol, 164(4), 546-59. Health effects of EMF – 2013-11-29 218

Otitoloju AA, Obe IA, Adewale OA, Otubanjo OA, Osunkalu VO (2010). Preliminary study on the induction of sperm head abnormalities in mice, Mus musculus, exposed to radiofrequency radiations from global system for mobile communication base stations. Bull Environ Contam Toxicol. 84(1), 51-4.

Ribeiro EP, Rhoden EL, Horn MM, Rhoden C, Lima LP, Toniolo L (2007). Effects of subchronic exposure to radio frequency from a conventional cellular telephone on testicular function in adult rats. J Urol, 177(1), 395-9.

Salama N, Kishimoto T and Kanayama HO (2010a). Effects of exposure to a mobile phone on testicular function and structure in adult rabbit. Int J Androl, 33(1), 88-94.

Salama N, Kishimoto T, Kanayama HO and Kagawa S (2009). The mobile phone decreases fructose but not citrate in rabbit semen: a longitudinal study. Syst Biol Reprod Med, 55(5-6), 181-7.

Salama N, Kishimoto T, Kanayama HO and Kagawa S (2010b). Effects of exposure to a mobile phone on sexual behavior in adult male rabbit: an observational study. Int J Impot Res, 22(2), 127-33.

BioInitiative Working Group; Mitochondrial Dysfunction and Disruption of Electrophysiology (Exhibit G); 2014

Exhibit G: Mitochondrial Dysfunction and Disruption of Electrophysiology

Mitochondria are broadly vulnerable, in part because the integrity of their membranes is vital to their optimal functioning – including channels and electrical gradients, and their membranes can be damaged by free radicals which can be generated in myriad ways. Moreover, just about every step in their metabolic pathways can be targeted by environmental agents, including toxicants and drugs, as well as mutations [1]. This supports a cumulative allostatic load model for conditions in which mitochondrial dysfunction is an issue, which includes autism as well as myriad other chronic conditions.

Mitochondria are commonly discussed in terms of the biochemical pathways and cascades of events by which they metabolize glucose and generate energy. But in parallel with this level of function there also appears to be a dimension of electromagnetic radiation that is part of the activity of these organelles. For example, electromagnetic radiation can be propagated through the mitochondrial reticulum, which along with the mitochondria has a higher refractive index than the surrounding cell and can serve to propagate electromagnetic radiation within the network [2]. [2]. It is also the case that *"The physiological domain is characterized by small-amplitude oscillations in mitochondrial membrane potential (delta psi(m)) showing correlated behavior over a wide range of frequencies…. Under metabolic stress, when the balance between ROS [reactive oxygen species, or free radicals] generation and ROS scavenging [as by antioxidants] is perturbed, the mitochondrial network throughout the cell locks to one main low-frequency, high-amplitude oscillatory mode. This behavior has major pathological implications because the energy dissipation and cellular redox changes that occur during delta psi(m) depolarization result in suppression of electrical excitability and Ca2+ handling…"* [3]. These electromagnetic aspects of mitochondrial physiology and pathophysiology could very well be impacted by EMF/RFR.

Other types of mitochondrial damage have been documented in at least some of the studies that have examined the impacts of EMF/RFR upon mitochondria. These include reduced or absent mitochondrial cristae [4-6], mitochondrial DNA damage [7], swelling and crystallization [5], alterations and decreases in various lipids suggesting an increase in their use in cellular energetics [8], damage to mitochondrial DNA [7], and altered mobility and lipid peroxidation after exposures [9]. Also noted has been enhancement of brain mitochondrial function in Alzheimer's transgenic mice and normal mice [10]. The existent of positive as well as negative effects gives an indication of the high context dependence of exposure impacts, including physical factors such as frequency, duration, and tissue characteristics [11].

Secondary mitochondrial dysfunction (i.e. environmentally triggered rather than rooted directly in genetic mutations) [15-18] could result among other things from the already discussed potential for EMF/RFR to damage channels, membranes and mitochondria themselves as well as from toxicant exposures and immune challenges. In a meta-analysis of studies of children with ASC and mitochondrial disorder, the spectrum of severity varied, and 79% of the cases were identified by laboratory findings without associated genetic abnormalities [16].

Electrophysiology

Nervous system electrophysiology when disrupted by ELF-EMF and RFR can produce alterations in molecular, cellular and systems physiological function. It occurs in the brain as well as in the body, and impacts the transduction into the electrical signaling activities of the brain and nervous

system. If the cells responsible for generating synapses and oscillatory signaling are laboring under cellular and oxidative stress, lipid peroxidation, impaired calcium and other signaling system abnormalities, then mitochondrial metabolism will fall short, all the more so because of the challenges from the immune system which in turn be triggered to a major extent by environment. How well will synaptic signals be generated? How well will immune-activated and thereby distracted glial cells be able to modulate synaptic and network activity? [19-22] Microglial activation can impact excitatory neurotransmission mediated by astrocytes [23]. Cortical innate immune response increases local neuronal excitability and can lead to seizures [24,25]. Inflammation can play an important role in epilepsy [26].

Seizures and epilepsy

Epileptic seizures can be both caused by and cause oxidative stress and mitochondrial dysfunction. Seizures can cause extravasation of plasma into brain parenchyma [27-31]. which can trigger a vicious circle of tissue damage from albumin and greater irritability, as discussed above. Evidence suggests that if a BBB is already disrupted, there will be greater sensitivity to EMF/RFR exposure than if the BBB were intact [32,33], suggesting that such exposures can further exacerbate vicious circles already underway. The combination of pathophysiological and electrophysiological vulnerabilities has been explored in relation to the impact of EMF/RFR on people with epilepsy EMF/RFR exposures from mobile phone emissions have been shown to modulate brain excitability and to increase interhemispheric functional coupling [34,35]. In a rat model the combination of picrotoxin and microwave exposure at mobile phone-like intensities led to a progressive increase in neuronal activation and glial reactivity, with regional variability in the fall-off of these responses three days after picrotoxin treatment [36], suggesting a potential for interaction between a hyperexcitable brain and EMF/RFR exposure.

One critical issue here is nonlinearity and context and parameter sensitivity of impact. In one study, rat brain slices exposed to EMF/RFR showed reduced synaptic activity and diminution of amplitude of evoked potentials, while whole body exposure to rats led to synaptic facilitation and increased seizure susceptibility in the subsequent analysis of neocortical slices [37]. Another study unexpectedly identified enhanced rat pup post-seizure mortality after perinatal exposure to a specific frequency and intensity of exposure, and concluded that apparently innocuous exposures during early development might lead to vulnerability to stimuli presented later in development [38].

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BioInitiative Working Group; Epidemiological Studies,
RF fields epidemiology, Comments by Drs. Lennart Hardell,
Fredrik Soderqvist PhD. and Michael Carlberg MSc. Section 3.5.1.1
Epidemiological Studies (Exhibit B); 2014

Exhibit B : Comment by Drs. Lennart Hardell, Fredrik Soderqvist, PhD and Michael Carlberg, MSc.

Section 3.5.1.1 Epidemiological Studies, RF fields epidemiology, *Pages 57-68*

We have read the SCENIHR 2013 Preliminary opinion on Potential health effects of exposure to electromagnetic fields (EMF), especially relating to epidemiological studies on neoplastic diseases. It is concluded at page 4 in the abstract that "*Based on the most recent cohort and incidence time trend studies, it appears that the evidence for glioma became weaker while the possibility of an association with acoustic neuroma remains open*".

This statement is not based on facts but on selective inclusion of studies with omission of the most recent publications, e.g. from our research group (the Hardell group). Our studies were well known to the Expert group since Dr Kjell Hansson Mild was one of these experts and also a co-author in most of the Hardell group studies. In fact he communicated our studies to the SCENIHR expert group obviously without response. If these studies had been included it would be apparent that the final conclusions on brain tumour risk in SCENIHR are not based on scientific facts. In contrast the evidence for glioma and acoustic neuroma would become stronger if recent publications had been included.

In the Terms of Reference (page 16) it is stated that the Committee is requested e.g.:

1. To update its opinions of 2009 in the light of newly available information
2. To give particular attention to issues affected by important gaps in knowledge in the previous opinions, especially:
    - The potential adverse effects of EMF on the nervous system, including neurobehavioral disorders, and on the risk of neo-plastic diseases;

It seems as if the Committee has been anxious to include 'newly available information' at least regarding some studies, e.g. **Benson VS, Pirie K, Schüz J, Reeves GK, Beral V, Green J. Int J Epidemiol 2013, Sep 27**, see page 64, not included in reference list. On the contrary our studies were excluded. In the following a summary is given.

Background:

The carcinogenic effect of RF-EMF on humans was evaluated at a meeting during 24 – 31 May 2011 at the International Agency for Research on Cancer (IARC) at WHO in Lyon, France. The Working Group consisted of 30 scientists representing four areas: 'animal cancer studies', 'epidemiology', 'exposure' and 'mechanistic and other relevant data' (http://monographs.iarc.fr/ENG/Meetings/vol102-participants.pdf). One of us, LH, was invited as an expert in the epidemiology group. On 31 May 2011 IARC categorised RF-EMFs from mobile phones, and from other devices that emit similar non-ionising electromagnetic fields, as a Group 2B, i.e. a 'possible', human carcinogen. The decision was almost unanimous.

The IARC decision on mobile phones was based mainly on two sets of case-control human studies on brain tumour risk; our studies from Sweden (the Hardell group) and the IARC Interphone study. Both provided complementary and supportive results on positive associations between two types of brain tumours; glioma and acoustic neuroma, and exposure to RF-EMF from mobile phones. No consistent evidence was found for

meningioma, a benign type of brain tumour. After the IARC meeting we have published further studies with new data, both overview of studies with meta-analysis (number 1 below) and our case-control study including brain tumour cases diagnosed during 2007-2009 (number 2-4 below). Furthermore we applied the Hill viewpoints on the risk for brain tumours associated with use of mobile and cordless phones (number 5 below). These criteria were developed in the 1960's during the height of the tobacco and lung cancer controversy.

Recent studies from the Hardell group not included in SCENIHR 2013:

1. **Hardell L, Carlberg M, Hansson Mild K. Use of mobile phones and cordless phones is associated with increased risk for glioma and acoustic neuroma. *Pathophysiology* 2013;20:85-110. Epub 2012 Dec 21.**

Abstract
The International Agency for Research on Cancer (IARC) at WHO evaluation of the carcinogenic effect of RF-EMF on humans took place during a 24-31 May 2011 meeting at Lyon in France. The Working Group consisted of 30 scientists and categorised the radiofrequency electromagnetic fields from mobile phones, and from other devices that emit similar non-ionising electromagnetic fields (RF-EMF), as Group 2B, i.e., a 'possible', human carcinogen. The decision on mobile phones was based mainly on the Hardell group of studies from Sweden and the IARC Interphone study. We give an overview of current epidemiological evidence for an increased risk for brain tumours including a meta-analysis of the Hardell group and Interphone results for mobile phone use. Results for cordless phones are lacking in Interphone. *The meta-analysis gave for glioma in the most exposed part of the brain, the temporal lobe, odds ratio (OR)=1.71, 95% confidence interval (CI)=1.04-2.81 in the ≥10 years (>10 years in the Hardell group) latency group. Ipsilateral mobile phone use ≥1640h in total gave OR=2.29, 95% CI=1.56-3.37. The results for meningioma were OR=1.25, 95% CI=0.31-4.98 and OR=1.35, 95% CI=0.81-2.23, respectively. Regarding acoustic neuroma ipsilateral mobile phone use in the latency group ≥10 years gave OR=1.81, 95% CI=0.73-4.45. For ipsilateral cumulative use ≥1640h OR=2.55, 95% CI=1.50-4.40 was obtained.* Also use of cordless phones increased the risk for glioma and acoustic neuroma in the Hardell group studies. Survival of patients with glioma was analysed in the Hardell group studies yielding in the >10 years latency period hazard ratio (HR)=1.2, 95% CI=1.002-1.5 for use of wireless phones. This increased HR was based on results for astrocytoma WHO grade IV (glioblastoma multiforme). Decreased HR was found for low-grade astrocytoma, WHO grades I-II, which might be caused by RF-EMF exposure leading to tumour-associated symptoms and earlier detection and surgery with better prognosis. *Some studies show increasing incidence of brain tumours whereas other studies do not. It is concluded that one should be careful using incidence data to dismiss results in analytical epidemiology.* The IARC carcinogenic classification does not seem to have had any significant impact on governments' perceptions of their responsibilities to protect public health from this widespread source of radiation.

2. **Carlberg M, Söderqvist F, Hansson Mild K, Hardell L. Meningioma patients diagnosed 2007-2009 and the association with use of mobile and cordless phones, *Environ. Health* 2013;12:60, doi:10.1186/1476-069X-12-60. Epub Jul 19, 2013**

Abstract
BACKGROUND: To study the association between use of wireless phones and meningioma.
METHODS: We performed a case--control study on brain tumour cases of both genders aged 18--75 years and diagnosed during 2007--2009. One population-based control matched on gender and age was used to each case. Here we report on meningioma cases including all available controls. Exposures were assessed by a questionnaire. Unconditional logistic regression analysis was performed.
RESULTS: In total 709 meningioma cases and 1,368 control subjects answered the questionnaire. *Mobile phone use in total produced odds ratio (OR) = 1.0, 95% confidence interval (CI) = 0.7-1.4 and cordless phone use gave OR = 1.1, 95% CI = 0.8-1.5.* The risk increased statistically significant per 100 h of cumulative use and highest OR was found in the fourth quartile (>2,376 hours) of cumulative use for all studied phone types. There was no statistically significant increased risk for ipsilateral mobile or cordless phone use, for meningioma in the temporal lobe or per year of latency. Tumour volume was not related to latency or cumulative use in hours of wireless phones.
CONCLUSIONS: *No conclusive evidence of an association between use of mobile and cordless phones and meningioma was found*. An indication of increased risk was seen in the group with highest cumulative use but

was not supported by statistically significant increasing risk with latency. Results for even longer latency periods of wireless phone use than in this study are desirable.

3. **Hardell L. Carlberg M, Söderqvist F, Hansson Mild K. Pooled analysis of case-control studies on acoustic neuroma diagnosed 1997-2003 and 2007-2009 and use of mobile and cordless phones*. Int J Oncol.* 2013;43:1036-1044. Epub 2013 Jul 22.**

Abstract
We previously conducted a case-control study of acoustic neuroma. Subjects of both genders aged 20-80 years, diagnosed during 1997-2003 in parts of Sweden, were included, and the results were published. We have since made a further study for the time period 2007-2009 including both men and women aged 18-75 years selected from throughout the country. These new results for acoustic neuroma have not been published to date. Similar methods were used for both study periods. In each, one population-based control, matched on gender and age (within five years), was identified from the Swedish Population Registry. Exposures were assessed by a self-administered questionnaire supplemented by a phone interview. Since the number of acoustic neuroma cases in the new study was low we now present pooled results from both study periods based on 316 participating cases and 3,530 controls. Unconditional logistic regression analysis was performed, adjusting for age, gender, year of diagnosis and socio-economic index (SEI). Use of mobile phones of the analogue type gave odds ratio (OR) = 2.9, 95% confidence interval (CI) = 2.0-4.3, increasing with >20 years latency (time since first exposure) to OR = 7.7, 95% CI = 2.8-21. Digital 2G mobile phone use gave OR = 1.5, 95% CI = 1.1-2.1, increasing with latency >15 years to an OR = 1.8, 95% CI = 0.8-4.2. The results for cordless phone use were OR = 1.5, 95% CI = 1.1-2.1, and, for latency of >20 years, OR = 6.5, 95% CI = 1.7-26. Digital type wireless phones (2G and 3G mobile phones and cordless phones) gave OR = 1.5, 95% CI = 1.1-2.0 increasing to OR = 8.1, 95% CI = 2.0-32 with latency >20 years. *For total wireless phone use, the highest risk was calculated for the longest latency time >20 years: OR = 4.4, 95% CI = 2.2-9.0.* Several of the calculations in the long latency category were based on low numbers of exposed cases. Ipsilateral use resulted in a higher risk than contralateral for both mobile and cordless phones. OR increased per 100 h cumulative use and per year of latency for mobile phones and cordless phones, though the increase was not statistically significant for cordless phones. *The percentage tumour volume increased per year of latency and per 100 h of cumulative use, statistically significant for analogue phones. This study confirmed previous results demonstrating an association between mobile and cordless phone use and acoustic neuroma.*

4. **Hardell L, Carlberg M, Söderqvist F, Hansson Mild K. Case-control study of the association between malignant brain tumors diagnosed 2007-2009 and mobile and cordless phone use. *Int J Oncol.* 2013;43:1833-1845. Epub 2013 Sep 24**

Abstract
Previous studies have shown a consistent association between long-term use of mobile and cordless phones and glioma and acoustic neuroma, but not for meningioma. When used these phones emit radiofrequency electromagnetic fields (RF-EMFs) and the brain is the main target organ for the handheld phone. The International Agency for Research on Cancer (IARC) classified in May, 2011 RF-EMF as a group 2B, i.e. a 'possible' human carcinogen. The aim of this study was to further explore the relationship between especially long-term (>10 years) use of wireless phones and the development of malignant brain tumours. We conducted a new case-control study of brain tumour cases of both genders aged 18-75 years and diagnosed during 2007-2009. One population-based control matched on gender and age (within 5 years) was used to each case. Here, we report on malignant cases including all available controls. Exposures on e.g. use of mobile phones and cordless phones were assessed by a self-administered questionnaire. Unconditional logistic regression analysis was performed, adjusting for age, gender, year of diagnosis and socio-economic index using the whole control sample. Of the cases with a malignant brain tumour, 87% (n=593) participated, and 85% (n=1,368) of controls in the whole study answered the questionnaire. *The odds ratio (OR) for mobile phone use of the analogue type was 1.8, 95% confidence interval (CI)=1.04-3.3, increasing with >25 years of latency (time since first exposure) to an OR=3.3, 95% CI=1.6-6.9. Digital 2G mobile phone use rendered an OR=1.6, 95% CI=0.996-2.7, increasing with latency >15-20 years to an OR=2.1, 95% CI=1.2-3.6. The results for cordless phone use were OR=1.7, 95% CI=1.1-2.9, and, for latency of 15-20 years, the OR=2.1, 95% CI=1.2-3.8. Few participants had used a cordless phone for >20-25 years.* Digital type of wireless phones (2G and 3G mobile phones, cordless phones) gave increased risk with latency >1-5 years, then a lower risk in the following latency groups, but again increasing risk with latency >15-20 years. Ipsilateral use resulted in a higher risk than contralateral mobile and cordless phone use. Higher ORs were calculated for tumours in the temporal and overlapping lobes. Using the meningioma cases in the same study as reference entity gave somewhat higher ORs indicating that the results were unlikely to be explained by recall or observational bias. This study confirmed previous results of an association between mobile and cordless phone use and malignant brain tumours. These findings provide support for the hypothesis that RF-EMFs play a role both in the initiation and promotion stages of carcinogenesis.

5. **Hardell L, Carlberg M. Using the Hill viewpoints from 1965 for evaluating strengths of evidence of the risk for brain tumors associated with use of mobile and cordless phones. *Rev Environ Health* 2013;38:97-106. doi: 10.1515/reveh-2013-0006.**

Abstract
BACKGROUND: Wireless phones, i.e., mobile phones and cordless phones, emit radiofrequency electromagnetic fields (RF-EMF) when used. An increased risk of brain tumors is a major concern. The International Agency for Research on Cancer (IARC) at the World Health Organization (WHO) evaluated the carcinogenic effect to humans from RF-EMF in May 2011. It was concluded that RF-EMF is a group 2B, i.e., a "possible", human carcinogen. Bradford Hill gave a presidential address at the British Royal Society of Medicine in 1965 on the association or causation that provides a helpful framework for evaluation of the brain tumor risk from RF-EMF.
METHODS: All nine issues on causation according to Hill were evaluated. Regarding wireless phones, only studies with long-term use were included. In addition, laboratory studies and data on the incidence of brain tumors were considered.
RESULTS: *The criteria on strength, consistency, specificity, temporality, and biologic gradient for evidence of increased risk for glioma and acoustic neuroma were fulfilled. Additional evidence came from plausibility and analogy based on laboratory studies. Regarding coherence, several studies show increasing incidence of brain tumors, especially in the most exposed area. Support for the experiment came from antioxidants that can alleviate the generation of reactive oxygen species involved in biologic effects, although a direct mechanism for brain tumor carcinogenesis has not been shown. In addition, the finding of no increased risk for brain tumors in subjects using the mobile phone only in a car with an external antenna is supportive evidence. Hill did not consider all the needed nine viewpoints to be essential requirements.*
CONCLUSION: Based on the Hill criteria*, glioma and acoustic neuroma should be considered to be caused by RF-EMF emissions from wireless phones and regarded as carcinogenic to humans,* classifying it as group 1 according to the IARC classification. Current guidelines for exposure need to be urgently revised.

SUMMARY

During 2013 our research group has published results from further studies on brain tumour risk associated with use of mobile and/or cordless desktop phones. We published data on tumour risk for use of these devices during 20 years or more. Clearly we find again an increased risk for malignant brain tumours including the most common type glioma ('brain cancer'). We find also increased risk of acoustic neuroma, a benign tumour of the hearing nerve (number VIII). These tumours usually lead to hearing problems (deafness), tinnitus and dizziness although rarely lethal. Still we find no clear increased risk for meningioma, even after 20 years use of the mobile phone.

Especially worrying is that we find highest risk for glioma and acoustic neuroma in subjects who started use of the wireless phone before the age of 20 years. We have also found that the prognosis of glioma (astrocytoma grade IV) is worse the longer time one has used the wireless phone. That means that long-term use shortens the survival.

Further research has thus strengthened the evidence in support of an increased risk of malignant brain tumours and acoustic neuroma associated with use of mobile phones. Based on the latest findings and using the so called Hill viewpoints from the 1960's exposure to RF-EMF from mobile phones may now be classified as a human cancer causing agent, Group 1, according to the definitions used by IARC.

It is unfortunate that SCENIHR has disregarded these findings and instead relies heavily on the much criticised Danish cohort study on mobile phone users with poor exposure data. We have discussed the many shortcomings in that study, see **Söderqvist F, Carlberg M, Hardell L. Review of four publications on the Danish cohort study on mobile phone subscribers and risk of brain tumors. *Reviews Environmental Health*. 2012; 27: 51-58.** SCENIHR lacks reference to our publication and accordingly also critical comments on the

Danish cohort study. The same lack of critical review applies to the study by Benson et al, included in SCENIHR, but without acknowledge of the limitations in that study.

The CEFALO study on brain tumour risk in children is included in SCENIHR, however without a critical review of the study. For example use of cordless phones was assessed only during the 3 first years of use, a most peculiar definition. Our review of that study is omitted from SCENIHR, see **Söderqvist F, Carlberg M, Hansson Mild K, Hardell L. Childhood brain tumour risk and its association with wireless phones: a commentary. *Environmental Health*. 2011; 10: 106.**

In addition to the Danish cohort study and the UK study by Benson et al SCENIHR relies heavily on time trend analyses. However the conclusion by IARC in the 2011 evaluation was that: "*Time-trend analyses did not show an increased rate of brain tumours after the increase in mobile phone use. However, these studies have substantial limitations because most of the analyses examined trends until the early 2000s only. Such analyses are uninformative if excess risk only manifests more than a decade after phone use begins, or if phone use only affects a small proportion of cases—eg, the most heavily exposed, or a subset of brain tumours*." See **Baan R, Grosse Y, Lauby-Secretan B, *et al*. Carcinogenicity of radiofrequency electromagnetic fields. *Lancet Oncology.* 2011; 12: 624-626.**

In our publication number 4 above, we presented restricted cubic spline plot of the relationship between latency of wireless phone use and malignant brain tumours, see figure below. The solid line indicates the OR estimate and the broken lines represent the 95% CI. Adjustment was made for age at diagnosis, gender, SEI-code and year of diagnosis. Obviously the latency is 20+ years for malignant brain tumours according to these results. Thus, it confirms the conclusion by IARC on incidence data that "*Such analyses are uninformative if excess risk only manifests more than a decade after phone use begins";* in fact it may even be two decades based on our data. Our results are also in agreement with de Vocht et al "*According to these ecological results the latency period is at least 11-12 years, but probably more than 20 years."* See **de Vocht F, Hannam K, Buchan I. Environmental risk factors for cancers of the brain and nervous system: the use of ecological data to generate hypotheses. *Occup Environ Med* 2013; 70: 349-356.**

In summary, the preliminary SCENIHR conclusion that glioma risk is weaker now is not scientifically justified. The only way that conclusion could be reached by SCENIHR is to exclude critical studies that present evidence to the contrary, i.e. studies that report the risk of glioma (and acoustic neuroma) is stronger now than in 2009. Including our studies would give different conclusions supported by critical review of the limitations in cohort studies and incidence data. The Preliminary Opinion should be sent back to the Committee for new evaluation of the scientific data, and should integrate the results of these published data.

1) Hardell L, Carlberg M, Hansson Mild K. Use of mobile phones and cordless phones is associated with increased risk for glioma and acoustic neuroma. *Pathophysiology* 2013;20:85-110. Epub 2012 Dec 21.

2. Carlberg M, Söderqvist F, Hansson Mild K, Hardell L. Meningioma patients diagnosed 2007-2009 and the association with use of mobile and cordless phones, *Environ. Health* 2013;12:60, doi:10.1186/1476-069X-12-60. Epub Jul 19, 2013

3. Hardell L. Carlberg M, Söderqvist F, Hansson Mild K. Pooled analysis of case-control studies on acoustic neuroma diagnosed 1997-2003 and 2007-2009 and use of mobile and cordless phones. *Int J Oncol.* 2013;43:1036-1044. Epub 2013 Jul 22.

4. Hardell L, Carlberg M, Söderqvist F, Hansson Mild K. Case-control study of the association between malignant brain tumors diagnosed 2007-2009 and mobile and cordless phone use. *Int J Oncol.* 2013;43:1833-1845. Epub 2013 Sep 24

5. Hardell L, Carlberg M. Using the Hill viewpoints from 1965 for evaluating strengths of evidence of the risk for brain tumors associated with use of mobile and cordless phones. *Rev Environ Health* 2013;38:97-106. doi: 10.1515/reveh-2013-0006.

Respectfully submitted

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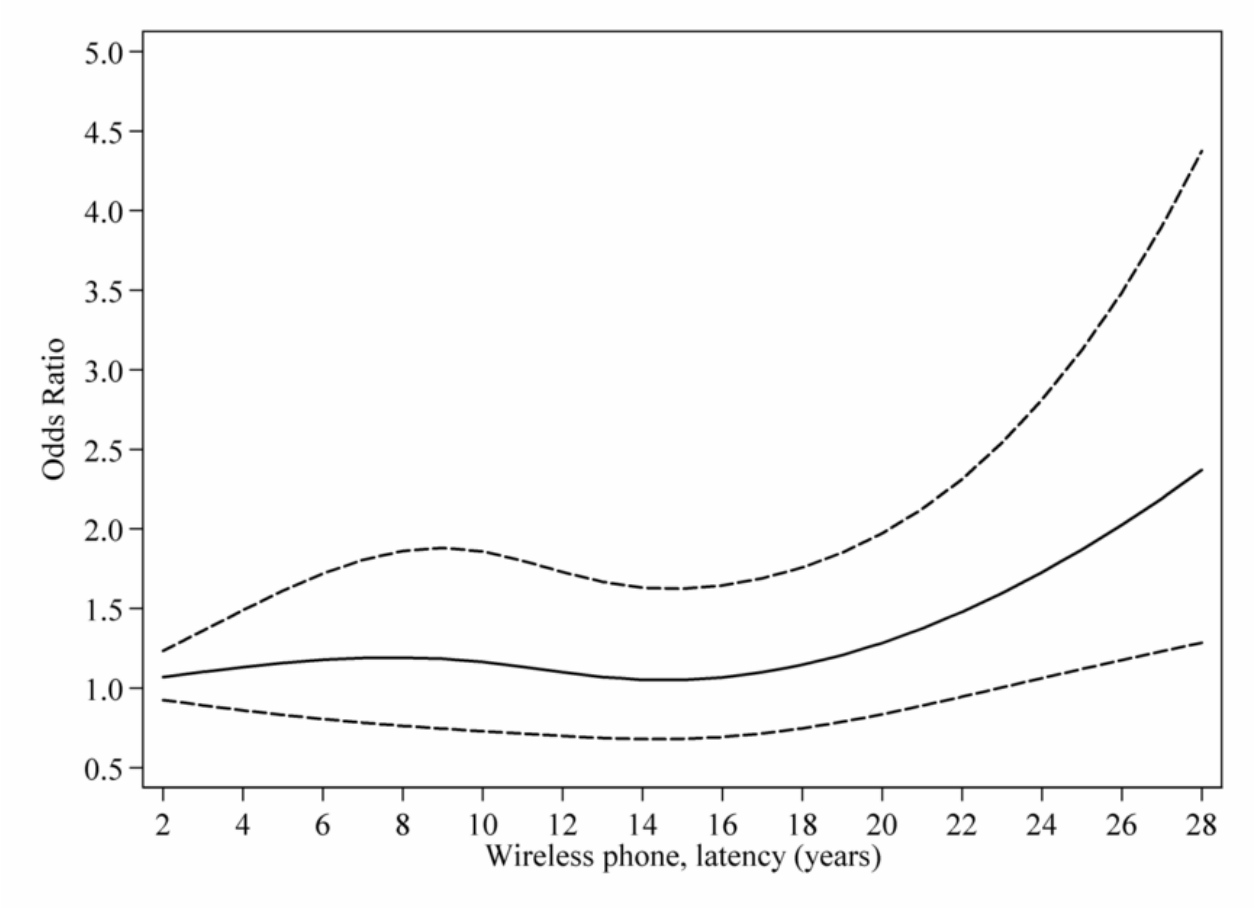
Attached Figure

In our publication **Hardell L, Carlberg M, Söderqvist F, Hansson Mild K. Case-control study of the association between malignant brain tumors diagnosed 2007-2009 and mobile and cordless phone use. Int J Oncol. 2013;43:1833-1845. Epub 2013 Sep 24,** we presented restricted cubic spline plot (see figure below) of the relationship between latency of wireless phone use and malignant brain tumours, see figure below. The solid line indicates the OR estimate and the broken lines represent the 95% CI. Adjustment was made for age at diagnosis, gender, SEI-code and year of diagnosis. Obviously the latency is 20+ years for malignant brain tumours according to these results. Thus, it confirms the conclusion by IARC on incidence data that "*Such analyses are uninformative if excess risk only manifests more than a decade after phone use begins";* in fact it may even be two decades based on our data. Our results are also in agreement with de Vocht et al "*According to these ecological results the latency period is at least 11-12 years, but probably more than 20 years."*



Restricted cubic spline plot of the relationship between latency of wireless phones and malignant brain tumours. The solid line indicates the OR estimate and the broken lines represent the 95% CI. Adjustment was made for age at diagnosis, gender, SEI-code and year of diagnosis. Population based controls were used. (Hardell et al *Int J Oncol.* 2013;43:1833-1845. Epub 2013 Sep 24)

BioInitiative Working Group; An Update on the Genetic Effects of Nonionizing Electromagnetic Fields by Prof. Henry Lai PhD; (Exhibit E); 2014

March, 2014

Exhibit E: An Update on the Genetic Effects of Nonionizing Electromagnetic Fields by Prof. Henry Lai, PhD, University of Washington, Emeritus

Introduction:

The following is an update of information and abstracts on research papers published since 2006/2007 on the genetic effects of nonionizing electromagnetic fields (EMF) in the radiofrequency (RF) and extremely-low frequency (ELF) ranges. Two static magnetic field papers (Jouni et al. 2012; Wang et al., 2009) are also included. Where additional information is relevant, some earlier papers, or papers not specifically related to genetic effects, are also included with citations contained within the discussion below. A list of abstracts, with summary sentences underlined for reader convenience, can be found at the end of this paper.

Analysis of these recent publications shows that there are more papers reporting effects than no effect. With E representing a biological effect, and NE representing no biological effects, the recent literature finds RFR-genetic effects at: E=74 publications (65%); NE=40 publications (35%); and ELF-genetic effects at: E=49 (83%); NE=10 (17%).

Discussion:

1. The effects of both RF and ELF fields are very similar. This is surprising because the energies carried by these EMFs are billions of folds different. An explanation for similar genetic effects has been provided by a recent paper by Blank and Goodman (Blank M, Goodman R. DNA is a fractal antenna in electromagnetic fields. Int. J. Radiat. Biol. 87(4):409-415, 2011) in which they stated that '…the wide frequency range of interaction with EMF is the functional characteristic of a fractal antenna, and DNA appears to possess the two structural characteristics of fractal antennas, electronic conduction and self symmetry.' However, similarities in effects between ELF and RF fields have also been reported in studies of other physiological processes, e.g., neurochemical and behavioral effects (Cf. Lai, H., Carino, M.A., Horita, A. and Guy, A.W. Opioid receptor subtypes that mediate a microwave-induced decrease in central cholinergic activity in the rat. Bioelectromagnetics 13:237-246, 1992; Lai, H. and Carino, M.A. Intracerebroventricular injections of mu and delta-opiate receptor antagonists block 60-Hz magnetic field-induced decreases in cholinergic activity in the frontal cortex and hippocampus of the rat. Bioelectromagnetics 19:433-437, 1998; Lai, H., Carino, M.A. and Ushijima, I. Acute exposure to a 60 Hz magnetic field affects rats' performance in the water maze. Bioelectromagnetics 19:117-122, 1998; Wang, B.M. and Lai, H. Acute exposure to pulsed 2450-MHz microwaves affects water maze learning in the rat. Bioelectromagnetics 21:52-56, 2000.) Thus, there is a basic interaction mechanism of biological tissues with electromagnetic fields that is independent of frequency.

Many studies have implicated the involvement of free radical processes in the genetic effects of EMF: ELF-EMF (Butdak et al., 2012; Jouni et al., 2012; Luukkonen et al., 2014; Tiwari et al.,2014); RFR (Agarwal et al., 2009; Atasoy et al., 2012; Burlaka et al., 2013; Campisi et al., 2010; De Iuliis et al., 2009; Esmekaya et al., 2011; Ferreira et al., 2006; Gajski and Garaj-Vrhovac, 2009; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Khalil et al., 2012; Kumar et al., 2010; Liu et al., 2013a,b; Luukkonan et al., 2009; Tomruk et al., 2010; Tkalec et al., 2013; Wu et al., 2008; Xu et al., 2010; Yao et al., 2003). Increase in free radical activity and changes in enzymes involved in cellular oxidative processes are the most consistent effects observed in cells and animals after EMF exposure. However, they are reports indicating that EMF could induce genetic effects without the involvement of free radicals (ELF-Alcaraz et al., 2013; RFR- Ferreira et al., 2006; Furtado-Filho et al., 2013) and increase in free radical after EMF exposure did not lead to genetic effects (Frahm et al., 2006). There are at least a couple of hundred published papers on the effects of EMF exposure on cellular oxidative processes. Many biological effects of EMF can be explained by intracellular changes in oxidative status, including the genetic effects reported in this review.

2. An important observation of the studies is that EMF can interact with other entities and synergistically cause genetic effects. These entities include: ELF-EMF- cisplastin (Buldak et al., 2012; El-Bialy et al., 2013), bleomycin (Cho et al., 2007), gadolinium (Cho et al., 2014); hydrogen peroxide and methyl methane sulfonate (Koyama et al., 2008), menadione (Luukkonan et al., 2011, 2014; Markkanen et al., 2008), ionizing radiation (Mairs et al., 2007; Journi et al., 2012 Yoon et al., 2014); RFR- chemical mutagens (Baohong et al., 2005), clastogens (Kim et al., 2008), x-rays (Manti et al., 2008), ultraviolet ray (Baohong et al., 2007), aphidicolin (Tiwari et al., 2008), picrotoxin (López-Martín et al., 2009), doxorubicin (Zhijian et al., 2010), and incoherent electromagnetic noise (Wu et al., 2008; Yao et al., 2008). Most of the compounds that interact with EMF are mutagens. This is important because in real life situations, a person is usually exposed to many different environmental factors simultaneously. Synergism of these factors with EMF should be considered more seriously.

3. Several long term/repeated exposure papers are included in this update: ELF-EMF (Borhani et al., 2011; Cuccurazzu et al., 2010; Erdal et al., 2007; Fedrowitz and Loscher, 2012; Mariucci et al., 2010; Panagopoulous et al., 2013; Udroiu et al., 2006), and RFR (Asasoy et al., 2012; Atli Serkeroglu et al., 2013; Burlaka et al., 2013; Chavdoula et al., 2010; Deshmukh et al., 2013; Ferreira et al., 2006; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Lakshmi et al., 2010; Paulraj and Behari, 2006; Tomruk et al., 2010; Yan et al., 2008). These data are important in the understanding of the biological effects of EMF exposure in real life situation, since human environmental EMF exposure is both chronic and intermittent. Within these long-term exposure studies, there are several that investigated the effect of EMF exposure on developing animals (ELF-EMF: Borhani et al., 2011; Cuccurazzu et al., 2010;

Panagopoulous et al., 2013; Udroiu et al., 2006, RFR: Burlaka et al., 2013; Ferreira et al., 2006; Guler et al., 2010, 2012; Serkeroglu et al., 2013; Tomruk et al., 2010; Zalata et al., In press). Data of effects of EMF exposure on growth and development of young animals are urgently needed. There are several studies indicating that RFR may affect reproduction, particularly with effects on sperm physiology and DNA (Agarwal et al., 2009; Atasoy et al., 2012; Avendano et al., 2012; Chavdoula et al., 2010; de Iuliis et al., 2009; Liu et al., 2013b; Panagopoulous et al., 2007). Similar effects of ELF-EMF on sperm have also been reported, e.g., Hong R, Zhang Y, Liu Y, Weng EQ. Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 23(6):414-417, 2005; Iorio R, Scrimaglio R, Rantucci E, Delle Monache S, Di Gaetano A, Finetti N, Francavilla F, Santucci R, Tettamanti E, Colonna R. A preliminary study of oscillating electromagnetic field effects on human spermatozoon motility. Bioelectromagnetics. 28(1):72-75, 2007; Iorio R, Delle Monache S, Bennato F, Di Bartolomeo C, Scrimaglio R, Cinque B, Colonna RC. Involvement of mitochondrial activity in mediating ELF-EMF stimulatory effect on human sperm motility. Bioelectromagnetics. 32(1):15-27, 2011.

4. Another area that needs more research is the biological effects of low-intensity exposure. This is particularly true for ELF-EMF, since intensities of ELF-EMF in the environment are in microtesla (μT) levels. There are many studies on biological effects of low-intensity RFR (see Table 1 in Levitt, B.B. and Lai, H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays. Environ. Rev. 18:369-395, 2010.) However, most cell and animal studies in ELF-EMF used fields in the millitesla (mT) level. Exceptions are the study of Sarimov et al. (2011) listed below in the reference section and the study of de Bruyn and de Jager (2010) (de Bruyn L and de Jager L. Effect of long-term exposure to a randomly varied 50 Hz power frequency magnetic field on the fertility of the mouse. Electromag. Biol. Med. 29(1-2):52-61, 2010).

5. Two other important findings of these recent studies are that the effects of EMF are shown to be waveform specific and cell-type specific. Regarding waveform specificity, Campisi et al. (2010) reported increases in free radical activity and DNA fragmentation in brain cells after acute exposure to a 50-Hz amplitude-modulated 900-MHz RFR, whereas a continuous-wave 9000-MHz field produced no effect. Franzellitti et al. (2010) showed increased DNA strand breaks in trophoblasts after exposure to a 217-Hz modulated 1.8 GHz-RFR, but a continuous-wave field of the same carrier frequency was without effect. Tkalec et al (2013) reported that AM-modulated (1 KHz sinusoidal) 900-MHz RFR is more potent than non-modulated field in causing DNA damage in coelomocytes of exposed earthworms. Luukkonen et al. (2009) reported a continuous-wave 872-MHz RFR increased chemically-induced DNA strand breaks and free radicals in human neuroblastoma cells, whereas a GSM-modulated 872-MHz field had no significant effect. Zhang et al. (2008) found that gene expression in rat neurons is more sensitive to intermittent than continuous exposure to a 1.8 GHz-RFR. López-Martín et al. (2009)

found that GSM and unmodulated RFR caused different effects on c-Fos gene expression in the rat brain. Regarding cell-type specificity, Nylund and Leszczynski (2006) and Remondini et al. (2006) reported different patterns of gene expression in different types of cells after exposure to RFR. Zhao et al. (2007) found than neurons are more sensitive to a 1.9 GHz cell phone radiation than astrocytes. Schwarz et al. (2008) reported DNA strand breaks and micronucleus formation in human fibroblasts, but not in lymphocytes, after exposure to a 1950-MHz UMTS field. Furthermore, Xu et al (2013) found DNA damages in some cell types and not in others after exposure to 1800-MHz RFR. Valbonesi et at. (2014) reported that HSP70 expression and MAPK signaling pathways in PC12 cells were affected by GSM-217 Hz signal and not by CW or GSM-talk signals. In ELF-EM research, Giorgi et al. (2011) found that DNA transposition in E. coli was *decreased* after exposure to a sinusoidal magnetic field and *increased* after exposure to a pulsed magnetic field. Kim et al. (2012) described DNA strand breaks in human fibroblasts after exposure to ELF magnetic field. They found that the pattern of changes depended on the eddy current and Lorentz force in the field. Nahab et al. (2007) reported that a square-continuous ELF magnetic field was more effective than sinusoidal-continuous or pulsed field in inducing sister chromatid exchange in human lymphocytes. These findings underscore the complicity of interaction of EMF with biological tissues and may partially explain why effects were observed in some studies and not others. It is essential to understand why and how certain wave-characteristics of an EMF are more effective than other characteristics in causing biological effects, and why certain types of cells are more susceptible to the effect of EMF? That there are different biological effects elicited by different EMF wave characteristics is critical proof for the existence of nonthermal effects.

6. Many biological/health effects have been reported in cells and animals after exposure to EMFs in both the ELF and RF ranges. (Sixty-five percent of the RFR papers and 82% of the ELF-EMF papers in the publication list below reported effects.) It is highly dishonest for a scientist to summarily deny the existence of biological effects of EMF. A biological effect of EMF can be detrimental to health, but can also be turned into a beneficial means for the treatment of human diseases. Denying any effects hampers the development of electromagnetic treatments for diseases. Examples of possible clinical uses of EMF are: Alzheimer's disease (Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C. Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. J Alzheimers Dis. 19(1):191-210, 2010); Parkinson's disease (Wang Z, Che PL, Du J, Ha B, Yarema KJ. Static magnetic field exposure reproduces cellular effects of the Parkinson's disease drug candidate ZM241385. PLoS One. 5(11):e13883, 2010); bone regeneration (Lee HM, Kwon UH, Kim H, Kim HJ, Kim B, Park JO, Moon ES, Moon SH. Pulsed eltromagnetic field stimulates cellular proliferation in human intervertebral disc cells. Yonsei Med. J. 51(6):954-959, 2010); cancer treatment (Costa FP, de Oliveira AC, Meirelles R, Machado MC, Zanesco T, Surjan R, Chammas MC, de Souza Rocha M, Morgan D, Cantor A, Zimmerman J, Brezovich I, Kuster N, Barbault A, Pasche B.

Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields. Br. J. Cancer. 105(5):640-648, 2011), and tissue regeneration (Gaetani R, Ledda M, Barile L, Chimenti I, De Carlo F, Forte E, Ionta V, Giuliani L, D'Emilia E, Frati G, Miraldi F, Pozzi D, Messina E, Grimaldi S, Giacomello A, Lisi A. Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic fields. Cardiovasc. Res. 82(3):411-420, 2009).

7. It must be pointed out that, consistent with previous research, not very much of the cellular and animal genetic research data directly indicate that EMF (both RF and ELF EMF) is a carcinogen. However, the data show that EMF can possibly alter genetic functions and thus it is advisable that one should limit one's exposure to EMF.

References and abstracts

Below is a key to abbreviations used throughout the following list of abstracts for recent papers published since 2006 and serve as my comments to help the reader quickly identify the significance of each work. The summary sentences by each author are underlined. The list is divided into RF effects papers, and ELF effects papers.

(E- effect observed; NE- no effect observed) (LE- long term exposure; GT- genotoxic effect, e.g., DNA damage, micronucleus formation, chromosome alterations; GE- gene expression; HU- human study; OX- oxidative effects, i.e., involvement of free radicals and oxidative enzymes; IA- interaction with other factors to cause genetic effects; DE- effects on developing animals; RP- reproduction, e.g., sperm damage; EH- compared with electro-hypersensitive subjects; WS- waveform specific effect, e.g., modulation and frequency; CS- cell type specific effect).

An update on the research on genetic effects of radiofrequency/cell phone radiation

(E) Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. Fertil Steril 92 1318-1325, 2009. (GT, RP, OX)

OBJECTIVE: To evaluate effects of cellular phone radiofrequency electromagnetic waves (RF-EMW) during talk mode on unprocessed (neat) ejaculated human semen. DESIGN: Prospective pilot study. SETTING: Center for reproductive medicine laboratory in tertiary hospital setting. SAMPLES: Neat semen samples from normal healthy donors (n = 23) and infertile patients (n = 9). INTERVENTION(S): After liquefaction, neat semen samples were divided into two aliquots. One aliquot (experimental) from each patient was exposed to cellular phone radiation (in talk mode) for 1 h, and the second aliquot (unexposed) served as the control sample under identical conditions. MAIN OUTCOME MEASURE(S): Evaluation of sperm parameters (motility, viability), reactive oxygen species (ROS), total antioxidant capacity (TAC) of semen, ROS-TAC score, and sperm DNA damage. RESULT(S): Samples exposed to RF-EMW showed a significant decrease in

sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score. Levels of TAC and DNA damage showed no significant differences from the unexposed group. CONCLUSION(S): Radiofrequency electromagnetic waves emitted from cell phones may lead to oxidative stress in human semen. We speculate that keeping the cell phone in a trouser pocket in talk mode may negatively affect spermatozoa and impair male fertility.

(E) Atasoy HI, Gunal MY, Atasoy P, Elgun S, Bugdayci G. Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices.J Pediatr Urol. 2012 Mar 30. [Epub ahead of print] (GT, OX, LE, RP)

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

(E) Atlı Şekeroğlu Z, Akar A, Sekeroğlu V. Evaluation of the cytogenotoxic damage in immature and mature rats exposed to 900 MHz radio frequency electromagnetic fields. Int J Radiat Biol. 89(11):985-992, 2013. [Epub ahead of print] (GT, DE, LE)

Abstract Purpose: One of the most important issues regarding radio frequency electromagnetic fields (RF-EMF) is their effect on genetic material. Therefore, we investigated the cytogenotoxic effects of 900 MHz radio frequency electromagnetic fields (RF-EMF) and the effect of a recovery period after exposure to RF-EMF on bone marrow cells of immature and mature rats. Materials and methods: The immature and mature rats in treatment groups were exposed to RF-EMF for 2 h/day for 45 days. Average electrical field values for immature and mature rats were 28.1±4.8 V/m and 20.0±3.2 V/m, respectively. Whole-body specific absorption rate (SAR) values for immature and mature rats were in the range of 0.38-0.78 W/kg, and 0.31-0.52 W/kg during the 45 days, respectively. Two recovery groups were kept for 15 days after RF-EMF exposure. Results: Significant differences were observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCE) in all

treatment and recovery groups. The cytogenotoxic damage in immature rats was statistically higher than the mature rats. The recovery period did not reduce the damage to the same extent as the corresponding control groups. Conclusions: The exposure of RF-EMF leads to cytotoxic and genotoxic damage in immature and mature rats. More sensitive studies are required to elucidate the possible carcinogenic risk of EMF exposure in humans, especially children.

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

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

(E) Baohong Wang, Jiliang H, Lifen J, Deqiang L, Wei Z, Jianlin L, Hongping D. Studying the synergistic damage effects induced by 1.8 GHz radiofrequency field radiation (RFR) with four chemical mutagens on human lymphocyte DNA using comet assay in vitro. Mutat Res 578:149-57, 2005. (GT, IA)

The aim of this investigation was to study the synergistic DNA damage effects in human lymphocytes induced by 1.8GHz radiofrequency field radiation (RFR, SAR of 3W/kg) with four chemical mutagens, i.e. mitomycin C (MMC, DNA crosslinker), bleomycin (BLM, radiomimetic agent), methyl methanesulfonate (MMS, alkylating agent), and 4-nitroquinoline-1-oxide (4NQO, UV-mimetic agent). The DNA damage of lymphocytes exposed to RFR and/or with chemical mutagens was detected at two incubation time (0 or 21h) after treatment with comet assay in vitro. Three combinative exposure ways were used. Cells were exposed to RFR and chemical mutagens for 2 and 3h, respectively. Tail length (TL) and tail moment (TM) were utilized as DNA damage indexes. The results showed no difference of DNA damage indexes between RFR group and control group at 0 and 21h incubation after exposure ($P>0.05$). There were significant

difference of DNA damage indexes between MMC group and RFR+MMC co-exposure group at 0 and 21h incubation after treatment (P<0.01). Also the significant difference of DNA damage indexes between 4NQO group and RFR+4NQO co-exposure group at 0 and 21h incubation after treatment was observed (P<0.05 or P<0.01). The DNA damage in RFR+BLM co-exposure groups and RFR+MMS co-exposure groups was not significantly increased, as compared with corresponding BLM and MMS groups (P>0.05). The experimental results indicated 1.8GHz RFR (SAR, 3W/kg) for 2h did not induce the human lymphocyte DNA damage effects in vitro, but could enhance the human lymphocyte DNA damage effects induced by MMC and 4NQO. The synergistic DNA damage effects of 1.8GHz RFR with BLM or MMS were not obvious.

(E) Baohong W, Lifen J, Lanjuan L, Jianlin L, Deqiang L, Wei Z, Jiliang H.Evaluating the combinative effects on human lymphocyte DNA damage induced by ultraviolet ray C plus 1.8GHz microwaves using comet assay in vitro. Toxicology. 232(3):311-316, 2007. (GT, IA)

The objective of this study was to observe whether 1.8GHz microwaves (MW) (SAR, 3 W/kg) exposure can influence human lymphocyte DNA damage induced by ultraviolet ray C (UVC). The lymphocytes, which were from three young healthy donors, were exposed to 254 nm UVC at the doses of 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 J m(-2), respectively. The lymphocytes were irradiated by 1.8GHz MW (SAR, 3 W/kg) for 0, 1.5 and 4 h. The combinative exposure of UVC plus MW was conducted. The treated cells were incubated for 0, 1.5 and 4 h. Finally, comet assay was used to measure DNA damage of above treated lymphocytes. The results indicated that the difference of DNA damage induced between MW group and control group was not significant (P>0.05). The MTLs induced by UVC were 1.71+/-0.09, 2.02+/-0.08, 2.27+/-0.17, 2.27+/-0.06, 2.25+/-0.12, 2.24+/-0.11 microm, respectively, which were significantly higher than that (0.96+/-0.05 microm) of control (P<0.01). MTLs of some sub-groups in combinative exposure groups at 1.5-h incubation were significantly lower that those of corresponding UVC sub-groups (P<0.01 or P<0.05). However, MTLs of some sub-groups in combinative exposure groups at 4-h incubation were significantly higher that those of corresponding UVC sub-groups (P<0.01 or P<0.05). In this experiment it was found that 1.8GHz (SAR, 3 W/kg) MW exposure for 1.5 and 4 h did not enhance significantly human lymphocyte DNA damage, but could reduce and increase DNA damage of human lymphocytes induced by UVC at 1.5-h and 4-h incubation, respectively.

(E) Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. Bioelectromagnetics 26:173-184, 2005. (GT, EH)

We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power frequency magnetic field (50 Hz, 15 muT peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were

analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock.

(E) Belyaev IY, Koch CB, Terenius O, Roxstrom-Lindquist K, Malmgren LO, H Sommer W, Salford LG, Persson BR. Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. Bioelectromagnetics 27:295-306, 2006. (GE)

We investigated whether exposure of rat brain to microwaves (MWs) of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression. An exposure installation was used based on a test mobile phone employing a GSM signal at 915 MHz, all standard modulations included, output power level in pulses 2 W, specific absorption rate (SAR) 0.4 mW/g. Rats were exposed or sham exposed to MWs during 2 h. After exposure, cell suspensions were prepared from brain samples, as well as from spleen and thymus. For analysis of gene expression patterns, total RNA was extracted from cerebellum. Changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependencies (AVTD). DNA double strand breaks (DSBs) were analyzed by pulsed-field gel electrophoresis (PFGE). Effects of MW exposure were observed on neither conformation of chromatin nor DNA DSBs. Gene expression profiles were obtained by Affymetrix U34 GeneChips representing 8800 rat genes and analyzed with the Affymetrix Microarray Suite (MAS) 5.0 software. In cerebellum from all exposed animals, 11 genes were upregulated in a range of 1.34-2.74 fold and one gene was downregulated 0.48-fold ($P < .0025$). The induced genes encode proteins with diverse functions including neurotransmitter regulation, blood-brain barrier (BBB), and melatonin production. The data shows that GSM MWs at 915 MHz did not induce PFGE-detectable DNA double stranded breaks or changes in chromatin conformation, but affected expression of genes in rat brain cells

(E) Belyaev IY, Markovà E, Hillert L, Malmgren LO, Persson BR. Microwaves from UMTS/GSM mobile phones induce long-lasting inhibition of 53BP1/gamma-H2AX DNA repair foci in human lymphocytes. Bioelectromagnetics 30:129-41, 2009. (GT, EH)

We have recently described frequency-dependent effects of mobile phone microwaves (MWs) of global system for mobile communication (GSM) on human lymphocytes from persons reporting hypersensitivity to electromagnetic fields and healthy persons. Contrary to GSM, universal global telecommunications system (UMTS) mobile phones emit wide-band MW signals. Hypothetically, UMTS MWs may result in higher biological effects compared to GSM signal because of eventual "effective" frequencies within the wideband. Here, we report for the first time that UMTS MWs affect chromatin and inhibit formation of DNA double-strand breaks co-localizing 53BP1/gamma-H2AX DNA repair foci in human lymphocytes from hypersensitive and healthy persons and confirm that effects of GSM MWs depend on carrier frequency. Remarkably, the effects of MWs on 53BP1/gamma-H2AX foci persisted up to 72 h following exposure of cells, even longer than the stress response following heat shock. The data are in line with the hypothesis that the type of signal, UMTS MWs, may have higher biological efficiency and possibly larger health risk effects compared to GSM radiation emissions. No significant differences in effects between groups of healthy and hypersensitive subjects were observed, except for the effects of UMTS MWs and GSM-915 MHz MWs on the formation of the DNA repair foci, which were different for hypersensitive ($P < 0.02$[53BP1]//0.01[gamma-H2AX]) but not for control subjects ($P > 0.05$). The non-parametric statistics used here did not indicate specificity of the differences revealed between the effects of GSM and UMTS MWs on cells from hypersensitive subjects and more data are needed to study the nature of these differences.

(NE) Bourthoumieu S, Joubert V, Marin B, Collin A, Leveque P, Terro F, Yardin C. Cytogenetic studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using R-banded karyotyping. Radiat Res 174:712-718, 2010. (GT)

It is important to determine the possible effects of exposure to radiofrequency (RF) radiation on the genetic material of cells since damage to the DNA of somatic cells may be linked to cancer development or cell death and damage to germ cells may lead to genetic damage in next and subsequent generations. The objective of this study was to investigate whether exposure to radiofrequency radiation similar to that emitted by mobile phones of second-generation standard Global System for Mobile Communication (GSM) induces genotoxic effects in cultured human cells. The cytogenetic effects of GSM-900 MHz (GSM-900) RF radiation were investigated using R-banded karyotyping after in vitro exposure of human cells (amniotic cells) for 24 h. The average specific absorption rate (SAR) was 0.25 W/kg. The exposures were carried out in wire-patch cells (WPCs) under strictly controlled conditions of temperature. The genotoxic effect was assessed immediately or 24 h after exposure using four different samples. One hundred metaphase cells were analyzed per assay. Positive controls were provided by using bleomycin. We found no direct cytogenetic effects of GSM-900 either 0 h or 24 h after exposure. To the best of our knowledge, our work is the first to study genotoxicity using

complete R-banded karyotyping, which allows visualizing all the chromosomal rearrangements, either numerical or structural.

(NE) Bourthoumieu S, Terro F, Leveque P, Collin A, Joubert V, Yardin C. Aneuploidy studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using FISH. Int J Radiat Biol 87:400-408, 2011. (GT)

PURPOSE: Since previous research found an increase in the rate of aneuploidies in human lymphocytes exposed to radiofrequencies, it seems important to perform further studies. The objective of this study was then to investigate whether the exposure to RF (radiofrequency) radiation similar to that emitted by mobile phones of a second generation standard, i.e., Global System for Mobile communication (GSM) may induce aneuploidy in cultured human cells. MATERIALS AND METHODS: The potential induction of genomic instability by GSM-900 MHz radiofrequency (GSM-900) was investigated after in vitro exposure of human amniotic cells for 24 h to average-specific absorption rates (SAR) of 0.25, 1, 2 and 4 W/kg in the temperature range of 36.3-39.7°C. The exposures were carried out in a wire-patch cell (WPC). The rate of aneuploidy of chromosomes 11 and 17 was determined by interphase FISH (Fluorescence In Situ Hybridisation) immediately after independent exposure of three different donors for 24 h. At least 100 interphase cells were analysed per assay. RESULTS: No significant change in the rate of aneuploidy of chromosomes 11 and 17 was found following exposure to GSM-900 for 24 h at average SAR up to 4 W/kg. CONCLUSION: Our study did not show any in vitro aneuploidogenic effect of GSM using FISH and is not in agreement with the results of previous research.

(NE) Bourthoumieu S, Magnaudeix A, Terro F, Leveque P, Collin A, Yardin C. Study of p53 expression and post-transcriptional modifications after GSM-900 radiofrequency exposure of human amniotic cells. Bioelectromagnetics. 2012 Jul 5. doi: 10.1002/bem.21744. [Epub ahead of print] (GE)

The potential effects of radiofrequency (RF) exposure on the genetic material of cells are very important to determine since genome instability of somatic cells may be linked to cancer development. In response to genetic damage, the p53 protein is activated and can induce cell cycle arrest allowing more time for DNA repair or elimination of damaged cells through apoptosis. The objective of this study was to investigate whether the exposure to RF electromagnetic fields, similar to those emitted by mobile phones of the second generation standard, Global System for Mobile Communications (GSM), may induce expression of the p53 protein and its activation by post-translational modifications in cultured human cells. The potential induction of p53 expression and activation by GSM-900 was investigated after in vitro exposure of human amniotic cells for 24 h to average specific absorption rates (SARs) of 0.25, 1, 2, and 4 W/kg in the temperature range of 36.3-39.7 °C. The exposures were carried out using a wire-patch cell (WPC) under strictly controlled conditions of temperature. Expression and activation of p53 by phosphorylation at serine 15 and 37 were studied using Western blot assay immediately after three independent exposures of cell cultures provided from three different

donors. Bleomycin-exposed cells were used as a positive control. According to our results, no significant changes in the expression and activation of the p53 protein by phosphorylation at serine 15 and 37 were found following exposure to GSM-900 for 24 h at average SARs up to 4 W/kg in human embryonic cells.

(E) Burlaka A, Tsybulin O, Sidorik E, Lukin S, Polishuk V, Tsehmistrenko S, Yakymenko I. Overproduction of free radical species in embryonal cells exposed to low intensity radiofrequency radiation. Exp Oncol. 35(3):219-225, 2013. (GT, LE, DE, OX)

Aim: Long-term exposure of humans to low intensity radiofrequency electromagnetic radiation (RF-EMR) leads to a statistically significant increase in tumor incidence. Mechanisms of such the effects are unclear, but features of oxidative stress in living cells under RF-EMR exposure were previously reported. Our study aims to assess a production of initial free radical species, which lead to oxidative stress in the cell. Materials and Methods: Embryos of Japanese quails were exposed in ovo to extremely low intensity RF-EMR of GSM 900 MHz (0.25 μW/cm2) during 158-360 h discontinuously (48 c - ON, 12 c - OFF) before and in the initial stages of development. The levels of superoxide (O2·-), nitrogen oxide (NO·), thiobarbituric acid reactive substances (TBARS), 8-oxo-2'-deoxyguanosine (8-oxo-dG) and antioxidant enzymes' activities were assessed in cells/tissues of 38-h, 5- and 10-day RF-EMR exposed and unexposed embryos. Results: The exposure resulted in a significant persistent overproduction of superoxide and nitrogen oxide in embryo cells during all period of analyses. As a result, significantly increased levels of TBARS and 8-oxo-dG followed by significantly decreased levels of superoxide dismutase and catalase activities were developed in the exposed embryo cells. Conclusion: Exposure of developing quail embryos to extremely low intensity RF-EMR of GSM 900 MHz during at least one hundred and fifty-eight hours leads to a significant overproduction of free radicals/reactive oxygen species and oxidative damage of DNA in embryo cells. These oxidative changes may lead to pathologies up to oncogenic transformation of cells.

(E) Buttiglione M, Roca L, Montemurno E, Vitiello F, Capozzi V, Cibelli G. Radiofrequency radiation (900 MHz) induces Egr-1 gene expression and affects cell-cycle control in human neuroblastoma cells. J Cell Physiol. 213(3):759-767, 2007. (GE)

Many environmental signals, including ionizing radiation and UV rays, induce activation of Egr-1 gene, thus affecting cell growth and apoptosis. The paucity and the controversial knowledge about the effect of electromagnetic fields (EMF) exposure of nerve cells prompted us to investigate the bioeffects of radiofrequency (RF) radiation on SH-SY5Y neuroblastoma cells. The effect of a modulated RF field of 900 MHz, generated by a wire patch cell (WPC) antenna exposure system on Egr-1 gene expression, was studied as a function of time. Short-term exposures induced a transient increase in Egr-1 mRNA level paralleled with activation of the

MAPK subtypes ERK1/2 and SAPK/JNK. The effects of RF radiations on cell growth rate and apoptosis were also studied. Exposure to RF radiation had an anti-proliferative activity in SH-SY5Y cells with a significant effect observed at 24 h. RF radiation impaired cell cycle progression, reaching a significant G2-M arrest. In addition, the appearance of the sub-G1 peak, a hallmark of apoptosis, was highlighted after a 24-h exposure, together with a significant decrease in mRNA levels of Bcl-2 and survivin genes, both interfering with signaling between G2-M arrest and apoptosis. Our results provide evidence that exposure to a 900 MHz-modulated RF radiation affect both Egr-1 gene expression and cell regulatory functions, involving apoptosis inhibitors like Bcl-2 and survivin, thus providing important insights into a potentially broad mechanism for controlling in vitro cell viability.

(E) Cam ST, Seyhan N. Single-strand DNA breaks in human hair root cells exposed to mobile phone radiation. Int J Radiat Biol 88(5):420-424, 2012 (GT, HU)

Purpose: To analyze the short term effects of radiofrequency radiation (RFR) exposure on genomic deoxyribonucleic acid (DNA) of human hair root cells. Subjects and methods: Hair samples were collected from 8 healthy human subjects immediately before and after using a 900-MHz GSM (Global System for Mobile Communications) mobile phone for 15 and 30 minutes. Single-strand DNA breaks of hair root cells from the samples were determined using the 'comet assay'. Results: The data showed that talking on a mobile phone for 15 or 30 minutes significantly increased ($p < .05$) single-strand DNA breaks in cells of hair roots close to the phone. Comparing the 15-min and 30-min data using the paired t-test also showed that significantly more damages resulted after 30 minutes than after 15 minutes of phone use. Conclusions: A short-term exposure (15 and 30 minutes) to RFR (900-MHz) from a mobile phone caused a significant increase in DNA single-strand breaks in human hair root cells located around the ear which is used for the phone calls.

(E) Campisi A, Gulino M, Acquaviva R, Bellia P, Raciti G, Grasso R, Musumeci F, Vanella A, Triglia A. Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. Neurosci Lett 473:52-55. 2010. (GT, OX, WS)

The exposure of primary rat neocortical astroglial cell cultures to acute electromagnetic fields (EMF) in the microwave range was studied. Differentiated astroglial cell cultures at 14 days in vitro were exposed for 5, 10, or 20 min to either 900 MHz continuous waves or 900 MHz waves modulated in amplitude at 50 Hz using a sinusoidal waveform and 100% modulation index. The strength of the electric field (rms value) at the sample position was 10V/m. No change in cellular viability evaluated by MTT test and lactate dehydrogenase release was observed. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated EMF for 20 min. No evident effects were detected when shorter time intervals or continuous waves were used. The irradiation conditions allowed the exclusion of any possible thermal effect. Our data demonstrate, for the first time, that even acute exposure to low intensity EMF induces ROS production and DNA fragmentation in astrocytes in primary cultures, which also represent the principal target of modulated EMF. Our findings also suggest

the hypothesis that the effects could be due to hyperstimulation of the glutamate receptors, which play a crucial role in acute and chronic brain damage. Furthermore, the results show the importance of the amplitude modulation in the interaction between EMF and neocortical astrocytes.

(E) 1 **Cervellati F, Valacchi G, Lunghi L, Fabbri E, Valbonesi P, Marci R, Biondi C, Vesce F. 17-β-estradiol counteracts the effects of high frequency electromagnetic fields on trophoblastic connexins and integrins. Oxid Med Cell Longev. 2013;2013:280850. doi: 10.1155/2013/280850. (GE)**

We investigated the effect of high-frequency electromagnetic fields (HF-EMFs) and 17-β-estradiol on connexins (Cxs), integrins (Ints), and estrogen receptor (ER) expression, as well as on ultrastructure of trophoblast-derived HTR-8/SVneo cells. HF-EMF, 17-β-estradiol, and their combination induced an increase of Cx40 and Cx43 mRNA expression. HF-EMF decreased Int alpha1 and β 1 mRNA levels but enhanced Int alpha5 mRNA expression. All the Ints mRNA expressions were increased by 17-β-estradiol and exposure to both stimuli. ER-β mRNA was reduced by HF-EMF but augmented by 17-β-estradiol alone or with HF-EMF. ER-β immunofluorescence showed a cytoplasmic localization in sham and HF-EMF exposed cells which became nuclear after treatment with hormone or both stimuli. Electron microscopy evidenced a loss of cellular contact in exposed cells which appeared counteracted by 17-β-estradiol. We demonstrate that 17-β-estradiol modulates Cxs and Ints as well as ER-β expression induced by HF-EMF, suggesting an influence of both stimuli on trophoblast differentiation and migration.

(NE) Chang SK, Choi JS, Gil HW, Yang JO, Lee EY, Jeon YS, Lee ZW, Lee M, Hong MY, Ho Son T, Hong SY. Genotoxicity evaluation of electromagnetic fields generated by 835-MHz mobile phone frequency band. Eur J Cancer Prev 14:175-179, 2005. (GT, IA) (Some interaction effects with chemicals are reported in this paper.)

It is still unclear whether the exposure to electromagnetic fields (EMFs) generated by mobile phone radiation is directly linked to cancer. We examined the biological effects of an EMF at 835 MHz, the most widely used communication frequency band in Korean CDMA mobile phone networks, on bacterial reverse mutation (Ames assay) and DNA stability (in vitro DNA degradation). In the Ames assay, tester strains alone or combined with positive mutagen were applied in an artificial mobile phone frequency EMF generator with continuous waveform at a specific absorption rate (SAR) of 4 W/kg for 48 h. In the presence of the 835-MHz EMF radiation, incubation with positive mutagen 4-nitroquinoline-1-oxide and cumene hydroxide further increased the mutation rate in Escherichia coli WP2 and TA102, respectively, while the contrary results in Salmonella typhimurium TA98 and TA1535 treated with 4-nitroquinoline-1-oxide and sodium azide, respectively, were shown as antimutagenic. However, these mutagenic or co-mutagenic effects of 835-MHz radiation were not significantly repeated in other relevant strains with same mutation type. In the DNA degradation test, the exposure to 835-MHz EMF

did not change the rate of degradation observed using plasmid pBluescriptSK(+) as an indicator. Thus, we suggest that 835-MHz EMF under the conditions of our study neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro.

(NE) Chauhan V, Mariampillai A, Bellier PV, Qutob SS, Gajda GB, Lemay E, Thansandote A, McNamee JP. Gene expression analysis of a human lymphoblastoma cell line exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Radiat Res. 165(4):424-429, 2006. (GE)

This study was designed to determine whether radiofrequency (RF) fields of the type used for wireless communications could elicit a cellular stress response. As general indicators of a cellular stress response, we monitored changes in proto-oncogene and heat-shock protein expression. Exponentially growing human lymphoblastoma cells (TK6) were exposed to 1.9 GHz pulse-modulated RF fields at average specific absorption rates (SARs) of 1 and 10 W/kg. Perturbations in the expression levels of the proto-oncogenes FOS, JUN and MYC after exposure to sham and RF fields were assessed by real-time RT-PCR. In addition, the transcript levels of the cellular stress proteins HSP27 and inducible HSP70 were also monitored. We demonstrated that transcript levels of these genes in RF-field-exposed cells showed no significant difference in relation to the sham treatment group. However, concurrent positive (heat-shock) control samples displayed a significant elevation in the expression of HSP27, HSP70, FOS and JUN. Conversely, the levels of MYC mRNA were found to decline in the positive (heat-shock) control. In conclusion, our study found no evidence that the 1.9 GHz RF-field exposure caused a general stress response in TK6 cells under our experimental conditions.

(NE) Chauhan V, Mariampillai A, Gajda GB, Thansandote A, McNamee JP. Analysis of proto-oncogene and heat-shock protein gene expression in human derived cell-lines exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Int J Radiat Biol. 82(5):347-354, 2006. (GE)

Purpose: Several studies have reported that radiofrequency (RF) fields, as emitted by mobile phones, may cause changes in gene expression in cultured human cell-lines. The current study was undertaken to evaluate this possibility in two human-derived immune cell-lines.Materials and methods: HL-60 and Mono-Mac-6 (MM6) cells were individually exposed to intermittent (5 min on, 10 min off) 1.9 GHz pulse-modulated RF fields at a average specific absorption rate (SAR) of 1 and 10 W/kg at 37 +/- 0.5 degrees C for 6 h. Concurrent negative and positive (heat-shock for 1 h at 43 degrees C) controls were conducted with each experiment. Immediately following RF field exposure (T = 6 h) and 18 h post-exposure (T = 24 h), cell pellets were collected from each of the culture dishes and analyzed for transcript levels of proto-oncogenes (c-jun, c-myc and c-fos) and the stress-related genes (heat shock proteins (HSP) HSP27 and HSP70B) by quantitative reverse transcriptase polymerase chain reaction (RT-PCR).Results: No significant effects were observed in mRNA expression of HSP27, HSP70, c-jun, c-myc or c-fos

between the sham and RF-exposed groups, in either of the two cell-lines. However, the positive (heat-shock) control group displayed a significant elevation in the expression of HSP27, HSP70, c-fos and c-jun in both cell-lines at T = 6 and 24 h, relative to the sham and negative control groups.Conclusion: This study found no evidence that exposure of cells to non-thermalizing levels of 1.9 GHz pulse-modulated RF fields can cause any detectable change in stress-related gene expression.

(NE) Chauhan V, Qutob SS, Lui S, Mariampillai A, Bellier PV, Yauk CL, Douglas GR, Williams A, McNamee JP. Analysis of gene expression in two human-derived cell lines exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. Proteomics. 7(21):3896-3905, 2007. (GE)

There is considerable controversy surrounding the biological effects of radiofrequency (RF) fields, as emitted by mobile phones. Previous work from our laboratory has shown no effect related to the exposure of 1.9 GHz pulse-modulated RF fields on the expression of 22,000 genes in a human glioblastoma-derived cell-line (U87MG) at 6 h following a 4 h RF field exposure period. As a follow-up to this study, we have now examined the effect of RF field exposure on the possible expression of late onset genes in U87MG cells after a 24 h RF exposure period. In addition, a human monocyte-derived cell-line (Mono-Mac-6, MM6) was exposed to intermittent (5 min ON, 10 min OFF) RF fields for 6 h and then gene expression was assessed immediately after exposure and at 18 h postexposure. Both cell lines were exposed to 1.9 GHz pulse-modulated RF fields for 6 or 24 h at specific absorption rates (SARs) of 0.1-10.0 W/kg. In support of our previous results, we found no evidence that nonthermal RF field exposure could alter gene expression in either cultured U87MG or MM6 cells, relative to nonirradiated control groups. However, exposure of both cell-lines to heat-shock conditions (43 degrees C for 1 h) caused an alteration in the expression of a number of well-characterized heat-shock proteins.

(E) Chavdoula ED, Panagopoulos DJ, Margaritis LH. Comparison of biological effects between continuous and intermittent exposure to GSM-900-MHz mobile phone radiation: detection of apoptotic cell-death features. Mutat Res 700:51-61, 2010. (RP, LE, GT)

In the present study we used a 6-min daily exposure of dipteran flies, Drosophila melanogaster, to GSM-900 MHz (Global System for Mobile Telecommunications) mobile phone electromagnetic radiation (EMR), to compare the effects between the continuous and four different intermittent exposures of 6min total duration, and also to test whether intermittent exposure provides any cumulative effects on the insect's reproductive capacity as well as on the induction of apoptotic cell death. According to our previous experiments, a 6-min continuous exposure per day for five days to GSM-900 MHz and DCS-1800 MHz (Digital Cellular System) mobile phone radiation, brought about a large decrease in the insect's reproductive capacity, as defined by the number of F pupae. This decrease was found to be non thermal and correlated with an increased percentage of induced fragmented DNA in the egg chambers' cells at early- and mid-oogenesis. In the present experiments we show that intermittent exposure also decreases the reproductive capacity and alters the actin cytoskeleton network of the egg chambers, another known aspect of cell death that was not investigated in previous

experiments, and that the effect is also due to DNA fragmentation. Intermittent exposures with 10-min intervals between exposure sessions proved to be almost equally effective as continuous exposure of the same total duration, whereas longer intervals between the exposures seemed to allow the organism the time required to recover and partly overcome the above-mentioned effects of the GSM exposure.

(E) Chen G, Lu D, Chiang H, Leszczynski D, Xu Z. Using model organism Saccharomyces cerevisiae to evaluate the effects of ELF-MF and RF-EMF exposure on global gene expression. Bioelectromagnetics. 33(7):550-560, 2012 . (GE)

The potential health hazard of exposure to electromagnetic fields (EMF) continues to cause public concern. However, the possibility of biological and health effects of exposure to EMF remains controversial and their biophysical mechanisms are unknown. In the present study, we used Saccharomyces cerevisiae to identify genes responding to extremely low frequency magnetic fields (ELF-MF) and to radiofrequency EMF (RF-EMF) exposures. The yeast cells were exposed for 6 h to either 0.4 mT 50 Hz ELF-MF or 1800 MHz RF-EMF at a specific absorption rate of 4.7 W/kg. Gene expression was analyzed by microarray screening and confirmed using real-time reverse transcription-polymerase chain reaction (RT-PCR). We were unable to confirm microarray-detected changes in three of the ELF-MF responsive candidate genes using RT-PCR ($P > 0.05$). On the other hand, out of the 40 potential RF-EMF responsive genes, only the expressions of structural maintenance of chromosomes 3 (SMC3) and aquaporin 2 (AQY2 (m)) were confirmed, while three other genes, that is, halotolerance protein 9 (HAL9), yet another kinase 1 (YAK1) and one function-unknown gene (open reading frame: YJL171C), showed opposite changes in expression compared to the microarray data ($P < 0.05$). In conclusion, the results of this study suggest that the yeast cells did not alter gene expression in response to 50 Hz ELF-MF and that the response to RF-EMF is limited to only a very small number of genes. The possible biological consequences of the gene expression changes induced by RF-EMF await further investigation. 1

(E) De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4:e6446, 2009. (GT, OX, RP)

BACKGROUND: In recent times there has been some controversy over the impact of electromagnetic radiation on human health. The significance of mobile phone radiation on male reproduction is a key element of this debate since several studies have suggested a relationship between mobile phone use and semen quality. The potential mechanisms involved have not been established, however, human spermatozoa are known to be particularly vulnerable to oxidative stress by virtue of the abundant availability of substrates for free radical attack and the lack of cytoplasmic space to accommodate antioxidant enzymes. Moreover, the induction of oxidative stress in these cells not only perturbs their capacity for fertilization but also

contributes to sperm DNA damage. The latter has, in turn, been linked with poor fertility, an increased incidence of miscarriage and morbidity in the offspring, including childhood cancer. In light of these associations, we have analyzed the influence of RF-EMR on the cell biology of human spermatozoa in vitro. PRINCIPAL FINDINGS: Purified human spermatozoa were exposed to radio-frequency electromagnetic radiation (RF-EMR) tuned to 1.8 GHz and covering a range of specific absorption rates (SAR) from 0.4 W/kg to 27.5 W/kg. In step with increasing SAR, motility and vitality were significantly reduced after RF-EMR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation were significantly elevated ($P<0.001$). Furthermore, we also observed highly significant relationships between SAR, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation after RF-EMR exposure. CONCLUSIONS: RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. These findings have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring.

(E) Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, Ardoino L, Lovisolo GA, Giardino L, Calzà L. Continuous exposure to 900MHz GSM-modulated EMF alters morphological maturation of neural cells. Neurosci Lett. 455(3):173-177, 2009. (GE, DE)

The effects of radiofrequency electromagnetic field (RF-EMF) exposure on neuronal phenotype maturation have been studied in two different in vitro models: murine SN56 cholinergic cell line and rat primary cortical neurons. The samples were exposed at a dose of 1W/kg at 900 MHz GSM modulated. The phenotype analysis was carried out at 48 and 72 h (24 and 48 h of SN56 cell line differentiation) or at 24, 72, 120 h (2, 4 and 6 days in vitro for cortical neurons) of exposure, on live and immunolabeled neurons, and included the morphological study of neurite emission, outgrowth and branching. Moreover, cortical neurons were studied to detect alterations in the expression pattern of cytoskeleton regulating factors, e.g. beta-thymosin, and of early genes, e.g. c-Fos and c-Jun through real-time PCR on mRNA extracted after 24h exposure to EMF. We found that RF-EMF exposure reduced the number of neurites generated by both cell systems, and this alteration correlates to increased expression of beta-thymosin mRNA.

(E) 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. (GT, LE)

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 × 10(-4) W/kg, Group III: Animals exposed to 1800 MHz at SAR 5.835 × 10(-4) W/kg and Group IV: Animals exposed to 2450 MHz at SAR 6.672 × 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.

(E) Engelmann JC, Deeken R, Müller T, Nimtz G, Roelfsema MR, Hedrich R. Is gene activity in plant cells affected by UMTS-irradiation? A whole genome approach. Adv Appl Bioinform Chem. 1:71-83, 2008. (GE)

Mobile phone technology makes use of radio frequency (RF) electromagnetic fields transmitted through a dense network of base stations in Europe. Possible harmful effects of RF fields on humans and animals are discussed, but their effect on plants has received little attention. In search for physiological processes of plant cells sensitive to RF fields, cell suspension cultures of Arabidopsis thaliana were exposed for 24 h to a RF field protocol representing typical microwave exposition in an urban environment. mRNA of exposed cultures and controls was used to hybridize Affymetrix-ATH1 whole genome microarrays. Differential expression analysis revealed significant changes in transcription of 10 genes, but they did not exceed a fold change of 2.5. Besides that 3 of them are dark-inducible, their functions do not point to any known responses of plants to environmental stimuli. The changes in transcription of these genes were compared with published microarray datasets and revealed a weak similarity of the microwave to light treatment experiments. Considering the large changes described in published experiments, it is questionable if the small alterations caused by a 24 h continuous microwave exposure would have any impact on the growth and reproduction of whole plants.

(E) Esmekaya MA, Aytekin E, Ozgur E, Güler G, Ergun MA, Omeroğlu S, Seyhan N. Mutagenic and morphologic impacts of 1.8GHz radiofrequency radiation on human peripheral blood lymphocytes (hPBLs) and possible protective role of pre-treatment with Ginkgo biloba (EGb 761). Sci Total Environ. 410-411:59-64, 2011. (GT, OX)

The mutagenic and morphologic effects of 1.8GHz Global System for Mobile Communications (GSM) modulated RF (radiofrequency) radiation alone and in combination with Ginkgo biloba (EGb 761) pre-treatment in human peripheral blood lymphocytes (hPBLs) were investigated in this study using Sister Chromatid Exchange (SCE) and electron microscopy. Cell viability was assessed with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay. The lymphocyte cultures were exposed to GSM modulated RF radiation at 1.8GHz for 6, 8, 24 and 48h with and without EGb 761. We observed morphological changes in pulse-modulated RF radiated lymphocytes. Longer exposure periods led to destruction of organelle and nucleus structures. Chromatin change and the loss of mitochondrial crista occurred in cells exposed to RF for 8h and 24h and were more pronounced in cells exposed for 48h. Cytoplasmic lysis and destruction of membrane integrity of cells and nuclei were also seen in 48h RF exposed cells. There was a significant increase ($p<0.05$) in SCE frequency in RF exposed lymphocytes compared to sham controls. EGb 761 pre-treatment significantly decreased SCE from RF radiation. RF radiation also inhibited cell viability in a time dependent manner. The inhibitory effects of RF radiation on the growth of lymphoctes were marked in longer exposure periods. EGb 761 pre-treatment significantly increased cell viability in RF+EGb 761 treated groups at 8 and 24h when compared to RF exposed groups alone. The results of our study showed that RF radiation affects cell morphology, increases SCE and inhibits cell proliferation. However, EGb 761 has a protective role against RF induced mutagenity. We concluded that RF radiation induces chromosomal damage in hPBLs but this damage may be reduced by EGb 761 pre-treatment.

(NE) Falzone N, Huyser C, Franken DR, Leszczynski D. Mobile phone radiation does not induce pro-apoptosis effects in human spermatozoa. Radiat Res 174:169-176, 2010. (GT, OX)

Abstract Recent reports suggest that mobile phone radiation may diminish male fertility. However, the effects of this radiation on human spermatozoa are largely unknown. The present study examined effects of the radiation on induction of apoptosis-related properties in human spermatozoa. Ejaculated, density-purified, highly motile human spermatozoa were exposed to mobile phone radiation at specific absorption rates (SARs) of 2.0 and 5.7 W/kg. At various times after exposure, flow cytometry was used to examine caspase 3 activity, externalization of phosphatidylserine (PS), induction of DNA strand breaks, and generation of reactive oxygen species. Mobile phone radiation had no statistically significant effect on any of the parameters studied. This suggests that the impairment of fertility reported in some studies was not caused by the induction of apoptosis in spermatozoa.

(E) Ferreira AR, Knakievicz T, de Bittencourt Pasquali MA, Gelain DP, Dal-Pizzol F, Fernandez CE, de Almeida de Salles AA, Ferreira HB, Moreira JC. Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring. Life Sci 80: 43-50, 2006. (GT, OX, LE, DE)

Mobile telephones and their base stations are an important ultra high frequency-electromagnetic field (UHF-EMF) source and their utilization is increasing all over the world. Epidemiological studies suggested that low energy UHF-EMF emitted from a cellular telephone

may cause biological effects, such as DNA damage and changes on oxidative metabolism. An in vivo mammalian cytogenetic test, the micronucleus (MN) assay, was used to investigate the occurrence of chromosomal damage in erythrocytes from rat offspring exposed to a non-thermal UHF-EMF from a cellular phone during their embryogenesis; the irradiated group showed a significant increase in MN occurrence. In order to investigate if UHF-EMF could also alter oxidative parameters in the peripheral blood and in the liver - an important hematopoietic tissue in rat embryos and newborns - we also measured the activity of antioxidant enzymes, quantified total sulfhydryl content, protein carbonyl groups, thiobarbituric acid-reactive species and total non-enzymatic antioxidant defense. No significant differences were found in any oxidative parameter of offspring blood and liver. The average number of pups in each litter has also not been significantly altered. Our results suggest that, under our experimental conditions, UHF-EMF is able to induce a genotoxic response in hematopoietic tissue during the embryogenesis through an unknown mechanism.

(NE) Finnie JW, Cai Z, Blumbergs PC, Manavis J, Kuchel TR. Expression of the immediate early gene, c-fos, in fetal brain after whole of gestation exposure of pregnant mice to global system for mobile communication microwaves. Pathology. 38(4):333-335, 2006. (GE, DE)

AIMS: To study immediate early gene, c-fos, expression as a marker of neural stress after whole of gestation exposure of the fetal mouse brain to mobile telephone-type radiofrequency fields. METHODS: Using a purpose-designed exposure system at 900 MHz, pregnant mice were given a single, far-field, whole body exposure at a specific absorption rate of 4 W/kg for 60 min/day from day 1 to day 19 of gestation. Pregnant control mice were sham-exposed or freely mobile in a cage without further restraint. Immediately prior to parturition on gestational day 19, fetal heads were collected, fixed in 4% paraformaldehyde and paraffin embedded. Any stress response in the brain was detected by c-fos immunohistochemistry in the cerebral cortex, basal ganglia, thalamus, hippocampus, midbrain, cerebellum and medulla. RESULTS: c-fos expression was of limited, but consistent, neuroanatomical distribution and there was no difference in immunoreactivity between exposed and control brains. CONCLUSION: In this animal model, no stress response was detected in the fetal brain using c-fos immunohistochemistry after whole of gestation exposure to mobile telephony.

(E) Franzellitti S, Valbonesi P, Ciancaglini N, Biondi C, Contin A, Bersani F, Fabbri E. Transient DNA damage induced by high-frequency electromagnetic fields (GSM 1.8 GHz) in the human trophoblast HTR-8/SVneo cell line evaluated with the alkaline comet assay. Mutat Res 683(1-2):35-42, 2010. (GT, WS)

One of the most controversial issue regarding high-frequency electromagnetic fields (HF-EMF) is their putative capacity to affect DNA integrity. This is of particular concern due to the increasing use of HF-EMF in communication technologies, including mobile phones. Although epidemiological studies report no detrimental effects on human health, the possible disturbance generated by HF-EMF on cell physiology remains controversial. In addition, the question remains as to whether cells are able to compensate their potential effects. We have previously reported that a 1-h exposure to amplitude-modulated 1.8 GHz sinusoidal waves

(GSM-217 Hz, SAR=2 W/kg) largely used in mobile telephony did not cause increased levels of primary DNA damage in human trophoblast HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations were considered of interest. In the present work, HTR-8/SVneo cells were exposed for 4, 16 or 24h to 1.8 GHz continuous wave (CW) and different GSM signals, namely GSM-217 Hz and GSM-Talk (intermittent exposure: 5 min field on, 10 min field off). The alkaline comet assay was used to evaluate primary DNA damages and/or strand breaks due to uncompleted repair processes in HF-EMF exposed samples. The amplitude-modulated signals GSM-217 Hz and GSM-Talk induced a significant increase in comet parameters in trophoblast cells after 16 and 24h of exposure, while the un-modulated CW was ineffective. However, alterations were rapidly recovered and the DNA integrity of HF-EMF exposed cells was similar to that of sham-exposed cells within 2h of recovery in the absence irradiation. Our data suggest that HF-EMF with a carrier frequency and modulation scheme typical of the GSM signal may affect the DNA integrity.

(E) Furtado-Filho OV, Borba JB, Dallegrave A, Pizzolato TM, Henriques JA, Moreira JC, Saffi J. Effect of 950 MHz UHF electromagnetic radiation on biomarkers of oxidative damage, metabolism of UFA and antioxidants in the livers of young rats of different ages. Int J Radiat Biol. 2013 Jul 25. [Epub ahead of print] (LE, GT, OX)

Purpose: To assess the effect of 950 MHz ultra-high-frequency electromagnetic radiation (UHF EMR) on biomarkers of oxidative damage, as well as to verify the concentration of unsaturated fatty acids (UFA) and the expression of the catalase in the livers of rats of different ages. Materials and methods: Twelve rats were equally divided into two groups as controls (CR) and exposed (ER), for each age (0, 6, 15 and 30 days). Radiation exposure lasted half an hour per day for up to 51 days (21 days of gestation and 6, 15 or 30 days of life outside the womb). The specific absorption rate (SAR) ranged from 1.3-1.0 W/kg. The damage to lipids, proteins and DNA was verified by thiobarbituric acid reactive substances (TBARS), protein carbonyls and comets, respectively. UFA were determined by gas chromatography with a flame ionization detector. The expression of catalase was by Western blotting. Results: The neonates had low levels of TBARS and concentrations of UFA after exposure. There was no age difference in the accumulation of protein carbonyls for any age. The DNA damage of ER 15 or 30 days was different. The exposed neonates exhibited lower expression of catalase. Conclusions: 950 MHz UHF EMR does not cause oxidative stress (OS), and it is not genotoxic to the livers of neonates or those of 6 and 15 day old rats, but it changes the concentrations of polyunsaturated fatty acid (PUFA) in neonates. For rats of 30 days, no OS, but it is genotoxic to the livers of ER to total body irradiation.

(E) Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (Apismellifera) against 915-MHz microwave radiation-induced DNA damage in wistar rat lymphocytes: in vitro study. Int J Toxicol 28:88-98, 2009. (GT, OX)

The aim of this study is to investigate the radioprotective effect of bee venom against DNA damage induced by 915-MHz microwave radiation (specific absorption rate of 0.6 W/kg) in

Wistar rats. Whole blood lymphocytes of Wistar rats are treated with 1 microg/mL bee venom 4 hours prior to and immediately before irradiation. Standard and formamidopyrimidine-DNA glycosylase (Fpg)-modified comet assays are used to assess basal and oxidative DNA damage produced by reactive oxygen species. Bee venom shows a decrease in DNA damage compared with irradiated samples. Parameters of Fpg-modified comet assay are statistically different from controls, making this assay more sensitive and suggesting that oxidative stress is a possible mechanism of DNA damage induction. Bee venom is demonstrated to have a radioprotective effect against basal and oxidative DNA damage. Furthermore, bee venom is not genotoxic and does not produce oxidative damage in the low concentrations used in this study.

(E) Gandhi G, Anita, Genetic damage in mobile phone users: some preliminary findings. Ind J Hum Genet 11:99-104, 2005. (GT, HU)

BACKGROUND: The impact of microwave (MW)/radio frequency radiation (RFR) on important biological parameters is probably more than a simply thermal one. Exposure to radio frequency (RF) signals generated by the use of cellular telephones have increased dramatically and reported to affect physiological, neurological, cognitive and behavioural changes and to induce, initiate and promote carcinogenesis. Genotoxicity of RFR has also been reported in various test systems after in vitro and/or in vivo exposure but none in mobile phone users. AIMS: In the present study, DNA and chromosomal damage investigations were carried out on the peripheral blood lymphocytes of individuals using mobile phones, being exposed to MW frequency ranging from 800 to 2000 MHz. METHODS: DNA damage was assessed using the single cell gel electrophoresis assay and aneugenic and clastogenic damage by the in vivo capillary blood micronucleus test (MNT) in a total of 24 mobile phone users. RESULTS: Mean comet tail length (26.76 ± 0.054 mm; 39.75% of cells damaged) in mobile phone users was highly significant from that in the control group. The in vivo capillary blood MNT also revealed highly significant (0.25) frequency of micronucleated (MNd) cells. CONCLUSIONS: These results highlight a correlation between mobile phone use (exposure to RFR) and genetic damage and require interim public health actions in the wake of widespread use of mobile telephony.

(E) Gandhi G, Singh P. Cytogenetic damage in mobile phone users: preliminary data. Int J Hum Genet 5:259-265, 2005. (GT, HU)

Mobile telephones, sometimes called cellular (cell) phones or handies, are now an integral part of modern life. The mobile phone handsets are low-powered radiofrequency transmitters, emitting maximum powers in the range of 0.2 to 0.6 watts. Scientific concenrns have increased sufficiently over the possible hazard to health from using cell phones. The reported adverse health effects include physiological, behavioural and cognitive changes as well as tumour formation and genetic damage. However findings are controversial and no consensus exists. Genotoxicity has been observed either in lower organisms or in vitro studies. The aim of the present study hence was to detect any cytogenertic damage in mobile phone users by analysing short term peripheral lymphocyte cultures for chromosomal aberrations and the buccal mucosal cells for micronuclei (aneugenicity and clastogenicity). The results revealed increased

number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes indicating the genotoxic response from mobile phone use.

(E) Garaj-Vrhovac V, Gajski G, Pažanin S, Sarolić A, Domijan AM, Flajs D, Peraica M. Assessment of cytogenetic damage and oxidative stress in personnel occupationally exposed to the pulsed microwave radiation of marine radar equipment. Int J Hyg Environ Health. 4(1):59-65, 2011. (GT, HU, OX)

Due to increased usage of microwave radiation, there are concerns of its adverse effect in today's society. Keeping this in view, study was aimed at workers occupationally exposed to pulsed microwave radiation, originating from marine radars. Electromagnetic field strength was measured at assigned marine radar frequencies (3 GHz, 5.5 GHz and 9.4 GHz) and corresponding specific absorption rate values were determined. Parameters of the comet assay and micronucleus test were studied both in the exposed workers and in corresponding unexposed subjects. Differences between mean tail intensity (0.67 vs. 1.22) and moment (0.08 vs. 0.16) as comet assay parameters and micronucleus test parameters (micronuclei, nucleoplasmic bridges and nuclear buds) were statistically significant between the two examined groups, suggesting that cytogenetic alterations occurred after microwave exposure. Concentrations of glutathione and malondialdehyde were measured spectrophotometrically and using high performance liquid chromatography. The glutathione concentration in exposed group was significantly lower than in controls (1.24 vs. 0.53) whereas the concentration of malondialdehyde was significantly higher (1.74 vs. 3.17), indicating oxidative stress. Results suggests that pulsed microwaves from working environment can be the cause of genetic and cell alterations and that oxidative stress can be one of the possible mechanisms of DNA and cell damage.

(E) Guler G, Tomruk A, Ozgur E, Seyhan N.The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. Gen Physiol Biophys 29:59-66, 2010. (GT, OX, LE, DE)

The concerns of people on possible adverse health effects of radiofrequency radiation (RFR) generated from mobile phones as well as their supporting transmitters (base stations) have increased markedly. RFR effect on oversensitive people, such as pregnant women and their developing fetuses, and older people is another source of concern that should be considered. In this study, oxidative DNA damage and lipid peroxidation levels in the brain tissue of pregnant and non-pregnant New Zealand White rabbits and their newborns exposed to RFR were investigated. Thirteen-month-old rabbits were studied in four groups as non-pregnant-control, non-pregnant-RFR exposed, pregnant-control and pregnant-RFR exposed. They were exposed to RFR (1800 MHz GSM; 14 V/m as reference level) for 15 min/day during 7 days. Malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were analyzed. MDA and 8-OHdG levels of non-pregnant and pregnant-RFR exposed animals significantly increased with respect to controls ($p < 0.001$, Mann-Whitney test). No difference was found in the newborns ($p > 0.05$, Mann-Whitney). There exist very few experimental studies on the

effects of RFR during pregnancy. It would be beneficial to increase the number of these studies in order to establish international standards for the protection of pregnant women from RFR.

(E) Güler G, Tomruk A, Ozgur E, Sahin D, Sepici A, Altan N, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in female and male infant rabbits. Int J Radiat Biol. 88(4):367-373, 2012. (LE, GT, OX, DE)

PURPOSE: We aimed to design a prolonged radiofrequency (RF) radiation exposure and investigate in an animal model, possible bio-effects of RF radiation on the ongoing developmental stages of children from conception to childhood. **MATERIALS AND METHODS:** A total of 72 New Zealand female and male white rabbits aged one month were used. Females were exposed to RF radiation for 15 min/day during 7 days, whereas males were exposed to the same level of radiation for 15 min/day during 14 days. Thirty-six female and 36 male infant rabbits were randomly divided into four groups: Group I [Intrauterine (IU) exposure (-); Extrauterine (EU) exposure (-)]: Sham exposure which means rabbits were exposed to 1800 MHz Global System for Mobile Telecommunication (GSM)-like RF signals neither in the IU nor in the EU periods. Group II [IU exposure (-); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals when they reached one month of age. Group III [IU exposure (+); EU exposure (-)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals in the IU period (between 15th and 22nd days of the gestational period). Group IV [IU exposure (+); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals both in the IU period (between 15th and 22nd days of the gestational period) and in the EU period when they reached one month of age. Biochemical analysis for lipid peroxidation and DNA damage were carried out in the livers of all rabbits. **RESULTS:** Lipid peroxidation levels in the liver tissues of female and male infant rabbits increased under RF radiation exposure. Liver 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of female rabbits exposed to RF radiation were also found to increase when compared with the levels of non-exposed infants. However, there were no changes in liver 8-OHdG levels of male rabbits under RF exposure. **CONCLUSION:** Consequently, it can be concluded that GSM-like RF radiation may induce biochemical changes by increasing free radical attacks to structural biomolecules in the rabbit as an experimental animal model.

(NE) Gurbuz N, Sirav B, Yuvaci HU, Turhan N, Coskun ZK, Seyhan N. Is there any possible genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 MHz GSM-like modulated radio frequency radiation (RFR)? Electromagn Biol Med. 29(3):98-104, 2010. (LE, GT)

People are exposed to many carcinogenic and mutagenic chemicals in their everyday lives. These include antineoplastic drugs, Polycyclic aromatic hydrocarbons (PAH)s, aromatic amines, nitrosamines, metals, and electromagnetic radiation. Based on the state of knowledge acquired during the last 50 years of research on possible biological effects of electromagnetic fields (EMF), the majority of the scientific community is convinced that exposure to EMF below the existing security limits does not cause a risk to the health of the general public. However, this

position is questioned by others, who are of the opinion that the available research data are contradictory or inconsistent and, therefore, unreliable. In this study, we aimed to investigate if there is any effect of 1800 MHz GSM modulated radio frequency radiation (RFR) on the number of micronucleus in exfoliated bladder cells of rat which will be informative about the genotoxic damage. Exposure period was 20 min/day, 5 days/week during a month. Six female Wistar rats were used for two groups: Group I (n=6): controls; Group II (n=6): 1.8 GHz exposed animals. 1800 MHz RFR did not showed a significant MN frequencies in rat bladder cells when compared with the control group (p>0.05). 1800 MHz RFR-exposed animals did not produce any genotoxic effect when compared with the control group (p>0.05). Kinetic studies are important for any biomarker, especially those in which tissue differentiation and maturation processes will heavily influence the time between induction of damage and collection of damaged cells for micronucleus analysis.

(NE) Gurbuz N, Sirav B, Colbay M, Yetkin I, Seyhan N. No genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 and 2100-MHz radio frequency radiation. Electromagn Biol Med. 2013 Nov 27. [Epub ahead of print] (GT, LE)

Abstract In this study, we aimed to investigate the effects of 1800 and 2100 MHz Radio Frequency (RF) radiation on the number of micronucleus (MN) in exfoliated bladder cells of rat which shows the genotoxic damage. Exposure period was 30 min/day, 6 days/week for a month and two months exposure periods. Thirty male wistar albino rats were used for five groups: Group I (n = 6): 1800 MHz RF exposed animals for one month, Group II (n = 6): 2100 MHz RF exposed animals for one month, Group III (n = 6): 2100 MHz RF exposed for two months, Group IV (n = 6): control group for one month, Group V (n = 6): control group for two months. Rats of the control groups were housed in their home cages during the entire experimental period without subjecting to any experimental manipulation. 1800 and 2100 MHz RF exposures did not result in any significant MN frequencies in rat bladder cells with respect to the control groups (p > 0.05). There was no statistically significant difference between 2100 MHz RF exposed groups, either. Further studies are needed to demonstrate if there is any genotoxic effect, micronucleus formation in other tissues of rats.

(NE) Hansteen IL, Lågeide L, Clausen KO, Haugan V, Svendsen M, Eriksen JG, Skiaker R, Hauger E, Vistnes AI, Kure EH. Cytogenetic effects of 18.0 and 16.5 GHz microwave radiation on human lymphocytes in vitro. Anticancer Res 29:2885-2892, 2009. (GT, IA, WS)

BACKGROUND: There are few cell studies on the direct genotoxic effects of microwave radiation. In this study, cytogenetic effects of microwave radiation alone or in combination with mitomycin C (MMC) were investigated. MATERIALS AND METHODS: Lymphocytes from two smoking and four non-smoking donors were exposed for 53 hours in vitro to 1.0 W/m continuous-wave radiation at 18.0 GHz or 10 W/m pulsed-wave at 16.5 GHz, alone or in

combination with MMC. DNA synthesis and repair were inhibited in vitro in some cultures. RESULTS: No synergistic effect was observed in cells exposed to combinations of microwave radiation and in vitro exposure to MMC, or to cells pre-exposed in vivo to tobacco smoke. For the 16.5 GHz pulsed exposure, a non-significant trend consisting of an increase in aberration frequencies with microwave radiation was shown for the DNA synthesis and repair inhibited cultures both with and without MMC. CONCLUSION: Neither 18.0 GHz continuous-wave nor 16.5 GHz pulsed-wave exposure to human lymphocytes in vitro induced statistically significant increases in chromosomal aberration frequencies. 16.5 GHz pulsed-wave exposure requires further documentation before a true negative conclusion can be drawn.

(NE) Hansteen IL, Clausen KO, Haugan V, Svendsen M, Svendsen MV, Eriksen JG, Skiaker R, Hauger E, Lågeide L, Vistnes AI, Kure EH. Cytogenetic effects of exposure to 2.3 GHz radiofrequency radiation on human lymphocytes in vitro. Anticancer Res 29:4323-4330, 2009. (GT, IA)

BACKGROUND: No previous in vitro studies have tested radio frequency radiation for at least one full cell cycle in culture. The aim was to test if exposure used in mobile phones and wireless network technologies would induce DNA damage in cultured human lymphocytes with and without a known clastogen. MATERIALS AND METHODS: Lymphocytes from six donors were exposed to 2.3 GHz, 10 W/m continuous waves, or 2.3 GHz, 10 W/m pulsed waves (200 Hz pulse frequency, 50% duty cycle). Mitomycin C was added to half of the cultures. DNA synthesis and repair were inhibited in one experiment. RESULTS: No statistically significant differences were observed between control and exposed cultures. A weak trend for more chromosomal damage with the interaction of pulsed fields with mitomycin C compared to a constant field was observed. CONCLUSION: Exposure during the whole cell cycle in inhibited cultures did not resulted in significant differences in chromosomal aberrations as compared to controls.

(E) Hekmat A, Saboury AA, Moosavi-Movahedi AA. The toxic effects of mobile phone radiofrequency (940MHz) on the structure of calf thymus DNA. Ecotoxicol Environ Saf. 2012 Nov 16. pii: S0147-6513(12)00368-5. doi: 10.1016/j.ecoenv.2012.10.016. [Epub ahead of print] (GT)

Currently, the biological effects of nonionizing electromagnetic fields (EMFs) including radiofrequency (RF) radiation have been the subject of numerous experimental and theoretical studies. The aim of this study is to evaluate the possible biological effects of mobile phone RF (940MHz, 15V/m and SAR=40mW/kg) on the structure of calf thymus DNA (ct DNA) immediately after exposure and 2h after 45min exposure via diverse range of spectroscopic instruments. The UV-vis and circular dichroism (CD) experiments depict that mobile phone EMFs can remarkably cause disturbance on ct DNA structure. In addition, the DNA samples, immediately after exposure and 2h after 45min exposure, are relatively thermally unstable compared to the DNA solution, which was placed in a small shielded box (unexposed ct DNA). Furthermore, the exposed DNA samples (the DNA samples that were exposed to 940MHz EMF) have more fluorescence emission when compared with the unexposed DNA, which may have occurred attributable to expansion of the exposed DNA structure. The results of dynamic light

scattering (DLS) and zeta potential experiments demonstrate that RF-EMFs lead to increment in the surface charge and size of DNA. The structure of DNA immediately after exposure is not significantly different from the DNA sample 2h after 45min exposure. In other words, the EMF-induced conformational changes are irreversible. Collectively, our results reveal that 940MHz can alter the structure of DNA. The displacement of electrons in DNA by EMFs may lead to conformational changes of DNA and DNA disaggregation. Results from this study could have an important implication on the health effects of RF-EMFs exposure. In addition, this finding could proffer a novel strategy for the development of next generation of mobile phone.

(NE) Hintzsche H, Stopper H. Micronucleus frequency in buccal mucosa cells of mobile phone users. Toxicol Lett. 193(1):124-130, 2010. (GT, HU)

Mobile phones are being used extensively throughout the world, with more than four billion accounts existing in 2009. This technology applies electromagnetic radiation in the microwave range. Health effects of this radiation have been subject of debate for a long time, both within the scientific community and within the general public. This study investigated the effect of mobile phone use on genomic instability of the human oral cavity's mucosa cells. 131 Individuals donated buccal mucosa cells extracted by slightly scraping the oral cavity with a cotton swab. Every participant filled out a questionnaire about mobile phone use including duration of weekly use, overall period of exposure and headset usage. 13 Individuals did not use mobile phones at all, 85 reported using the mobile phone for three hours per week or less, and 33 reported use of more than three hours per week. Additionally, information on age, gender, body weight, smoking status, medication and nutrition was retrieved. For staining of the cells a procedure using alpha-tubulin-antibody and chromomycin A(3) was applied. Micronuclei and other markers were evaluated in 1000 cells per individual at the microscope. A second scorer counted another 1000 cells, resulting in 2000 analyzed cells per individual. Mobile phone use did not lead to a significantly increased frequency of micronuclei.

(NE) Hintzsche H, Jastrow C, Kleine-Ostmann T, Schrader T, Stopper H. 900 MHz radiation does not induce micronucleus formation in different cell types. Mutagenesis. 27(4):477-483, 2012 . (GT)

The exposure of the population to non-ionising electromagnetic radiation is still increasing, mainly due to mobile communication. Whether low-intensity electromagnetic fields can cause other effects apart from heating has been a subject of debate. One of the effects, which were proposed to be caused by mobile phone radiation, is the occurrence of mitotic disturbances. The aim of this study was to investigate possible consequences of these mitotic disturbances as manifest genomic damage, i.e. micronucleus induction. Cells were irradiated at a frequency of 900 MHz, which is located in one of the main frequency bands applied for mobile communication. Two cell types were used, HaCaT cells as human cells and A(L) cells (human-hamster hybrid cells), in which mitotic disturbances had been reported to occur. After different post-exposure incubation periods, cells were fixed and micronucleus frequencies were evaluated. Both cell types did not show any genomic damage after exposure. To adapt the

protocol for the micronucleus test into the direction of the protocol for mitotic disturbances, the post-exposure incubation period was reduced and exposure time was extended to one cell cycle length. This did not result in any increase of the genomic damage. In conclusion, micronucleus induction was not observed as a consequence of exposure to non-ionising radiation, even though this agent was reported to cause mitotic disturbances under similar experimental conditions.

(NE) Hirose H, Sakuma N, Kaji N, Suhara T, Sekijima M, Nojima T, Miyakoshi J. Phosphorylation and gene expression of p53 are not affected in human cells exposed to 2.1425 GHz band CW or W-CDMA modulated radiation allocated to mobile radio base stations. Bioelectromagnetics 27:494-504, 2006. (GT)

A large-scale in vitro study focusing on low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields induce apoptosis or other cellular stress response that activate p53 or the p53-signaling pathway. First, we evaluated the response of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and wideband code division multiple access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced apoptosis or any signs of stress. Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg, and CW radiation at 80 mW/kg for 24 or 48 h. Human IMR-90 fibroblasts from fetal lungs were exposed to both W-CDMA and CW radiation at a SAR of 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the percentage of apoptotic cells were observed between the test groups exposed to RF signals and the sham-exposed negative controls, as evaluated by the Annexin V affinity assay. No significant differences in expression levels of phosphorylated p53 at serine 15 or total p53 were observed between the test groups and the negative controls by the bead-based multiplex assay. Moreover, microarray hybridization and real-time RT-PCR analysis showed no noticeable differences in gene expression of the subsequent downstream targets of p53 signaling involved in apoptosis between the test groups and the negative controls. Our results confirm that exposure to low-level RF signals up to 800 mW/kg does not induce p53-dependent apoptosis, DNA damage, or other stress response in human cells.

(NE) Hirose H, Sakuma N, Kaji N, Nakayama K, Inoue K, Sekijima M, Nojima T, Miyakoshi J. Mobile phone base station-emitted radiation does not induce phosphorylation of Hsp27. Bioelectromagnetics 28:99-108, 2007. (GE)

An in vitro study focusing on the effects of low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields act to induce phosphorylation and overexpression of heat shock protein hsp27. First, we evaluated the

responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced activation or gene expression of hsp27 and other heat shock proteins (hsps). Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80 and 800 mW/kg for 2-48 h, and CW radiation at 80 mW/kg for 24 h. Human IMR-90 fibroblasts from fetal lungs were exposed to W-CDMA at 80 and 800 mW/kg for 2 or 28 h, and CW at 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the expression levels of phosphorylated hsp27 at serine 82 (hsp27[pS82]) were observed between the test groups exposed to W-CDMA or CW signal and the sham-exposed negative controls, as evaluated immediately after the exposure periods by bead-based multiplex assays. Moreover, no noticeable differences in the gene expression of hsps were observed between the test groups and the negative controls by DNA Chip analysis. Our results confirm that exposure to low-level RF field up to 800 mW/kg does not induce phosphorylation of hsp27 or expression of hsp gene family.

(NE) Huang TQ, Lee MS, Oh E, Zhang BT, Seo JS, Park WY. Molecular responses of Jurkat T-cells to 1763 MHz radiofrequency radiation.Int J RadiatBiol 84:734-741, 2008. (GT, GE)

PURPOSE: The biological effects of exposure to mobile phone emitted radiofrequency (RF) radiation are the subject of intense study, yet the hypothesis that RF exposure is a potential health hazard remains controversial. In this paper, we monitored cellular and molecular changes in Jurkat human T lymphoma cells after irradiating with 1763 MHz RF radiation to understand the effect on RF radiation in immune cells. MATERIALS AND METHODS: Jurkat T-cells were exposed to RF radiation to assess the effects on cell proliferation, cell cycle progression, DNA damage and gene expression. Jurkat cells were exposed to 1763 MHz RF radiation at 10 W/kg specific absorption rate (SAR) and compared to sham exposed cells. RESULTS: RF exposure did not produce significant changes in cell numbers, cell cycle distributions, or levels of DNA damage. In genome-wide analysis of gene expressions, there were no genes changed more than two-fold upon RF-radiation while ten genes change to 1.3 approximately 1.8-fold. Among ten genes, two cytokine receptor genes such as chemokine (C-X-C motif) receptor 3 (CXCR3) and interleukin 1 receptor, type II (IL1R2) were down-regulated upon RF radiation, but they were not directly related to cell proliferation or DNA damage responses. CONCLUSION: These results indicate that the alterations in cell proliferation, cell cycle progression, DNA integrity or global gene expression was not detected upon 1763 MHz RF radiation under 10 W/kg SAR for 24 h to Jurkat T cells.

(NE) Huang TQ, Lee MS, Oh EH, Kalinec F, Zhang BT, Seo JS, Park WY. Characterization of biological effect of 1763 MHz radiofrequency exposure on auditory hair cells.Int J Radiat Biol 84:909-915, 2008. (GT, GE)

Purpose: Radiofrequency (RF) exposure at the frequency of mobile phones has been reported not to induce cellular damage in in vitro and in vivo models. We chose HEI-OC1 immortalized mouse auditory hair cells to characterize the cellular response to 1763 MHz RF exposure, because auditory cells could be exposed to mobile phone frequencies. Materials and methods: Cells were exposed to 1763 MHz RF at a 20 W/kg specific absorption rate (SAR) in a code division multiple access (CDMA) exposure chamber for 24 and 48 h to check for changes in cell cycle, DNA damage, stress response, and gene expression. Results: Neither of cell cycle changes nor DNA damage was detected in RF-exposed cells. The expression of heat shock proteins (HSP) and the phosphorylation of mitogen-activated protein kinases (MAPK) did not change, either. We tried to identify any alteration in gene expression using microarrays. Using the Applied Biosystems 1700 full genome expression mouse microarray, we found that only 29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure. Conclusion: From these results, we could not find any evidence of the induction of cellular responses, including cell cycle distribution, DNA damage, stress response and gene expression, after 1763 MHz RF exposure at an SAR of 20 W/kg in HEI-OC1 auditory hair cells.

(E) Jiang B, Nie J, Zhou Z, Zhang J, Tong J, Cao Y. Adaptive response in mice exposed to 900 MHz radiofrequency fields: primary DNA damage. PLoS One. 7(2):e32040, 2012. (LE, GT, IA)

The phenomenon of adaptive response (AR) in animal and human cells exposed to ionizing radiation is well documented in scientific literature. We have examined whether such AR could be induced in mice exposed to non-ionizing radiofrequency fields (RF) used for wireless communications. Mice were pre-exposed to 900 MHz RF at 120 µW/cm(2) power density for 4 hours/day for 1, 3, 5, 7 and 14 days and then subjected to an acute dose of 3 Gy γ-radiation. The primary DNA damage in the form of alkali labile base damage and single strand breaks in the DNA of peripheral blood leukocytes was determined using the alkaline comet assay. The results indicated that the extent of damage in mice which were pre-exposed to RF for 1 day and then subjected to γ-radiation was similar and not significantly different from those exposed to γ-radiation alone. However, mice which were pre-exposed to RF for 3, 5, 7 and 14 days showed progressively decreased damage and was significantly different from those exposed to γ-radiation alone. Thus, the data indicated that RF pre-exposure is capable of inducing AR and suggested that the pre-exposure for more than 4 hours for 1 day is necessary to elicit such AR.

(NE) Juutilainen J, Heikkinen P, Soikkeli H, Mäki-Paakkanen J. Micronucleus frequency in erythrocytes of mice after long-term exposure to radiofrequency radiation. Int J Radiat Biol. 83(4):213-220, 2007. (LE, GT)

PURPOSE: The aim of the study was to investigate genotoxicity of long-term exposure to radiofrequency (RF) electromagnetic fields by measuring micronuclei in erythrocytes. The blood samples were collected in two animal studies evaluating possible cocarcinogenic effects of RF fields. METHODS: In study A, female CBA/S mice were exposed for 78 weeks (1.5 h/d, 5 d/week) to either a continuous 902.5 MHz signal similar to that emitted by analog NMT (Nordic Mobile Telephone) phones at a whole-body specific absorption rate (SAR) of 1.5 W/kg, or to a

pulsed 902.4 MHz signal similar to that of digital GSM (Global System for Mobile Communications) phones at 0.35 W/kg. A third group was sham-exposed, and a fourth group served as cage controls. All but the cage control animals were exposed to 4 Gy of x-rays during three first weeks of the experiment. In study B, female transgenic mice (line K2) and their nontransgenic littermates were exposed for 52 weeks (1.5 h/d, 5 d/week). Two digital mobile phone signals, GSM and DAMPS (Digital Advanced Mobile Phone System), were used at 0.5 W/kg. All but the cage-control animals were exposed 3 times per week to an ultraviolet radiation dose of 1.2 MED (minimum erythema dose). RESULTS AND CONCLUSIONS: The results did not show any effects of RF fields on micronucleus frequency in polychromatic or normochromatic erythrocytes. The results were consistent in two mouse strains (and in a transgenic variant of the second strain), after 52 or 78 weeks of exposure, at three SAR levels relevant to human exposure from mobile phones, and for three different mobile signals.

(E) Karaca E, Durmaz B, Altug H, Yildiz T, Guducu C, Irgi M, Koksal MG, Ozkinay F, Gunduz C, Cogulu O. The genotoxic effect of radiofrequency waves on mouse brain. J Neurooncol 106:53-58, 2012. (GT, GE)

Erratum: J Neurooncol 2012 May;107:665.

Concerns about the health effects of radiofrequency (RF) waves have been raised because of the gradual increase in usage of cell phones, and there are scientific questions and debates about the safety of those instruments in daily life. The aim of this study is to evaluate the genotoxic effects of RF waves in an experimental brain cell culture model. Brain cell cultures of the mice were exposed to 10.715 GHz with specific absorbtion rate (SAR) 0.725 W/kG signals for 6 h in 3 days at 25°C to check for the changes in the micronucleus (MNi) assay and in the expression of 11 proapoptotic and antiapoptotic genes. It was found that MNi rate increased 11-fold and STAT3 expression decreased 7-fold in the cell cultures which were exposed to RF. Cell phones which spread RF may damage DNA and change gene expression in brain cells.

(E) Kesari KK, Behari J. Fifty-gigahertz Microwave exposure effect of radiations on rat brain. Appl Biochem Biotechnol 158:126-139, 2009. (GT, OX, LE)

The object of this study is to investigate the effects of 50-GHz microwave radiation on the brain of Wistar rats. Male rats of the Wistar strain were used in the study. Animals of 60-day age were divided into two groups-group 1, sham-exposed, and group 2, experimental (microwave-exposed). The rats were housed in a temperature-controlled room (25 degrees C) with constant humidity (40-50%) and received food and water ad libitum. During exposure, rats were placed in Plexiglas cages with drilled ventilation holes and kept in an anechoic chamber. The animals were exposed for 2 h a day for 45 days continuously at a power level of 0.86 muW/cm with nominal specific absorption rate 8.0 x 10(-4) w/kg. After the exposure period, the rats were killed and homogenized, and protein kinase C (PKC), DNA double-strand break, and antioxidant enzyme activity [superoxides dismutase (SOD), catalase, and glutathione peroxidase (GPx)] were estimated in the whole brain. Result shows that the chronic exposure to these radiations causes DNA double-strand break (head and tail length, intensity and tail migration) and a

significant decrease in GPx and SOD activity (p = <0.05) in brain cells, whereas catalase activity shows significant increase in the exposed group of brain samples as compared with control (p = <0.001). In addition to these, PKC decreased significantly in whole brain and hippocampus (p < 0.05). All data are expressed as mean +/- standard deviation. We conclude that these radiations can have a significant effect on the whole brain.

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

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

(E) Khalil AM, Gagaa M, Alshamali A. 8-Oxo-7, 8-dihydro-2'-deoxyguanosine as a biomarker of DNA damage by mobile phone radiation. Hum ExpToxicol 31(7):734-740, 2012. (GT, OX)

We examined the effect of exposure to mobile phone 1800 MHz radio frequency radiation (RFR) upon the urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG), one major form of oxidative DNA damage, in adult male Sprague-Dawley rats. Twenty-four rats were used in three independent experiments (RFR exposed and control, 12 rats, each). The animals were exposed to RFR for 2 h from Global System for Mobile Communications (GSM) signal generator with whole-body-specific absorption rate of 1.0 W/kg. Urine samples were collected from the rat while housed in a metabolic cage during the exposure period over a 4-h period at 0.5, 1.0, 2.0 and 4.0 h from the beginning of exposure. In the control group, the signal generator was left in the turn-off position. The creatinine-standardized concentrations of 8-oxodG were measured. With the exception of the urine collected in the last half an hour of exposure, significant elevations were noticed in the levels of 8-oxodG in urine samples from rats exposed to RFR when compared to control animals. Significant differences were seen overall across time

points of urine collection with a maximum at 1 h after exposure, suggesting repair of the DNA lesions leading to 8-oxodG formation.

(E) Kim JY, Hong SY, Lee YM, Yu SA, Koh WS, Hong JR, Son T, Chang SK, Lee M.In vitro assessment of clastogenicity of mobile-phone radiation (835 MHz) using the alkaline comet assay and chromosomal aberration test. Environ Toxicol 23:319-327, 2008. (GT, IA)

Recently we demonstrated that 835-MHz radiofrequency radiation electromagnetic fields (RF-EMF) neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro. Here, two kinds of cytogenetic endpoints were further investigated on mammalian cells exposed to 835-MHz RF-EMF (the most widely used communication frequency band in Korean CDMA mobile phone networks) alone and in combination with model clastogens: in vitro alkaline comet assay and in vitro chromosome aberration (CA) test. No direct cytogenetic effect of 835-MHz RF-EMF was found in the in vitro CA test. The combined exposure of the cells to RF-EMF in the presence of ethylmethanesulfonate (EMS) revealed a weak and insignificant cytogenetic effect when compared to cells exposed to EMS alone in CA test. Also, the comet assay results to evaluate the ability of RF-EMF alone to damage DNA were nearly negative, although showing a small increase in tail moment. However, the applied RF-EMF had potentiation effect in comet assay when administered in combination with model clastogens (cyclophosphamide or 4-nitroquinoline 1-oxide). Thus, our results imply that we cannot confidently exclude any possibility of an increased risk of genetic damage, with important implications for the possible health effects of exposure to 835-MHz electromagnetic fields.

(E) Kumar S, Kesari KK, Behari J. Evaluation of genotoxic effects in male Wistar rats following microwave exposure. Indian J Exp Biol 48:586-592, 2010. (GT, OX)

Wistar rats (70 days old) were exposed for 2 h a day for 45 days continuously at 10 GHz [power density 0.214 mW/cm2, specific absorption rate (SAR) 0.014 W/kg] and 50 GHz (power density 0.86 microW/cm2, SAR 8.0 x10(-4) W/kg). Micronuclei (MN), reactive oxygen species (ROS), and antioxidant enzymes activity were estimated in the blood cells and serum. These radiations induce micronuclei formation and significant increase in ROS production. Significant changes in the level of serum glutathione peroxidase, superoxide dismutase and catalase were observed in exposed group as compared with control group. It is concluded that microwave exposure can be affective at genetic level. This may be an indication of tumor promotion, which comes through the overproduction of reactive oxygen species.

(E) Lakshmi NK, Tiwari R, Bhargava SC, Ahuja YR. Investigations on DNA damage and frequency of micronuclei in occupational exposure to electromagnetic fields (EMFs) emitted from video display terminals (VDTs). Gen MolBiol 33, 154-158, 2010. (GT, HU, LE)

The potential effect of electromagnetic fields (EMFs) emitted from video display terminals (VDTs) to elicit biological response is a major concern for the public. The software professionals are subjected to cumulative EMFs in their occupational environments. This study was undertaken to evaluate DNA damage and incidences of micronuclei in such professionals. To the best of our knowledge, the present study is the first attempt to carry out cytogenetic

investigations on assessing bioeffects in personal computer users. The study subjects (n = 138) included software professionals using VDTs for more than 2 years with age, gender, socioeconomic status matched controls (n = 151). DNA damage and frequency of micronuclei were evaluated using alkaline comet assay and cytochalasin blocked micronucleus assay respectively. Overall DNA damage and incidence of micronuclei showed no significant differences between the exposed and control subjects. With exposure characteristics, such as total duration (years) and frequency of use (minutes/day) sub-groups were assessed for such parameters. Although cumulative frequency of use showed no significant changes in the DNA integrity of the classified sub-groups, the long-term users (> 10 years) showed higher induction of DNA damage and increased frequency of micronuclei and micro nucleated cells.

(E) Liu C, Duan W, Xu S, Chen C, He M, Zhang L, Yu Z, Zhou Z. Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line. Toxicol Lett 218(1): 2-9, 2013a. (GT, OX, RP)

Whether exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from mobile phones can induce DNA damage in male germ cells remains unclear. In this study, we conducted a 24 h intermittent exposure (5 min on and 10 min off) of a mouse spermatocyte-derived GC-2 cell line to 1800 MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at specific absorption rates (SAR) of 1 W/kg, 2 W/kg or 4 W/kg. Subsequently, through the use of formamidopyrimidine DNA glycosylase (FPG) in a modified comet assay, we determined that the extent of DNA migration was significantly increased at a SAR of 4 W/kg. Flow cytometry analysis demonstrated that levels of the DNA adduct 8-oxoguanine (8-oxoG) were also increased at a SAR of 4 W/kg. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS); these phenomena were mitigated by co-treatment with the antioxidant α-tocopherol. However, no detectable DNA strand breakage was observed by the alkaline comet assay. Taking together, these findings may imply the novel possibility that RF-EMR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells.

(E) Liu C, Gao P, Xu SC, Wang Y, Chen CH, He MD, Yu ZP, Zhang L, Zhou Z. Mobile phone radiation induces mode-dependent DNA damage in a mouse spermatocyte-derived cell line: a protective role of melatonin. Int J Radiat Biol. 2013b Aug 19. [Epub ahead of print] (GT, OX, RP)

Purpose: To evaluate whether exposure to mobile phone radiation (MPR) can induce DNA damage in male germ cells. Materials and methods: A mouse spermatocyte-derived GC-2 cell line was exposed to a commercial mobile phone handset once every 20 minutes in standby, listen, dialed or dialing modes for 24 h. DNA damage was determined using an alkaline comet assay. Results: The levels of DNA damage were significantly increased following exposure to MPR in the listen, dialed and dialing modes. Moreover, there were significantly higher increases

in the dialed and dialing modes than in the listen mode. Interestingly, these results were consistent with the radiation intensities of these modes. However, the DNA damage effects of MPR in the dialing mode were efficiently attenuated by melatonin pretreatment. Conclusions: These results regarding mode-dependent DNA damage have important implications for the safety of inappropriate mobile phone use by males of reproductive age and also suggest a simple preventive measure, keeping our body from mobile phones as far away as possible, not only during conversations but during "dialed" and "dialing" operation modes as well. Since the "dialed" mode is actually part of the standby mode, mobile phones should be kept at a safe distance from our body even during standby operation. Furthermore, the protective role of melatonin suggests that it may be a promising pharmacological candidate for preventing mobile phone use-related reproductive impairments.

(E) Lixia S, Yao K, Kaijun W, Deqiang L, Huajun H, Xiangwei G, Baohong W, Wei Z, Jianling L, Wei W. Effects of 1.8GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells. Mutat Res 602(1-2):135-42, 2006. (GT, GE)

To investigate the DNA damage, expression of heat shock protein 70 (Hsp70) and cell proliferation of human lens epithelial cells (hLEC) after exposure to the 1.8GHz radiofrequency field (RF) of a global system for mobile communications (GSM). An Xc-1800 RF exposure system was used to employ a GSM signal at 1.8GHz (217Hz amplitude-modulated) with the output power in the specific absorption rate (SAR) of 1, 2 and 3W/kg. After 2h exposure to RF, the DNA damage of hLEC was accessed by comet assay at five different incubation times: 0, 30, 60, 120 and 240min, respectively. Western blot and RT-PCR were used to determine the expression of Hsp70 in hLECs after RF exposure. The proliferation rate of cells was evaluated by bromodeoxyuridine incorporation on days 0, 1 and 4 after exposure. The results show that the difference of DNA-breaks between the exposed and sham-exposed (control) groups induced by 1 and 2W/kg irradiation were not significant at any incubation time point ($P>0.05$). The DNA damage caused by 3W/kg irradiation was significantly increased at the times of 0 and 30min after exposure ($P<0.05$), a phenomenon that could not be seen at the time points of 60, 120 or 240min ($P>0.05$). Detectable mRNA as well as protein expression of Hsp70 was found in all groups. Exposure at SARs of 2 and 3W/kg for 2h exhibited significantly increased Hsp70 protein expression ($P<0.05$), while no change in Hsp70 mRNA expression could be found in any of the groups ($P>0.05$). No difference of the cell proliferation rate between the sham-exposed and exposed cells was found at any exposure dose tested ($P>0.05$). The results indicate that exposure to non-thermal dosages of RF for wireless communications can induce no or repairable DNA damage and the increased Hsp70 protein expression in hLECs occurred without change in the cell proliferation rate. The non-thermal stress response of Hsp70 protein increase to RF exposure might be involved in protecting hLEC from DNA damage and maintaining the cellular capacity for proliferation.

(E) López-Martín E, Bregains J, Relova-Quinteiro JL, Cadarso-Suárez C, Jorge-Barreiro FJ, Ares-Pena FJ. The action of pulse-modulated GSM radiation increases regional changes in brain

activity and c-Fos expression in cortical and subcortical areas in a rat model of picrotoxin-induced seizure proneness. J Neurosci Res. 87(6):1484-1499, 2009. (AS, GE, WS, IA)

The action of the pulse-modulated GSM radiofrequency of mobile phones has been suggested as a physical phenomenon that might have biological effects on the mammalian central nervous system. In the present study, GSM-exposed picrotoxin-pretreated rats showed differences in clinical and EEG signs, and in c-Fos expression in the brain, with respect to picrotoxin-treated rats exposed to an equivalent dose of unmodulated radiation. Neither radiation treatment caused tissue heating, so thermal effects can be ruled out. The most marked effects of GSM radiation on c-Fos expression in picrotoxin-treated rats were observed in limbic structures, olfactory cortex areas and subcortical areas, the dentate gyrus, and the central lateral nucleus of the thalamic intralaminar nucleus group. Nonpicrotoxin-treated animals exposed to unmodulated radiation showed the highest levels of neuronal c-Fos expression in cortical areas. These results suggest a specific effect of the pulse modulation of GSM radiation on brain activity of a picrotoxin-induced seizure-proneness rat model and indicate that this mobile-phone-type radiation might induce regional changes in previous preexcitability conditions of neuronal activation.

(E) Luukkonen J, Hakulinen P, Mäki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872MHz radiofrequency radiation. Mutat Res 662:54-58, 2009. (GT, OX, WS)

The objective of the study was to investigate effects of 872 MHz radiofrequency (RF) radiation on intracellular reactive oxygen species (ROS) production and DNA damage at a relatively high SAR value (5W/kg). The experiments also involved combined exposure to RF radiation and menadione, a chemical inducing intracellular ROS production and DNA damage. The production of ROS was measured using the fluorescent probe dichlorofluorescein and DNA damage was evaluated by the Comet assay. Human SH-SY5Y neuroblastoma cells were exposed to RF radiation for 1h with or without menadione. Control cultures were sham exposed. Both continuous waves (CW) and a pulsed signal similar to that used in global system for mobile communications (GSM) mobile phones were used. Exposure to the CW RF radiation increased DNA breakage ($p<0.01$) in comparison to the cells exposed only to menadione. Comparison of the same groups also showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60 min after the end of exposure ($p<0.05$ and $p<0.01$, respectively). No effects of the GSM signal were seen on either ROS production or DNA damage. The results of the present study suggest that 872MHz CW RF radiation at 5W/kg might enhance chemically induced ROS production and thus cause secondary DNA damage. However, there is no known mechanism that would explain such effects from CW RF radiation but not from GSM modulated RF radiation at identical SAR.

(NE) Luukkonen J, Juutilainen J, Naarala J. Combined effects of 872 MHz radiofrequency radiation and ferrous chloride on reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells. Bioelectromagnetics 31:417-424, 2010. (GT, OX)

The aim of the present study was to investigate possible cooperative effects of radiofrequency (RF) radiation and ferrous chloride (FeCl) on reactive oxygen species (ROS) production and DNA damage. In order to test intracellular ROS production as a possible underlying mechanism of DNA damage, we applied the fluorescent probe DCFH-DA. Integrity of DNA was quantified by alkaline comet assay. The exposures to 872 MHz RF radiation were conducted at a specific absorption rate (SAR) of 5 W/kg using continuous waves (CW) or a modulated signal similar to that used in Global System for Mobile Communications (GSM) phones. Four groups were included: Sham exposure (control), RF radiation, Chemical treatment, Chemical treatment, and RF radiation. In the ROS production experiments, human neuroblastoma (SH-SY5Y) cells were exposed to RF radiation and 10 microg/ml FeCl for 1 h. In the comet assay experiments, the exposure time was 3 h and an additional chemical (0.015% diethyl maleate) was used to make DNA damage level observable. The chemical treatments resulted in statistically significant responses, but no effects from either CW or modulated RF radiation were observed on ROS production, DNA damage or cell viability.

(NE) Maes A, Van Gorp U, Verschaeve L. Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation. Mutagenesis 21:139-42, 2006. (GT, IA)

Nowadays, virtually everybody is exposed to radiofrequency radiation (RFR) from mobile phone base station antennas or other sources. At least according to some scientists, this exposure can have detrimental health effects. We investigated cytogenetic effects in peripheral blood lymphocytes from subjects who were professionally exposed to mobile phone electromagnetic fields in an attempt to demonstrate possible RFR-induced genetic effects. These subjects can be considered well suited for this purpose as their RFR exposure is 'normal' though rather high, and definitely higher than that of the 'general population'. The alkaline comet assay, sister chromatid exchange (SCE) and chromosome aberration tests revealed no evidence of RFR-induced genetic effects. Blood cells were also exposed to the well known chemical mutagen mitomycin C in order to investigate possible combined effects of RFR and the chemical. No cooperative action was found between the electromagnetic field exposure and the mutagen using either the comet assay or SCE test.

(E) Manti L, Braselmann H, Calabrese ML, Massa R, Pugliese M, Scampoli P, Sicignano G, Grossi G. Effects of modulated microwave radiation at cellular telephone frequency (1.95 GHz) on X-ray-induced chromosome aberrations in human lymphocytes in vitro. Radiat Res 169:575-583, 2008. (GT, IA)

The case for a DNA-damaging action produced by radiofrequency (RF) signals remains controversial despite extensive research. With the advent of the Universal Mobile Telecommunication System (UMTS) the number of RF-radiation-exposed individuals is likely to escalate. Since the epigenetic effects of RF radiation are poorly understood and since the

potential modifications of repair efficiency after exposure to known cytotoxic agents such as ionizing radiation have been investigated infrequently thus far, we studied the influence of UMTS exposure on the yield of chromosome aberrations induced by X rays. Human peripheral blood lymphocytes were exposed in vitro to a UMTS signal (frequency carrier of 1.95 GHz) for 24 h at 0.5 and 2.0 W/kg specific absorption rate (SAR) using a previously characterized waveguide system. The frequency of chromosome aberrations was measured on metaphase spreads from cells given 4 Gy of X rays immediately before RF radiation or sham exposures by fluorescence in situ hybridization. Unirradiated controls were RF-radiation- or sham-exposed. No significant variations due to the UMTS exposure were found in the fraction of aberrant cells. However, the frequency of exchanges per cell was affected by the SAR, showing a small but statistically significant increase of 0.11 exchange per cell compared to 0 W/kg SAR. We conclude that, although the 1.95 GHz signal (UMTS modulated) does not exacerbate the yield of aberrant cells caused by ionizing radiation, the overall burden of X-ray-induced chromosomal damage per cell in first-mitosis lymphocytes may be enhanced at 2.0 W/kg SAR. Hence the SAR may either influence the repair of X-ray-induced DNA breaks or alter the cell death pathways of the damage response.

(E) Mazor R, Korenstein-Ilan A, Barbul A, Eshet Y, Shahadi A, Jerby E, Korenstein R. Increased levels of numerical chromosome aberrations after in vitro exposure of human peripheral blood lymphocytes to radiofrequency electromagnetic fields for 72 hours. Radiat Res. 169(1):28-37, 2008. (GT)

We investigated the effects of 72 h in vitro exposure of 10 human lymphocyte samples to radiofrequency electromagnetic fields (800 MHz, continuous wave) on genomic instability. The lymphyocytes were exposed in a specially designed waveguide resonator at specific absorption rates (SARs) of 2.9 and 4.1 W/kg in a temperature range of 36-37 degrees C. The induced aneuploidy of chromosomes 1, 10, 11 and 17 was determined by interphase FISH using semi-automated image analysis. We observed increased levels of aneuploidy depending on the chromosome studied as well as on the level of exposure. In chromosomes 1 and 10, there was increased aneuploidy at the higher SAR, while for chromosomes 11 and 17, the increases were observed only for the lower SAR. Multisomy (chromosomal gains) appeared to be the primary contributor to the increased aneuploidy. The effect of temperature on the level of aneuploidy was examined over the range of 33.5-40 degrees C for 72 h with no statistically significant difference in the level of aneuploidy compared to 37 degrees C. These findings suggest the possible existence of an athermal effect of RF radiation that causes increased levels of aneuploidy. These results contribute to the assessment of potential health risks after continuous chronic exposure to RF radiation at SARs close to the current levels set by ICNIRP guidelines.

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

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

(E) Nittby H, Widegren B, Krogh M, Grafström G, Berlin H, Rehn G, Eberhardt JL, Malmgren L, Persson BRR, Salford L. Exposure to radiation from global system for mobile communications at 1,800 MHz significantly changes gene expression in rat hippocampus and cortex. Environmentalist 28(4), 458-465, 2008. (GE)

We have earlier shown that radio frequency electromagnetic fields can cause significant leakage of albumin through the blood–brain barrier of exposed rats as compared to non-exposed rats, and also significant neuronal damage in rat brains several weeks after a 2 h exposure to a mobile phone, at 915 MHz with a global system for mobile communications (GSM) frequency modulation, at whole-body specific absorption rate values (SAR) of 200, 20, 2, and 0.2 mW/kg. We have now studied whether 6 h of exposure to the radiation from a GSM mobile test phone at 1,800 MHz (at a whole-body SAR-value of 13 mW/kg, corresponding to a brain SAR-value of 30 mW/kg) has an effect upon the gene expression pattern in rat brain cortex and hippocampus—areas where we have observed albumin leakage from capillaries into neurons and neuronal damage. Microarray analysis of 31,099 rat genes, including splicing variants, was performed in cortex and hippocampus of 8 Fischer 344 rats, 4 animals exposed to global system for mobile communications electromagnetic fields for 6 h in an anechoic chamber, one rat at a time, and 4 controls kept as long in the same anechoic chamber without exposure, also in this case one rat at a time. Gene ontology analysis (using the gene ontology categories biological processes, molecular functions, and cell components) of the differentially expressed genes of the exposed animals versus the control group revealed the following highly significant altered gene categories in both cortex and hippocampus: extracellular region, signal transducer activity, intrinsic to membrane, and integral to membrane. The fact that most of

these categories are connected with membrane functions may have a relation to our earlier observation of albumin transport through brain capillaries.

(E) Nylund R, Leszczynski D. Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome- and proteome-dependent. Proteomics 6:4769-4780, 2006. (GE, CS)

We have examined in vitro cell response to mobile phone radiation (900 MHz GSM signal) using two variants of human endothelial cell line: EA.hy926 and EA.hy926v1. Gene expression changes were examined in three experiments using cDNA Expression Arrays and protein expression changes were examined in ten experiments using 2-DE and PDQuest software. Obtained results show that gene and protein expression were altered, in both examined cell lines, in response to one hour mobile phone radiation exposure at an average specific absorption rate of 2.8 W/kg. However, the same genes and proteins were differently affected by the exposure in each of the cell lines. This suggests that the cell response to mobile phone radiation might be genome- and proteome-dependent. Therefore, it is likely that different types of cells and from different species might respond differently to mobile phone radiation or might have different sensitivity to this weak stimulus. Our findings might also explain, at least in part, the origin of discrepancies in replication studies between different laboratories.

(E) Panagopoulos DJ, Chavdoula ED, Nezis IP, Margaritis LH. Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation. Mutat Res 626:69-78, 2007. (GT, RP)

In the present study, the TUNEL (Terminal deoxynucleotidetransferasedUTP Nick End Labeling) assay - a well known technique widely used for detecting fragmented DNA in various types of cells - was used to detect cell death (DNA fragmentation) in a biological model, the early and mid stages of oogenesis of the insect Drosophila melanogaster. The flies were exposed in vivo to either GSM 900-MHz (Global System for Mobile telecommunications) or DCS 1800-MHz (Digital Cellular System) radiation from a common digital mobile phone, for few minutes per day during the first 6 days of their adult life. The exposure conditions were similar to those to which a mobile phone user is exposed, and were determined according to previous studies of ours [D.J Panagopoulos, A. Karabarbounis, L.H. Margaritis, Effect of GSM 900-MHz mobile phone radiation on the reproductive capacity of D. melanogaster, Electromagn. Biol Med 23 (2004) 29-43; D.J Panagopoulos, N. Messini, A. Karabarbounis, A.L. Philippetis, L.H. Margaritis, Radio frequency electromagnetic radiation within "safety levels" alters the physiological function of insects, in: P. Kostarakis, P. Stavroulakis (Eds.), Proceedings of the Millennium International Workshop on Biological Effects of Electromagnetic Fields, Heraklion, Crete, Greece, October 17-20, 2000, pp. 169-175, ISBN: 960-86733-0-5; D.J Panagopoulos, L.H. Margaritis, Effects of electromagnetic fields on the reproductive capacity of D. melanogaster, in: P. Stavroulakis (Ed.), Biological Effects of Electromagnetic Fields, Springer, 2003, pp. 545-578], which had shown a large decrease in the oviposition of the same insect caused by GSM radiation. Our present results suggest that the decrease in oviposition previously reported, is due to degeneration of large numbers of egg chambers after DNA fragmentation of their constituent cells, induced by both types of mobile telephony radiation. Induced cell death is

recorded for the first time, in all types of cells constituting an egg chamber (follicle cells, nurse cells and the oocyte) and in all stages of the early and mid-oogenesis, from germarium to stage 10, during which programmed cell death does not physiologically occur. Germarium and stages 7-8 were found to be the most sensitive developmental stages also in response to electromagnetic stress induced by the GSM and DCS fields and, moreover, germarium was found to be even more sensitive than stages 7-8.

(NE) Paparini A, Rossi P, Gianfranceschi G, Brugaletta V, Falsaperla R, De Luca P, Romano Spica V. No evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal. Bioelectromagnetics. 29(4):312-323, 2008. (GE)

To analyze possible effects of microwaves on gene expression, mice were exposed to global system for mobile communication (GSM) 1800 MHz signal for 1 h at a whole body SAR of 1.1 W/kg. Gene expression was studied in the whole brain, where the average SAR was 0.2 W/kg, by expression microarrays containing over 22,600 probe sets. Comparison of data from sham and exposed animals showed no significant difference in gene expression modulation. However, when less stringent constraints were adopted to analyze microarray results, 75 genes were found to be modulated following exposure. Forty-two probes showed fold changes ranging from 1.5 to 2.8, whereas 33 were down-regulated from 0.67- to 0.29-fold changes, but these differences in gene expression were not confirmed by real-time PCR. Under these specific limited conditions, no consistent indication of gene expression modulation in whole mouse brain was found associated to GSM 1800 MHz exposure.

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

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

(E) Pesnya DS, Romanovsky AV. Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the Allium cepa test. Mutat Res. 2012 Oct 8. pii: S1383-5718(12)00291-4. doi: 10.1016/j.mrgentox.2012.08.010. [Epub ahead of print] (GT)

The goal of this study was to compare the cytotoxic and genotoxic effects of plutonium-239 alpha particles and GSM 900 modulated mobile phone radiation in the Allium cepa test. Three

groups of bulbs were exposed to mobile phone radiation during 0 (sham), 3 and 9hours. A positive control group was treated during 20 min with plutonium-239 alpha-radiation. Mitotic abnormalities, chromosome aberrations, micronuclei and mitotic index were analyzed. Exposure to alpha-radiation from plutonium-239 and exposure to modulated radiation from mobile phone during 3 and 9h significantly increased the mitotic index. GSM 900 mobile phone radiation as well as alpha-radiation from plutonium-239 induced both clastogenic and aneugenic effects. However, the aneugenic activity of mobile phone radiation was more pronounced. After 9 hours of exposure to mobile phone radiation, polyploid cells, three-groups metaphases, amitoses and some unspecified abnormalities were detected, which were not registered in the other experimental groups. Importantly, GSM 900 mobile phone radiation increased the mitotic index, the frequency of mitotic and chromosome abnormalities, and the micronucleus frequency in a time-dependent manner. Due to its sensitivity, the Allium cepa test can be recommended as a useful cytogenetic assay to assess cytotoxic and genotoxic effects of radiofrequency electromagnetic fields.

(NE) Qutob SS, Chauhan V, Bellier PV, Yauk CL, Douglas GR, Berndt L, Williams A, Gajda GB, Lemay E, Thansandote A, McNamee JP. Microarray gene expression profiling of a human glioblastoma cell line exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. Radiat Res 165:636-644, 2006. (GE)

The widespread use of mobile phones has led to public concerns about the health effects associated with exposure to radiofrequency (RF) fields. The paramount concern of most persons relates to the potential of these fields to cause cancer. Unlike ionizing radiation, RF fields used for mobile telecommunications (800-1900 MHz) do not possess sufficient energy to directly damage DNA. Most rodent bioassay and in vitro genotoxicity/mutation studies have reported that RF fields at non-thermal levels have no direct mutagenic, genotoxic or carcinogenic effects. However, some evidence has suggested that RF fields may cause detectable postexposure changes in gene expression. Therefore, the purpose of this study was to assess the ability of exposure to a 1.9 GHz pulse-modulated RF field for 4 h at specific absorption rates (SARs) of 0.1, 1.0 and 10.0 W/kg to affect global gene expression in U87MG glioblastoma cells. We found no evidence that non-thermal RF fields can affect gene expression in cultured U87MG cells relative to the nonirradiated control groups, whereas exposure to heat shock at 43 degrees C for 1 h up-regulated a number of typical stress-responsive genes in the positive control group. Future studies will assess the effect of RF fields on other cell lines and on gene expression in the mouse brain after in vivo exposure.

(E) Remondini D, Nylund R, Reivinen J, Poulletier de Gannes F, Veyret B, Lagroye I, Haro E, Trillo MA, Capri M, Franceschi C, Schlatterer K, Gminski R, Fitzner R, Tauber R, Schuderer J, Kuster N, Leszczynski D, Bersani F, Maercker C. Gene expression changes in human cells after exposure to mobile phone microwaves. Proteomics 6:4745-4754, 2006. (GE, CS)

Possible biological effects of mobile phone microwaves were investigated in vitro. In this study, which was part of the 5FP EU project REFLEX (Risk Evaluation of Potential Environmental Hazards From Low-Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods), six

human cell types, immortalized cell lines and primary cells, were exposed to 900 and 1800 MHz. RNA was isolated from exposed and sham-exposed cells and labeled for transcriptome analysis on whole-genome cDNA arrays. The results were evaluated statistically using bioinformatics techniques and examined for biological relevance with the help of different databases. NB69 neuroblastoma cells, T lymphocytes, and CHME5 microglial cells did not show significant changes in gene expression. In EA.hy926 endothelial cells, U937 lymphoblastoma cells, and HL-60 leukemia cells we found between 12 and 34 up- or down-regulated genes. Analysis of the affected gene families does not point towards a stress response. However, following microwave exposure, some but not all human cells might react with an increase in expression of genes encoding ribosomal proteins and therefore up-regulating the cellular metabolism.

(NE) Ros-Llor I, Sanchez-Siles M, Camacho-Alonso F, Lopez-Jornet P. Effect of mobile phones on micronucleus frequency in human exfoliated oral mucosal cells. Oral Dis. 18:786-792, 2012. (GT)

Objective: In the last two decades, the use of mobile phones has increased enormously all over the world. The controversy regarding whether radiofrequency (RF) fields exert effects upon biological systems is a concern for the general population. An evaluation is made of DNA damage and cytokinetic defects, proliferative potential, and cell death because of RF radiation emitted by mobile phones in healthy young users. Study design: This cohort study was carried out in 50 Caucasian mobile phone users. We collected two cell samples from each subject (a total of 100 cell samples), corresponding to the right and left cheek mucosa, respectively. Case histories and personal information were assessed, including age, gender, body height and weight, history of cancer, smoking and alcohol consumption, exposure to chemical carcinogens or radiation, and dietary habits. Sampling comprised cell collection from both cheeks with a cytobrush, centrifugation, slide preparation, fixation, and staining, followed by fluorescent microscopic analysis. A total of 2000 exfoliated cells were screened for nuclear abnormalities, especially micronucleus. Results: No statistically significant changes were recorded in relation to age, gender, body mass index, or smoking status. A comparison of the results vs the control area according to the side of the face on which the mobile phone was placed, and in relation to the duration of exposure (years) to mobile phone radiation in the total 100 samples, yielded no significant differences. Conclusions: No genotoxic effects because of RF exposure were observed in relation to any of the study parameters.

(NE) Sakuma N, Komatsubara Y, Takeda H, Hirose H, Sekijima M, Nojima T, Miyakoshi J. DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. Bioelectromagnetics 27:51-57, 2006. (CT)

We conducted a large-scale in vitro study focused on the effects of low level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system in order to test the hypothesis that modulated RF fields may act as a DNA damaging agent. First, we evaluated the responses of

human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced different levels of DNA damage. Human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs were exposed to mobile communication frequency radiation to investigate whether such exposure produced DNA strand breaks in cell culture. A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg and CW radiation at 80 mW/kg for 2 and 24 h, while IMR-90 cells were exposed to both W-CDMA and CW radiations at a SAR of 80 mW/kg for the same time periods. Under the same RF field exposure conditions, no significant differences in the DNA strand breaks were observed between the test groups exposed to W-CDMA or CW radiation and the sham exposed negative controls, as evaluated immediately after the exposure periods by alkaline comet assays. Our results confirm that low level exposures do not act as a genotoxicant up to a SAR of 800 mW/kg.

(NE) Sakurai T, Kiyokawa T, Narita E, Suzuki Y, Taki M, Miyakoshi J. Analysis of gene expression in a human-derived glial cell line exposed to 2.45 GHz continuous radiofrequency electromagnetic fields. J Radiat Res. 52(2):185-192, 2011. (GE)

The increasing use of mobile phones has aroused public concern regarding the potential health risks of radiofrequency (RF) fields. We investigated the effects of exposure to RF fields (2.45 GHz, continuous wave) at specific absorption rate (SAR) of 1, 5, and 10 W/kg for 1, 4, and 24 h on gene expression in a normal human glial cell line, SVGp12, using DNA microarray. Microarray analysis revealed 23 assigned gene spots and 5 non-assigned gene spots as prospective altered gene spots. Twenty-two genes out of the 23 assigned gene spots were further analyzed by reverse transcription-polymerase chain reaction to validate the results of microarray, and no significant alterations in gene expression were observed. Under the experimental conditions used in this study, we found no evidence that exposure to RF fields affected gene expression in SVGp12 cells.

(NE) Sannino A, Di Costanzo G, Brescia F, Sarti M, Zeni O, Juutilainen J, Scarfì MR. Human fibroblasts and 900 MHz radiofrequency radiation: evaluation of DNA damage after exposure and co-exposure to 3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-furanone (MX). Radiat Res 171:743-751, 2009. (NT, IA)

Abstract Sannino, A., Di Costanzo, G., Brescia, F., Sarti, M., Zeni, O., Juutilainen, J and Scarfì, M. R. Human Fibroblasts and 900 MHz Radiofrequency Radiation: Evaluation of DNA Damage after Exposure and Co-exposure to 3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-furanone (MX). Radiat Res 171, 743-751 (2009). The aim of this study was to investigate DNA damage in human dermal fibroblasts from a healthy subject and from a subject affected by Turner's syndrome that were exposed for 24 h to radiofrequency (RF) radiation at 900 MHz. The RF-radiation exposure was carried out alone or in combination with 3-chloro-4-(dichloromethyl)-5-hydroxy-

2(5H)-furanone (MX), a well-known environmental mutagen and carcinogen produced during the chlorination of drinking water. Turner's syndrome fibroblasts were also exposed for a shorter time (1 h). A signal similar to that emitted by Global System for Mobile Communications (GSM) mobile phones was used at a specific absorption rate of 1 W/kg under strictly controlled conditions of temperature and dosimetry. To evaluate DNA damage after RF-radiation exposure alone, the alkaline comet assay and the cytokinesis-block micronucleus assay were used. In the combined-exposure experiments, MX was given at a concentration of 25 microM for 1 h immediately after the RF-radiation exposure, and the effects were evaluated by the alkaline comet assay. The results revealed no genotoxic and cytotoxic effects from RF radiation alone in either cell line. As expected, MX treatment induced an increase in DNA migration in the comet assay, but no enhancement of the MX-induced DNA damage was observed in the cells exposed to RF radiation.

(E) Schwarz C, Kratochvil E, Pilger A, Kuster N, Adlkofer F, Rüdiger HW. Radiofrequency electromagnetic fields (UMTS, 1,950 MHz) induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes. Int Arch Occup Environ Health 81:755-767, 2008. (GT, CS)

OBJECTIVE: Universal Mobile Telecommunication System (UMTS) was recently introduced as the third generation mobile communication standard in Europe. This was done without any information on biological effects and genotoxic properties of these particular high-frequency electromagnetic fields. This is discomforting, because genotoxic effects of the second generation standard Global System for Mobile Communication have been reported after exposure of human cells in vitro. METHODS: Human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures were exposed to 1,950 MHz UMTS below the specific absorption rate (SAR) safety limit of 2 W/kg. The alkaline comet assay and the micronucleus assay were used to ascertain dose and time-dependent genotoxic effects. Five hundred cells per slide were visually evaluated in the comet assay and comet tail factor (CTF) was calculated. In the micronucleus assay 1,000 binucleated cells were evaluated per assay. The origin of the micronuclei was determined by fluorescence labeled anticentromere antibodies. All evaluations were performed under blinded conditions. RESULTS: UMTS exposure increased the CTF and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way. Incubation for 24 h at a SAR of 0.05 W/kg generated a statistically significant rise in both CTF and MN (P = 0.02). At a SAR of 0.1 W/kg the CTF was significantly increased after 8 h of incubation (P = 0.02), the number of MN after 12 h (P = 0.02). No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with Phytohemagglutinin. CONCLUSION: UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

(E) Sekeroğlu V, Akar A, Sekeroğlu ZA. Cytotoxic and genotoxic effects of high-frequency electromagnetic fields (GSM 1800 MHz) on immature and mature rats. Ecotoxicol Environ Saf. 80:140-144, 2012. (LE, GT, DE)

We investigated the cytogenotoxic effects of high frequency electromagnetic fields (HF-EMF) for 45 day and the effect of a recovery period of 15 day after exposure to EMF on bone marrow cells of immature and mature rats. The animals in treatment groups were exposed to 1800 MHz EMF at SAR of 0.37 W/kg and 0.49 W/kg for 2h/day for 45 day. Two recovery groups were kept for a recovery period of 15 day without EMF after exposure to HF-EMF. Two control groups for both immature and mature rats were also included. Significant differences were also observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCEs) in all treatment groups. The cytogenotoxic damage was more remarkable in immature rats and, the recovery period did not improve this damage in immature rats. Because much higher and irreversible cytogenotoxic damage was observed in immature rats than in mature rats, further studies are needed to understand effects of EMF on DNA damage and DNA repair, and to determine safe limits for environment and human, especially for children.

(NE) Sekijima M, Takeda H, Yasunaga K, Sakuma N, Hirose H, Nojima T, Miyakoshi J. 2-GHz band CW and W-CDMA modulated radiofrequency fields have no significant effect on cell proliferation and gene expression profile in human cells. J Radiat Res. 51(3):277-284, 2010. (GE)

We investigated the mechanisms by which radiofrequency (RF) fields exert their activity, and the changes in both cell proliferation and the gene expression profile in the human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung) following exposure to 2.1425 GHz continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) RF fields at three field levels. During the incubation phase, cells were exposed at the specific absorption rates (SARs) of 80, 250, or 800 mW/kg with both CW and W-CDMA RF fields for up to 96 h. Heat shock treatment was used as the positive control. No significant differences in cell growth or viability were observed between any test group exposed to W-CDMA or CW radiation and the sham-exposed negative controls. Using the Affymetrix Human Genome Array, only a very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression in each experiment. The results confirm that low-level exposure to 2.1425 GHz CW and W-CDMA RF fields for up to 96 h did not act as an acute cytotoxicant in either cell proliferation or the gene expression profile. These results suggest that RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines under these conditions.

(E) Souza LD, Cerqueira ED, Meireles JR. Assessment of nuclear abnormalities in exfoliated cells from the oral epithelium of mobile phone users. Electromagn Biol Med. 2013 May 28. [Epub ahead of print] (GE, HU)

Abstract Transmission and reception of mobile telephony signals take place through electromagnetic wave radiation, or electromagnetic radiofrequency fields, between the mobile

terminal and the radio base station. Based on reports in the literature on adverse effects from exposure to this type of radiation, the objective of this study was to evaluate the genotoxic and cytotoxic potential of such exposure, by means of the micronucleus test on exfoliated cells from the oral epithelium. The sample included 45 individuals distributed in 3 groups according to the amount of time in hours per week (t) spent using mobile phones: group I, t>5 h; group II, t>1 h and ≤5 h; and group III, t≤1 h. Cells from the oral mucosa were analyzed to assess the numbers of micronuclei, broken egg structures and degenerative nuclear abnormalities indicative of apoptosis (condensed chromatin, karyorrhexis and pyknosis) or necrosis (karyolysis in addition to these changes). The occurrences of micronuclei and degenerative nuclear abnormalities did not differ between the groups, but the number of broken egg (structures that may be associated with gene amplification) was significantly greater in the individuals in group I ($p<0.05$).

(NE) Speit G, Schütz P, Hoffmann H. Genotoxic effects of exposure to radiofrequency electromagnetic fields (RF-EMF) in cultured mammalian cells are not independently reproducible. Mutat Res. 626(1-2):42-47, 2007. (GT)

Conflicting results have been published regarding the induction of genotoxic effects by exposure to radiofrequency electromagnetic fields (RF-EMF). Using the comet assay, the micronucleus test and the chromosome aberration test with human fibroblasts (ES1 cells), the EU-funded "REFLEX" project (Risk Evaluation of Potential Environmental Hazards From Low Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods) reported clearly positive effects for various exposure conditions. Because of the ongoing discussion on the biological significance of the effects observed, it was the aim of the present study to independently repeat the results using the same cells, the same equipment and the same exposure conditions. We therefore exposed ES1 cells to RF-EMF (1800 MHz; SAR 2 W/kg, continuous wave with intermittent exposure) for different time periods and then performed the alkaline (pH>13) comet assay and the micronucleus test (MNT). For both tests, clearly negative results were obtained in independently repeated experiments. We also performed these experiments with V79 cells, a sensitive Chinese hamster cell line that is frequently used in genotoxicity testing, and also did not measure any genotoxic effect in the comet assay and the MNT. Appropriate measures of quality control were considered to exclude variations in the test performance, failure of the RF-EMF exposure or an evaluation bias. The reasons for the difference between the results reported by the REFLEX project and our experiments remain unclear.

(NE) Stronati L, Testa A, Moquet J, Edwards A, Cordelli E, Villani P, Marino C, Fresegna AM, Appolloni M, Lloyd D. 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes. Int J Radiat Biol 82:339-346, 2006. (GT, IA)

Purpose: The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with x-rays was investigated in vitro using several assays on human lymphocytes.

The chosen specific absorption rate (SAR) values are near the upper limit of actual energy absorption in localized tissue when persons use some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen.Methods: Blood specimens from 14 donors were exposed continuously for 24 h to a Global System for Mobile Communications (GSM) basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in vitro cell cycling.Results: By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did not modify any measured effects of the x-radiation. Conclusions: This study has used several standard in vitro tests for chromosomal and DNA damage in Go human lymphocytes exposed in vitro to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic per se or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed.

(E) Sun LX, Yao K, He JL, Lu DQ, Wang KJ, Li HW.[Effect of acute exposure to microwave from mobile phone on DNA damage and repair of cultured human lens epithelial cells in vitro.] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing ZaZhi. 24:465-467, 2006. [Article in Chinese] (GT)

OBJECTIVE: To investigate the DNA damage of human lens epithelial cells (LECs) caused by acute exposure to low-power 217 Hz modulated 1.8 GHz microwave radiation and DNA repair. METHODS: Cultured LECs were exposed to 217 Hz modulated 1.8 GHz microwave radiation at SAR (specific absorption rate) of 0, 1, 2, 3 and 4 W/kg for 2 hours in an sXc-1800 incubator and irradiate system. The DNA single strand breaks were detected with comet assay in sham-irradiated cells and irradiated cells incubated for varying periods: 0, 30, 60, 120 and 240 min after irradiation. Images of comets were digitized and analyzed using an Imagine-pro plus software, and the indexes used in this study were tail length (TL) and tail moment (TM). RESULTS: The difference in DNA-breaks between the exposure and sham exposure groups induced by 1 and 2 W/kg irradiation was not significant at every detect time ($P > 0.05$). As for the dosage of 3 and 4 W/kg there was difference in both groups immediately after irradiation ($P < 0.01$). At the time of 30 min after irradiation the difference went on at both group ($P < 0.01$). However, the difference disappeared after one hour's incubation in 3 W/kg group ($P > 0.05$), and existed in 4 W/kg group. CONCLUSION: No or repairable DNA damage was observed after 2 hour irradiation of 1.8 GHz microwave on LECs when SAR </= 3 W/kg. The DNA damages caused by 4 W/kg irradiation were irreversible.

(E) Tiwari R, Lakshmi NK, Surender V, Rajesh AD, Bhargava SC, Ahuja YR. Combinative exposure effect of radio frequency signals from CDMA mobile phones and aphidicolin on DNA integrity. Electromagn Biol Med 27:418-425, 2008. (GT, IA)

The aim of present study is to assess DNA integrity on the effect of exposure to a radio frequency (RF) signal from Code Division Multiple Access (CDMA) mobile phones. Whole blood samples from six healthy male individuals were exposed for RF signals from a CDMA mobile phone for 1 h. Alkaline comet assay was performed to assess the DNA damage. The combinative exposure effect of the RF signals and APC at two concentrations on DNA integrity was studied. DNA repair efficiency of the samples was also studied after 2 h of exposure. The RF signals and APC (0.2 microg/ml) alone or in synergism did not have any significant DNA damage as compared to sham exposed. However, univariate analysis showed that DNA damage was significantly different among combinative exposure of RF signals and APC at 0.2 microg/ml ($p < 0.05$) and at 2 microg/ml ($p < 0.02$). APC at 2 microg/ml concentration also showed significant damage levels ($p < 0.05$) when compared to sham exposed. DNA repair efficiency also varied in a significant way in combinative exposure sets ($p < 0.05$). From these results, it appears that the repair inhibitor APC enhances DNA breaks at 2 microg/ml concentration and that the damage is possibly repairable. Thus, it can be inferred that the in vitro exposure to RF signals induces reversible DNA damage in synergism with APC.

(E) Tkalec M, Stambuk A, Srut M, Malarić K, Klobučar GI. Oxidative and genotoxic effects of 900MHz electromagnetic fields in the earthworm Eisenia fetida. Ecotoxicol Environ Saf. 90:7-12, 2013. (GT, OX, WS)

Accumulating evidence suggests that exposure to radiofrequency electromagnetic field (RF-EMF) can have various biological effects. In this study the oxidative and genotoxic effects were investigated in earthworms Eisenia fetida exposed in vivo to RF-EMF at the mobile phone frequency (900MHz). Earthworms were exposed to the homogeneous RF-EMF at field levels of 10, 23, 41 and 120Vm(-1) for a period of 2h using a Gigahertz Transversal Electromagnetic (GTEM) cell. At the field level of 23Vm(-1) the effect of longer exposure (4h) and field modulation (80% AM 1kHz sinusoidal) was investigated as well. All exposure treatments induced significant genotoxic effect in earthworms coelomocytes detected by the Comet assay, demonstrating DNA damaging capacity of 900MHz electromagnetic radiation. Field modulation additionally increased the genotoxic effect. Moreover, our results indicated the induction of antioxidant stress response in terms of enhanced catalase and glutathione reductase activity as a result of the RF-EMF exposure, and demonstrated the generation of lipid and protein oxidative damage. Antioxidant responses and the potential of RF-EMF to induce damage to lipids, proteins and DNA differed depending on the field level applied, modulation of the field and duration of E. fetida exposure to 900MHz electromagnetic radiation. Nature of detected DNA lesions and oxidative stress as the mechanism of action for the induction of DNA damage are discussed.

(E) Tomruk A, Guler G, Dincel AS. The influence of 1800 MHz GSM-like signals on hepatic oxidative DNA and lipid damage in nonpregnant, pregnant, and newly born rabbits. Cell Biochem Biophys 56:39-47, 2010. (GT, OX, DE, LE)

The aim of our study is to evaluate the possible biological effects of whole-body 1800 MHz GSM-like radiofrequency (RF) radiation exposure on liver oxidative DNA damage and lipid peroxidation levels in nonpregnant, pregnant New Zealand White rabbits, and in their newly borns. Eighteen nonpregnant and pregnant rabbits were used and randomly divided into four groups which were composed of nine rabbits: (i) Group I (nonpregnant control), (ii) Group II (nonpregnant-RF exposed), (iii) Group III (pregnant control), (iv) Group IV (pregnant-RF exposed). Newborns of the pregnant rabbits were also divided into two groups: (v) Group V (newborns of Group III) and (vi) Group VI (newborns of Group III). 1800 MHz GSM-like RF radiation whole-body exposure (15 min/day for a week) was applied to Group II and Group IV. No significant differences were found in liver 8 OHdG/10 dG levels of exposure groups (Group II and Group IV) compared to controls (Group I and Group III). However, in Group II and Group IV malondialdehyde (MDA) and ferrous oxidation in xylenol orange (FOX) levels were increased compared to Group I ($P < 0.05$, Mann-Whitney). No significant differences were found in liver tissue of 8 OHdG/10 dG and MDA levels between Group VI and Group V ($P > 0.05$, Mann-Whitney) while liver FOX levels were found significantly increased in Group VI with respect to Group V ($P < 0.05$, Mann-Whitney). Consequently, the whole-body 1800 MHz GSM-like RF radiation exposure may lead to oxidative destruction as being indicators of subsequent reactions that occur to form oxygen toxicity in tissues.

(E) Trivino Pardo JC, Grimaldi S, Taranta M, Naldi I, Cinti C. Microwave electromagnetic field regulates gene expression in T-lymphoblastoid leukemia CCRF-CEM cell line exposed to 900 MHz. Electromagn Biol Med. 31(1):1-18, 2012. (GE)

Electric, magnetic, and electromagnetic fields are ubiquitous in our society, and concerns have been expressed regarding possible adverse effects of these exposures. Research on Extremely Low-Frequency (ELF) magnetic fields has been performed for more than two decades, and the methodology and quality of studies have improved over time. Studies have consistently shown increased risk for childhood leukemia associated with ELF magnetic fields. There are still inadequate data for other outcomes. More recently, focus has shifted toward Radio Frequencies (RF) exposures from mobile telephony. There are no persuasive data suggesting a health risk, but this research field is still immature with regard to the quantity and quality of available data. This technology is constantly changing and there is a need for continued research on this issue. To investigate whether exposure to high-frequency electromagnetic fields (EMF) could induce adverse health effects, we cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of 900 MHz MW-EMF generated by a transverse electromagnetic (TEM) cell at short and long exposure times. We evaluated the effect of high-frequency EMF on gene expression and we identified functional pathways influenced by 900 MHz MW-EMF exposure.

(E) Trosić I, Pavicić I, Milković-Kraus S, Mladinić M, Zeljezić D. Effect of electromagnetic radiofrequency radiation on the rats' brain, liver and kidney cells measured by comet assay. Coll Antropol 35:1259-1264, 2011. (GT)

The goal of study was to evaluate DNA damage in rat's renal, liver and brain cells after in vivo exposure to radiofrequency/microwave (Rf/Mw) radiation of cellular phone frequencies range. To determine DNA damage, a single cell gel electrophoresis/comet assay was used. Wistar rats (male, 12 week old, approximate body weight 350 g) (N = 9) were exposed to the carrier frequency of 915 MHz with Global System Mobile signal modulation (GSM), power density of 2.4 W/m2, whole body average specific absorption rate SAR of 0.6 W/kg. The animals were irradiated for one hour/day, seven days/week during two weeks period. The exposure set-up was Gigahertz Transversal Electromagnetic Mode Cell (GTEM--cell). Sham irradiated controls (N = 9) were apart of the study. The body temperature was measured before and after exposure. There were no differences in temperature in between control and treated animals. Comet assay parameters such as the tail length and tail intensity were evaluated. In comparison with tail length in controls (13.5 +/- 0.7 microm), the tail was slightly elongated in brain cells of irradiated animals (14.0 +/- 0.3 microm). The tail length obtained for liver (14.5 +/- 0.3 microm) and kidney (13.9 +/- 0.5 microm) homogenates notably differs in comparison with matched sham controls (13.6 +/- 0.3 microm) and (12.9 +/- 0.9 microm). Differences in tail intensity between control and exposed animals were not significant. The results of this study suggest that, under the experimental conditions applied, repeated 915 MHz irradiation could be a cause of DNA breaks in renal and liver cells, but not affect the cell genome at the higher extent compared to the basal damage.

(NE) Valbonesi P, Franzellitti S, Piano A, Contin A, Biondi C, Fabbri E. Evaluation of HSP70 Expression and DNA damage in cells of a human trophoblast cell line exposed to 1.8 GHz amplitude-modulated radiofrequency fields. Radiat Res 169:270-279, 2008. (GT, GE)

The aim of this study was to determine whether high-frequency electromagnetic fields (EMFs) could induce cellular effects. The human trophoblast cell line HTR-8/SVneo was used as a model to evaluate the expression of proteins (HSP70 and HSC70) and genes (HSP70A, B, C and HSC70) of the HSP70 family and the primary DNA damage response after nonthermal exposure to pulse-modulated 1817 MHz sinusoidal waves (GSM-217 Hz; 1 h; SAR of 2 W/kg). HSP70 expression was significantly enhanced by heat, which was applied as the prototypical stimulus. The HSP70A, B and C transcripts were differentially expressed under basal conditions, and they were all significantly induced above basal levels by thermal stress. Conversely, HSC70 protein and gene expression was not influenced by heat. Exposing HTR-8/SVneo cells to high-frequency EMFs did not change either HSP70 or HSC70 protein or gene expression. A significant increase in DNA strand breaks was caused by exposure to HO, which was used as a positive stimulus; however, no effect was observed after exposure of cells to high-frequency EMFs. Overall, no evidence was found that a 1-h exposure to GSM-217 Hz induced a HSP70-mediated stress response or primary DNA damage in HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations are needed.

(E) Valbonesi P, Franzellitti S, Bersani F, Contin A, Fabbri E. Effects of the exposure to intermittent 1.8 GHz radio frequency electromagnetic fields on HSP70 expression and MAPK signaling pathways in PC12 cells. Int J Radiat Biol. 2014 Feb 11. [Epub ahead of print] (GE, WS)

Purpose: We previously reported effects on heat shock protein 70 (HSP70) mRNA expression, a cytoprotective protein induced under stressful condition, in human trophoblast cells exposed to amplitude-modulated Global System for Mobile Communication (GSM) signals. In the present work the same experimental conditions were applied to the rat PC12 cells, in order to assess the stress responses mediated by HSP70 and by the Mitogen Activated Protein Kinases (MAPK) in neuronal-like cells, an interesting model to study possible effects of mobile phone frequencies exposure. Materials and methods: HSP70 gene expression level was evaluated by reverse transcriptase polymerase chain reaction, HSP70 protein expression and MAPK phosphorylation were assessed by Western blotting. PC12 cells were exposed for 4, 16 or 24 h to 1.8 GHz continuous wave signal (CW, carrier frequency without modulation) or to two different GSM modulation schemes, GSM-217Hz and GSM-Talk (which generates temporal changes between two different GSM signals, active during talking or listening phases respectively, thus simulating a typical conversation). Specific adsorption rate (SAR) was 2 W/kg. Results: After PC12 cells exposure to the GSM-217Hz signal for 16 or 24 h, HSP70 transcription significantly increased, whereas no effect was observed in cells exposed to the CW or GSM-Talk signals. HSP70 protein expression and three different MAPK signaling pathways were not affected by the exposure to any of the three different 1.8 GHz signals. Conclusion: The positive effect on HSP70 mRNA expression, observed only in cells exposed to the GSM-217Hz signal, is a repeatable response previously reported in human trophoblast cells and now confirmed in PC12 cells. Further investigations towards a possible role of 1.8 GHz signal modulation are therefore advisable.

(NE) Verschaeve L, Heikkinen P, Verheyen G, Van Gorp U, Boonen F, Vander Plaetse F, Maes A, Kumlin T, Maki-Paakkanen J, Puranen L, Juutilainen J. Investigation of co-genotoxic effects of radiofrequency electromagnetic fields in vivo. Radiat Res 165:598-607, 2006. (GT, LE, IA)

We investigated the possible combined genotoxic effects of radiofrequency (RF) electromagnetic fields (900 MHz, amplitude modulated at 217 Hz, mobile phone signal) with the drinking water mutagen and carcinogen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). Female rats were exposed to RF fields for a period of 2 years for 2 h per day, 5 days per week at average whole-body specific absorption rates of 0.3 or 0.9 W/kg. MX was given in the drinking water at a concentration of 19 mug/ml. Blood samples were taken at 3, 6 and 24 months of exposure and brain and liver samples were taken at the end of the study (24 months). DNA damage was assessed in all samples using the alkaline comet assay, and micronuclei were determined in erythrocytes. We did not find significant genotoxic activity of MX in blood and liver cells. However, MX induced DNA damage in rat brain. Co-exposures to MX and RF radiation did not significantly increase the response of blood, liver and brain cells compared to MX exposure only. In conclusion, this 2-year animal study involving long-term

exposures to RF radiation and MX did not provide any evidence for enhanced genotoxicity in rats exposed to RF radiation.

(NE) Vijayalaxmi. Cytogenetic studies in human blood lymphocytes exposed in vitro to 2.45 GHz or 8.2 GHz radiofrequency radiation. Radiat Res 166, 532–538, 2006. (GT)

Peripheral blood samples collected from healthy human volunteers were exposed in vitro to 2.45 GHz or 8.2 GHz pulsed-wave radiofrequency (RF) radiation. The net forward power, average power density, mean specific absorption rate, and the temperature maintained during the 2-h exposure of the cells to 2.45 GHz or 8.2 GHz were, respectively, 21 W or 60 W, 5 mW/cm2 or 10 mW/cm2, 2.13 W/kg or 20.71 W/kg, and 36.9 ± 0.1°C or 37.5 ± 0.2°C. Aliquots of the same blood samples that were either sham-exposed or exposed in vitro to an acute dose of 1.5 Gy γ radiation were used as unexposed and positive controls, respectively. Cultured lymphocytes were examined to determine the extent of cytogenetic damage assessed from the incidence of chromosomal aberrations and micronuclei. Under the conditions used to perform the experiments, the levels of damage in RF-radiation-exposed and sham-exposed lymphocytes were not significantly different. Also, there were no significant differences in the response of unstimulated lymphocytes and lymphocytes stimulated with phytohemagglutinin when exposed to 8.2 GHz RF radiation. In contrast, the positive control cells that had been subjected to γ irradiation exhibited significantly more damage than RF-radiation- and sham-exposed lymphocytes.

(NE) Waldmann P, Bohnenberger S, Greinert R, Hermann-Then B, Heselich A, Klug SJ, Koenig J, Kuhr K, Kuster N, Merker M, Murbach M, Pollet D, Schadenboeck W, Scheidemann-Wesp U, Schwab B, Volkmer B, Weyer V, Blettner M. Influence of GSM Signals on Human Peripheral Lymphocytes: Study of Genotoxicity. Radiat Res. 2013 Jan 14. [Epub ahead of print] (GT)

Exposure to radiofrequency (RF) electromagnetic fields (EMF) is continuously increasing worldwide. Yet, conflicting results of a possible genotoxic effect of RF EMF continue to be discussed. In the present study, a possible genotoxic effect of RF EMF (GSM, 1,800 MHz) in human lymphocytes was investigated by a collaboration of six independent institutes (institutes a, b, c, d, e, h). Peripheral blood of 20 healthy, nonsmoking volunteers of two age groups (10 volunteers 16-20 years old and 10 volunteers 50-65 years old) was taken, stimulated and intermittently exposed to three specific absorption rates (SARs) of RF EMF (0.2 W/kg, 2 W/kg, 10 W/kg) and sham for 28 h (institute a). The exposures were performed in a setup with strictly controlled conditions of temperature and dose, and randomly and automatically determined waveguide SARs, which were designed and periodically maintained by ITIS (institute h). Four genotoxicity tests with different end points were conducted (institute a): chromosome aberration test (five types of structural aberrations), micronucleus test, sister chromatid exchange test and the alkaline comet assay (Olive tail moment and % DNA). To demonstrate the validity of the study, positive controls were implemented. The genotoxicity end points were evaluated independently by three laboratories blind to SAR information (institute c = laboratory 1; institute d = laboratory 2; institute e = laboratory 3). Statistical analysis was carried out by

institute b. Methods of primary statistical analysis and rules to adjust for multiple testing were specified in a statistical analysis plan based on a data review before unblinding. A linear trend test based on a linear mixed model was used for outcomes of comet assay and exact permutation test for linear trend for all other outcomes. It was ascertained that only outcomes with a significant SAR trend found by at least two of three analyzing laboratories indicated a substantiated suspicion of an exposure effect. On the basis of these specifications, none of the nine end points tested for SAR trend showed a significant and reproducible exposure effect. Highly significant differences between sham exposures and positive controls were detected by each analyzing laboratory, thus validating the study. In conclusion, the results show no evidence of a genotoxic effect induced by RF EMF (GSM, 1,800 MHz).

(E) Wu W, Yao K, Wang KJ, Lu DQ, He JL, Xu LH, Sun WJ. [Blocking 1800 MHz mobile phone radiation-induced reactive oxygen species production and DNA damage in lens epithelial cells by noise magnetic fields.]Zhejiang Da XueXueBao Yi Xue Ban 37:34-38, 2008. [Article in Chinese] (GT, IA, OX)

OBJECTIVE: To investigate whether the exposure to the electromagnetic noise can block reactive oxygen species (ROS) production and DNA damage of lens epithelial cells induced by 1800 MHz mobile phone radiation. METHODS: The DCFH-DA method and comet assay were used respectively to detect the intracellular ROS and DNA damage of cultured human lens epithelial cells induced by 4 W/kg 1800 MHz mobile phone radiation or/and 2microT electromagnetic noise for 24 h intermittently. RESULT: 1800 MHz mobile phone radiation at 4 W/kg for 24 h increased intracellular ROS and DNA damage significantly (P<0.05). However, the ROS level and DNA damage of mobile phone radiation plus noise group were not significant enhanced (P>0.05) as compared to sham exposure group. Conclusion: Electromagnetic noise can block intracellular ROS production and DNA damage of human lens epithelial cells induced by 1800 MHz mobile phone radiation.

(E) Xu S, Zhong M, Zhang L, Zhou Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Yu Z. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res 1311:189-196. 2010. (GT, OX)

Increasing evidence indicates that oxidative stress may be involved in the adverse effects of radiofrequency (RF) radiation on the brain. Because mitochondrial DNA (mtDNA) defects are closely associated with various nervous system diseases and mtDNA is highly susceptible to oxidative stress, the purpose of this study was to determine whether radiofrequency radiation can cause oxidative damage to mtDNA. In this study, we exposed primary cultured cortical neurons to pulsed RF electromagnetic fields at a frequency of 1800 MHz modulated by 217 Hz at an average special absorption rate (SAR) of 2 W/kg. At 24h after exposure, we found that RF radiation induced a significant increase in the levels of 8-hydroxyguanine (8-OHdG), a common biomarker of DNA oxidative damage, in the mitochondria of neurons. Consistent with this finding, the copy number of mtDNA and the levels of mitochondrial RNA (mtRNA) transcripts showed an obvious reduction after RF exposure. Each of these mtDNA disturbances could be reversed by pretreatment with melatonin, which is known to be an efficient in the brain.

Together, these results suggested that 1800 MHz RF radiation could cause oxidative damage to mtDNA in primary cultured neurons. Oxidative damage to mtDNA may account for the neurotoxicity of RF radiation in the brain.

(E) Xu S, Chen G, Chen C, Sun C, Zhang D, Murbach M, Kuster N, Zeng Q, Xu Z. Cell Type-Dependent Induction of DNA Damage by 1800 MHz Radiofrequency Electromagnetic Fields Does Not Result in Significant Cellular Dysfunctions. PLoS One. 8(1):e54906, 2013. (GT, CS)

BACKGROUND: Although IARC clarifies radiofrequency electromagnetic fields (RF-EMF) as possible human carcinogen, the debate on its health impact continues due to the inconsistent results. Genotoxic effect has been considered as a golden standard to determine if an environmental factor is a carcinogen, but the currently available data for RF-EMF remain controversial. As an environmental stimulus, the effect of RF-EMF on cellular DNA may be subtle. Therefore, more sensitive method and systematic research strategy are warranted to evaluate its genotoxicity. **OBJECTIVES:** To determine whether RF-EMF does induce DNA damage and if the effect is cell-type dependent by adopting a more sensitive method γH2AX foci formation; and to investigate the biological consequences if RF-EMF does increase γH2AX foci formation. **METHODS:** Six different types of cells were intermittently exposed to GSM 1800 MHz RF-EMF at a specific absorption rate of 3.0 W/kg for 1 h or 24 h, then subjected to immunostaining with anti-γH2AX antibody. The biological consequences in γH2AX-elevated cell type were further explored with comet and TUNEL assays, flow cytometry, and cell growth assay. **RESULTS:** Exposure to RF-EMF for 24 h significantly induced γH2AX foci formation in Chinese hamster lung cells and Human skin fibroblasts (HSFs), but not the other cells. However, RF-EMF-elevated γH2AX foci formation in HSF cells did not result in detectable DNA fragmentation, sustainable cell cycle arrest, cell proliferation or viability change. RF-EMF exposure slightly but not significantly increased the cellular ROS level. **CONCLUSIONS:** RF-EMF induces DNA damage in a cell type-dependent manner, but the elevated γH2AX foci formation in HSF cells does not result in significant cellular dysfunctions.

(NE) Yadav AS, Sharma MK. Increased frequency of micronucleated exfoliated cells among humans exposed in vivo to mobile telephone radiations. Mutat Res.650(2):175-180, 2008. (LE, GT, HU)

The health concerns have been raised following the enormous increase in the use of wireless mobile telephones throughout the world. This investigation had been taken, with the motive to find out whether mobile phone radiations cause any in vivo effects on the frequency of micronucleated exfoliated cells in the exposed subjects. A total of 109 subjects including 85 regular mobile phone users (exposed) and 24 non-users (controls) had participated in this study. Exfoliated cells were obtained by swabbing the buccal-mucosa from exposed as well as sex-age-matched controls. One thousand exfoliated cells were screened from each individual for nuclear anomalies including micronuclei (MN), karyolysis (KL), karyorrhexis (KH), broken egg (BE) and binucleated (BN) cells. The average daily duration of exposure to mobile phone radiations is 61.26 min with an overall average duration of exposure in term of years is 2.35

years in exposed subjects along with the 9.84+/-0.745 micronucleated cells (MNCs) and 10.72+/-0.889 total micronuclei (TMN) as compared to zero duration of exposure along with average 3.75+/-0.774 MNC and 4.00+/-0.808 TMN in controls. The means are significantly different in case of MNC and TMN at 0.01% level of significance. The mean of KL in controls is 13.17+/-2.750 and in exposed subjects is 13.06+/-1.793. The value of means of KH in exposed subjects (1.84+/-0.432) is slightly higher than in controls (1.42+/-0.737). Mean frequency of broken egg is found to be more in exposed subjects (0.65+/-0.276) as compared to controls (0.50+/-0.217). Frequency of presence of more than one nucleus in a cell (binucleated) is also higher in exposed (2.72+/-0.374) in comparison to controls (0.67+/-0.231). Although there is a slight increase in mean frequency of KH, BE and BN in exposed subjects but the difference is not found statistically significant. Correlation between 0-1, 1-2, 2-3 and 3-4 years of exposure and the frequency of MNC and TMN has been calculated and found to be positively correlated.

(E) Yan JG, Agresti M, Zhang LL, Yan Y, Matloub HS. Upregulation of specific mRNA levels in rat brain after cell phone exposure. Electromagn Biol Med. 27(2):147-154, 2008. (LE, GE)

Adult Sprague-Dawley rats were exposed to regular cell phones for 6 h per day for 126 days (18 weeks). RT-PCR was used to investigate the changes in levels of mRNA synthesis of several injury-associated proteins. Calcium ATPase, Neural Cell Adhesion Molecule, Neural Growth Factor, and Vascular Endothelial Growth Factor were evaluated. The results showed statistically significant mRNA up-regulation of these proteins in the brains of rats exposed to cell phone radiation. These results indicate that relative chronic exposure to cell phone microwave radiation may result in cumulative injuries that could eventually lead to clinically significant neurological damage.

(E) Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, Ye J, Sun L. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol Vis 14:964-969, 2008. (GT, IA, OX)

PURPOSE: The goal of this study was to investigate whether superposing of electromagnetic noise could block or attenuate DNA damage and intracellular reactive oxygen species (ROS) increase of cultured human lens epithelial cells (HLECs) induced by acute exposure to 1.8 GHz radiofrequency field (RF) of the Global System for Mobile Communications (GSM). METHODS: An sXc-1800 RF exposure system was used to produce a GSM signal at 1.8 GHz (217 Hz amplitude-modulated) with the specific absorption rate (SAR) of 1, 2, 3, and 4 W/kg. After 2 h of intermittent exposure, the ROS level was assessed by the fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DNA damage to HLECs was examined by alkaline comet assay and the phosphorylated form of histone variant H2AX (gammaH2AX) foci formation assay. RESULTS: After exposure to 1.8 GHz RF for 2 h, HLECs exhibited significant intracellular ROS increase in the 2, 3, and 4 W/kg groups. RF radiation at the SAR of 3 W/kg and 4 W/kg could induce significant DNA damage, examined by alkaline comet assay, which was used to detect mainly single strand breaks (SSBs), while no statistical difference in double strand breaks (DSBs), evaluated by gammaH2AX foci, was found between RF exposure (SAR: 3

and 4 W/kg) and sham exposure groups. When RF was superposed with 2 muT electromagnetic noise could block RF-induced ROS increase and DNA damage. CONCLUSIONS: DNA damage induced by 1.8 GHz radiofrequency field for 2 h, which was mainly SSBs, may be associated with the increased ROS production. Electromagnetic noise could block RF-induced ROS formation and DNA damage.

(NE) Yildirim MS, Yildirim A, Zamani AG, Okudan N. Effect of mobile phone station on micronucleus frequency and chromosomal aberrations in human blood cells. Genet Couns. 21(2):243-251, 2010. (HU, LE, GT)

The use of mobile telephones has rapidly increased worldwide as well as the number of mobile phone base stations that lead to rise low level radiofrequency emissions which may in turn have possible harm for human health. The national radiation protection board has published the known effects of radio waves exposure on humans living close to mobile phone base stations. However, several studies have claimed that the base station has detrimental effects on different tissues. In this study, we aimed to evaluate the effects of mobile phone base stations on the micronucleus (MN) frequency and chromosomal aberrations on blood in people who were living around mobile phone base stations and healthy controls. Frequency of MN and chromosomal aberrations in study and control groups was 8.96 +/- 3.51 and 6.97 +/- 1.52 (p: 0.16); 0.36 +/- 0.31 and 0.75 +/- 0.61 (p: 0.07), respectively. Our results show that there was not a significant difference of MN frequency and chromosomal aberrations between the two study groups. The results claim that cellular phones and their base stations do not produce important carcinogenic changes.

(E) Zalata, A., A. Z. El-Samanoudy, D. Shaalan, Y. El-Baiomy, and T. Mostafa. In vitro effect of cell phone radiation on motility, DNA fragmentation and clusterin gene expression of sperm. Int J Fertil Steril, In Press. Published online ahead of print. (GT, GE, RP)

Background: Use of cellular phones that emits radiofrequency electromagnetic field (RF-EMF) has been increased exponentially and became a part of everyday life. This study aimed to investigate the effects of RF-EMF radiation emitted from cellular phones on sperm motility variables, sperm DNA fragmentation and clusterin (CLU) gene expression. Materials and Methods: 124 semen samples were grouped into; normozoospermia (N, n=26), asthenozoospermia (A, n=32), asthenoteratozoospermia (AT, n=31) and oligoasthenoteratozoospermia (OAT, n=35). Semen samples were divided into two aliquots; samples not exposed to cell phone and samples exposed to cell phone radiation (850 MHz, maximum power < 1 watt; SAR 1.46 W/kg at 10 cm distance) for 1 hr. Before and immediately after exposure both aliquots were subjected to assessment of sperm motility, acrosin activity, sperm DNA fragmentation and CLU gene expression. Statistical differences were analyzed using paired t-student test for comparisons where P< 0.05 was set as significant. Results: There was significant decrease in sperm motility, sperm linear velocity, sperm linearity index, sperm

acrosin activity and significant increase in sperm DNA fragmentation percent, CLU gene expression and CLU protein levels in the exposed semen samples to RF-EMF compared with non- exposed samples in OAT > AT > A > N groups (P<0.05).
Conclusions: Cell phone emissions have a negative impact on exposed sperm motility indices, sperm acrosin activity, sperm DNA fragmentation and CLU gene expression especially in OAT cases.

(NE) Zeni O, Schiavoni A, Perrotta A, Forigo D, Deplano M, Scarfi MR. Evaluation of genotoxic effects in human leukocytes after in vitro exposure to 1950 MHz UMTS radiofrequency field. Bioelectromagnetics 29:177-184, 2008. (GT)

In the present study the third generation wireless technology of the Universal Mobile Telecommunication System (UMTS) signal was investigated for the induction of genotoxic effects in human leukocytes. Peripheral blood from six healthy donors was used and, for each donor, intermittent exposures (6 min RF on, 2 h RF off) at the frequency of 1950 MHz were conducted at a specific absorption rate of 2.2 W/kg. The exposures were performed in a transverse electro magnetic (TEM) cell hosted in an incubator under strictly controlled conditions of temperature and dosimetry. Following long duration intermittent RF exposures (from 24 to 68 h) in different stages of the cell cycle, micronucleus formation was evaluated by applying the cytokinesis block micronucleus assay, which also provides information on cell division kinetics. Primary DNA damage (strand breaks/alkali labile sites) was also investigated following 24 h of intermittent RF exposures, by applying the alkaline single cell gel electrophoresis (SCG)/comet assay. Positive controls were included by treating cell cultures with Mitomycin-C and methylmethanesulfonate for micronucleus and comet assays, respectively. The results obtained indicate that intermittent exposures of human lymphocytes in different stages of cell cycle do not induce either an increase in micronucleated cells, or change in cell cycle kinetics; moreover, 24 h intermittent exposures also fail to affect DNA structure of human leukocytes soon after the exposures, likely indicating that repairable DNA damage was not induced.

(E) Zhang DY, Xu ZP, Chiang H, Lu DQ, Zeng QL. [Effects of GSM 1800 MHz radiofrequency electromagnetic fields on DNA damage in Chinese hamster lung cells.] Zhonghua Yu Fang Yi XueZaZhi 40:149-152, 2006. [Article in Chinese] (GT)

OBJECTIVE: To study the effects of GSM 1800 MHz radiofrequency electromagnetic fields (RF EMF) on DNA damage in Chinese hamster lung (CHL) cells. METHODS: The cells were intermittently exposed or sham-exposed to GSM 1800 MHz RF EMF (5 minutes on/10 minutes off) at a special absorption rate (SAR) of 3.0 W/kg for 1 hour or 24 hours. Meanwhile, cells exposed to 2-acetaminofluorene, a DNA damage agent, at a final concentration of 20 mg/L for 2 hours were used as positive control. After exposure, cells were fixed by using 4% paraformaldehyde and processed for phosphorylated form of H2AX (gammaH2AX) immunofluorescence measurement. The primary antibody used for immunofluorescence was mouse monoclonal antibody against gammaH2AX and the secondary antibody was fluorescein

isothiocyanate (FITC)-conjugated goat anti-mouse IgG. Nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI). The gammaH2AX foci and nuclei were visualized with an Olympus AX70 fluorescent microscope. Image Pro-Plus software was used to count the gammaH2AX foci in each cell. For each exposure condition, at least 50 cells were selected to detect gammaH2AX foci. Cells were classified as positive when more than five foci were detected. The percentage of gammaH2AX foci positive cells was adopted as the index of DNA damage. RESULTS: The percentage of gammaH2AX foci positive cell of 1800 MHz RF EMF exposure for 24 hours (37.9 +/- 8.6)% or 2-acetylaminofluorene exposure (50.9 +/- 9.4)% was significantly higher compared with the sham-exposure (28.0 +/- 8.4)%. However, there was no significant difference between the sham-exposure and RF EMF exposure for 1 hour (31.8 +/- 8.7)%. CONCLUSION: 1800 MHz RF EMF (SAR, 3.0 W/kg) for 24 hours might induce DNA damage in CHL cells.

(E) Zhang SZ, Yao GD, Lu DQ, Chiang H, Xu ZP. [Effect of 1.8 GHz radiofrequency electromagnetic fields on gene expression of rat neurons]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 26(8):449-452, 2008. [Article in Chinese] (GE, WS)

OBJECTIVE: To investigate the changes of gene expression in rat neuron induced by 1.8 GHz radiofrequency electromagnetic fields (RF EMF) to screen for RF EMF-responsive genes and the effect of different exposure times and modes on the gene expression in neuron. METHODS: Total RNA was extracted immediately and purified from the primary culture of neurons after intermittent exposed or sham-exposed to a frequency of 1.8 GHz RF EMF for 24 hours at an average special absorption rate (SAR) of 2 W/kg. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron. Differentially expressed genes (Egr-1, Mbp and Plp) were further confirmed by semi-quantitative revere transcription polymerase chain reaction (RT PCR). The expression levels of Egr-1, Mbp and Plp were observed at different exposure times (6, 24 h) and modes (intermittent and continuous exposure). RESULTS: Among 1200 candidate genes, 24 up-regulated and 10 down-regulated genes were found by using Affymetrix microarray suite software 5.0 which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. Under 24 h and 6 h intermittent exposure, Egr-1 and Plp in experiment groups showed statistic significance ($P < 0.05$) compared with the control groups, while expression of Mbp did not change significantly ($P > 0.05$). After 24 h continuous exposure, Egr-1 and Mbp in experiment groups showed statistic significance ($P < 0.05$) compared with the control group, while expression of Plp did not change significantly ($P > 0.05$). Under the same exposure mode 6 h, expression of all the 3 genes did not change significantly. Different times (6, 24 h) and modes (intermittent and continuous exposure) of exposure exerted remarkable different influences on the expression of Egr-1, Mbp, Plp genes ($P < 0.01$). CONCLUSION: The changes of many genes transcription were involved in the effect of 1.8 GHz RF EMF on rat neurons; Down-regulation of Egr-1 and up-regulation of Mbp, Plp indicated the negative effects of RF EMF on neurons; The effect of RF intermittent exposure on gene expression was more obvious than that of continuous exposure; The effect of 24 h RF exposure (both intermittent and continuous) on gene expression was more obvious than that of 6 h (both intermittent and continuous).

(E) Zhao R, Zhang S, Xu Z, Ju L, Lu D, Yao G. Studying gene expression profile of rat neuron exposed to 1800MHz radiofrequency electromagnetic fields with cDNA microassay. Toxicology 235:167-175, 2007. (GE)

A widespread use of mobile phone (MP) evokes a growing concern for their possible adverse effects on human, especially the brain. Gene expression is a unique way of characterizing how cells and organism adapt to changes in the external environment, so the aim of this investigation was to determine whether 1800 MHz radiofrequency electromagnetic fields (RF EMF) can influence the gene expression of neuron. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron after exposed to the pulsed RF EMF at a frequency of 1800 MHz modulated by 217 Hz which is commonly used in MP. Among 1200 candidate genes, 24 up-regulated genes and 10 down-regulated genes were identified after 24-h intermittent exposure at an average special absorption rate (SAR) of 2 W/kg, which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. The results were further confirmed by quantitative real-time polymerase chain reaction (RT PCR). The present results indicated that the gene expression of rat neuron could be altered by exposure to RF EMF under our experimental conditions.

(E) Zhao TY, Zou SP, Knapp PE. Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosci Lett. 412(1):34-38, 2007. (GE, CS)

The health effects of cell phone radiation exposure are a growing public concern. This study investigated whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to a working Global System for Mobile Communication (GSM) cell phone rated at a frequency of 1900MHz. Primary cultures were exposed to cell phone emissions for 2h. We used array analysis and real-time RT-PCR to show up-regulation of caspase-2, caspase-6 and Asc (apoptosis associated speck-like protein containing a card) gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The effects are specific since up-regulation was not seen for other genes associated with apoptosis, such as caspase-9 in either neurons or astrocytes, or Bax in neurons. The results show that even relatively short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

(E) Zhijian C, Xiaoxue L, Yezhen L, Shijie C, Lifen J, Jianlin L, Deqiang L, Jiliang H. Impact of 1.8-GHz radiofrequency radiation (RFR) on DNA damage and repair induced by doxorubicin in human B-cell lymphoblastoid cells. Mutat Res. 695(1-2):16-21, 2010. (GT, IA)

In the present in vitro study, a comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR, SAR of 2W/kg) can influence DNA repair in human B-cell

lymphoblastoid cells exposed to doxorubicin (DOX) at the doses of 0microg/ml, 0.05microg/ml, 0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml. The combinative exposures to RFR with DOX were divided into five categories. DNA damage was detected at 0h, 6h, 12h, 18h and 24h after exposure to DOX via the comet assay, and the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage. The results demonstrated that (1) RFR could not directly induce DNA damage of human B-cell lymphoblastoid cells; (2) DOX could significantly induce DNA damage of human B-cell lymphoblastoid cells with the dose-effect relationship, and there were special repair characteristics of DNA damage induced by DOX; (3) E-E-E type (exposure to RFR for 2h, then simultaneous exposure to RFR and DOX, and exposure to RFR for 6h, 12h, 18h and 24h after exposure to DOX) combinative exposure could obviously influence DNA repair at 6h and 12h after exposure to DOX for four DOX doses (0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml) in human B-cell lymphoblastoid cells.

(NE) Zhijian C, Xiaoxue L, Yezhen L, Deqiang L, Shijie C, Lifen J, Jianlin L, Jiliang H. Influence of 1.8-GHz (GSM) radiofrequency radiation (RFR) on DNA damage and repair induced by X-rays in human leukocytes in vitro. Mutat Res. 677(1-2):100-104, 2009. (GT, IA)

In the present study, the in vitro comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR) can influence DNA repair in human leukocytes exposed to X-rays. The specific energy absorption rate (SAR) of 2 W/kg (the current European safety limit) was applied. The leukocytes from four young healthy donors were intermittently exposed to RFR for 24 h (fields on for 5 min, fields off for 10 min), and then irradiated with X-rays at doses of 0.25, 0.5, 1.0 and 2.0 Gy. DNA damage to human leukocytes was detected using the comet assay at 0, 15, 45, 90, 150 and 240 min after exposure to X-rays. Using the comet assay, the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage; the DNA repair percentage (DRP) served as the indicator of the DNA repair speed. The results demonstrated that (1) the DNA repair speeds of human leukocytes after X-ray exposure exhibited individual differences among the four donors; (2) the intermittent exposures of 1.8-GHz RFR at the SAR of 2 W/kg for 24 h did not directly induce DNA damage or exhibit synergistic effects with X-rays on human leukocytes.

(NE) Ziemann C, Brockmeyer H, Reddy SB, Vijayalaxmi, Prihoda TJ, Kuster N, Tillmann T, Dasenbrock C. Absence of genotoxic potential of 902 MHz (GSM) and 1747 MHz (DCS) wireless communication signals: In vivo two-year bioassay in B6C3F1 mice. Int J Radiat Biol. 85(5):454-464, 2009. (GT, LE)

PURPOSE: The aim of the present investigation was to determine the incidence of micronuclei in peripheral blood erythrocytes of B6C3F1 mice that had been chronically exposed to radiofrequencies (RF) used for mobile communication. MATERIALS AND METHODS: 'Ferris wheels' were used to expose tube-restrained male and female mice to simulated environmental RF signals of the Global System for Mobile Communications (GSM, 902 MHz) or Digital Cellular System (DCS, 1747 MHz). RF signals were applied to the mice for 2 hours/day on 5 days/week for two years, at maximal whole-body-averaged specific absorption rates of 0.4, 1.3, and 4.0 W/kg body weight. Concurrent sham-exposed mice, cage controls, and positive controls injected with mitomycin C were included in this investigation. At necropsy, peripheral blood smears were prepared, and coded slides were stained using May-Grunwald-Giemsa or acridine orange. The incidence of micronuclei was recorded for each mouse in 2000 polychromatic and 2000 normochromatic erythrocytes. RESULTS: There were no significant differences in the frequency of micronuclei between RF-exposed, sham-exposed, and cage control mice, irrespective of the staining/counting method used. Micronuclei were, however, significantly increased in polychromatic erythrocytes of the positive control mice. CONCLUSIONS: In conclusion, the data did not indicate RF-induced genotoxicity in mice after two years of exposure.

Update on genetic effects of extremely-low frequency electromagnetic fields

(NE) Albert GC, McNamee JP, Marro L, Bellier PV, Prato FS, Thomas AW. Assessment of genetic damage in peripheral blood of human volunteers exposed (whole-body) to a 200 muT, 60 Hz magnetic field. Int J Radiat Biol. 85(2):144-152, 2009. (GT, IA)

AIM: To investigate the extent of damage in nucleated cells in peripheral blood of healthy human volunteers exposed to a whole-body 60 Hz, 200 microT magnetic field. **MATERIALS AND METHODS:** In this study, 10 male and 10 female healthy human volunteers received a 4 h whole-body exposure to a 200 microT, 60 Hz magnetic field. In addition, five males and five females were treated in a similar fashion, but were exposed to sham conditions. For each subject, a blood sample was obtained prior to the exposure period and aliquots were used as negative- (pre-exposure) and positive- [1.5 Gray (Gy) (60)Cobalt ((60)Co) gamma-irradiation] controls. At the end of the 4 h exposure period, a second blood sample was obtained. The extent of DNA damage was assessed in peripheral human blood leukocytes from all samples using the alkaline comet assay. To detect possible clastogenic effects, the incidence of micronuclei was assessed in phytohemagglutinin (PHA)-stimulated lymphocytes using the cytokinesis-block micronucleus assay. **RESULTS:** There was no evidence of either increased DNA damage, as indicated by the alkaline comet assay, or increased incidence of micronuclei (MN) in the magnetic field exposed group. However, an in vitro exposure of 1.5 Gy gamma-irradiation caused a significant increase in both DNA damage and MN induction. **CONCLUSIONS:** This study found no evidence that an acute, whole-body exposure to a 200 microT, 60 Hz magnetic field for 4 hours could cause DNA damage in human blood.

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

Abstract In recent years extremely low-frequency magnetic fields (ELF-EMF) have become widely used in human activities, leading to an increased chance of exposure to ELF-EMF. There are few reports on in vivo mammalian genotoxic effects using micronucleus (MN) assays, which generally have been used as a short-term screening system. We analyzed the possible genotoxic effect induced by long-term exposure (7, 14, 21, 28 d) of a 50 Hz ELM-MF to mice by measuring the increase in frequency of micronucleated polychromatic erythrocyte in their bone marrow (MNPCEs) and we compared it with that induced by 50 cGy of X-rays. Subsequently, we tried to reduce this chromosomal damage by administering four antioxidants substances with radioprotective capacities: dimethyl sulfoxide (DMSO), 6-n-propyl-2-thiouracil (PTU), grape-

procyanidins (P) and citrus flavonoids extract (CE). The increase in micronucleated cells was higher in both physical treatments (Control < ELF-EMF ($p < 0.01$) <X-rays ($p > 0.001$)); however, the antioxidant substances only showed a genoprotective capacity against the damage induced by ionizing radiation (Ci > PTU = DMSO ($p < 0.001$) > P = CE ($p < 0.001$). The 50 Hz ELM-MF increased MNPCEs in mouse bone marrow, expressing a genotoxic capacity. Administration of antioxidant substances with radioprotective capacities known to act through the elimination of free radicals did not diminish the genotoxic effect induced by ELM-MF.

(E) Balamuralikrishnan B, Balachandar V, Kumar SS, Stalin N, Varsha P, Devi SM, Arun M, Manikantan P, Venkatesan C, Sasikala K, Dharwadkar SN. Evaluation of Chromosomal Alteration in Electrical Workers Occupationally Exposed to Low Frequency of Electro Magnetic Field (EMFs) in Coimbatore Population, India. Asian Pac J Cancer Prev. 13(6):2961-2966, 2012. (HU, LE, GT)

Extremely low frequency electromagnetic fields (EMFs) have been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer. An increased number of chromosomal alterations in peripheral lymphocytes are correlated with elevated incidence of cancer. The aim of the present study was to assess occupationally induced chromosomal damage in EMF workers exposed to low levels of radiation. We used conventional metaphase chromosome aberration (CA) analysis and the micronucleus (MN) assay as biological indicators of nonionizing radiation exposure. In the present study totally 70 subjects were selected including 50 exposed and 20 controls. Informed written consent was obtained from all participants and the study was performed in accordance with the Declaration of Helsinki and the approval of the local ethical committee. A higher degree of CA and MN was observed in exposed subjects compared to controls, the frequency of CA being significantly enhanced with long years of exposure ($P<0.05$). Moreover increase in CA and MN with age was noted in both exposed subjects and controls, but was significantly greater in the former. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers occupationally exposed to EMFs in electric transformer and distribution stations. In conclusion, our findings suggest that EMFs possess genotoxic capability, as measured by CA and MN assays; CA analysis appeared more sensitive than other cytogenetic end-points. It can be concluded that chronic occupational exposure to EMFs may lead to an increased risk of genetic damage among electrical workers.

(E) Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. Bioelectromagnetics 26:173-184, 2005. (GT, EH)

We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power frequency

magnetic field (50 Hz, 15 muT peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock.

(E) Borhani N, Rajaei F, Salehi Z, Javadi A. Analysis of DNA fragmentation in mouse embryos exposed to an extremely low-frequency electromagnetic field. Electromagn Biol Med. 30(4):246-252, 2011. (GT, DE, LE)

Effects of extremely low-frequency electromagnetic fields (ELF-EMFs) on DNA damage in biological systems are still a matter of dispute. The aim of the present study was to investigate the possible effect of electromagnetic field exposure on DNA fragmentation in cells (blastomers) of mouse blastocysts. Eighty female NMRI mice were randomly divided into 2 groups of 40 animals each. The control group was left unexposed whereas the animals in the EMF-group were exposed to a 50-Hz EMF at 0.5 mT 4 h per day, 6 days a week for a duration of 2 weeks. After the 8(th) day of exposure, the female mice in both groups were superovulated (with injections of pregnant mare serum gonadotropin and human chorionic gonadotropin) and then mated overnight. At approximately 4 days after mating (102 h after the human chorionic gonadotropin treatment), blastocysts were obtained by flushing the uterus horns. The mean numbers of pregnant mice, blastocysts after flushing, blastomers within the blastocysts, and the DNA fragmentation index following staining in both groups were compared using statistical methods (SPSS, the Chi-square test, the Student's t-test and the Mann-Whitney U-test, $P < 0.05$). The results showed that the mean number of blastocysts after flushing was significantly decreased in the EMF-group compared to that of the control group ($P < 0.03$). The DNA fragmentation index was significantly increased in the EMF-group compared to control (10.53% vs. 7.14%; $P < 0.001$). However, there was no significant difference in the mean numbers of blastomers and numbers of pregnant mice between the EMF-exposed and control group. Our findings indicate that the EMF exposure in preimplantation stage could have detrimental effects

on female mouse fertility and embryo development by decreasing the number of blastocysts and increasing the blastocysts DNA fragmentation.

(E) Bułdak RJ, Polaniak R, Bułdak L, Zwirska-Korczala K, Skonieczna M, Monsiol A, Kukla M, Duława-Bułdak A, Birkner E. Short-term exposure to 50 Hz ELF-EMF alters the cisplatin-induced oxidative response in AT478 murine squamous cell carcinoma cells. Bioelectromagnetics. 2012 Apr 25. doi: 10.1002/bem.21732. [Epub ahead of print] (GT, IA, OX)

The aim of this study was to assess the influence of cisplatin and an extremely low frequency electromagnetic field (ELF-EMF) on antioxidant enzyme activity and the lipid peroxidation ratio, as well as the level of DNA damage and reactive oxygen species (ROS) production in AT478 carcinoma cells. Cells were cultured for 24 and 72 h in culture medium with cisplatin. Additionally, the cells were irradiated with 50 Hz/1 mT ELF-EMF for 16 min using a solenoid as a source of the ELF-EMF. The amount of ROS, superoxide dismutase (SOD) isoenzyme activity, glutathione peroxidase (GSH-Px) activity, DNA damage, and malondialdehyde (MDA) levels were assessed. Cells that were exposed to cisplatin exhibited a significant increase in ROS and antioxidant enzyme activity. The addition of ELF-EMF exposure to cisplatin treatment resulted in decreased ROS levels and antioxidant enzyme activity. A significant reduction in MDA concentrations was observed in all of the study groups, with the greatest decrease associated with treatment by both cisplatin and ELF-EMF. Cisplatin induced the most severe DNA damage; however, when cells were also irradiated with ELF-EMF, less DNA damage occurred. Exposure to ELF-EMF alone resulted in an increase in DNA damage compared to control cells. ELF-EMF lessened the effects of oxidative stress and DNA damage that were induced by cisplatin; however, ELF-EMF alone was a mild oxidative stressor and DNA damage inducer. We speculate that ELF-EMF exerts differential effects depending on the exogenous conditions. This information may be of value for appraising the pathophysiologic consequences of exposure to ELF-EMF.

(E) Calabrò E, Condello S, Magazù S, Ientile, R. Static and 50 Hz electromagnetic fields effects on human neuronal-like cells vibration bands in the mid-infrared region. J Electromagnetic Analysis and Applications 3(2) 69-78, 2011. (GT)

Human neuronal-like cells were exposed to static and 50 Hz electromagnetic fields at the intensities of 2 mT and 1 mT, respectively. The effects of exposure were investigated in the mid-infrared region by means of Fourier self deconvolution spectroscopic analysis. After exposure of 3 hours to static and 50 Hz electromagnetic fields, the vibration bands of CH2 methilene group increased significantly after both exposures, suggesting a relative increase of lipid related to conformational changes in the cell membrane due to electromagnetic fields. In addition, PO2- stretching phosphate bands decreased after both exposures, suggesting that alteration in DNA/RNA can be occurred. In particular, exposure of 3 hours to 50 Hz electromagnetic fields produced significant increases in β-sheet contents in amide I, and around the 1740 cm^{-1} band

assigned to non-hydrogen-bonded ester carbonyl stretching mode, that can be related to unfolding processes of proteins structure and cells death. Further exposure up to 18 hours to static magnetic field produced an increase in β-sheet contents as to α-helix components of amide I region, as well.

(E) Celikler S, Aydemir N, Vatan O, Kurtuldu S, Bilaloglu R. A biomonitoring study of genotoxic risk to workers of transformers and distribution line stations. Int J Environ Health Res. 19(6):421-430, 2009. (GT, HU)

A cytogenetic monitoring study was carried out on a group of workers from transformer and distribution line stations in the Bursa province of Turkey, to investigate the genotoxic risk of occupational exposure to extremely low frequency electric (ELF) and magnetic fields (EMF). Cytogenetic analysis, namely chromosomal aberrations (CAs) and micronucleus (MN) tests were performed on a strictly selected group of 55 workers and compared to 17 controls. CA and MN frequencies in electrical workers appeared significantly higher than in controls ($p < 0.001$, 0.05, respectively). The frequency of CA in exposed groups were significantly enhanced with the years of exposure ($p < 0.01$). The effect of smoking on the level of CA and MN was not significant in the control and exposure groups. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers engaged to occupational exposure to ELMF in electric transformer and distribution stations.

(E) Chen GD, Lu DQ, Jiang H, Xu ZP.[Effects of 50 Hz magnetic fields on gene expression in MCF-7 cells]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):15-22, 2008. [Article in Chinese] **(GT, GE)**

OBJECTIVE: To investigate whether 50 Hz magnetic fields (MF) can change the gene expression profile in MCF-7 cells and to screen MF responsive genes. **METHODS:** In vitro cultured MCF-7 cells were continuously exposed or sham-exposed to 0.4 mT of 50 Hz MF for 24 hours. Affymetrix Human Genome Genechips (U133A) were applied to analyze gene expression profiles in MF exposed and sham-exposed MCF-7 cells and the data were processed with Genechip data analysis software MAS 5.0 and DMT 3.0. Real-time RT-PCR assay was employed to examine the differentially expressed genes. **RESULT:** Thirty differentially expressed genes were screened with 100 % consistency change calls in the MF exposed MCF-7 cells. Six independent real-time RT-PCR analyses showed that SCNN1A, METTL3 and GPR137B were slightly but statistically significantly changed in MCF-7 cells after exposure to 50 Hz MF ($P<0.05$), while other analyzed genes exhibited slight up-and down-fluctuations in expressions and no increase or decrease in each gene expression reached statistical significance ($P>0.05$). **CONCLUSION:** The present study identified three 50 Hz MF responsive genes in MCF-7 cells and the biological consequences of expression changes in these MF responsive genes need to be further investigated.0.4 mT 50 Hz MF exposure for longer duration might induce DNA double-strand breaks in human lens epithelial cells in vitro.

(NE) Chen G, Lu D, Chiang H, Leszczynski D, Xu Z. Using model organism Saccharomyces cerevisiae to evaluate the effects of ELF-MF and RF-EMF exposure on global gene expression. Bioelectromagnetics. 33(7):550-560, 2012. (GE)

The potential health hazard of exposure to electromagnetic fields (EMF) continues to cause public concern. However, the possibility of biological and health effects of exposure to EMF remains controversial and their biophysical mechanisms are unknown. In the present study, we used Saccharomyces cerevisiae to identify genes responding to extremely low frequency magnetic fields (ELF-MF) and to radiofrequency EMF (RF-EMF) exposures. The yeast cells were exposed for 6 h to either 0.4 mT 50 Hz ELF-MF or 1800 MHz RF-EMF at a specific absorption rate of 4.7 W/kg. Gene expression was analyzed by microarray screening and confirmed using real-time reverse transcription-polymerase chain reaction (RT-PCR). We were unable to confirm microarray-detected changes in three of the ELF-MF responsive candidate genes using RT-PCR ($P > 0.05$). On the other hand, out of the 40 potential RF-EMF responsive genes, only the expressions of structural maintenance of chromosomes 3 (SMC3) and aquaporin 2 (AQY2 (m)) were confirmed, while three other genes, that is, halotolerance protein 9 (HAL9), yet another kinase 1 (YAK1) and one function-unknown gene (open reading frame: YJL171C), showed opposite changes in expression compared to the microarray data ($P < 0.05$). In conclusion, the results of this study suggest that the yeast cells did not alter gene expression in response to 50 Hz ELF-MF and that the response to RF-EMF is limited to only a very small number of genes. The possible biological consequences of the gene expression changes induced by RF-EMF await further investigation.

(E) Cho S, Lee Y, Lee S, Choi YJ, Chung HW. Enhanced cytotoxic and genotoxic effects of gadolinium following ELF-EMF irradiation in human lymphocytes. Drug Chem Toxicol. 2014 Jan 30. [Epub ahead of print] (GT, IA)

Gadolinium (Gd) and its chelated derivatives are widely utilized for various industrial and medical purposes, particularly as a contrast agent for magnetic resonance imaging (MRI). There are many studies of Gd nephrotoxicity and neurotoxicity, whereas research on cyto- and genotoxicity in normal human lymphocytes is scarce. It is important to investigate the effect of extremely low-frequency electromagnetic fields (ELF-EMF) on Gd toxicity, as patients are co-exposed to Gd and ELF-EMF generated by MRI scanners. We investigated the cytotoxicity and genotoixcity of Gd and the possible enhancing effect of ELF-EMF on Gd toxicity in cultured human lymphocytes by performing a micronuclei (MN) assay, trypan blue dye exclusion, single cell gel electrophoresis, and apoptosis analyses using flow cytometry. Isolated lymphocytes were exposed to 0.2-1.2 mM of Gd only or in combination with a 60-Hz ELF-EMF of 0.8-mT field strength. Exposing human lymphocytes to Gd resulted in a concentration- and time-dependent decrease in cell viability and an increase in MN frequency, single strand DNA breakage, apoptotic cell death, and ROS production. ELF-EMF (0.8 mT) exposure also increased cell death,

MN frequency, olive tail moment, and apoptosis induced by Gd treatment alone. These results suggest that Gd induces DNA damage and apoptotic cell death in human lymphocytes and that ELF-EMF enhances the cytotoxicity and genotoxicity of Gd.[1]

(E) Cho YH, Jeon HK, Chung HW. Effects of extremely low-frequency electromagnetic fields on delayed chromosomal instability induced by bleomycin in normal human fibroblast cells. J Toxicol Environ Health A. 70(15-16):1252-1258, 2007. (GT, IA)

This study was carried out to examine the interaction of extremely low-frequency electromagnetic fields (ELF-EMF) on delayed chromosomal instability by bleomycin (BLM) in human fibroblast cells. A micronucleus-centromere assay using DNA probes for chromosomes 1 and 4 was performed and a 60-Hz ELF-EMF of 0.8 mT field strength was applied either alone or with BLM throughout the culture period. The frequencies of micronuclei (MN) and aneuploidy were analyzed at 28, 88, and 240 h after treatment with BLM. The coexposure of cells to BLM and ELF-EMF led to a significant increase in the frequencies of MN and aneuploidy compared to the cells treated with BLM alone. No difference was observed between field-exposed and sham-exposed control cells. The frequency of MN induced by BLM was increased at 28 h, and further analysis showed a persistent increase up to 240 h, but the new levels were not significantly different from the level at 28 h. BLM increased the frequencies of aneuploidy at 28, 88, and 240 h, and significantly higher frequency of aneuploidy was observed in the cells analyzed at 240 h compared to the cells examined at 28 h. No interaction of ELF-EMF on delayed chromosomal instability by BLM was observed. Our results suggest that ELF-EMF enhances the cytotoxicity of BLM. BLM might induce delayed chromosomal instability, but no effect of ELF-EMF was observed on the BLM-induced delayed chromosomal instability in fibroblast cells.

(E) Collard JF, Lazar C, Nowé A, Hinsenkamp M. Statistical validation of the acceleration of the differentiation at the expense of the proliferation in human epidermal cells exposed to extremely low frequency electric fields. Prog Biophys Mol Biol. 111(1):37-45, 2013. (GE)

An acceleration of differentiation at the expense of proliferation is observed in our previous publications and in the literature after exposure of various biological models to low frequency and low-amplitude electric and electromagnetic fields. This observation is related with a significant modification of genes expression. We observed and compared over time this modification. This study use microarray data obtained on epidermis cultures harvested from human abdominoplasty exposed to ELF electric fields. This protocol is repeated with samples collected on three different healthy patients. The sampling over time allows comparison of the effect of the stimulus at a given time with the evolution of control group. After 4 days, we observed a significant difference of the genes expression between control (D4C) and stimulated (D4S) ($p < 0.05$). On the control between day 4 and 7, we observed another group of genes with significant difference ($p < 0.05$) in their expression. We identify the common genes between these two groups and we select from them those expressing no difference between stimulate

at 4 days (D4S) and control after 7 days (D7C). The same analysis was performed with D4S-D4C-D12C and D7S-D7C-D12C. The lists of genes which follow this pattern show acceleration in their expressions under stimulation appearing on control at a later time. In this list, genes such as DKK1, SPRR3, NDRG4, and CHEK1 are involved in cell proliferation or differentiation. Numerous other genes are also playing a function in mitosis, cell cycle or in the DNA replication transcription and translation. 1

(E) Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, Azzena GB, Grassi C. Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. Exp Neurol. 226(1):173-182, 2010. (LE, GE, DE)

Throughout life, new neurons are continuously generated in the hippocampus, which is therefore a major site of structural plasticity in the adult brain. We recently demonstrated that extremely low-frequency electromagnetic fields (ELFEFs) promote the neuronal differentiation of neural stem cells in vitro by up-regulating Ca(v)1-channel activity. The aim of the present study was to determine whether 50-Hz/1 mT ELFEF stimulation also affects adult hippocampal neurogenesis in vivo, and if so, to identify the molecular mechanisms underlying this action and its functional impact on synaptic plasticity. ELFEF exposure (1 to 7 h/day for 7 days) significantly enhanced neurogenesis in the dentate gyrus (DG) of adult mice, as documented by increased numbers of cells double-labeled for 5-bromo-deoxyuridine (BrdU) and double cortin. Quantitative RT-PCR analysis of hippocampal extracts revealed significant ELFEF exposure-induced increases in the transcription of pro-neuronal genes (Mash1, NeuroD2, Hes1) and genes encoding Ca(v)1.2 channel α(1C) subunits. Increased expression of NeuroD1, NeuroD2 and Ca(v)1 channels was also documented by Western blot analysis. Immunofluorescence experiments showed that, 30 days after ELFEF stimulation, roughly half of the newly generated immature neurons had survived and become mature dentate granule cells (as shown by their immunoreactivity for both BrdU and NeuN) and were integrated into the granule cell layer of the DG. Electrophysiological experiments demonstrated that the new mature neurons influenced hippocampal synaptic plasticity, as reflected by increased long-term potentiation. Our findings show that ELFEF exposure can be an effective tool for increasing in vivo neurogenesis, and they could lead to the development of novel therapeutic approaches in regenerative medicine.

(E) Di Campli E, Di Bartolomeo S, Grande R, Di Giulio M, Cellini L. Effects of extremely low-frequency electromagnetic fields on Helicobacter pylori biofilm. Curr Microbiol. 60(6):412-418, 2010. (GE)

The aim of this work was to investigate the effects of exposure to extremely low-frequency electromagnetic fields (ELF-EMF) both on biofilm formation and on mature biofilm of Helicobacter pylori. Bacterial cultures and 2-day-old biofilm of H. pylori ATCC 43629 were exposed to ELF-EMF (50 Hz frequency-1 mT intensity) for 2 days to assess their effect on the cell adhesion and on the mature biofilm detachment, respectively. All the exposed cultures and the

respective sham exposed controls were studied for: the cell viability status, the cell morphological analysis, the biofilm mass measurement, the genotypic profile, and the luxS and amiA gene expression. The ELF-EMF acted on the bacterial population during the biofilm formation displaying significant differences in cell viability, as well as, in morphotypes measured by the prevalence of spiral forms (58.41%) in respect to the controls (33.14%), whereas, on mature biofilm, no significant differences were found when compared to the controls. The measurement of biofilm cell mass was significantly reduced in exposed cultures in both examined experimental conditions. No changes in DNA patterns were recorded, whereas a modulation in amiA gene expression was detected. An exposure to ELF-EMF of H. pylori biofilm induces phenotypic changes on adhering bacteria and decreases the cell adhesion unbalancing the bacterial population therefore reducing the H. pylori capability to protect itself.

(E) Dominici L, Villarini M, Fatigoni C, Monarca S, Moretti M. Genotoxic hazard evaluation in welders occupationally exposed to extremely low-frequency magnetic fields (ELF-MF). Int J Hyg Environ Health. 215(1):68-75, 2011. (GT, HU)

Electric arc welding is known to involve considerable exposure to extremely low-frequency magnetic fields (ELF-MF). A cytogenetic monitoring study was carried out in a group of welders to investigate the genotoxic risk of occupational exposure to ELF-MF. This study assessed individual occupational exposure to ELF-MF using a personal magnetic-field dosimeter, and the cytogenetic effects were examined by comparing micronuclei (MN) and sister chromatid exchange (SCE) frequencies in the lymphocytes of the exposed workers with those of non-exposed control subjects (blood donors) matched for age and smoking habit. Cytogenetic analyses were carried out on 21 workers enrolled from two different welding companies in Central Italy and compared to 21 controls. Some differences between the groups were observed on analysis of SCE and MN, whereas replication indices in the exposed were found not to differ from the controls. In particular, the exposed group showed a significantly higher frequency of MN (group mean±SEM: 6.10±0.39) compared to the control group (4.45±0.30). Moreover, the increase in MN is associated with a proportional increase in ELF-MF exposure levels with a dose-response relationship. A significant decrease in SCE frequency was observed in exposed subjects (3.73±0.21) compared to controls (4.89±0.12). The hypothesis of a correlation between genotoxic assays and ELF-MF exposure value was partially supported, especially as regards MN assay. Since these results are derived from a small-scale pilot study, a larger scale study should be undertaken.

(E) Du XG, Xu SS, Chen Q, Lu DQ, Xu ZP, Zeng QL. [Effects of 50 Hz magnetic fields on DNA double-strand breaks in human lens epithelial cells]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):9-14, 2008. [Article in Chinese] (GT)

OBJECTIVE: To investigate the effects of 50 Hz magnetic fields (MF) on DNA double-strand breaks in human lens epithelial cells (hLECs). **METHODS:** The cultured human lens epithelial cells were exposed to 0.4 mT 50 Hz MF for 2 h, 6 h, 12 h, 24 h and 48 h. Cells exposed to 4-nitroquinoline-1-oxide, a DNA damage agent, at a final concentration of 0.1 micromol/L for 1 h

were used as positive controls.After exposure, cells were fixed with 4 % paraformaldehyde and for H2AX (gamma H2AX) immunofluorescence measurement. gamma H2AX foci were detected at least 200 cells for each sample. Cells were classified as positive when more than three foci per cell were observed. Mean values of foci per cell and percentage of foci positive cells were adopted as indexes of DNA double-strand breaks. **RESULT:** The mean value of foci per cell and the percentage of gamma H2AX foci positive cells in 50 Hz MF exposure group for 24 h were (2.93 +/-0.43) and (27.88 +/-2.59)%, respectively, which were significantly higher than those of sham-exposure group [(1.77 +/-0.37) and (19.38+/-2.70)%, P <0.05], and the mean value of foci per cell and the percentage of gamma H2AX foci positive cells in 50 Hz MF exposure group for 48 h were (3.14 +/-0.35) and (31.00 +/-3.44)%, which were significantly higher than those of sham-exposure group (P <0.01). However there was no significant difference between 50 Hz MF exposure groups for 2 h, 6 h, 12 h and sham-exposure group for above two indexes (P >0.05). **CONCLUSION:** 0.4 mT 50 Hz MF exposure for longer duration might induce DNA double-strand breaks in human lens epithelial cells in vitro.

(E) El-Bialy NS, Rageh MM. Extremely low-frequency magnetic field enhances the therapeutic efficacy of low-dose cisplatin in the treatment of Ehrlich carcinoma. Biomed Res Int. 2013;2013:189352. doi: 10.1155/2013/189352. Epub 2013 Jan 14. (GT, IA)

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

(E) Erdal N, Gürgül S, Celik A. Cytogenetic effects of extremely low frequency magnetic field on Wistar rat bone marrow. Mutat Res. 630(1-2):69-77, 2007. (GT, LE)

In this study, the genotoxic and cytotoxic potential of extremely low frequency magnetic fields (ELF-MF) was investigated in Wistar rat tibial bone marrow cells, using the chromosomal aberration (CA) and micronucleus (MN) test systems. In addition to these test systems, we also investigated the mitotic index (MI), and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs). Wistar rats were exposed to acute (1 day for 4h) and long-term (4h/day for 45 days) to a horizontal 50Hz, 1mT uniform magnetic field generated by a

Helmholtz coil system. Mitomycin C (MMC, 2mg/kg BW) was used as positive control. Results obtained by chromosome analysis do not show any statistically significant differences between the negative control and both acute and long-term ELF-MF exposed samples. When comparing the group mean CA of long-term exposure with the negative control and acute exposure, the group mean of the long-term exposed group was higher, but this was not statistically significant. However, the mean micronucleus frequency of the longer-term exposed group was considerably higher than the negative control and acutely exposed groups. This difference was statistically significant ($p<0.01$). The results of the MI in bone marrow showed that the averages of both A-MF and L-MF groups significantly decreased when compared to those in the negative control ($p<0.001$ and $p<0.01$, respectively). No significant differences were found between the group mean MI of A-MF exposure with L-MF. We found that the average of PCEs/NCEs ratios of A-MF exposed group was significantly lower than the negative control and L-MF exposed groups ($p<0.001$ and $p<0.01$, respectively). In addition, the group mean of the PCEs/NCEs ratios of L-MF was significantly lower than negative control ($p<0.01$). We also found that the MMC treated group showed higher the number of CA and the frequency of MN formation when compared to those in all other each groups (p-values of all each groups <0.01) and also MMC treated group showed lower MI and the PCEs/NCEs ratios when compared to those in all other each groups (p-values of all groups <0.01). These observations indicate the in vivo suspectibility of mammals to the genotoxicity potential of ELF-MF.

(E) Fedrowitz M, Löscher W. Gene expression in the mammary gland tissue of female Fischer 344 and Lewis rats after magnetic field exposure (50 Hz, 100 µT) for 2 weeks. Int J Radiat Biol. 88(5):425-429, 2012. (GE, LE) See also: Fedrowitz M, Hass R, Löscher W. Effects of 50 Hz magnetic field exposure on the stress marker α-amylase in the rat mammary gland. Int J Radiat Biol. 88(7):556-564, 2012.

PURPOSE: The issue of whether exposure to environmental power-frequency magnetic fields (MF) has impact on breast cancer development still remains equivocal. Previously, we observedrat strain differences in the MF response of breast tissue, so that the genetic background plays a role in MF effects. The present experiment aimed to elucidate candidate genes involved in MF effects by comparison of MF-susceptible Fischer 344 (F344) rats and MF-insensitive Lewis rats. **MATERIALS AND METHODS:** Female F344 and Lewis rats were exposed to MF (50 Hz, 100 µT) for two weeks, and a whole genome microarray analysis in the mammary gland tissue was performed. **RESULTS:** A remarkably decreased α-amylase gene expression, decreases in carbonic anhydrase 6 and lactoperoxidase, both relevant for pH regulation, and an increased gene expression of cystatin E/M, a tumor suppressor, were observed in MF-exposed F344, but not in Lewis rats. **CONCLUSION:** The MF-exposed F344 breast tissue showed alterations in gene expression, which were absent in Lewis and may therefore be involved in the MF-susceptibility of F344. Notably α-amylase might serve as a promising target to study MF effects, because first experiments indicate that MF exposure alters the functionality of this enzyme in breast tissue.

(E) Focke F, Schuermann D, Kuster N, Schär P. DNA fragmentation in human fibroblasts under extremely low frequency electromagnetic field exposure. Mutat Res. 683(1-2):74-83, 2010. (GT)

Extremely low frequency electromagnetic fields (ELF-EMFs) were reported to affect DNA integrity in human cells with evidence based on the Comet assay. These findings were heavily debated for two main reasons; the lack of reproducibility, and the absence of a plausible scientific rationale for how EMFs could damage DNA. Starting out from a replication of the relevant experiments, we performed this study to clarify the existence and explore origin and nature of ELF-EMF induced DNA effects. Our data confirm that intermittent (but not continuous) exposure of human primary fibroblasts to a 50 Hz EMF at a flux density of 1 mT induces a slight but significant increase of DNA fragmentation in the Comet assay, and we provide first evidence for this to be caused by the magnetic rather than the electric field. Moreover, we show that EMF-induced responses in the Comet assay are dependent on cell proliferation, suggesting that processes of DNA replication rather than the DNA itself may be affected. Consistently, the Comet effects correlated with a reduction of actively replicating cells and a concomitant increase of apoptotic cells in exposed cultures, whereas a combined Fpg-Comet test failed to produce evidence for a notable contribution of oxidative DNA base damage. Hence, ELF-EMF induced effects in the Comet assay are reproducible under specific conditions and can be explained by minor disturbances in S-phase processes and occasional triggering of apoptosis rather than by the generation of DNA damage.

(E) Frisch P, Li GC, McLeod K, Laramee CB. Induction of heat shock gene expression in RAT1 primary fibroblast cells by ELF electric fields. Bioelectromagnetics. 34(5):405-413, 2013. (GE)

Recent studies have demonstrated that the Ku70 gene fragment can be placed in the anti-sense orientation under the control of a heat-inducible heat shock protein 70 (HSP70) promoter and activated through heat shock exposure. This results in attenuation of the Ku70 protein expression, inhibiting cellular repair processes, and sensitizing the transfected cells to exposures such as the ionizing radiation exposures used clinically. However, achieving the tissue temperatures necessary to thermally induce the HSP70 response presents significant limitations to the clinical application of this strategy. Previous findings suggest an alternative approach to inducing a heat shock response, specifically through the use of extremely low frequency (ELF) electrical field stimulation. To further pursue this approach, we investigated HSP70 responses in transfected rat primary fibroblast (RAT1) cells exposed to 10 Hz electric fields at intensities of 20-500 V/m. We confirmed that low frequency electric fields can induce HSP70 heat shock expression, with peak responses obtained at 8 h following a 2 h field exposure. However, the approximate threefold increase in expression is substantially lower than that obtained using thermal stimulation, raising questions of the clinical utility of the response.

(E) Giorgi G, Marcantonio P, Bersani F, Gavoçi E, Del Re B. Effect of extremely low frequency magnetic field exposure on DNA transposition in relation to frequency, wave shape and exposure time. Int J Radiat Biol. 87(6):601-608, 2011. (GT, WS)

PURPOSE: To examine the effect of extremely low frequency magnetic field (ELF-MF) exposure on transposon (Tn) mobility in relation to the exposure time, the frequency and the wave shape of the field applied. **MATERIALS AND METHODS:** Two Escherichia coli model systems were used: (1) Cells unable to express β-galactosidase (LacZ(-)), containing a mini-transposon Tn10 element able to give ability to express β-galactosidase (LacZ(+)) upon its transposition; therefore in these cells transposition activity can be evaluated by analysing LacZ(+) clones; (2) cells carrying Fertility plasmid (F(+)), and a Tn5 element located on the chromosome; therefore in these cells transposition activity can be estimated by a bacterial conjugation assay. Cells were exposed to sinusoidal (SiMF) or pulsed-square wave (PMF) magnetic fields of various frequencies (20, 50, 75 Hz) and for different exposure times (15 and 90 min). **RESULTS:** Both mini-Tn10 and Tn5 transposition decreased under SiMF and increased under PMF, as compared to sham exposure control. No significant difference was found between frequencies and between exposure times. **CONCLUSIONS:** ELF-MF exposure affects transposition activity and the effects critically depend on the wave shape of the field, but not on the frequency and the exposure time, at least in the range observed.

(E) Heredia-Rojas JA, Rodríguez de la Fuente AO, Alcocer González JM, Rodríguez-Flores LE, Rodríguez-Padilla C, Santoyo-Stephano MA, Castañeda-Garza E, Taméz-Guerra RS. Effect of 60 Hz magnetic fields on the activation of hsp70 promoter in cultured INER-37 and RMA E7 cells. In Vitro Cell Dev Biol Anim. 46(9):758-63, 2010. (GE)

It has been reported that 50-60 Hz magnetic fields (MF) with flux densities ranging from microtesla to millitesla are able to induce heat shock factor or heat shock proteins in various cells. In this study, we investigated the effect of 60 Hz sinusoidal MF at 8 and 80 μT on the expression of the luciferase gene contained in a plasmid labeled as electromagnetic field-plasmid (pEMF). This gene construct contains the specific sequences previously described for the induction of hsp70 expression by MF, as well as the reporter for the luciferase gene. The pEMF vector was transfected into INER-37 and RMA E7 cell lines that were later exposed to either MF or thermal shock (TS). Cells that received the MF or TS treatments and their controls were processed according to the luciferase assay system for evaluate luciferase activity. An increased luciferase gene expression was observed in INER-37 cells exposed to MF and TS compared with controls ($p < 0.05$), but MF exposure had no effect on the RMA E7 cell line.

(NE) Huwiler SG, Beyer C, Fröhlich J, Hennecke H, Egli T, Schürmann D, Rehrauer H, Fischer HM. Genome-wide transcription analysis of Escherichia coli in response to extremely low-frequency magnetic fields. Bioelectromagnetics. 2012 Feb 13. doi: 10.1002/bem.21709. [Epub ahead of print] (GE)

The widespread use of electricity raises the question of whether or not 50 Hz (power line frequency in Europe) magnetic fields (MFs) affect organisms. We investigated the transcription of Escherichia coli K-12 MG1655 in response to extremely low-frequency (ELF) MFs. Fields generated by three signal types (sinusoidal continuous, sinusoidal intermittent, and power line intermittent; all at 50 Hz, 1 mT) were applied and gene expression was monitored at the transcript level using an Affymetrix whole-genome microarray. Bacterial cells were grown continuously in a chemostat (dilution rate D = 0.4 h(-1)) fed with glucose-limited minimal medium and exposed to 50 Hz MFs with a homogenous flux density of 1 mT. For all three types of MFs investigated, neither bacterial growth (determined using optical density) nor culturable counts were affected. Likewise, no statistically significant change (fold-change > 2, P ≤ 0.01) in the expression of 4,358 genes and 714 intergenic regions represented on the gene chip was detected after MF exposure for 2.5 h (1.4 generations) or 15 h (8.7 generations). Moreover, short-term exposure (8 min) to the sinusoidal continuous and power line intermittent signal neither affected bacterial growth nor showed evidence for reliable changes in transcription. In conclusion, our experiments did not indicate that the different tested MFs (50 Hz, 1 mT) affected the transcription of E. coli.

(NE) Jin YB, Kang GY, Lee JS, Choi JI, Lee JW, Hong SC, Myung SH, Lee YS. Effects on micronuclei formation of 60-Hz electromagnetic field exposure with ionizing radiation, hydrogen peroxide, or c-Myc overexpression. Int J Radiat Biol. 88(4):374-380, 2012. (GT, IA)

PURPOSE: Epidemiological studies have demonstrated a possible correlation between exposure to extremely low-frequency magnetic fields (ELF-MF) and cancer. However, this correlation has yet to be definitively confirmed by epidemiological studies. The principal objective of this study was to assess the effects of 60 Hz magnetic fields in a normal cell line system, and particularly in combination with various external factors, via micronucleus (MN) assays. **MATERIALS AND METHODS:** Mouse embryonic fibroblast NIH3T3 cells and human lung fibroblast WI-38 cells were exposed for 4 h to a 60 Hz, 1 mT uniform magnetic field with or without ionizing radiation (IR, 2 Gy), H(2)O(2) (100 µM) and cellular myelocytomatosis oncogene (c-Myc) activation. **RESULTS:** The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic effects were observed when ELF-MF was combined with IR, H(2)O(2), and c-Myc activation. **CONCLUSIONS:** Our results demonstrate that ELF-MF did not enhance MN frequency by IR, H(2)O(2) and c-Myc activation.

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

The principal objective of this study was to assess the DNA damage in a normal cell line system after exposure to 60 Hz of extremely low frequency magnetic field (ELF-MF) and particularly in combination with various external factors, via comet assays. NIH3T3 mouse fibroblast cells, WI-38 human lung fibroblast cells, L132 human lung epithelial cells, and MCF10A human mammary gland epithelial cells were exposed for 4 or 16 h to a 60-Hz, 1 mT uniform magnetic field in the presence or absence of ionizing radiation (IR, 1 Gy), H_2O_2 (50 µM), or c-Myc oncogenic activation. The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic or additive effects were observed after 4 or 16 h of pre-exposure to 1 mT ELF-MF or simultaneous exposure to ELF-MF combined with IR, H_2O_2, or c-Myc activation.

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

The investigation was performed to evaluate the influence of the static magnetic field on oxidative stress in Vicia faba cultivated in soil from high background natural radioactivity in Iran. Soil samples were collected from Ramsar, Iran where the annual radiation absorbed dose from background radiation is substantially higher than 20 mSv/year. The soil samples were then divided into 2 separate groups including high and low natural radioactivity. The plants were continuously exposed to static magnetic field of 15 mT for 8 days, each 8h/day. The results showed that in the plants cultivated in soils with high background natural radioactivity and low background natural radioactivity the activity of antioxidant enzymes as well as flavonoid content were lower than those of the control. Treatment of plants with static magnetic field showed similar results in terms of lowering of antioxidant defense system and increase of peroxidation of membrane lipids. Accumulation of ROS also resulted in chromosomal aberration and DNA damage. This phenomenon was more pronounced when a combination of natural radiation and treatment with static magnetic field was applied. The results suggest that exposure to static magnetic field causes accumulation of reactive oxygen species in V. faba and natural radioactivity of soil exaggerates oxidative stress.

(E) Kim J, Ha CS, Lee HJ, Song K. Repetitive exposure to a 60-Hz time-varying magnetic field induces DNA double-strand breaks and apoptosis in human cells. Biochem Biophys Res Commun. 400(4):739-744, 2010. (GT)

We investigated the effects of extremely low frequency time-varying magnetic fields (MFs) on human normal and cancer cells. Whereas a single exposure to a 60-Hz time-varying MF of 6 mT for 30min showed no effect, repetitive exposure decreased cell viability. This decrease was accompanied by phosphorylation of γ-H2AX, a common DNA double-strand break (DSB) marker, and checkpoint kinase 2 (Chk2), which is critical to the DNA damage checkpoint pathway. In addition, repetitive exposure to a time-varying MF of 6 mT for 30 min every 24 h for 3 days led to p38 activation and induction of apoptosis in cancer and normal cells. Therefore, these results demonstrate that repetitive exposure to MF with extremely low

frequency can induce DNA DSBs and apoptosis through p38 activation. These results also suggest the need for further evaluation of the effects of repetitive exposure to environmental time-varying MFs on human health.

(E) Kim J, Yoon Y, Yun S, Park GS, Lee HJ, Song K. Time-varying magnetic fields of 60 Hz at 7 mT induce DNA double-strand breaks and activate DNA damage checkpoints without apoptosis. Bioelectromagnetics. 33(5):383-393, 2012. (GT, WS)

The potential genotoxic effect of a time-varying magnetic field (MF) on human cells was investigated. Upon continuous exposure of human primary fibroblast and cervical cancer cells to a 60 Hz MF at 7 mT for 10-60 min, no significant change in cell viability was observed. However, deoxyribonucleic acid (DNA) double-strand breaks (DSBs) were detected, and the DNA damage checkpoint pathway was activated in these cells without programmed cell death (called apoptosis). The exposure of human cells to a 60 Hz MF did not induce intracellular reactive oxygen species (ROS) production, suggesting that the observed DNA DSBs are not directly caused by ROS. We also compared the position and time dependency of DNA DSBs with numerical simulation of MFs. The Lorentz force and eddy currents in these experiments were numerically calculated to investigate the influence of each factor on DNA DSBs. The DNA DSBs mainly occurred at the central region, where the MF was strongest, after a 30-min exposure. After 90 min, however, the amount of DNA DSBs increased rapidly in the outer regions, where the eddy current and Lorentz force were strong.

(NE) Kirschenlohr H, Ellis P, Hesketh R, Metcalfe J. Gene Expression Profiles in White Blood Cells of Volunteers Exposed to a 50 Hz Electromagnetic Field. Radiat Res. 178(3): 138-149, 2012. (GE, HU)

Consistent and independently replicated laboratory evidence to support a causative relationship between environmental exposure to extremely low-frequency electromagnetic fields (EMFs) at power line frequencies and the associated increase in risk of childhood leukemia has not been obtained. In particular, although gene expression responses have been reported in a wide variety of cells, none has emerged as robust, widely replicated effects. DNA microarrays facilitate comprehensive searches for changes in gene expression without a requirement to select candidate responsive genes. To determine if gene expression changes occur in white blood cells of volunteers exposed to an ELF-EMF, each of 17 pairs of male volunteers age 20-30 was subjected either to a 50 Hz EMF exposure of 62.0 ± 7.1 μT for 2 h or to a sham exposure (0.21 ± 0.05 μT) at the same time (11:00 a.m. to 13:00 p.m.). The alternative regime for each volunteer was repeated on the following day and the two-day sequence was repeated 6 days later, with the exception that a null exposure (0.085 ± 0.01 μT) replaced the sham exposure. Five blood samples (10 ml) were collected at 2 h intervals from 9:00 to 17:00 with five additional samples during the exposure and sham or null exposure periods on each study day. RNA samples were pooled for the same time on each study day for

the group of 17 volunteers that were subjected to the ELF-EMF exposure/sham or null exposure sequence and were analyzed on Illumina microarrays. Time courses for 16 mammalian genes previously reported to be responsive to ELF-EMF exposure, including immediate early genes, stress response, cell proliferation and apoptotic genes were examined in detail. No genes or gene sets showed consistent response profiles to repeated ELF-EMF exposures. A stress response was detected as a transient increase in plasma cortisol at the onset of either exposure or sham exposure on the first study day. The cortisol response diminished progressively on subsequent exposures or sham exposures, and was attributable to mild stress associated with the experimental protocol.

(E) Koyama S, Sakurai T, Nakahara T, Miyakoshi J. Extremely low frequency (ELF) magnetic fields enhance chemically induced formation of apurinic/apyrimidinic (AP) sites in A172 cells. Int J Radiat Biol. 84(1):53-59, 2008. (GT, IA)

PURPOSE: To detect the effects of extremely low frequency (ELF) magnetic fields, the number of apurinic/apyrimidinic (AP) sites in human glioma A172 cells was measured following exposure to ELF magnetic fields. **MATERIALS AND METHODS:** The cells were exposed to an ELF magnetic field alone, to genotoxic agents (methyl methane sulfonate (MMS) and hydrogen peroxide (H2O2)) alone, or to an ELF magnetic field with the genotoxic agents. After exposure, DNA was extracted, and the number of AP sites was measured. **RESULTS:** There was no difference in the number of AP sites between cells exposed to an ELF magnetic field and sham controls. With MMS or H2O2 alone, the number of AP sites increased with longer treatment times. Exposure to an ELF magnetic field in combination with the genotoxic agents increased AP-site levels compared with the genotoxic agents alone. **CONCLUSIONS:** Our results suggest that the number of AP sites induced by MMS or H2O2 is enhanced by exposure to ELF magnetic fields at 5 millitesla (mT). This may occur because such exposure can enhance the activity or lengthen the lifetime of radical pairs.

(E) Lee JW, Kim MS, Kim YJ, Choi YJ, Lee Y, Chung HW. Genotoxic effects of 3 T magnetic resonance imaging in cultured human lymphocytes. Bioelectromagnetics. 32(7):535-542, 2011. (GT)

The clinical and preclinical use of high-field intensity (HF, 3 T and above) magnetic resonance imaging (MRI) scanners have significantly increased in the past few years. However, potential health risks are implied in the MRI and especially HF MRI environment due to high-static magnetic fields, fast gradient magnetic fields, and strong radiofrequency electromagnetic fields. In this study, the genotoxic potential of 3 T clinical MRI scans in cultured human lymphocytes in vitro was investigated by analyzing chromosome aberrations (CA), micronuclei (MN), and single-cell gel electrophoresis. Human lymphocytes were exposed to electromagnetic fields generated during MRI scanning (clinical routine brain examination protocols: three-channel head coil) for 22, 45, 67, and 89 min. We observed a significant increase in the frequency of single-strand DNA breaks following exposure to a 3 T MRI. In addition, the frequency of both CAs and MN in exposed cells increased in a time-dependent manner. The frequencies of MN in

lymphocytes exposed to complex electromagnetic fields for 0, 22, 45, 67, and 89 min were 9.67, 11.67, 14.67, 18.00, and 20.33 per 1000 cells, respectively. Similarly, the frequencies of CAs in lymphocytes exposed for 0, 45, 67, and 89 min were 1.33, 2.33, 3.67, and 4.67 per 200 cells, respectively. These results suggest that exposure to 3 T MRI induces genotoxic effects in human lymphocytes.

(E) Leone L, Fusco S, Mastrodonato A, Piacentini R, Barbati SA, Zaffina S, Pani G, Podda MV, Grassi C. Epigenetic Modulation of Adult Hippocampal Neurogenesis by Extremely Low-Frequency Electromagnetic Fields. Mol Neurobiol. 2014 Feb 16. [Epub ahead of print] (GE)

Throughout life, adult neurogenesis generates new neurons in the dentate gyrus of hippocampus that have a critical role in memory formation. Strategies able to stimulate this endogenous process have raised considerable interest because of their potential use to treat neurological disorders entailing cognitive impairment. We previously reported that mice exposed to extremely low-frequency electromagnetic fields (ELFEFs) showed increased hippocampal neurogenesis. Here, we demonstrate that the ELFEF-dependent enhancement of hippocampal neurogenesis improves spatial learning and memory. To gain insights on the molecular mechanisms underlying ELFEFs' effects, we extended our studies to an in vitro model of neural stem cells (NSCs) isolated from the hippocampi of newborn mice. We found that ELFEFs enhanced proliferation and neuronal differentiation of hippocampal NSCs by regulation of epigenetic mechanisms leading to pro-neuronal gene expression. Upon ELFEF stimulation of NSCs, we observed a significant enhancement of expression of the pro-proliferative gene hairy enhancer of split 1 and the neuronal determination genes NeuroD1 and Neurogenin1. These events were preceded by increased acetylation of H3K9 and binding of the phosphorylated transcription factor cAMP response element-binding protein (CREB) on the regulatory sequence of these genes. Such ELFEF-dependent epigenetic modifications were prevented by the Ca_v1-channel blocker nifedipine, and were associated with increased occupancy of CREB-binding protein (CBP) to the same loci within the analyzed promoters. Our results unravel the molecular mechanisms underlying the ELFEFs' ability to improve endogenous neurogenesis, pointing to histone acetylation-related chromatin remodeling as a critical determinant. These findings could pave the way to the development of novel therapeutic approaches in regenerative medicine.

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

Extremely low frequency electromagnetic field (ELF-EMF) exposure is attracting increased attention as a possible disease-inducing factor. The in vivo effects of short-term and long-term ELF-EMF exposure on male Drosophila melanogaster were studied using transcriptomic analysis for preliminary screening and QRT-PCR for further verification. Transcriptomic analysis indicated that 439 genes were up-regulated and 874 genes were down-regulated following short-term exposures and that 514 genes were up-regulated and 1206 genes were down-

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

(E) Lupke M, Frahm J, Lantow M, Maercker C, Remondini D, Bersani F, Simkó M. Gene expression analysis of ELF-MF exposed human monocytes indicating the involvement of the alternative activation pathway. Biochim Biophys Acta. 1763(4):402-12, 2006. (GE)

This study focused on the cell activating capacity of extremely low frequency magnetic fields (ELF-MF) on human umbilical cord blood-derived monocytes. Our results confirm the previous findings of cell activating capacity of ELF-MF (1.0 mT) in human monocytes, which was detected as an increased ROS release. Furthermore, gene expression profiling (whole-genome cDNA array Human Unigene RZPD-2) was performed to achieve a comprehensive view of involved genes during the cell activation process after 45 min ELF-MF exposure. Our results indicate the alteration of 986 genes involved in metabolism, cellular physiological processes, signal transduction and immune response. Significant regulations could be analyzed for 5 genes (expression >2- or <0.5-fold): IL15RA (Interleukin 15 receptor, alpha chain), EPS15R (Epidermal growth factor receptor pathway substrate 15 - like 1), DNMT3A (Hypothetical protein MGC16121), DNMT3A (DNA (cytosine-5) methyltransferase 3 alpha), and one gene with no match to known genes, DKFZP586J1624. Real-time RT-PCR analysis of the kinetic of the expression of IL15RA, and IL10RA during 45 min ELF-MF exposure indicates the regulation of cell activation via the alternative pathway, whereas the delayed gene expression of FOS, IL2RA

and the melatonin synthesizing enzyme HIOMT suggests the suppression of inflammatory processes. Accordingly, we suggest that ELF-MF activates human monocytes via the alternative pathway.

(E) Luukkonen J, Liimatainen A, Höytö A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced genotoxic effects in human SH-SY5Y neuroblastoma cells. PLoS One. 2011 Mar 23;6(3):e18021. (GT, IA)

BACKGROUND: Extremely low frequency (ELF) magnetic fields (MF) are generated by power lines and various electric appliances. They have been classified as possibly carcinogenic by the International Agency for Research on Cancer, but a mechanistic explanation for carcinogenic effects is lacking. A previous study in our laboratory showed that pre-exposure to ELF MF altered cancer-relevant cellular responses (cell cycle arrest, apoptosis) to menadione-induced DNA damage, but it did not include endpoints measuring actual genetic damage. In the present study, we examined whether pre-exposure to ELF MF affects chemically induced DNA damage level, DNA repair rate, or micronucleus frequency in human SH-SY5Y neuroblastoma cells. **METHODOLOGY/PRINCIPAL FINDINGS:** Exposure to 50 Hz MF was conducted at 100 µT for 24 hours, followed by chemical exposure for 3 hours. The chemicals used for inducing DNA damage and subsequent micronucleus formation were menadione and methyl methanesulphonate (MMS). Pre-treatment with MF enhanced menadione-induced DNA damage, DNA repair rate, and micronucleus formation in human SH-SY5Y neuroblastoma cells. Although the results with MMS indicated similar effects, the differences were not statistically significant. No effects were observed after MF exposure alone. **CONCLUSIONS:** The results confirm our previous findings showing that pre-exposure to MFs as low as 100 µT alters cellular responses to menadione, and show that increased genotoxicity results from such interaction. The present findings also indicate that complementary data at several chronological points may be critical for understanding the MF effects on DNA damage, repair, and post-repair integrity of the genome.

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

Epidemiological studies have suggested that exposure to 50Hz magnetic fields (MF) increases the risk of childhood leukemia, but there is no mechanistic explanation for carcinogenic effects. In two previous studies we have observed that a 24-h pre-exposure to MF alters cellular responses to menadione-induced DNA damage. The aim of this study was to investigate the cellular changes that must occur already during the first 24h of exposure to MF, and to explore whether the MF-induced changes in DNA damage response can lead to genomic instability in the progeny of the exposed cells. In order to answer these questions, human SH-SY5Y neuroblastoma cells were exposed to a 50-Hz, 100-µT MF for 24h, followed by 3-h exposure to menadione. The main finding was that MF exposure was associated with increased level of

micronuclei, used as an indicator of induced genomic instability, at 8 and 15d after the exposures. Other delayed effects in MF-exposed cells included increased mitochondrial activity at 8d, and increased reactive oxygen species (ROS) production and lipid peroxidation at 15d after the exposures. Oxidative processes (ROS production, reduced glutathione level, and mitochondrial superoxide level) were affected by MF immediately after the exposure. In conclusion, the present results suggest that MF exposure disturbs oxidative balance immediately after the exposure, which might explain our previous findings on MF altered cellular responses to menadione-induced DNA damage. Persistently elevated levels of micronuclei were found in the progeny of MF-exposed cells, indicating induction of genomic instability.

(E) Ma Q, Deng P, Zhu G, Liu C, Zhang L, Zhou Z, Luo X, Li M, Zhong M, Yu Z, Chen C, Zhang Y. Extremely low-frequency electromagnetic fields affect transcript levels of neuronal differentiation-related genes in embryonic neural stem cells. PLoS One. 2014 Mar 3;9(3):e90041. doi: 10.1371/journal.pone.0090041. eCollection 2014. (GE)

Previous studies have reported that extremely low-frequency electromagnetic fields (ELF-EMF) can affect the processes of brain development, but the underlying mechanism is largely unknown. The proliferation and differentiation of embryonic neural stem cells (eNSCs) is essential for brain development during the gestation period. To date, there is no report about the effects of ELF-EMF on eNSCs. In this paper, we studied the effects of ELF-EMF on the proliferation and differentiation of eNSCs. Primary cultured eNSCs were treated with 50 Hz ELF-EMF; various magnetic intensities and exposure times were applied. Our data showed that there was no significant change in cell proliferation, which was evaluated by cell viability (CCK-8 assay), DNA synthesis (Edu incorporation), average diameter of neurospheres, cell cycle distribution (flow cytometry) and transcript levels of cell cycle related genes (P53, P21 and GADD45 detected by real-time PCR). When eNSCs were induced to differentiation, real-time PCR results showed a down-regulation of Sox2 and up-regulation of Math1, Math3, Ngn1 and Tuj1 mRNA levels after 50 Hz ELF-EMF exposure (2 mT for 3 days), but the percentages of neurons (Tuj1 positive cells) and astrocytes (GFAP positive cells) were not altered when detected by immunofluorescence assay. Although cell proliferation and the percentages of neurons and astrocytes differentiated from eNSCs were not affected by 50 Hz ELF-EMF, the expression of genes regulating neuronal differentiation was altered. In conclusion, our results support that 50 Hz ELF-EMF induce molecular changes during eNSCs differentiation, which might be compensated by post-transcriptional mechanisms to support cellular homeostasis.

(E) Mairs RJ, Hughes K, Fitzsimmons S, Prise KM, Livingstone A, Wilson L, Baig N, Clark AM, Timpson A, Patel G, Folkard M, Angerson WJ, Boyd M. Microsatellite analysis for determination of the mutagenicity of extremely low-frequency electromagnetic fields and ionising radiation in vitro. Mutat Res. 626(1-2):34-41, 2007. (GT, IA)

Extremely low-frequency electromagnetic fields (ELF-EMF) have been reported to induce lesions in DNA and to enhance the mutagenicity of ionising radiation. However, the significance

of these findings is uncertain because the determination of the carcinogenic potential of EMFs has largely been based on investigations of large chromosomal aberrations. Using a more sensitive method of detecting DNA damage involving microsatellite sequences, we observed that exposure of UVW human glioma cells to ELF-EMF alone at a field strength of 1 mT (50 Hz) for 12 h gave rise to 0.011 mutations/locus/cell. This was equivalent to a 3.75-fold increase in mutation induction compared with unexposed controls. Furthermore, ELF-EMF increased the mutagenic capacity of 0.3 and 3 Gy gamma-irradiation by factors of 2.6 and 2.75, respectively. These results suggest not only that ELF-EMF is mutagenic as a single agent but also that it can potentiate the mutagenicity of ionising radiation. Treatment with 0.3 Gy induced more than 10 times more mutations per unit dose than irradiation with 3 Gy, indicating hypermutability at low dose.

(E) Mariucci G, Villarini M, Moretti M, Taha E, Conte C, Minelli A, Aristei C, Ambrosini MV. Brain DNA damage and 70-kDa heat shock protein expression in CD1 mice exposed to extremely low frequency magnetic fields. Int J Radiat Biol. 86(8):701-710, 2010. (GT, LE)

PURPOSE: The question of whether exposure to extremely low frequency magnetic fields (ELF-MF), may contribute to cerebral cancer and neurodegeneration is of current interest. In this study we investigated whether exposure to ELF-MF (50 Hz-1 mT) harms cerebral DNA and induces expression of 70-kDa heat shock protein (hsp70). **MATERIALS AND METHODS:** CD1 mice were exposed to a MF (50 Hz-1 mT) for 1 or 7 days (15 h/day) and sacrificed either at the end of exposure or after 24 h. Unexposed and sham-exposed mice were used as controls. Mouse brains were dissected into cerebral cortex-striatum, hippocampus and cerebellum to evaluate primary DNA damage and hsp70 gene expression. Food intake, weight gain, and motor activity were also evaluated. **RESULTS:** An increase in primary DNA damage was detected in all cerebral areas of the exposed mice sacrificed at the end of exposure, as compared to controls. DNA damage, as can be evaluated by the comet assay, appeared to be repaired in mice sacrificed 24 h after a 7-day exposure. Neither a short (15 h) nor long (7 days) MF-exposure induced hsp70 expression, metabolic and behavioural changes. **CONCLUSIONS:** These results indicate that in vivo ELF-MF induce reversible brain DNA damage while they do not elicit the stress response.

(E) Markkanen A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced DNA damage response in murine L929 cells. Int J Radiat Biol. 84(9):742-751, 2008. (IA)

PURPOSE: Effects on DNA damage response were investigated in murine L929 cells exposed to 50 Hz magnetic fields (MF) with or without ultraviolet B (UVB, wavelength 280-320 nm) radiation or menadione (MQ). **MATERIALS AND METHODS:** Cells were exposed to MF at 100 or 300 microT combined with MQ (150 microM, 1 hour) or UVB radiation (160 J/m(2)) using various exposure schedules. The samples were stained with propidium iodide (PI) and analysed by flow cytometer for cell cycle stages. Apoptotic cells were defined as sub G(1) events. **RESULTS:** In cells first exposed to 100 microT MF for 24 h, the response to subsequent MQ

treatment was significantly altered so that the proportion of sub G(1) cells was decreased and the proportion of cells in the G(2)/M phase was increased. When a 300 microT MF was used, also the proportion of cells in the G(1) phase was decreased. MF exposures after MQ treatment did not alter responses to MQ. No effects were found from MF exposure alone or from MF combined with UVB radiation. **CONCLUSIONS:** The results strengthen previous findings suggesting that pre-exposure to MF can alter cellular responses to other agents, and indicate that MF as low as 100 microT has measurable impacts on cancer-relevant cellular processes such as DNA-damage.

(NE) Mizuno K, Narita E, Yamada M, Shinohara N, Miyakoshi J. ELF magnetic fields do not affect cell survival and DNA damage induced by ultraviolet B. Bioelectromagnetics. 35(2):108-115, 2014. (GT, IA)

We investigated whether extremely low frequency (ELF) magnetic field exposure has modification effects on cell survival after ultraviolet B (UV-B) irradiation and on repair process of DNA damage induced by UV-B irradiation in WI38VA13 subcloned 2RA and XP2OS(SV) cells. The ELF magnetic field exposure was conducted using a Helmholtz coil-based system that was designed to generate a sinusoidal magnetic field at 5 mT and 60 Hz. Cell survival was assessed by WST assay after UV-B irradiation at 20-80 J/m(2) , ELF magnetic field exposure for 24 h, followed by incubation for 48 h. DNA damage was assessed by quantification of cyclobutane pyrimidine dimer formation and 6-4 photoproduct formation using ELISA after UV-B irradiation at 20-80 J/m(2) followed by ELF magnetic field exposure for 24 h. No significant changes were observed in cell survival between ELF magnetic field and sham exposures. Similarly, DNA damage induced by UV-B irradiation did not change significantly following ELF magnetic field exposure. Our results suggest that ELF magnetic field exposure at 5 mT does not have modification effect on cell survival after UV-B irradiation and on repair process of DNA damage induced by UV-B irradiation.

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

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

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

(NE) Okudan N, Celik I, Salbacak A, Cicekcibasi AE, Buyukmumcu M, Gökbel H. Effects of long-term 50 Hz magnetic field exposure on the micro nucleated polychromatic erythrocyte and blood lymphocyte frequency and argyrophilic nucleolar organizer regions in lymphocytes of mice. Neuro Endocrinol Lett. 31(2):208-214, 2010. (GT)

OBJECTIVES: We aimed to investigate the effects of weak extremely low frequency electromagnetic fields (ELF-EMFs) on the nucleus size, the silver staining nucleolar organizer regions (AgNORs), the frequency of micro nucleated peripheral blood lymphocytes (MPBLs) and the micro nucleated polychromatic erythrocytes (MPCEs).**METHODS:** One hundred and twenty Swiss albino mice were equally divided into 6 groups. The study groups were exposed to 1, 2, 3, 4 and 5 microT 50 Hz-EMFs for 40 days. Micronucleus number (MN) per PBL was determined. **RESULTS:** ELF-EMF exposure caused a nonlinear decline of nucleus area. A sharp drop occurred in AgNOR area of 1 microT group, and following it gained an insignificantly higher level than that of the control group. The field did not change mean AgNOR numbers per nucleus of the groups. Relative AgNOR area had the highest level in 1 microT-exposure group, and the level was quite similar to that of the 5 microT-exposure group. The remaining groups had significantly lower values quite similar to that of the control level. The field exposure at any intensity did not affect significantly the frequency of either MPBLs or MPCEs. The number of MN per PBL in the 4 and 5 microT-exposure groups were significantly higher than those of the lower intensity exposure groups. The males in 4 microT-exposure group displayed the highest MN number per PBL, whereas values changed in a nonlinear manner. **CONCLUSIONS:** The results of the present study suggest that </=5 microT intensities of 50 Hz EMFs did not cause genotoxic effect on the mouse.

(E) Panagopoulos DJ, Karabarbounis A, Lioliousis C. ELF alternating magnetic field decreases reproduction by DNA damage induction. Cell Biochem Biophys. 67(2):703-16, 2013. (LE, GT, RP)

In the present experiments, the effect of 50-Hz alternating magnetic field on Drosophila melanogaster reproduction was studied. Newly eclosed insects were separated into identical groups of ten males and ten females and exposed to three different intensities of the ELF magnetic field (1, 11, and 21 G) continuously during the first 5 days of their adult lives. The

reproductive capacity was assessed by the number of F1 pupae according to a well-defined protocol of ours. The magnetic field was found to decrease reproduction by up to 4.3%. The effect increased with increasing field intensities. The decline in reproductive capacity was found to be due to severe DNA damage (DNA fragmentation) and consequent cell death induction in the reproductive cells as determined by the TUNEL assay applied during early and mid-oogenesis (from germarium to stage 10) where physiological apoptosis does not occur. The increase in DNA damage was more significant than the corresponding decrease in reproductive capacity (up to ~7.5%). The TUNEL-positive signal denoting DNA fragmentation was observed exclusively at the two most sensitive developmental stages of oogenesis: the early and mid-oogenesis checkpoints (i.e. region 2a/2b of the germarium and stages 7-8 just before the onset of vitellogenesis)-in contrast to exposure to microwave radiation of earlier work of ours in which the DNA fragmentation was induced at all developmental stages of early and mid-oogenesis. Moreover, the TUNEL-positive signal was observed in all three types of egg chamber cells, mainly in the nurse and follicle cells and also in the oocyte, in agreement with the microwave exposure of our earlier works. According to previous reports, cell death induction in the oocyte was observed only in the case of microwave exposure and not after exposure to other stress factors as toxic chemicals or food deprivation. Now it is also observed for the first time after ELF magnetic field exposure. Finally, in contrast to microwave exposure of previous experiments of ours in which the germarium checkpoint was found to be more sensitive than stage 7-8, in the magnetic field exposure of the present experiments the mid-oogenesis checkpoint was found to be more sensitive than the germarium.

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

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

(E) Reyes-Guerrero G, Guzmán C, García DE, Camacho-Arroyo I, Vázquez-García M. Extremely low-frequency electromagnetic fields differentially regulate estrogen receptor-alpha and -beta expression in the rat olfactory bulb. Neurosci Lett. 471(2):109-13, 2010. (GE)

Recently, the effects of extremely low-frequency electromagnetic fields (ELF EMF) on biological systems have been extensively investigated. In this report, the influence of ELF EMF on olfactory bulb (OB) estrogen receptor-alpha (ER alpha) mRNA and -beta (ER beta) mRNA expression was studied by RT-PCR in adult female and male rats. Results reveal for the first time that ELF EMF exerted a biphasic effect on female OB ER beta mRNA gene expression, which increased during diestrous and decreased during estrous. We did not observe any influence of ELF EMF on female OB ER alpha mRNA expression. Our data demonstrate a fluctuating pattern of ER-alpha and -beta mRNA expression in the female OB throughout the phases of the estrous cycle in non-ELF EMF-exposed animals. Thus the highest ER alpha expression was observed in diestrous and the lowest in proestrous. The pattern of ER beta mRNA was less variable, the lowest expression was observed in diestrous. ER-alpha mRNA and -beta mRNA expression level in the male OB did not exhibit any variation either in ELF EMF-exposed or non-ELF EMF-exposed animals. In summary, ELF EMF modulate ER beta gene expression in the OB of female adult rats but not in males.

(E) Ruiz-Gómez MJ, Sendra-Portero F, Martínez-Morillo M. Effect of 2.45 mT sinusoidal 50 Hz magnetic field on Saccharomyces cerevisiae strains deficient in DNA strand breaks repair. Int J Radiat Biol. 86(7):602-611, 2010. (GT)

PURPOSE: To investigate whether extremely-low frequency magnetic field (MF) exposure produce alterations in the growth, cell cycle, survival and DNA damage of wild type (wt) and mutant yeast strains. **MATERIALS AND METHODS:** wt and high affinity DNA binding factor 1 (hdf1), radiation sensitive 52 (rad52), rad52 hdf1 mutant Saccharomyces cerevisiae strains were exposed to 2.45 mT, sinusoidal 50 Hz MF for 96 h. MF was generated by a pair of Helmholtz coils. During this time the growth was monitored by measuring the optical density at 600 nm and cell cycle evolution were analysed by microscopic morphological analysis. Then, yeast survival was assayed by the drop test and DNA was extracted and electrophoresed. **RESULTS:** A significant increase in the growth was observed for rad52 strain (P = 0.005, Analysis of Variance [ANOVA]) and close to significance for rad52 hdf1 strain (P = 0.069, ANOVA). In addition, the surviving fraction values obtained for MF-exposed samples were in all cases less than for the controls, being the P value obtained for the whole set of MF-treated strains close to significance (P = 0.066, Student's t-test). In contrast, the cell cycle evolution and the DNA pattern obtained for wt and the mutant strains were not altered after exposure to MF. **CONCLUSIONS:** The data presented in the current report show that the applied MF (2.45 mT, sinusoidal 50 Hz, 96 h) induces alterations in the growth and survival of S. cerevisiae strains deficient in DNA strand breaks repair. In contrast, the MF treatment does not induce alterations in the cell cycle and does not cause DNA damage.

(E) Sarimov R, Alipov ED, Belyaev IY. Fifty hertz magnetic fields individually affect chromatin conformation in human lymphocytes: dependence on amplitude, temperature, and initial chromatin state. Bioelectromagnetics. 32(7):570-579, 2011. (GT)

Effects of magnetic field (MF) at 50 Hz on chromatin conformation were studied by the method of anomalous viscosity time dependence (AVTD) in human lymphocytes from two healthy donors. MF within the peak amplitude range of 5-20 μT affected chromatin conformation. These MF effects differed significantly between studied donors, and depended on magnetic flux density and initial condensation of chromatin. While the initial state of chromatin was rather stable in one donor during one calendar year of measurements, the initial condensation varied significantly in cells from another donor. Both this variation and the MF effect depended on temperature during exposure. Despite these variations, the general rule was that MF condensed the relaxed chromatin and relaxed the condensed chromatin. Thus, in this study we show that individual effects of 50 Hz MF exposure at peak amplitudes within the range of 5-20 μT may be observed in human lymphocytes in dependence on the initial state of chromatin and temperature.

(E) Tiwari R, Lakshmi NK, Bhargava SC, Ahuja YR. Epinephrine, DNA integrity and oxidative stress in workers exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) at 132 kV substations. Electromagn Biol Med. 2014 Jan 24. [Epub ahead of print] (LE, GT, HU, OX)

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

(E) Udroiu I, Cristaldi M, Ieradi LA, Bedini A, Giuliani L, Tanzarella C. Clastogenicity and aneuploidy in newborn and adult mice exposed to 50 Hz magnetic fields. Int J Radiat Biol. 82(8):561-567, 2006. (GT, DE, LE)

PURPOSE: To detect possible clastogenic and aneugenic properties of a 50 Hz, 650 muT magnetic field. **MATERIALS AND METHODS:** The micronucleus test with CREST (Calcinosis,

Raynaud's phenomenon, Esophageal dismotility, Sclerodactility, Telangectasia) antibody staining was performed on liver and peripheral blood sampled from newborn mice exposed to an ELF (Extremely Low Frequency) magnetic field during the whole intra-uterine life (21 days), and on bone marrow and peripheral blood sampled from adult mice exposed to the same magnetic field for the same period. **RESULTS:** Data obtained in newborn mice show a significant increase in micronuclei frequencies. In absolute terms, most of the induced micronuclei were CREST-negative (i.e., formed by a chromosome fragment). However, in relative terms, ELF exposure caused a two-fold increase in CREST-negative micronuclei and a four-fold increase in CREST-positive micronuclei (i.e., formed by a whole chromosome). No significant effect was recorded on exposed adults. **CONCLUSIONS:** These findings suggest the need for investigation of aneugenic properties of ELF magnetic fields in order to establish a possible relationship to carcinogenesis.

(NE) Verschaeve L, Anthonissen R, Grudniewska M, Wudarski J, Gevaert L, Maes A. Genotoxicity investigation of ELF-magnetic fields in Salmonella typhimurium with the sensitive SOS-based VITOTOX test. Bioelectromagnetics. 32(7):580-584, 2011. (GT, IA)

We performed a genotoxicity investigation of extremely low-frequency (ELF) magnetic fields (MFs, 50 Hz, 100 and 500 µT, 1 and 2 h exposure) alone and in combination with known chemical mutagens using the VITOTOX test. This test is a very sensitive reporter assay of Salmonella typhimurium bacteria based on the SOS response. Our study showed that ELF-MFs do not induce SOS-based mutagenicity in S. typhimurium bacteria and do not show any synergetic effect when combined with chemical mutagens.

(E) Villarini M, Ambrosini MV, Moretti M, Dominici L, Taha E, Piobbico D, Gambelunghe C, Mariucci G. Brain hsp70 expression and DNA damage in mice exposed to extremely low frequency magnetic fields: a dose-response study. Int J Radiat Biol. 89(7):562-570, 2013. (LE, GT)

Purpose: To determine whether a dose-response relationship exists among exposure to extremely low frequency magnetic fields (ELF-MF) at different densities and 70-kDa heat shock protein (hsp70) expression and DNA damage in mouse brain. Materials and Methods: Male CD1 mice were exposed to ELF-MF (50 Hz; 0.1, 0.2, 1 or 2 mT) for 7 days (15 hours/day) and sacrificed either at the end of exposure or after 24 h. Hsp70 expression was determined in cerebral cortex-striatum, hippocampus and cerebellum by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and western blot analysis. Primary DNA damage was evaluated in the same tissues by comet assay. Sham-exposed mice were used as controls. Results: No changes in both hsp70 mRNA and corresponding protein occurred following exposure to ELF-MF, except for a weak increase in the mRNA in hippocampus of exposed mice to 0.1 mT ELF-MF. Only mice exposed to 1 or 2 mT and sacrificed immediately after exposure presented DNA strand breaks higher than controls in all the cerebral areas; such DNA breakage reverted to baseline in the mice sacrificed 24 h after exposure. Conclusions: These data show

that high density ELF-MF only induce reversible brain DNA damage while they do not affect hsp70 expression.

(E) Wahab MA, Podd JV, Rapley BI, Rowland RE. Elevated sister chromatid exchange frequencies in dividing human peripheral blood lymphocytes exposed to 50 Hz magnetic fields. Bioelectromagnetics. 28(4):281-288, 2007. (GT, WS)

The in vitro cytomolecular technique, sister chromatid exchange (SCE), was applied to test the clastogenic potentiality of extremely low frequency (ELF) electromagnetic fields (EMFs) on human peripheral blood lymphocytes (HPBLs). SCE frequencies were scored in dividing peripheral blood lymphocytes (PBLs) from six healthy male blood donors in two rounds of experiments, R1 and R2, to determine reproducibility. Lymphocyte cultures in the eight experiments conducted in each round were exposed to 50 Hz sinusoidal (continuous or pulsed) or square (continuous or pulsed) MFs at field strengths of 1 microT or 1 mT for 72 h. A significant increase in the number of SCEs/cell in the grouped experimental conditions compared to the controls was observed in both rounds. The highest SCE frequency in R1 was 10.03 for a square continuous field, and 10.39 for a square continuous field was the second highest frequency in R2. DNA crosslinking at the replication fork is proposed as a model which could explain the mechanistic link between ELF EMF exposure and increased SCE frequency.

(E) Wang Z, Sarje A, Che PL, Yarema KJ. Moderate strength (0.23-0.28 T) static magnetic fields (SMF) modulate signaling and differentiation in human embryonic cells. BMC Genomics. 10:356, 2009. (GE)

BACKGROUND: Compelling evidence exists that magnetic fields modulate living systems. To date, however rigorous studies have focused on identifying the molecular-level biosensor (e.g., radical ion pairs or membranes) or on the behavior of whole animals leaving a gap in understanding how molecular effects are translated into tissue-wide and organism-level responses. This study begins to bridge this gulf by investigating static magnetic fields (SMF) through global mRNA profiling in human embryonic cells coupled with software analysis to identify the affected signaling pathways. **RESULTS:** Software analysis of gene expression in cells exposed to 0.23-0.28 T SMF showed that nine signaling networks responded to SMF; of these, detailed biochemical validation was performed for the network linked to the inflammatory cytokine IL-6. We found the short-term (<24 h) activation of IL-6 involved the coordinate up-regulation of toll-like receptor-4 (TLR4) with complementary changes to NEU3 and ST3GAL5 that reduced ganglioside GM3 in a manner that augmented the activation of TLR4 and IL-6. Loss of GM3 also provided a plausible mechanism for the attenuation of cellular responses to SMF that occurred over longer exposure periods. Finally, SMF-mediated responses were manifest at the cellular level as morphological changes and biochemical markers indicative of pre-oligodendrocyte differentiation. **CONCLUSION:** This study provides a framework describing how magnetic exposure is transduced from a plausible molecular biosensor (lipid membranes) to cell-level responses that include differentiation toward neural lineages. In addition, SMF provided a stimulus that uncovered new relationships - that exist even in the absence of magnetic

fields - between gangliosides, the time-dependent regulation of IL-6 signaling by these glycosphingolipids, and the fate of embryonic cells.

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

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

(E) Yokus B, Akdag MZ, Dasdag S, Cakir DU, Kizil M. Extremely low frequency magnetic fields cause oxidative DNA damage in rats. Int J Radiat Biol. 84(10):789-795, 2008. (GT)

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

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

Purpose: Genotoxic effects have been considered the gold standard to determine if an environmental factor is a carcinogen, but the currently available data for extremely low frequency time-varying magnetic fields (ELF-MFs) remain controversial. As an environmental stimulus, the effect of ELF-MF on cellular DNA may be subtle. Therefore, a more sensitive method and systematic research strategy are warranted to evaluate genotoxicity. Materials and methods: We investigated the effect of ELF-MFs in combination with ionizing radiation (IR) or H_2O_2 on the DNA damage response of expression of phosphorylated H2AX (γ-H2AX) and production of γ-H2AX foci in non-tumorigenic human cell systems consisting of human lung fibroblast WI38 cells and human lung epithelial L132 cells. Results: Exposure to a 60-Hz, 2 mT ELF-MFs for 6 h produced increased γ-H2AX expression, as well as γ-H2AX foci production, a common DNA double-strand break (DSB) marker. However, exposure to a 1 mT ELF-MFs did not have the same effect. Moreover, 2 mT ELF-MFs exposure potentiated the expression of γ-H2AX and γ-H2AX foci production when combined with IR, but not when combined with H_2O_2. Conclusions: ELF-MFs could affect the DNA damage response and, in combination with different stimuli, provide different effects on γ-H2AX.

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BioInitiative Working Group; An Update on Physical and Biological Variables, Cancer and Safety Standards by Prof. Igor Belyaev Dr. Sc., (Exhibit F); 2014

Exhibit F: An Update on Physical and Biological Variables, Cancer and Safety Standards by Igor Belyaev, Dr.Sc., Cancer Research Institute Slovak Academy of Sciences, Slovak Republic

This review is divided into comments on two separate sections, one for extremely-low frequency (ELF) and the other for radiofrequency (RFR) studies. Comments are presented to address deficiencies in the Preliminary Opinion on EMF issued by the SCENIHR Committee. The comments are relevant to sections of the BioInitiative Working Group letter including brain tumors, oxidative damage, genomic instability, mitochrondrial damage, carcinogenic classifications, biological plausibility and methodological deficiencies.

Comments on ELF Sections

ELF Carcinogenicity

Page 131 of the SCENIHR provides misleading and flawed conclusions on ELF and neoplastic diseases. As a matter of fact, the increased risk of childhood leukemia with daily average exposure above 0.3 to 0.4 μT is as strong as never before. All available studies from Europe, America and Asia consistently show such correlation. It has been further supported by recent meta-analysis by Zhao et al. (Zhao, Liu et al. 2014). The statement of lack of mechanisms for ELF effects is wrong. Recent studies provided more evidence for such mechanisms even if they have not been comprehensively studied, see below. Considerations of ELF carcinogenicity in the SCENIHR report did not use standard methods such as the Bradford Hill criteria which do not require complete knowledge of mechanisms in case when epidemiological evidence is overwhelming as in case of childhood leukemia (Zhao, Liu et al. 2014).

Similar to effects of MW, the ELF effects depend on variety of parameters that should be taken into account and have not been considered by the SCENIHR report when comparing data from different studies.

Baldi et al analyzed the relationship between residential and occupational exposure to electromagnetic field and brain tumors in adults (Baldi, Coureau et al. 2010). A case-control study was carried out in southwestern France between May 1999 and April 2001. A total of 221 central nervous system tumors (105 gliomas, 67 meningiomas, 33 neurinomas and 16 others) and 442 individually age- and sex-matched controls selected from general population were included. Electromagnetic field exposure to ELF and radiofrequency separately was assessed in occupational settings through expert judgment based on complete job calendar, and at home by assessing the distance to power lines. Confounders such as education, use of home pesticide, residency in a rural area and occupational exposure to chemicals were taken into account. Separate analyses were performed for gliomas, meningiomas and acoustic neurinomas. A nonsignificant increase in risk was found for occupational exposure to electromagnetic fields [odds ratio (OR = 1.52, 0.92-2.51)]. This increase became significant for meningiomas, especially when considering ELF separately [OR = 3.02; 95 percent confidence interval (95% CI) =1.10-8.25]. The risk of meningioma was also higher in subjects living in the vicinity of power lines (<100 m), even if not significant (OR = 2.99, 95% CI 0.86-10.40). These data suggest that occupational or residential exposure to ELF may play a role in the occurrence of meningioma. The insignificance of data obtained in group RF+ELF is well explained by majority of RF data showing no significant relationship of RF exposure with increased risks of meningioma (Carlberg, Soderqvist et al. 2013).

Exhibit F: An Update on Physical and Biological Variables, Cancer and Safety Standards by Igor Belyaev, Dr.Sc., Cancer Research Institute Slovak Academy of Sciences, Slovak Republic

Comments on RFR Sections

Main conclusions on health effects from RFR fields

1. The positive and negative studies were selected by unclear criteria, which (i) are different from those generally accepted and used by IARC and (ii) resulted in omission of majority of positive findings and almost all laboratory studies which were performed using conditions of EMF exposure similar as general public is exposed (see text below and reference list).
2. The report shows fundamental flaw in assessment of mechanisms for non-thermal EMF effects.
3. Analysis of data seem to be biased in favor of negative studies and negative interpretations.

Flawed assessment of negative studies

The main fundamental flaw of the report is neglecting the mechanistic data on non-thermal (NT) effects of microwaves (MW). As reported in multiple studies, these effects depend on variety of biological and physical parameters including polarization, frequency, modulation and environmental EMF (see (Belyaev 2010) and (IARC 2013). The *in vitro* and *in vivo* studies included in the preliminary Opinion are largely negative studies only. Moreover, negative studies cannot be directly compared to positive studies if the exposure was performed under different conditions as it almost always done. Thus, obtained so far data of negative studies cannot be extrapolated to all real cell phone signals. The negative studies cannot neither dismiss positive studies, which were performed under other conditions, nor provide evidence for safety of majority of signals used for mobile communication. The reported" inconsistency" of *in vitro* and *in vivo* data (see for example page 120) and "conflicting results" (see for example page 121) has at least one simple explanation because the studies were performed under different conditions. Thus, results of most studies cannot be directly compared and conclusion by the SCENIHR report on inconsistency. Conflicting results instead reflect the level of superficial analysis. Another fundamental flaw deals with neglecting many studies showing dependence of the NT MW effects on exposure duration or dose (defined in radiation physics as multiplication of SAR on exposure duration), see (Belyaev 2010). In addition to laboratory studies, when brain caner risk was epidemiologically examined as a function of dose received in different time windows before diagnosis, increasing trend was observed with increasing RFR dose, for exposures 7 years or more in the past (Cardis, Armstrong et al. 2011). This study provided straightforward evidence for one of most important Bradford Hill criteria - dependence on dose.

Another important parameter is intermittence of exposure which involves interaction with adaptation mechanisms and accumulative effects of NT MW. Chavdoula et al. used a 6 min daily exposure of dipteran flies, Drosophila melanogaster, to GSM-900 MHz mobile phone electromagnetic radiation (EMR), to compare the effects between continuous and four different intermittent exposures of 6 min total duration on the insect's reproductive capacity as well as on the induction of apoptosis (Chavdoula, Panagopoulos et al. 2010). It was found that intermittent exposure, similar to continuous exposure, decreases the reproductive capacity and alters the actin-cytoskeleton network of the egg chambers, another known aspect of cell death, and that this effect is due to DNA fragmentation. Intermittent exposures with l0-min intervals between exposure sessions proved to be nearly equally effective as continuous exposure of the same total duration, however, longer intervals between the exposures seemed

to allow the organism the time required to recover and partly overcome the described effects of the GSM exposure.

The preliminary Opinion bases it's conclusions mostly on SAR value, which is a main parameter for thermal MW effects but has much less value for NT MW to which general public is exposed to (Belyaev 2010; Panagopoulos, Johansson et al. 2013).

RFR epidemiologic evidence for carcinogenicity

The SCENIHR preliminary Opinion has conclusions on brain cancer that are heavily based on the Danish subscriber cohort study of mobile phone subscribers. However this study has not assessed exposure, has been heavily criticized an thus far is inconclusive. This study is not informative even according to the requirement of this SCENIHR reports : " *The minimum requirement for exposure assessment for an epidemiological study to be informative is to include reasonably accurate individual exposure characterization over a relevant period of time capturing all major sources of exposure for the pertinent part of the body"* (page 10). The preliminary Opinion is internally inconsistent with this requirement as the authors have based their review largely on epidemiological studies, where individual exposure was not accurately accessed. These studies include those coauthored by Dr Schüz who is one of the authors for this SCENIHR report. For example, the UK Million women study (Benson et al 2013) included only two simple questions regarding usage of mobile phone which cannot estimate individual exposure in any reasonable degree. Following the general bias of this report in favor of negative finding, the authors forgot to state that this study found statistically significant increase of acoustic neuroma for long term users vs never users (10+ years: RR = 2.46, 95% CI = 1.07–5.64, $P = 0.03$), the risk increasing with duration of use (trend among users, $P = 0.03$).

Another example is the underestimation of importance of the positive findings of de Vocht et al (2013) on global link of mobile phone usage and brain cancer. "*The study is not informative for causal inference, as popular use of mobile phones can also reflect standard of living, which is also associated with, for example, availability of diagnostic services"*. The SCENIHR's preliminary Opinion did not mention that this statement is relevant to most negative studies and especially to the Danish subscriber cohort study upon which this preliminary Opinion heavily relies. In contrast, the meta-analyses of studies which included only data on ipsilateral tumors in subjects using mobile phones for at least 10 years, show large and statistically significant increases in risk of ipsilateral brain gliomas and acoustic neuromas (Levis, Minicuci et al. 2011). The risk of head tumors was nearly doubled and was induced by long-term mobile phone use.

Consideration of the data on childhood cancers in relation to base stations is also biased in favor of weighting negative studies. While limitation of positive study by (Li et al. 2012) is provided, no limitations of negative study by (Elliott et al. 2010) is considered in contrast to about one-page description of such limitations provided by the authors (Elliott et al. 2010). In addition, the report did not provide the main positive result of the (Li et al. 2012) study which has shown increased (brain+leukemia) incidence related to base stations.

Brain cancer time trend analysis

The SCENIHR report provides biased consideration of available information. It should be noted that histology analysis and localization of tumors in respect to irradiation from mobile phone is of key importance for this analysis.

At the time of IARC meeting in 2011 the following data were available and included into the IARC monograph (IARC 2013):

USA

According to data collected by the Surveillance, Epidemiology, and End Results (SEER) Program, age- and sex-specific trends and overall temporal trends in rates of incidence of brain cancer in the USA were flat or downward between 1992 and 2006, with the exception of women aged 20–29 years (Inskip *et al.*, 2010). In this age group, a statistically significant increasing trend was driven by the rising incidence in tumors of the frontal lobe. [It is the temporal lobe that is most heavily exposed to radiation when using a mobile phone at the ear (Cardis *et al.*, 2008).] Incidence of brain cancer in USA "could be consistent with the modest excess risks in the Interphone study" (Little, Rajaraman et al. 2012).

UK

Overall rates of incidence of cancer of the brain in males or females, or in any specific age group were not increased in England between 1998 and 2007 (de Vocht, Burstyn et al. 2011). For men and women, the incidence of tumors (primarily glioma) was increased ($p<0.01$) in the temporal lobe that is most heavily exposed to radiation when using a mobile phone at the ear (Cardis, Deltour et al. 2008). The incidence increased also in frontal lobe for men ($p < 0.01$) and in the frontal lobe for women, although not statistically significant ($p = 0.07$). The incidence decreased in other parts of the brain. In a subsequent paper, the same authors reported separate time trends for cancers of the temporal lobe in the periods 1979–99 and 2000–08 (de Vocht, Burstyn et al. 2011). For men, a linear regression of age-adjusted rates showed an overall annual increase in 2000 –2008 of 3.3% (95% CI, 1.1–5.4), whereas it was lower 2.0% (95% CI, 1.4–2.6) for 1979–1999. For women, a linear regression of age-adjusted rates showed an overall annual increase in 2000 –2008 of 2.8% (95% CI, 0.9–4.8), whereas it was lower 1.4% (95% CI, 0.7–2.2) for 1979–1999. This change may be suggestive of increased rates for brain cancers of the temporal lobe in the recent years. [The linear regression used for this analysis was not an appropriate method and therefore the 95% confidence intervals reported may not be reliable.] p.190

After the IARC meeting in 2011 the following data were available

USA

Zada et al. studied incidence trends of primary malignant brain tumors in the Los Angeles area during 1992-2006 (Zada, Bond et al. 2012). Incidence data for histologically-confirmed brain tumors were obtained from the Los Angeles County Cancer Surveillance Program (LAC), the California Cancer Registry (CCR), and the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program for 1992 to 2006. Annual percentage change (APC) was calculated for microscopically confirmed histological subtypes and anatomic sub sites. The overall incidence of primary malignant brain tumors decreased over the time period with the exception of glioblastoma multiforme (GBM) (astrocytoma grade IV). The annual age adjusted incidence rate of that tumor type increased statistically significant in the frontal lobe with APC +2.4 % to +3.0 % ($p < 0.001$) and temporal lobe APC +1.3 % to +2.3 % ($p < 0.027$) across all registries. In the California Cancer Registry the incidence of glioblastoma multiforme increased also in cerebellum, APC +11.9 % ($p < 0.001$). In the parietal and occipital lobes or in overlapping lobes no statistically significant changes in incidence were seen. For lower grade astrocytoma decreases of annual age adjusted incidence rates were observed. The authors concluded that despite decreased incidences in other brain regions there was an increase in the incidence of glioblastoma multiforme in

frontal and temporal lobes and cerebellum. These parts of the brain are characterized by highest absorbed dose of radiation from mobile phones (Cardis, Deltour et al. 2008; Deltour, Wiart et al. 2011).

China

Ding et al. (Ding and Wang 2011) investigated time trends in the incidence of brain and nervous tumor in urban Shanghai, from 1983 to 2007, applying joinpoint regression models to analyze the annual incidence rates. From 1983 to 2007, the age-adjusted incidence rate of brain and nervous tumors increased gradually by 1.2% per year (95% confidence interval [CI] = 0.4% to 1.9%) among men and 2.8% per year (95% CI =2.1 to 3.4) among women. While the authors concluded that this study did not support an association between cellular telephone use and increased risk of brain and nervous tumors, the conclusion was made on assumption about *latency periods shorter 5-10 years.* Authors themselves recognize that this conclusion is not valid for longer latency periods, which are indeed predictable for gliomas and acoustic neuromas. Thus, authors do not take into account that radiation induced glioma (RIG) studies would reasonably not show so soon, given significantly higher latency periods. Common conclusions reached across diverse cases on RIG is that mean latency time was in the order of many years *(range: 9–17 years)* (Prasad and Haas-Kogan 2009). Thus, while the incidence rate has been shown to be increased in urban Shanghai, the conclusion of the authors on lack of association with mobile phones is flawed.

Australia

A multicenter study was performed to determine the brain cancer incidence in Australia (the state of New South Wales (NSW) and the Australian Capital Territory (ACT)) with age-, sex-, and benign-versus-malignant histology-specific analyses (Dobes, Shadbolt et al. 2011). One hundred percent of tumors were histologically confirmed. Data were weighted for patient outflow and *data completeness.* Incidence rates were age standardized and trends analyzed using joinpoint analysis. An overall significant increase in primary malignant brain tumors was observed over the study period from 2000 to 2008 (APC, 3.9; 95%CI, 2.4–5.4). Overall increasing trend in malignant tumors was consistent for both males (APC, 2.3; 95% CI, 0.4–4.2) and females (APC, 2.3; 95% CI, 0.3– 4.3). This increase appears to be largely due to an increase in malignant tumor incidence in the >/=65-year age group. The same authors reported an analysis of incidence by tumor subtype (Dobes, Khurana et al. 2011). A significant increasing incidence in glioblastoma multiforme (GBM) was observed in the study period (annual percentage change [APC], 2.5; 95% confidence interval [CI], 0.4-4.6, n = 2275), particularly after 2006. In GBM patients in the >/=65-year group, a significantly increasing incidence for men and women combined (APC, 3.0; 95% CI, 0.5-5.6) and men only (APC, 2.9; 95% CI, 0.1-5.8) was seen. Rising trends in incidence were also seen for meningioma in the total male population (APC, 5.3; 95% CI, 2.6-8.1, n = 515) and males aged 20-64 years (APC, 6.3; 95% CI, 3.8-8.8). Significantly decreasing incidence trends were observed for Schwannoma for the total study population (APC, -3.5; 95% CI, -7.2 to -0.2, n = 492), significant in women (APC, -5.3; 95% CI, -9.9 to -0.5) but not men.

Korea

Recent data from Korea has shown increase in brain cancer incidence (Jung, Won et al. 2013). Tumors of the brain and nervous system increased APC 1.0% per year for men and 0.5% per year for women during 1999 - 2010. The rate of increase was statistically significant for men (p <0.05%), while was not statistically significant for women. It should be noted that key parameters for the NT MW effects include sex and age (Belyaev 2010; IARC 2013). For both sexes, combined statistically significant rate of increase was 0.8% annually.

Nordic national cancer registers

In Denmark, the Danish cancer register has reported increase in brain cancer incidence of 40% in men, and by 29% in women during 2001-2010. (http://www.sst.dk/publ/Publ2011/DAF/Cancer/Cancerregisteret2010.pdf)

Finland

In Finland, age-adjusted (world) brain cancer incidence rates per 100,000 person-years has not changed significantly since 1997 (http://www.kreftregisteret.no/no/Registrene/Kreftstatistikk/). Age-adjusted (world) incidence rates per 100 000 person-years by primary site and five-year period was in females 12,0 in 1992-96, 13,6 in 1997-01, 14,2 in 2002-06, 13,7 in 2007-11 (http://stats.cancerregistry.fi/stats/eng/veng0006i0.html)
Age-adjusted (world) incidence rates per 100,000 person-years by primary site and five-year period was in males 10,7 in 1992-96, 10,6 in 1997-01, 11,7 in 2002-06, 11,2 in 2007-11. (http://stats.cancerregistry.fi/stats/eng/veng0005i0.html)

Norway

In Norway, age-adjusted (world) brain cancer incidence rates per 100 000 person-years has grown since 1997 (http://www.kreftregisteret.no/no/Registrene/Kreftstatistikk/). Age-adjusted (world) incidence rates per 100,000 person-years by primary site and five-year period was in females 10.6 in 1992-96, 13.3 in 1997-01, 17.3 in 2002-06, and 16.4 in 2007-11. Age-adjusted (world) incidence rates per 100,000 person-years by primary site and five-year period was in males 10.7 in 1992-96, 12.2 in 1997-01, 14.1 in 2002-06, and 14.2 in 2007-11.

Sweden

In Sweden, no statistically significant changes in brain cancer incidence per 100,000 person was shown in Cancer Register (Socialstyrelsens Cancerregister) during 1996 -2011. (http://www.socialstyrelsen.se/statistik/statistikdatabas/cancer). There is a scientifically reasonable suspicion that underreporting of brain cancers masks the brain cancer incidence in Sweden (Barlow, Westergren et al. 2009).

All Nordic countries. NORDCAN

Nordic cancer register (NORDCAN) shows increases in brain cancer incidence. NORDCAN project presents the incidence, mortality, prevalence and survival statistics from 41 major cancers in the Nordic countries (http://www-dep.iarc.fr/NORDCAN/english/frame.asp). In Denmark, a statistically significant increase in incidence rate per year for brain and central nervous system tumors (combined) was seen during 2001-2011 both in men, *annual percentage change (APC)*, 3.77, [95% CI 2.90; 4.64] and in women 3.68, [95% CI 2.29; 5.10]. While no statistically significant changes are observed in incidence rate per year for brain and central nervous system tumors during last 10 years in other Nordic countries (Finland, Iceland, Norway, and Sweden), a statistically significant increase is seen during last 10 years in men 1.02, 95%CI [0.40;1.65] and women, 1.05, 95%CI [0.35;1.74] in all Nordic countries combined.

Quality and completeness of cancer registers

The SCENIHR preliminary Opinion reaches an indefensible and highly controversial conclusion on brain cancer: *"That renders all studies reporting increased risks of such magnitude implausible. The reason for the increases are methodological artefacts"*. First, the time trends for brain cancer incidence is positive according to at least some data shown

above. Second, it generally accepted that if two pieces of data do not fit each other both pieces should be scientifically analyzed. As a matter of fact, the utility of Cancer registries depends heavily on their quality including the *completeness* with which patients eligible for registration are ascertained (Bray and Parkin 2009; Parkin and Bray 2009). The *completeness* of cancer registry data – the extent to which all of the incident cancers occurring in the population are included in the registry database – is an extremely important attribute of a cancer registry (Parkin and Bray 2009). However, registries rarely report their completeness because it is difficult to measure (Bullard, Coleman et al. 2000).

Incompleteness was found in the Swedish Cancer Register (Barlow, Westergren et al. 2009). Underreporting of brain cancers including gliomas in Swedish Cancer Register was about 3.7% of the cases reported in 1998 (Barlow, Westergren et al. 2009).
It was estimated, that the Thames Cancer Registry (UK) attains 92.1% completeness 5 years after diagnosis for all cancers (Bullard, Coleman et al. 2000). Recent data have confirmed relatively low completeness of the Thames Cancer Registry with estimates ranging from around 78% (female melanoma) to 95% (female stomach cancer) (Robinson, Sankila et al. 2007). The Finnish data appeared to be more complete, with estimates ranging from around 96% completeness for prostate cancer to 100% for ovarian cancer (Robinson, Sankila et al. 2007).

The best characterized is the Cancer Register of Norway (CRN) (Larsen, Smastuen et al. 2009). A total of 93.8% of the cancer cases registered in the period 2001–2005 were morphologically verified. The proportion of DCO (death certificate only) cases 2001–2005 was only 0.9%, and only 2.2% were registered with primary site unknown (PSU). The overall completeness for the period 2001–2005, estimated by the capture/recapture method, was 98.8%. The lowest completeness was estimated for pancreas (95.7%), multiple myeloma (95.5%), leukemia (94.6%) and central nervous system (93.8%). Authors recognize that cancers of the central nervous system did not meet the highest standards. Nevertheless, recent registration data from Norway are among the most complete among the European Registries (Larsen, Smastuen et al. 2009).

Recent study has indicated the US cancer registries data may be incomplete as related to cancer mortality (German, Fink et al. 2011). Confirmation rate was estimated as 93.4 (95% CI, 92.6–94.2) (per 100 deaths) = the number of individuals who died sometime in 2002–2004 and had been diagnosed with brain cancer sometime in 1993–2004 for whom the cancer site listed in the population-based cancer registry matched the site (underlying cause) on their death certificate, divided by the total number of these decedents (both matched and unmatched). Detection rate was estimated 93.7 (95% CI, 90.5–96.9) = the number of individuals diagnosed with brain cancer (ICD-10, the International Statistical Classification of Diseases and Related Health Problems, 10th Revision) sometime in 1993–1995 who died sometime in 1993–2004 for whom the cancer site listed in the population-based cancer registry matched the site (underlying cause) on their death certificate, divided by the total number of these decedents (both matched and unmatched).

Similar incompleteness has been reported by Meguerditchian et al for the National Cancer Data Base (NCDB) (Meguerditchian, Stewart et al. 2010). Claims for patients with breast cancer surgery from one payer in Western New York (WNY) were matched with NCDB for participating hospitals for 2001-2003 using available identifiers (reporting hospital, gender, birth date, ZIP code). Four hundred seventy patients with health insurance provided by IHA with a breast procedure and a diagnosis code for breast cancer between January 1, 2001 and

January 1, 2003 at the participating institutions were identified by ICD-9 and CPT codes. These patients were matched to all breast cancers reported to the NCDB from the CoC-approved hospitals during the same period and in the same geographic area. The final match rate between the two datasets was 93.4% (430 patients). Forty cases identified by IHA remained unmatched to the registries.

The time trends for *incompleteness* of the Cancer Registers is not known. Finally, Cancer Register's data should be questioned if no consistence is observed between them and epidemiological data on mobile phone usage.

Conclusion on brain cancer time trend data and mobile phones

Cancer incidence data are derived from cancer registries and quality of these data dependent on quality and completeness of cancer registers. Completeness and quality of most cancer registries are not comprehensively characterized and vary between cancer registers. At least some cancer registries including better described Nordic Cancer Register show increased time trends in brain cancer incidence, especially in those parts of brain which are mostly exposed to radiation from mobile phones. Taking into account the IARC statement regarding the role of incidence data in phone risk assessment, the incidence data do not contradict to the increased cancer risk seen in epidemiological studies at latencies more than 10-25 years (Carlberg, Soderqvist et al. 2013; Hardell and Carlberg 2013; Hardell, Carlberg et al. 2013; Hardell, Carlberg et al. 2013). The IARC Working Group further noted that these descriptive analyses would be null if an excess in cancer risk from mobile-phone use became manifest only decades after phone use began, or if an increase affected only a small proportion of the cases by location.

On page 68 the SCENIHR report states: "it appears the evidence for glioma became weaker". This conclusion is in evident contradiction with available data. Recent publications including those omitted in the SCENHIR report and mentioned in these comments make this evidence much stronger then during the last IARC meeting in 2011 and demands IARC classification "carcinogen, group 1" for EMF exposures from mobile phones.

In vivo studies

Similar to other parts of this report, the conclusions from *In vivo* studies, p 68- , are fundamentally flawed because they are not based on mechanistic studies and consideration of important physical and biological parameters (IARC 2013).
As a matter of fact, only negligible amount of real signals (frequency, modulation, polarizaton) were tested in mentioned *in vivo* studies. Thus, the statement, p 68, "*Overall, it was concluded that RFR fields such as those emitted by mobile phones were not carcinogenic in laboratory rodents*" may be relevant only to these limited number of tested signals.
Similarly the statement: *" Overall, because a considerable number of well-performed studies using a wide variety of animal models have been mostly negative in outcome, the animal studies are considered to provide strong evidence for the absence of an effect*" deals with only minority of real signals and cannot be used as an argument against overwhelming evidence for increased cancer risks following from epidemiological studies, which involved all possible signals. What is even more important, most positive studies involved exposure to the more realistic exposure that includes combined signals from real mobile phones. These are the most relevant for health risk assessment, but were omitted in the SCENIHR report (see below).

It is fundamentally flawed to question results of epidemiological studies obtained with exposure to all signals from mobile phones by *in vivo* or *in vitro* negative studies obtained with negligible number of mobile phone-like signals.

Genotoxic RFR effects, p. 70

These studies were omitted from review in the preliminary Opinion and should be incorporated. Positive studies on RFR/mobile phone genotoxicity include but are not limited to (Guler, Tomruk et al. 2010; Cam and Seyhan 2012; Guler, Tomruk et al. 2012; Karaca, Durmaz et al. 2012; Sekeroglu, Akar et al. 2012; Atasoy, Gunal et al. 2013; Atli Şekero□lu, Akar et al. 2013; Hanci, Odaci et al. 2013; Liu, Duan et al. 2013; Liu, Gao et al. 2013; Pesnya and Romanovsky 2013).

Considering Belyaev's group studies (Belyaev, Markova et al. 2009; Markova, Malmgren et al. 2010) the SCENIHR preliminary opinion stated, page 72, that effects at 905 MHz were inconsistent. It should be noted that this "inconsistency" was actually individual variability, which nature has recently been established to be dependant on individual state of chromatin at time of exposure (Sarimov, Alipov et al. 2011). One of the main results following from the Belyaev's group studies including those unmentioned neither in this nor in previous SCENIHR report (Sarimov, Malmgren et al. 2004; Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005) is strong dependence of effects from mobile phones on carrier frequency/frequency channel. Effects at 905 MHz/GSM channel 74 on DNA repair foci were consistently lower compared to effects at 915 MHz/GSM channel 124 regardless cell type, human lymphocytes, fibroblasts or stem cells. In addition, the data indicated stronger effects of exposure to RF from UMTS mobile phone at frequency at 1947.4 MHz, middle channel. Importantly, human stem cells (not "steam cells" as spelled in the SCENIHR preliminary opinion on page 72, line 16) were most sensitive to MW exposure providing a mechanistic link to carcinogenesis. This is because stem cells are the generally accepted cellular target for origination of different types of tumors and leukemia. These data provided evidence that different frequency channels of different types of mobile communications should be separately tested for health effects and that primary human stem cells are an key cellular focus for in vitro EMF studies dealing with carcinogenesis.

Mechanisms for non-thermal MW effects below ICNIRP safety levels

It is generally accepted now that MW induce effects under non-thermal intensities which are generally called non-thermal effects. The SCENIHR preliminary opinion states that: *"(I)n view of the lack of verification of any proposed non-thermal interaction mechanism, established knowledge does not suggest effects accumulating with time".*

First, this statement is in contradiction with generally accepted Bradford Hill criteria: *"Plausibility: It will be helpful if the causation we suspect is biologically plausible. But this is a feature I am convinced we cannot demand. What is biologically plausible depends upon the biological knowledge of the day. '*. . . no biological knowledge to support (or to refute) Pott's observation in the 18th century of the excess of cancer in chimney sweeps. It was lack of biological knowledge in the 19th that led a prize essayist writing on the value and the fallacy of statistics to conclude, amongst other "absurd" associations, that "*it could be no more ridiculous for the stranger who passed the night in the steerage of an emigrant ship to ascribe the typhus, which he there contracted, to the vermin with which bodies of the sick might be infected*". And coming to nearer times, in the 20th century there was no biological knowledge to support the evidence against rubella.' ..... the association we observe may be one new to science or medicine and we must not dismiss it too light-heartedly as just too odd. As

Sherlock Holmes advised Dr Watson, *'when you have eliminated the impossible, whatever remains, however improbable, must be the truth*.' "(Hill 1965).

Second, there are a number of studies showing accumulation of effects with time (Belyaev 2010).

Third, the majority of scientists consider NT MW effects within the frame of mechanisms using quantum mechanics and physics of nonlinear systems in biological non-equilibrium systems, which are relevant for mechanisms of NT MW in biological systems (Belyaev 2010). It is generally accepted that more then one physical theory may describe the same phenomena (compare for example Debye model of phonons in a box and Einstein model of quantum harmonic oscillators for solids). Thus, the demand of a generally accepted mechanism is not scientifically justified and represents methodological flaw. Most representative so far international IARC expert panel has concluded: "*Although it has been argued that RF radiation cannot induce physiological effects at exposure intensities that do not cause an increase in tissue temperature, it is likely that not all mechanisms of interaction between weak RF-EMF (with the various signal modulations used in wireless communications) and biological structures have been discovered or fully characterized*", see page 104 (IARC 2013). Thus, the IARC Working Group does not reject physical mechanisms for mobile phone exposure and recognizes that either new mechanisms may come or already known mechanisms may be better characterized to explain the non-thermal effects.
Among other mechanisms, radical pairs mechanisms is widely accepted. In many recent reports unmentioned by the SCENIHR preliminary opinion it has been shown that ROS may be involved in radical pair reactions, thus, radical pairs may be considered one of the mechanisms of transduction able to initiate cell oxidative stress (Georgiou 2010; Apollonio, Liberti et al. 2013; Bodera, Stankiewicz et al. 2013; Burlaka, Tsybulin et al. 2013). Furthermore, many of the changes observed in RF-exposed cells were prevented by (pre)treatment with antioxidants (IARC 2013). In addition, recent review has summarized studies on EMFs exposure and oxidative stress in brain (Consales, Merla et al. 2012). While the data from different studies should be compared with care in view of variation in physical and biological parameters, most part of collected data have shown effects of ELF and RF EMF on oxidative stress in brain (Consales, Merla et al. 2012). IARC monograph states: "*even small effects on radical concentration could potentially affect multiple biological functions*", page 103 (IARC 2013).

One of the main arguments against NT MW effects, so called kT-paradox, has further been challenged by consideration of biological processes far from thermodynamic equilibrium (Cifra, Fields et al. 2011). Subculture structures such as molecular motors operate, in general, under conditions far from thermodynamic equilibrium and, therefore, the formalism of non-equilibrium thermodynamics, which was generally used in critics of mechanisms for NT MW effects, for coupled mechano-chemical processes is not applicable (Chowdhury 2013). Therefore, one has to use the more sophisticated toolbox of stochastic processes and nonequilibrium statistical mechanics for theoretical treatment of molecular motors. Theoretical studies by Srobar in development of fundamental theory by H. Fröhlich have not been considered neither in this nor in previous SCENIHR Opinion on EMF (Srobar 2009; Srobar 2009).

Effects of RFR exposure on oxidative stress, p 177

This chapter provides a biased record of very minor part of oxidative stress studies without definition how these studies have been chosen for analysis. Recent positive studies on

RF/mobile phone oxidative stress and genotoxicity have not been included (Haghani, Shabani et al. 2013) (Tomruk, Guler et al. 2010; Esmekaya, Ozer et al. 2011; Kumar, Behari et al. 2012) (Lu, Huang et al. 2012) (Tkalec, Stambuk et al. 2013) (Deshmukh, Banerjee et al. 2013) (Shahin, Singh et al. 2013) (Eser, Songur et al. 2013) (Burlaka, Tsybulin et al. 2013) (Esmekaya, Aytekin et al. 2011) (Avci, Akar et al. 2012) (Ceyhan, Akkaya et al. 2012) (Sokolovic, Djordjevic et al. 2013) (Oksay, Naziroglu et al. 2012) (Sisodia, Rifat et al. 2013) (Liu, Duan et al. 2013) (Jelodar, Akbari et al. 2013) (Liu, Gao et al. 2013) (Ghanbari, Mortazavi et al. 2013) (Guler, Tomruk et al. 2010; Guler, Tomruk et al. 2012) (Imge, Kilicoglu et al. 2010) (Jelodar, Akbari et al. 2013) (Liu, Duan et al. 2013) (Naziroglu, Cig et al. 2012) (Ni, Yu et al. 2013) (Ozgur, Gler et al. 2010) (Park, Seo et al. 2013)

Replication studies

The most representative so far international IARC panel have included in the RF monograph, pages 101-102: "*The reproducibility of reported effects may be influenced by exposure characteristics (including SAR or power density, duration of exposure, carrier frequency, type of modulation, polarization, continuous versus intermittent exposures, pulsed-field variables, and background electromagnetic environment), biological parameters (including cell type, growth phase, cell density, sex, and age) and environmental conditions (including culture medium, aeration, and antioxidant levels)*" (IARC 2013). IARC admits also that some of the discrepancies between EMF replication studies could be due to differences in species, page 416 (IARC 2013). And at the page 104: "*Biological systems are complex and factors such as metabolic activity, growth phase, cell density, and antioxidant level might alter the potential effects of RF radiation*". Physical factors that affect interpretation of study results are considered in the IARC monograph in more detail on pages 385-387 (IARC 2013).

The SCENIHR preliminary Opinion requires "*replication studies in a strict sense*" for positive findings (page 101). Furthermore, those studies which consistently showed positive findings were criticized for deviations in protocols (p 101, lines 41-49). No such criticism was applied to studies which failed to "replicate" original positive finding (for example page 102, lines 39-49) even if the key parameters of experiments were or might be different between original studies and "replications". At many occasions, the SCENIHR preliminary Opinion states that replication of positive findings is essential before weight is given to positive results. However, the SCENIHR preliminary Opinion has never applied the same criteria to negative studies even if statistical power was not evaluated in most of them and thus the value of possibly missed effects is not known. As a matter of fact, not one of the negative studies has been replicated "*in a strict sense*" and not one of positive studies has been "unreplicated"/dismissed in "*in a strict sense*". Application of double standards for assessment of positive and negative studies is methodologically flawed and makes the SCENIHR preliminary Opinion internally inconsistent.

The SCENIHR report missed successful replications of positive studies (Grigoriev, Grigoriev et al. 2010; Havas and Marrongelle 2013).

In addition to aforementioned omitted studies reporting positive effects, this preliminary Opinion omitted many other recent positive studies which include but not limited to:

(Fragopoulou, Samara et al. 2012) (Karaca, Durmaz et al. 2012) (Dasdag, Akdag et al. 2012) (Celikozlu, Ozyurt et al. 2012) (Sharma, Sisodia et al. 2013) (Lv, Chen et al. 2014) (Jin, Zong et al. 2012) (Trivino Pardo, Grimaldi et al. 2012) (Aboul Ezz, Khadrawy et al. 2013) (Kesari, Kumar et al. 2011) (Redmayne, Smith et al. 2013) (Deshmukh, Banerjee et al. 2013; Deshmukh, Megha et al. 2013) (Aboul Ezz, Khadrawy et al. 2013) (Cam and Seyhan 2012)

(Cervellati, Valacchi et al. 2013) (Finnie, Cai et al. 2010) (Jorge-Mora, Misa-Agustino et al. 2011) (Kwon, Vorobyev et al. 2011) (Panagopoulos, Chavdoula et al. 2010; Panagopoulos and Margaritis 2010; Panagopoulos and Margaritis 2010) (Shckorbatov, Pasiuga et al. 2010) (Suhhova, Bachmann et al. 2013) (Vishnu, Nithyaja et al. 2011) (Sun, Shen et al. 2013) (Tomruk, Guler et al. 2010) (Wu, Wang et al. 2012) (Xu, Chen et al. 2013)

Negative studies were preferentially included into the report even if the same group published both positive and negative studies analyzing different endpoints. An example is the group of Lopez-Martin, which has published negative study on apoptosis in adult male Sprague-Dawley rats exposed for 1 hour to 900 MHz. This negative study was included to the SCENIHR report on page 157. However, the same group has published study revealing that similar exposure at 900 MHz and intensities lower then those from mobile phones induces c-fos proto-oncogene and glial fibrillary acid protein (GFAP) marker in brain of exposed male Sprague-Dawley rats (Carballo-Quintas, Martinez-Silva et al. 2011). This positive study has not been included in the SCENIHR report.

Omission of positive studies showing detrimental effects of RFR exposure and their possible mechanisms especially negatively affects conclusions of the SCENIHR report. An example is data from by Deshmukh et al., which show effects of RFR on cognitive function, DNA damage and oxidative stress in rats exposed under the same conditions (Deshmukh, Banerjee et al. 2013; Deshmukh, Megha et al. 2013).

Exclusion of positive studies questions the conclusions of the SCENIHR report on RFR health effects because some of them describe critical effects which were not considered by the SCENIHR report. Example is study by Aboul Ezz (Aboul Ezz, Khadrawy et al. 2013) which investigated the effect of RFR (frequency 1800 MHz, specific absorption rate 0.843 W/kg, power density 0.02 mW/cm2, modulated at 217 Hz) on the concentrations of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in the hippocampus, hypothalamus, midbrain and medulla oblongata of adult rats. Adult rats were exposed daily to EMR and sacrificed after 1, 2 and 4 months of daily RFR exposure and 1 month after 4 months of daily RFR exposure. RFR exposure induced significant changes in DA, NE and 5-HT in all studied areas of adult rat brain. The authors concluded that exposure of adult rats to RFR may cause disturbances in monoamine neurotransmitters and this may underlie many of the adverse effects reported after RFR including memory, learning, and stress. In a recent German study, 24 out of 60 participants were exposed to MW from a base station (cell tower) at a power density of < 60 $\mu W/m^2$, 20 participants to 60 - 100 $\mu W/m^2$, and 16 participants to more than 100 $\mu W/m^2$ (Buchner and Eger 2011). The values of the stress hormones adrenaline and noradrenalin increased significantly during the first 6 months after exposure to the GSM base station; the values of the precursor substance dopamine substantially decreased in this time period. The subject's initial endocrine state was not restored even after 1.5 years. Due to the non-regulable chronic difficulties of the stress balance, the phenylethylamine levels dropped until the end of the investigation period. These effects show a dose response relationship.

Provocation studies, p. 108

In view of complex dependence of NT MEW effects on physiological state of the object, individual sensitivity, physical parameters of exposure, duration and time after exposure the provocation studies should not be considered as informative regarding exposure to all real mobile communication systems including cellphones because only minor part of these parameters (frequency, modulation, duration of exposure et cetera) have been analyzed.

Conclusions on symptoms. p. 115

Similar to other conclusions on RFR health effects, conclusions on symptoms on page 115 do not take into account dependence of RFR effects on physical parameters such as frequency and modulation. In contrast to this flawed approach by the SCENIHR report, in recent study Redmayne et al. evaluated associations between New Zealand early-adolescents' subjective well-being and self-reported use of, or exposure to diffrent types of wireless phones and internet technology (Redmayne, Smith et al. 2013). In this cross-sectional survey, participants completed questionnaires in class about their cellphone and cordless phone use, their self-reported well-being, and possible confounding information such as whether they had had influenza recently or had a television in the bedroom. Parental questionnaires provided data on whether they had WiFi at home and cordless phone ownership and model. Data were analysed with Ordinal Logistic Regression adjusting for common confounders. Odds ratios (OR) and 95% confidence intervals were calculated. The number and duration of cellphone and cordless phone calls were associated with increased risk of headaches (>6 cellphone calls over 10 minutes weekly, adjusted OR 2.4, CI 1.2-4.8; >15 minutes cordless use daily adjusted OR 1.74, CI 1.1-2.9)). Using a wired cellphone headset was associated with tinnitus (adjusted OR 1.8, CI 1.0-3.3), while wireless headsets were associated with headache (adjusted OR 2.2, CI 1.1-4.5), feeling down/depressed (adjusted OR 2.0, CI 1.1-3.8), and waking in the night (adjusted OR 2.4, CI 1.2-4.8). Several cordless phone frequencies bands were related to tinnitus, feeling down/depressed and sleepiness at school, while the last of these was also related to modulation. The only significant negative regression was less likely Waking nightly for those with Wi-Fi at home (adjusted OR 0.7, CI 0.4-0.99). Being woken at night by a cellphone was strongly related to tiredness at school (OR 3.49, CI 1.97-6.2).
There were more statistically significant associations (36%) than could be expected by chance (5%). Several were dose-dependent relationships. The obtained data were in line with previous findings of others and suggested limiting use of cellphones and cordless phones to less than 15 minutes daily, and employing a speaker-phone device for longer daily use.

Methodological flaw in assessment

In contrast to generally accepted methodology used by IARC, this SCENIHR report subjectively divides studies into informative and non-informative (page 83-84). As a result the same studies SCENIHR report assess differently as compared to IARC : *" For in vivo studies our assessment of evidence is weaker than IARC, based on the same studies as used in the IARC evaluation".* While the SCENIHR report requires statistical power for negative studies (page 17), the majority of negative studies which the preliminary Opinion relies upon did not analyze statistical power and were not able to determine at what level of sensitivity the RFR effects might be missed. It is not stated in the SCENIHR preliminary Opinion how many experts evaluated each study and whether experts were allowed to evaluate own studies. The SCENIHR report inconsistently uses criteria for replication studies and verification of results. Strict following to generally accepted key biological and physical parameters the conditions is demanded at some occasions of the SCENIHR report. On the other hand, the effects of gender and biological efficiency of low SAR values is used to question validity of results (lines 3-4, page 103). Effects of low SARs and gender were described in many papers (Belyaev 2010; IARC 2013) and thus cannot be used as argument against NT MW effects.

Exclusion of studies with exposure to real mobile phones, which are most relevant for assessment of health effects from mobile telephony p. 117

On Page 117 the SCENIHR report states that studies with exposure to real mobile phones "*are of no use for health risk assessment, as the exposures would have been highly complex and very variable, especially if the animals were unrestrained and free to move in their*

cages". This is fundamentally flawed statement which results in excluding mostly important for health risk assessment studies and thus masking health risks from mobile communication. As a matter of fact, the studies with real mobile phones, given the EMF field was measured from the phone, represent most valuable type of studies for assessment of risks from mobile telephony. The reasons were recently analyzed in review by Belyaev that has not been included in the SCENIHR report (Belyaev 2010). In brief, real signals contain multiple (hundreds and even thousands, in dependence on type of mobile communication) components, such as carrier frequencies or frequency bands, different types of modulations. It is generally accepted that all these parameters are important for effects of MW (IARC 2013). Exposure to mobile phone may reproduce the majority of real signals during the same exposure session and thus provide the best possibility to asses detrimental effects from mobile telephony. Another type of exposure, to which the SCENIHR report has chosen to rely upon, is exposure to one fixed frequency and fixed modulation which reproduces one from thousands possible signals. While one RFR frequency/frequency band/modulation can induce detrimental effect, another one can be inactive (Belyaev 2010). In addition, mobile phones emit not only MW but also ELF fields, which have also been shown to produce detrimental effects (www.bioititive.org) and to interfere with MW effects (Belyaev 2010; Sun, Shen et al. 2013). Importantly, most of aforementioned studies with mobile phones as source of EMF exposure and omitted by the SCENIHR report show detrimental effects and most importantly indicate mechanism of these effects based on induction of ROS.

Data obtained with selected frequency/frequency band/modulation provides possibility to asses only this specific signal and may be important for consideration of biophysical mechanisms for NT MW effects. However, these studies are evidently less important for health risk assessment by the reasons provided above.

Recommendations

The main issue of further research is to promote studies on biophysical mechanisms that will provide a mechanistic basis for risk assessment. Such parameters as frequency, modulation, polarization should be given priority for mechanistic studies so that physical and biological variables that influence study outcome can be taken into account.

For risks assessment in laboratory studies, the complexity and interplay of variables from real systems of mobile communication should also be taken into account. In other words, to assess health risks from any type of mobile communication, all specific frequency channels and all specific modulations should be investigated in combinations as at real exposures.

Recent studies indicated that financial interests may affect the outcome of EMF laboratory studies (Huss, Egger et al. 2007; Huss, Egger et al. 2008). Also recent review reports that the negative results produced by studies funded by the cell-phone companies are affected by many biases and flaws, giving rise to a systematic underestimate of the risk (Levis, Minicuci et al. 2011). On the contrary, studies producing positive results - without errors and financial conditioning - indicate a cause/effect relationship supported by biological plausibility (Levis, Minicuci et al. 2011). In view if these facts, it is recommended to take into account the source of funding in evaluation of the results.

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Human Health Effects of EMFs: The Cost of Doing Nothing

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Abstract. Everyone is exposed to electromagnetic fields (EMFs) from electricity (extremely low frequency, ELF), communication frequencies and wireless devices (radiofrequency, RF), as well as naturally occurring EMFs. Concern of health hazards from EMFs has increased as the use of mobile phones and other wireless devices has grown in all segments of the population, especially children. While there has been strong evidence for an association between leukemia and residential or occupational exposure to ELF EMFs for many years, the standards in existence are not sufficiently stringent to protect from an increased risk of cancer. ELF EMFs also increase risk of at least two types of neurodegenerative diseases. For RF EMFs, standards are set at levels designed to avoid tissue heating, in spite of many reports of biological effects at intensities too low to cause significant heating. Recent evidence demonstrates elevations in risk of brain cancer and acoustic neuroma only on the side of the head where individuals used their mobile phone. Individuals who begin exposure at younger ages are more vulnerable. These data indicate that the existing standards for radiofrequency exposure are not adequate. While there are many unanswered questions, the cost of doing nothing may result in an increasing number of people, many of them young, developing these diseases.

1. Introduction

It has been known for many years that high energy EMFs (X-rays, gamma rays, cosmic rays) have sufficient energy to directly break chemical bonds, causing damage to molecules ranging from water to DNA that results in cancer and birth defects [1]. Thus these forms of EMF are “ionizing”. There is less consensus as to whether lower energy forms of EMFs, such as radiofrequency and ELF EMFs, can cause disease. In spite of strong evidence for such relationships from the biomedical community, most national and international bodies have discounted this evidence, based on the belief that lower energy EMFs cannot possibly cause serious disease [2]. This particular point of view is held by many in the physics and engineering communities, individuals not known for their detailed knowledge of medicine. There are legitimate concerns as to what mechanisms might explain these relationships. The purpose of this review is to provide an overview of the issues, explore both the associations between exposure and disease and the mechanisms that might explain them, and to propose biologically-based standards of exposure which, although difficult to achieve, would be more protective of human health.

While there are a variety of diseases of possible concern, this review will focus on only two major classes, cancer and neurodegenerative disease. This is for two reasons. The evidence for an association with EMF is strongest for these diseases, and these are very serious diseases that cause significant morbidity and mortality in humans.

2. Health Effects of ELF EMFs

There has been evidence that residential exposure to elevated residential magnetic fields results in an increased risk for leukemia since the pioneering studies of Wertheimer and Leeper [3]. Most subsequent studies have confirmed elevated risks of leukemia [4-6], and several meta-analyses have shown significantly elevated odds ratios (ORs) whether exposure was determined through use of wire codes or measured magnetic fields [7-9]. In addition there is evidence that leukemia is elevated in adults employed in occupations that involve elevated exposure to EMFs from electricity [10]. Meta-analyses of occupational exposure have also reported elevated risks for leukemia, with less strong evidence for relations to other kinds of cancer [11]. There has also been a meta-analysis reporting a significant elevation in rates of brain cancer among adults working in "electrical" occupations [12].

There is also strong evidence for a relationship between occupational exposure to EMFs and neurodegenerative diseases, Alzheimer's and amyotrophic lateral sclerosis (ALS). In a meta-analysis of EMFs and Alzheimer's disease, Garcia et al. [13] report an OR ratio of 2.03 (95% CL = 1.38-3.00) for case-control studies, and 1.62 (95% CL = 1.16-2.80) for cohort studies. Ahlbom [14] reviewed seven studies of EMF exposure and ALS, and found an overall significant OR of 1.5 (95% CL = 1.2-1.7). More recent studies of Hakansson et al. [15] found an OR of 2.2 (95% CL = 1.0-4.7) among welders and other workers exposed to elevated magnetic fields.

3. Human Disease from Exposure to RF EMFs

Until recently there has been relatively little attention to RF exposures and human health. Older studies have reported elevations in both leukemia and brain tumors among individuals with occupational exposures to RF (see [16] for references), but results were not very consistent across studies. Recent reports have found elevated rates of leukemia among children who live near AM radio transmitter sites [17-19]. This is the same cancer elevated with exposure to powerline frequency EMFs, suggesting that leukemia is the cancer most likely to show elevated risk with whole body exposure to a variety of EMFs frequencies.

With the advent of enormous increases in the use of mobile phones, we now have a situation in which a very large segment of society is regularly exposed to high levels of RF. In addition, the whole population has increased exposure through the placement of mobile phone towers, wireless buildings and even wireless cities. The strongest evidence for hazards has come from Europe, especially Scandinavia, where mobile phones were initially manufactured, and have been in wide use for a longer period of time as compared to other parts of the world.

Recent studies have found an elevated risk of brain tumors and acoustic neurons in individuals who have used mobile phones regularly for ten years or longer. A meta-analysis by Hardell et al. [20] based on four studies finds an OR of 2.0 (95% CL = 1.2-3.4) for glioma among individuals who have used a mobile phone for ten years or more, but only on the side of the head where the phone was used. There was also an OR of 2.4 (95%CL = 1.1-5.3) for acoustic neuroma among long-term users. Risks for meningioma were elevated, but not statistically significant. Kundi [21] has reported on 33 epidemiological studies, and finds that the combined ORs from these studies show an OR of 1.5 (95% CL = 1.2-1.8) for glioma. There was also a nonsignificant elevation in ORs for acoustic neuroma but no relationship with meningioma. Hopefully, additional information will come from the pooled results of the INTERPHONE study, a 13-nation investigation coordinated by the World Health Organization, which should be available in the near future. Interestingly, the Israeli component of this study has found an elevated risk of parotid gland cancer with long-term mobile phone use [22], but results from the full INTERPHONE study of parotid gland cancers are also not yet available.

There is a particular concern about risks to children exposed to RF. Hardell et al. [23] studied relative risk based on the age when a person began to use a mobile phone, and found that individuals whose use began while they were in their 20's displayed ORs that were higher than those of older

persons for both analog and cordless phones when assessed at either >1 or >5 year latency. Later Hardell reported at a meeting (personal communication) that children who began use of a mobile phone prior to the age of 20 had an OR of developing glioma of 5.2 (95% CL = 2.2-12) after only one plus year of mobile phone use, while for all ages the OR was 1.4 (95% CL = 1.1-1.7). The same relative relationship was seen with use of a cordless phone, where use before the age of 20 years gave an OR of 4.4 (95% CL = 1.9-10), whereas for all ages the OR was 1.4 (95% CL = 1.1-1.8). These studies support the conclusion that cordless phones increase both exposure levels and disease by about the same magnitude as do mobile phones, and that use of either results in an increased risk of gliomas.

4. Why Have These Results not been Reflected in New Standards of Exposure?

In spite of this consistency in observations relating to ELF EMFs and leukemia, and the developing evidence for a relationship between mobile phone use and elevated risk of brain cancer and acoustic neuroma, there has been a general failure of governments and international advisory bodies to accept the reported relationships as being cause and effect, and to follow through with standards designed to reduce exposure. This is a consequence of two major scientific problems, as well as the public excitement and support of wireless technologies and the political power of the industry. Animal studies have not consistently demonstrated cancer as a result of exposure to ELF EMFs. In addition, no single mechanism has been identified to be the basis for the development of cancer following exposure to EMFs.

In spite of the widespread belief that most cancer is genetic, recent studies of identical twins have convincingly shown that most cancers result from some environmental exposure and are not primarily genetic [24]. This is not to say that genetics is unimportant, since genetic susceptibility will determine whether or not environmentally-induced cancers develop and become life-threatening. There is also the widespread and mistaken belief that all carcinogens act by causing direct DNA damage, as is the case with ionizing radiation. However, many proven human carcinogens do not cause direct DNA damage. These are identified as “non-mutagenic carcinogens” by the USEPA, and include such well-documented carcinogens as arsenic [25-26] and dioxins [27]. Exact mechanisms are not known to explain the carcinogenicity of either, although a number of possible factors are known. Thus the fact that ELF and RF EMFs are “non-ionizing” does not mean they are not carcinogens. Both ELF and RF EMFs are known to cause gene induction, generate reactive oxygen species, trigger formation of heat shock proteins and cause other alterations in cellular function, any one of which might lead to cancer (see [16] for references and detailed discussion).

The causes of Alzheimer’s disease and ALS are also not known. In the case of Alzheimer’s disease there is the accumulation in the brain of two different deposits that come from normal proteins, called amyloid plaques and neurofibulary tangles [28]. There is uncertainty whether these deposits cause the disease or are only associated with the disease. Recent evidence suggests that reactive oxygen species formation may be a major factor in causing cell death in Alzheimer’s disease [29]. ALS is even less well understood, but the disease is the result of death of upper and lower motoneurons [30]. Both reactive oxygen species [31] and heat-shock proteins [32] have been implicated in the resulting cell death. As with cancer, genetics plays a minor role in etiology of these diseases. There is no reason to discount an association with exposure to EMFs on the basis of lack of firm knowledge of a mechanism, as the mechanisms causing both diseases are uncertain. It is worth noting that reactive oxygen species and heat-shock proteins are implicated in both non-mutagenic carcinogen actions and these neurodegenerative diseases, and that both are altered by EMFs.

5. Proposed EMF Standards that are Based on Studies of Human Health after Exposure

The Bioinitiative Report [33] presents recommendations for standards of EMF exposure that are based on the epidemiological evidence in human populations. For ELF EMFs the proposed standard is 1 mG (0.1 μT), to be compared with the current International Commission on Non-ionizing Radiation Protection (ICNIRP) standard of 1,000 mG (100 μT). For RF radiation the proposed standard is 0.1

$\mu W/cm^2$, to be compared with the US Federal Communications Commission standard of 583 $\mu W/cm^2$ for 875 MHz cell phone frequency, and 1,000 $\mu W/cm^2$ in the frequency range of 1,800 - 1,950 MHz. The difference between these numbers show the magnitude of the problem. There is no question that a sudden imposition of standards so drastically different from those existing would impose severe hardship. However, there is also no question that the human studies clearly indicate that the existing standards are not protective of human health.

The benefits to society derived from electricity and wireless communications are significant, and certainly none of us is willing to return to the pre-electric age. However it is imperative that society at least acknowledge the disparities between current standards and current evidence of adverse health effects. Rigid and sudden imposition of the standards we propose is certainly unrealistic at the present time, but these levels are appropriate goals that could at least be approached by a combination of development of new technology and voluntary changes in behaviors.

6. The Costs of Being Wrong

At present we do not know precisely to what degree risk of cancer and neurodegenerative diseases is increased by excessive exposure to EMFs. Human studies are difficult under any circumstances, but those difficulties are even greater when studying the effects of EMFs. Levels of exposure for each of us vary over the course of every day as we move through our environment and use appliances and mobile phones. This makes exposure assessment extremely difficult. Under the circumstances of poor exposure assessment there is a great likelihood that the total risk is underappreciated.

There is considerable evidence that the developing organism is more vulnerable to several environmental insults than are adults [34-35]. The reality is that children throughout the world are using mobile phones at increasing rates and for long durations. Therefore, if there are real risks, and especially if children are more susceptible, we may be facing an epidemic of brain and other cancers. The concern is increased because, to date, there are few warnings to parents and physicians advising restrictions on use of mobile phones by children. While the evidence at present is certainly less than total proof of a relationship between exposure and elevated risk of cancer, it is sufficiently strong so as to demand precaution. The alternative may be significant increases in certain cancers, especially leukemia and brain cancer. It is not clear whether other kinds of cancer are also at increased risk following exposure, since there has not been much study of, for example, the possible health hazards of wearing a cell phone on your belt and pelvic cancers.

Fortunately, the rates of leukemia and brain cancer are not high. There have recently been significant improvements in treatment of leukemia, especially among children. Kundi [21] has hypothesized that use of mobile phones may increase rates of some forms of brain cancer, such as gliomas, by as much as 50%. Even if this is true, this certainly does not mean that every exposed person will develop brain cancer. Such an increase in brain cancer would still have a significant impact, not only on the individuals affected but also on society, especially given that much of this increase is likely to occur among young people.

Application of the Precautionary Principle is appropriate under the circumstances where there is a demonstrated elevation in rates of serious diseases in humans following elevated EMF exposure, but has many unanswered questions as to mechanisms responsible. We need additional research, of course, and much better exposure assessment. The evidence that we have at present is too convincing to be ignored. Our national and international standards are obsolete, and ignore evidence reported by many different investigators. The lack of certainty with regard to mechanisms and animal models is no reason to ignore studies of human health. Similar lack of certainty regarding mechanisms also exists for some chemicals, yet precautionary measures are commonly taken to reduce exposure. We need the electric and communications industries to be proactive in developing products that can be used with reduced exposures. We need governments and international organizations to set standards that are based on the evidence of whether there are hazards to humans, not on a hypothesis that is not

credible based on the evidence from animal and cellular studies. Most importantly, we need individuals to understand that personal decisions will significantly impact the level to which they are exposed to both ELF and RF EMFs.

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BioInitiative Author; Statement of Prof. Martin Blank PhD. PhD.; 2016

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January 28, 2016

Declaration by MARTIN BLANK, PhD

1. My name is Martin Blank PhD. Given my expertise as a cellular biologist and experience as a professor and researcher having authored over 90 scientific papers and a recent book "Overpowered" regarding electromagnetic fields effects on cells, I strongly recommend that the City of Los Angeles, the Mayor, the City Council and the Office of the City Attorney **NOT** proceed with the Citywide WiFi/Citylink LA program due to the damage to public health. The research attached and cited shows that, at the power levels required for WiFi to operate reliably over the project's large areas, the Radio Frequency (RF) Radiation will have significant biological health effects, especially on electrosensitive individuals and children. Many research studies have documented the damage that will result from the ongoing exposure to electromagnetic fields and corresponding Radio Frequency Radiation emitted by the wireless transmitters used by Citywide WiFi. The radiation may not cause thermal or heating effects but will certainly cause non-thermal biological effects that are not being accounted for, and are not protected by our current FCC safety standards.

From 1962 to 2011, I was a professor in the Department of Physiology and Cellular Biophysics at Columbia University, New York, NY where I both taught and conducted research. Currently, I am a Special Lecturer in that department.

My formal education included a Bachelor of Science degree (Magna Cum Laude) in Chemistry from City College of New York, a PhD in Physical Chemistry from Columbia University, and a PhD in Colloid Science from Cambridge University, England. The focus in the Colloid Science department, under the direction of Professor F.J. W. Roughton was on the electrical properties of biological surfaces and membranes. This provided unique training for research on electric and magnetic field effects in biological cells.

My research has focused on living cells, their components (e.g. DNA, proteins, ions, electrons) and their interactions with the environment. The research (detailed in my Curriculum Vitae in Attachment 1) has concentrated on electric and magnetic field effects on electron transfer reactions, enzymes, DNA and fluxes in the ion channels of excitable membranes. This entailed determining how electrically charged components (ions and electrons) of cells are affected by external fields. Studies of electric field effects on proteins, lipids and ions provide insight into the effects of electric and magnetic fields (EMF) on cells in living organisms.

2. To render a professional opinion regarding the health risks associated with exposure to EMF (Electromagnetic Fields) from many sources, including ELF (extremely low frequency) from power lines, and RF (radio frequency) from cell phones, WiFi, smart meters, etc. I have reviewed the relevant information and commented on the reported harmful effects, as well as protective biological reactions of cells to this un-natural (i.e., man-made) radiation in the environment.

3. In addition to teaching and research, I have been involved in EMF related activities for many years. I served as President of the Bioelectromagnetics Society 1989-1990, and was selected to open the First Congress of the European Bioelectromagnetics Association in Belgium in 1992. I was Editor-in-Chief of the First World Congress on "Electricity and Magnetism in Biology and Medicine" proceedings, and Plenary Lecturer on Bioelectromagnetics for international conferences in Brazil, Canada, India, Israel, Italy and Japan. I also served on the "Bioelectrochemistry and Bioenergetics" Editorial Board and as Biology Divisional Editor of the Journal of the Electrochemical Society for thirteen years. In 2015, I was a consultant for the Canadian Parliament regarding EMF safety standards, and was spokesperson for the over 200 published EMF scientists who petitioned the UN and the World Health Organization (W.H.O.) regarding the strong scientific evidence showing the need for stricter control of EMF exposure to protect the public. (See Attachment 2)

I was one of the organizers of the Bioinitiative Report (BIR) and wrote online reviews on the Cellular Stress Response in both 2007 and 2012 editions (See

Attachments 3 and 4). The most recent report, http://www.bioinitiative.org/freeaccess/report/docs/report.pdf, summarizes over 1800 recent epidemiology studies, as well as cell and molecular biology research (See Attachment 5). A key summary from the report states:

> '**Bioeffects are clearly established and occur at very low levels of exposure to electromagnetic fields and radiofrequency radiation**. **Bioeffects can occur in the first few minutes of exposure to power lines as well as at levels associated with cell and cordless phone use.** Bioeffects can also occur from just minutes of exposure to mobile phone masts (cell towers), WiFi, and wireless utility 'smart' meters. Chronic base station level exposures can result in illness.'

I recently published **Overpowered (2014)**, a book to introduce the public to the potentially harmful effects of EMF in the environment and how to protect oneself.

4. Based on a wide range of research studies, I conclude that:

- Electricity and magnetism are fundamental forces that interact with charged particles, i.e., primarily with electrons in our cells. The organism, in reaction to these conditions, produces **the cellular stress response, a DNA mechanism that is activated by many potentially harmful stimuli** (e.g., high and low temperature, changes in pH, toxic metals). In other words, ***cells react to EMF as potentially harmful***.
- Stress protein synthesis starts with activation of DNA. **Higher RF-EMF levels can cause chemical changes in DNA that lead to mutations and cancer** and other abnormal biological processes (e.g., development and growth of tumors).
- **Biological systems are affected by a wide range of EMF frequencies, including ELF, RF and MW (microwave) ranges.** Because of the many sources in the environment (cell phone towers, WiFi, smart meters) the effects are additive. Unfortunately, the divisions of the EM (electromagnetic) spectrum were created by engineers and physicists who assumed arbitrary frequency boundaries that do not relate to the biology. **Human cells do not recognize EM spectrum divisions. They react to electromagnetic fields across the spectrum**.

- Furthermore, the same engineers and physicists assumed that the biological response was caused by the energy of the EMF stimulus, and could be measured by an increase in temperature. ***The biological response is stimulation of stress protein synthesis in DNA, and the stress response occurs across the EM spectrum.*** **When stress protein synthesis is stimulated by EMF, the body is essentially telling us that exposure is harmful to living cells.**
- ***The stress protein synthesis occurs*** **at field strength and duration thresholds that are very low and *below the temperature-based thresholds set by safety standards.* (This is especially true in the ELF range where epidemiology studies indicate increased risk of leukemia at 3-4mG and the U.S. Standards are at 1000mG)** This means that cells in the body respond at very low exposure levels.

Because cells activate the stress response to a wide range of EMF frequencies, this reaction would appear to be highly relevant to the setting of safety standards. However, the stress response has been ignored in the setting of safety standards. **Safety standards have been set based on the ability of EMF to heat tissue!** Both non-thermal and thermal EMF signals activate the stress response, (See Attachment 6 - Blank, Goodman. 2004) but thresholds triggering stress on biological systems occur at levels on the order of 0.5 to 1.0 μT (5-10 mG) for ELF, **thousands of times lower energy than the 'safe levels' in the RF range.** However, this information has not been included in prior scientific reviews because **insufficient attention was paid to the relevant cell biology**.

5. The stress response has provided vital evidence about cellular defense mechanisms – it shows that the reaction starts when EMF interacts with DNA.

Protein synthesis begins when the two chains of DNA come apart and make an mRNA copy of the amino acid code (that is in the DNA composition) for a particular protein. This normally is initiated when a particular chemical stimulus (transcription factor) binds to a specific DNA, and in forming a bond changes the electron distribution. Research has shown electron conduction in DNA (See Attachment 7 - Wan et al, 1999)

enables communication along the molecule, and so EMF affects electron distribution and movement in DNA and enables the two chains of the double helix to come apart to initiate protein synthesis. During this coming apart, along with normal functioning, abnormal processes can also occur (See Attachment 8 - Blank, Goodman. 2001).

Several studies have reported both single and double strand breaks in the DNA 'double helix', and other chromosome damage after exposure to extremely low frequency (ELF) fields (See Attachment 9 - Lai, Singh. 1997). Similar malfunction has also been reported after exposure to higher frequency, radiofrequency (RF) fields. The REFLEX Project, a collaboration of twelve laboratories in the European Union (Attachment 10 - REFLEX. 2004), found that **both ELF and RF exposures modified the expression of many genes and proteins well below the safety limits**.

For a long time, agencies such as ICNIRP (International Commission on non-Ionizing Radiation Protection) and WHO maintained that an EMF must increase the temperature in order to cause changes in cells. Many lines of research now point to changes in DNA without elevation of temperature. The thresholds for a number of biological systems are shown in Table 1 (below), and many are in the range of 0.5 to 1.0 μT (5-10mG), not very much higher than the usual environmental backgrounds of ~0.1μT (1mG). The effects occur in basic cellular systems at relatively low field strengths, similar to those in the environment. **Non-thermal ELF and RF fields can cause DNA damage, and therefore represent health and safety concerns**.

Table 1. Cells React to Very Low EMF (well below safety limit)

Biological System	Threshold	Reference
Stress proteins in cells		
HL60, Sciara, yeast	**<8mG**	**Goodman, Blank, 1998**
breast (HTB124, MCF7)	**<8mG**	**Lin et al, 1999**
chick embryo (anoxia)	**~20mG**	**DiCarlo et al, 1999**
Accelerate electron transfer		
Na,K-ATPase	**2-3mG**	**Blank & Soo, 2001**
cytochrome oxidase	**5-6mG**	**Blank & Soo, 2001**
ornithine decarboxylase	**~20mG**	**Litovitz et al, 1991**

Belousov-Zhabotinsky	**<5mG**	**Blank & Soo, 2003**
Disease related		
leukemia epidemiology	**3-4mG**	**Ahlbom et al, 2000**
		Greenland et al, 2000
ELF Safety Limit	**1000mG**	**ICNIRP, 1997**

All of the reported thresholds are well below the safety limit!!!

In the RF range, research by Lai and Singh (1997) and Litovitz et al. (1991) have shown similar effects (See Attachments 9 and 11). As articulated by the Bioinitiative Report citing over 130 sources showing Radio Frequency radiation emitted by wireless transmitters has biological effects at levels millions of times lower than current FCC Safety Standards (see Attachment 22 Reported Biological Effects from RFR at Low-Intensity Exposure). In addition to very low thresholds, exposure durations in the RF range do not have to be very long to be effective.

Table 2. Cells React to Very Low RF (well below safety limit). Excerpted from the Bioinitiative Report 2012 (see Attachment 23 for the full chart)

Power Density (microWatts/cm^2)	Observed Effects	Reference
As low as (10^{-13}) or 100 femtowatts/cm2	Super-low intensity RFR effects at MW resonant frequencies resulted in changes in genes; problems with chromatin conformation (DNA)	Belyaev, 1997
0.00034 uW/cm2	Chronic exposure to mobile phone pulsed RF significantly reduced sperm count,	Behari, 2006
0.0005 uW/cm2	RFR decreased cell proliferation at 960 MHz GSM 217 Hz for 30-min exposure	Velizarov, 1999
0.0006 - 0.0128 uW/cm2	Fatigue, depressive tendency, sleeping disorders, concentration difficulties, cardio-vascular problems reported with exposure to GSM 900/1800 MHz cell phone signal at base station level exposures.	Oberfeld, 2004
0.003 - 0.02 uW/cm2	In children and adolescents (8-17 yrs) short-term exposure caused headache, irritation, concentration difficulties in school.	Heinrich, 2010

0.003 to 0.05 uW/cm2	In children and adolescents (8-17 yrs) short-term exposure caused conduct problems in school (behavioral problems)	Thomas, 2010
0.005 uW/cm2	In adults (30-60 yrs) chronic exposure caused sleep disturbances, (but not significantly increased across the entire population)	Mohler, 2010
0.005 - 0.04 uW/cm2	Adults exposed to short-term cell phone radiation reported headaches, concentration difficulties (differences not significant, but elevated)	Thomas, 2008
0.006 - 0.01 uW/cm2	Chronic exposure to base station RF (whole-body) in humans showed increased stress hormones; dopamine levels substantially decreased; higher levels of adrenaline and nor-adrenaline; dose-response seen; produced chronic physiological stress in cells even after 1.5 years.	Buchner, 2012
1000 uW/cm^2	FCC RF Safety Limit	

Litovitz et al (1991), working with the enzyme ornithine decarboxylase, have shown **a full response to EMF when cells were exposed for only 10 sec** (See Attachment 11)**.** This occurred with ELF sine waves or ELF modulated 915 MHz sine waves. (915MHz is RF but the ELF modulation was effective!) Kultz (2005) summarized the evidence that specific groups of genes are activated along with stress genes and are involved in sensing and repairing damage to DNA and proteins.

The stress response is a natural defense mechanism activated by molecular damage caused by environmental forces. The response involves reaction with DNA, i.e., stimulating stress genes as well as genes that sense and repair damage to DNA and proteins. At high EMF intensities, the interaction with DNA can lead to DNA strand breaks that can result in mutations, an initiating step in the development of cancer. (See Attachment 12 - Blank, Electromagnetic Biology and Medicine, 2008).

EMF have been shown to cause other potentially harmful biological effects, such as leakage of the blood brain barrier that can lead to damage of neurons in the brain,

increased micronuclei (DNA fragments) in human blood lymphocytes, all at exposures well below the limits in the current FCC guidelines in the US.

In summary, the human health consequences of long-term exposure to high EMF levels lead to molecular damage, including DNA. If the molecular damage is not fully repaired and the damaged cells are not eliminated by apoptosis (cell suicide), the diseases that are most likely to develop are: (a) cancer, primarily leukemia in children and breast cancer in women; (b) neurodegenerative diseases such as Alzheimer's disease and ALS; (c) immunological disorders, including electrohypersensitivity (EHS).

6. Epidemiology studies

Epidemiology research, that is, research on large populations over time, has served as a key guide for EMF policy on health risks associated with ELF (power lines) and RF (cell phones). These studies, which show the effects of long term exposures demonstrate quantitative dose-response relations (i.e., the health effects are proportional to the EMF dose).

The paper published in 1979 by Wertheimer and Leeper (1979) showed a dose-response link between EMF and leukemia (See Attachment 13). Since then, there have been many studies on the relation between EMF and human disease. Among the key studies are two pooled analyses by Greenland et al (2000) and Ahlbom et al (2000) which confirmed a statistically significant doubling of the risk of leukemia in children when exposures exceed 3-4mG (See Attachments 14 and 15). The link between DNA damage and development of cancer is further supported by Yang et al (2009) who correlated **a significantly increased risk of leukemia in children with a deficiency in DNA repair genes** (See Attachment 16). (i.e., when repair genes were present, they appear to be able to repair some of the damage and prevent disease.)

Dr. Neil Cherry found strong corroborating evidence for these effects in the archives of public health statistics of all childhood cancers around the Sutro Broadcasting Tower in San Francisco between the years 1937 and 1988. The **50 years of data from the archives** involved a total of 123 cases of childhood cancer from a population of 50,686 children, and included 51 cases of leukaemia, 35 cases of brain cancer and 37 cases of lymphatic cancer. The risk ratio (RR) for all childhood cancers was elevated in

the area studied. **The risk declined with radial distance from the antennas, but it was still above a risk ratio of 5 even at a distance of 3km where the field was measured to be 1μW/cm^2, comparable to what has been measured near cellphone towers (See Attachment 17). (Similar results have been reported around RF broadcasting antennas in Sydney, Australia and Rome, Italy, and there are now studies of effects of cellphones on brain cancer and cancer of the salivary glands.)**

There is also evidence that EMF plays a role in breast cancer in women by inhibiting the ability of normally secreted melatonin to slow the growth of breast cancer cells. Liburdy et al. (1993) showed that the threshold for inhibiting melatonin lies between 2-12mG (See Attachment 18).

Inhibition of melatonin secretion by the pineal gland is also associated with sleep disorders and disturbances of the immune system through various allergic and inflammatory responses and effects on tissue repair processes. The pineal gland also secretes serotonin, and a deficiency in serotonin due to EMF is also associated with insomnia, as well as memory and mood disorders.

In addition to the risk of cancer and effects on the immune system, Huss et al (2009) found an increased risk of Alzheimer's disease and death from neurodegenerative diseases for people who live within 50 meters of 220-380 kV power lines compared with people who live 600 meters or more, where the fields were about 1mG. **The estimated fields at 50 meters for the 220kV line are about 5mG and for the 380kV line are about 8mG. The fields would be very much higher at 20 meters.** (See Attachment 19)

After reviewing the full range of studies, the International Agency for Research on Cancer (IARC) in 2002 found that there is reliable scientific evidence that EMFs in the ELF range are a possible human carcinogen (In 2011, IARC made a similar evaluation regarding the RF range.) (See Attachments 20 and 21). Since 2002, additional evidence has supported the IARC statement. Hence, like the cell biology studies, epidemiological studies show adverse biological changes on exposure to EMF. The EMF interactions with DNA and the low levels at which these reactions occur offer a plausible mechanism connecting environmental exposure and human carcinogenesis (See Attachment 12 - Blank, Electromagnetic Biology and Medicine, 2008).

7. Mechanism of EMF Interaction with DNA as a Fractal Antenna

The responses of deoxyribonucleic acid (DNA) to electromagnetic fields (EMF) in different frequency ranges can be understood in terms of the double helical structure of the DNA and the electronic conduction within the DNA molecule and its compact structure in the nucleus. Human DNA is 2 meters long and it is coiled many fold in order to fit into a nucleus that is only microns in size. The need to fit into this cramped space results in the DNA being coiled many times, and a molecule having electron conduction paths of many different lengths. The many different lengths mean that the DNA can act as an antenna that is sensitive to many non-ionizing frequencies in the extremely low frequency (ELF) and radio frequency (RF) ranges.

The wide frequency range of interaction with EMF is the functional characteristic of a fractal antenna, and **DNA appears to possess the two structural characteristics of fractal antennas, electronic conduction and self symmetry. These properties contribute to greater reactivity of DNA with EMF in the environment, and the DNA damage could account for increases in cancer epidemiology**, as well as variations in the rate of chemical evolution in early geologic history.

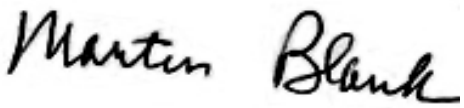
8. The **Growing Presence of EMF in the Environment**

All of the studies cited above occurred when EMF levels were lower than they are today. Increasingly, people are exposed to a much wider range of EMF as a result of advancing technological developments, such as cell phones, WiFi, smart meters, radiation from installation of cell towers, etc. Also, the scientists (primarily engineers and physicists) who set the divisions of the EM spectrum, selected frequency boundaries that do not relate to the biology. For example, they incorrectly assumed that the only dangerous range was EMF that caused the body temperature to increase. Human cells do not recognize EM spectrum divisions. The same biological reactions (including the cellular stress response), can be stimulated in more than one subdivision of the EM spectrum and in subdivisions that do not cause temperature increases.

There are now sufficient scientific data about the biological effects of EMF to limit human exposure. We can state unequivocally that EMF can cause damage (single and double strand breaks) to DNA at exposure levels that are considered safe under the FCC guidelines in the USA (See Attachment 9 - Lai and Singh, 1997). Further, these

guidelines do not take into account the accumulation of changes or mutations in DNA that occur with prolonged exposure—and the actual use of the various devices involves prolonged exposure, indeed increasingly prolonged exposure.

In conclusion, given my expertise as a cellular biologist and experience as a professor and researcher authoring over 90 scientific papers and a recent book "Overpowered" regarding EMF effects on the cells, I strongly recommend that the city of Los Angeles, the Mayor, the City Council and the office of the City Attorney **NOT** proceed with the Citywide WiFi/Citylink LA program due to the damage to public health, that will result from the ongoing exposure to electromagnetic fields and corresponding Radio Frequency Radiation emitted by the wireless transmitters used by citywide WiFi. The proposed system may not cause thermal or heating effects but will certainly cause non-thermal biological effects that are not being accounted for or protected by our current FCC safety standards. It is clear that the safety standards must be revised to take into account the potentially harmful non-thermal biological processes that occur. I'm available for further consultation or questions.



Martin Blank, PhD.

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FCC 13-39

Before the Federal Communications Commission

Washington, D.C. 20554

In the Matter of

Reassessment of Federal Communications Commission Radiofrequency Exposure Limits and Policies	)	ET Docket No. 13-84
Proposed Changes in the Commission's Rules Regarding Human Exposure to Radiofrequency Electromagnetic Fields	)	ET Docket No. 03-137

To: Office of the Secretary
Federal Communications Commission , Washington, DC 20554

As officially presented in the Federal Register/ Vol. 78, No. 107 / Tuesday, June 4, 2013 / Proposed Rules. Federal Communications Commission, 47 CFR Parts 1, 2, 15, 24, 25, 27, 73, 90, 95, 97, and 101 [ET Docket Nos. 03–137 and 13–84; FCC 13–39], Reassessment of Exposure to Radiofrequency Electromagnetic Fields Limits and Policies, Federal Communications Commission

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1. New, biologically-based public exposure standards should be developed under the direction of experts in the biological effects and adverse health effects of chronic exposures to radiofrequency electromagnetic radiation (RFR), drawing upon the substantial international body of scientific and public health literature, and not be limited to individuals in electrical and electronic engineering.

2. A rapidly accumulating body of scientific evidence of harm to health and well-being constitute warnings that adverse health effects can occur with prolonged exposures to very low-intensity EMF at biologically active frequencies or frequency combinations.

3. The BioInitiative 2012 Report reports biological effects at exposure levels significantly below the 2007 recommended goal of 0.1 uW/cm2. Since 2007, five new studies of base-station level RFR at intensitites ranging from less than 0.001 uW/cm2 to 0.05 uW/cm2 report headaches, concentration difficulties and behavioral problems in children and adolescents; and sleep disturbances, headaches and concentration problems in adults. Exhibit A presents some representative studies (peer-reviewed and published in reputable scientific journals) that report biological effects and adverse health effects at levels that are clearly non-thermal (low-intensity). New biologically-based public exposure limits are critically needed in light of the vast rollout of wireless technologies that expose billions of people globally to elevated, artificial RFR (particularly pulsed RFR) in daily life. These studies are representative of several thousand studies over four decades that constitute emerging scientific evidence of risk to very low-intensity RFR with chronic exposure.

4. As new studies are completed and published on the effects of chronic, low-intensity RFR exposure across populations (from cell towers and wireless devices, for example) the results indicate adverse health impacts occur from on-going disruption of normal metabolism, endocrine function, male fertility parameters, fetal brain development, immune function, mental abilities, electrophysiology, and neural synchrony. Disruption of basic neural function due to artificial EMF/RFR exposures can disrupt weak-field effects that are necessary to guide non-linear biological oscillations and other cellular communications necessary for normal biological functioning, and result in unacceptable burdens on human health.

5. Evidence for Damage to Sperm and Reproduction

Evidence for damage to sperm and male reproduction parameters include adverse effects on sperm quality, motility and pathology in men who use and particularly those who wear a cell phone, PDA or pager on their belt or in a pocket (Agarwal et al, 2008; Agarwal et al, 2009; Wdowiak et al, 2007; De Iuliis et al, 2009; Fejes et al, 2005; Aitken et al, 2005; Kumar, 2012). Other studies conclude that usage of cell phones, exposure to cell phone radiation, or storage of a mobile phone close to the testes of human males affect sperm counts, motility, viability and structure (Aitken et al, 2004; Agarwal et al, 2007; Erogul et al, 2006). Animal studies have demonstrated oxidative and DNA damage, pathological changes in the testes of animals, decreased sperm mobility and viability, and other measures of deleterious damage to the male germ line (Dasdag et al, 1999; Yan et al, 2007; Otitoloju et al, 2010; Salama et al, 2008; Behari et al, 2006; Kumar et al, 2012). There are fewer animal studies that have studied effects of cell phone radiation on female fertility parameters. Panagopoulous et al (2012) report decreased ovarian development and size of ovaries, and premature cell death of ovarian follicles and nurse cells in *Drosophila melanogaster*. Gul et al (2009) reported rats exposed to stand-by level RFR (phones on but not transmitting calls) had a decrease in the number of ovarian follicles in pups born to these exposed dams. Magras and Xenos (1997) reported irreversible infertility in mice after five (5) generations of exposure to RFR at cell phone tower exposure levels of less than one

microwatt per centimeter squared (μW/cm2). See www.bioinitiative.org Section 18 for references.

HUMAN SPERM AND THEIR DNA ARE DAMAGED

Human sperm are damaged by cell phone radiation at very low intensities (0.00034 – 0.07 μW/cm2). Many new studies in the last decade report sperm damage in humans and animals, leading to substantial concerns for fertility, reproduction and health of the offspring (unrepaired de novo mutations in sperm). Exposure levels are similar to those resulting from wearing a cell phone on the belt, or in the pants pocket, or using a wireless laptop computer on the lap. Sperm lack the ability to repair DNA damage.

6. Evidence for Brain Tumors

Based on epidemiological studies there is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of mobile phones and cordless phones. The evidence comes mainly from two study centres, the Hardell group in Sweden and the Interphone Study Group. No consistent pattern of an increased risk is seen for meningioma. A systematic bias in the studies that explains the results would also have been the case for meningioma. The different risk pattern for tumor type strengthens the findings regarding glioma and acoustic neuroma. Meta-analyses of the Hardell group and Interphone studies show an increased risk for glioma and acoustic neuroma. Supportive evidence comes also from anatomical localisation of the tumor to the most exposed area of the brain, cumulative exposure in hours and latency time that all add to the biological relevance of an increased risk. In addition risk calculations based on estimated absorbed dose give strength to the findings. See www.bioinitiative.org Section 11 for references.

- There is reasonable basis to conclude that RF-EMFs are bioactive and have a potential to cause health impacts.
- There is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of wireless phones (mobile phones and cordless phones) mainly based on results from case-control studies from the Hardell group and Interphone Final Study results.
- Epidemiological evidence gives that RF-EMF should be classified as a human carcinogen.
- The existing FCC/IEE and ICNIRP public safety limits and reference levels are not adequate to protect public health based on evidence for brain tumors and RFR exposure.
- New public health standards and limits are needed.

7. Evidence for Adverse Fetal and Neonatal Effects

Effects on the developing fetus from in-utero exposure to cell phone radiation have been observed in both human and animal studies since 2006. Sources of fetal and neonatal exposures of concern include cell phone radiation (both paternal use of wireless devices worn on the body and maternal use of wireless phones during pregnancy). Sources include exposure to whole-body RFR from base stations and WI-FI, use of wireless laptops, use of incubators for newborns with excessively high ELF-EMF levels resulting in altered heart rate variability and reduced melatonin levels in newborns, fetal exposures to MRI of the pregnant mother, and greater susceptibility to

leukemia and asthma in the child where there have been maternal exposures to ELF-EMF. Divan et al (2008) found that children born to mothers who used cell phones during pregnancy develop more behavioral problems by the time they have reached school age than children whose mothers did not use cell phones during pregnancy. Children whose mothers used cell phones during pregnancy had 25% more emotional problems, 35% more hyperactivity, 49% more conduct problems and 34% more peer problems (Divan et al, 2008). Aldad et al (2012) showed that cell phone radiation significantly altered fetal brain development and produced ADHD-like behavior in the offspring of pregnant mice. Exposed mice had a dose-dependent impaired glutamatergic synaptic transmission onto Layer V pyramidal neurons of the prefrontal cortex. The authors conclude the behavioral changes were the result of altered neuronal developmental programming in utero. Offspring mice were hyperactive and had impaired memory function and behavior problems, much like the human children in Divan et al (2008). Fragopoulou et al (2012) reports that brain astrocyte development followed by proteomic studies is adversely affected by DECT (cordless phone radiation) and mobile phone radiation.
See www.bioinitiative.org Section 19 and 20 for references.

Fetal (in-utero) and early childhood exposures to cell phone radiation and wireless technologies in general may be a risk factor for hyperactivity, learning disorders and behavioral problems in school.

8. Evidence for Effects on Autism (Autism Spectrum Disorders)

"*Autism spectrum disorder (ASD), the fastest-growing complex neurodevelopment disorder, continues to rise in its prevalence, now affecting up to 1 in 50 children in the USA, and averaging 1% globally, according to the latest CDC report. More children will be diagnosed with ASD this year than with AIDS, diabetes & cancer combined in the USA.* ***ASD costs the nation $137 billion a year and this debt is expected to increase in the next decade.*** *Hence, ASD has become a huge healthcare burden and global threat, categorized by the CDC as a national public health crisis*." (Special Issue on Autism, North American Journal of Medicine and Science, Vol 6, Issue 3, July 2013, Harvard Medical School).

Several thousand scientific studies over four decades point to serious biological effects and health harm from EMF and RFR. These studies report genotoxicity, single-and double-strand DNA damage, chromatin condensation, loss of DNA repair capacity in human stem cells, reduction in free-radical scavengers (particularly melatonin), abnormal gene transcription, neurotoxicity, carcinogenicity, damage to sperm morphology and function, effects on behavior, and effects on brain development in the fetus of human mothers that use cell phones during pregnancy. Cell phone exposure has been linked to altered fetal brain development and ADHD-like behavior in the offspring of pregnant mice.

Many disrupted physiological processes and impaired behaviors in people with ASDs closely resemble those related to biological and health effects of EMF/RFR exposure. Biomarkers and indicators of disease and their clinical symptoms have striking similarities. At the cellular and molecular level many studies of people with ASDs have identified oxidative stress and evidence of free-radical damage, as well as deficiencies of antioxidants such as glutathione. Elevated intracellular calcium in ASDs can be associated with genetic mutations but more often may be downstream of inflammation or chemical exposures. Lipid peroxidation of cell membranes, disruption of calcium metabolism, altered brain wave activity and consequent sleep, behavior and

immune disfunction, pathological leakage of critical barriers between gut and blood or blood and brain may also occur. Mitochondria may function poorly, and immune system disturbances of various kinds are common. Changes in brain and autonomic nervous system electrophysiology can be measured and seizures are far more common in ASCs than in the population at large. Sleep disruption and high levels of stress are close to universal in ASCs. All of these phenomena have also been documented to result from or be modulated by EMF/RFR exposure. Reducing or removing EMF and wireless RFR stressors from the environment is a reasonable precautionary action given the overall weight of evidence for a link to ASDs. The FCCs thermal safety limits do not address low-intensity (non-thermal) effects. The evidence is now overwhelming that limiting exposures to those causing thermal injury alone does not address the much broader array of risks and harm now clearly evident with chronic exposure to low-intensity (non-thermal) EMF/RFR. The now well-documented genotoxic impacts of EMF/RFR, placed in parallel with the huge rise in reported cases of ASCs as well as with the de novo mutations associated with some cases of ASCs (as well as other conditions), make it urgent to address the issue of (environmental) acquired as well as inherited genetic damage. With the rising numbers people with ASCs and other childhood health and developmental disorders, and with emerging evidence that EMF/RFR is a preventable environmental exposure of consequence to ASCs; public safety limits must be rethought in terms of fetal, neonatal and childhood neurological and electrophysiological development. The evidence is sufficient to warrant new public exposure standards benchmarked to low-intensity (non-thermal) exposure levels causing biological disruption and strong, interim precautionary practices are advocated. See www.bioinitiative.org Section 20 for references.

9. FCC Dockets 13-84, 03-137 and 13-39 propose to significantly relax rather than tighten exposure standards, in stark contrast to what the scientific evidence suggests is needed to protect public health from RFR. IEEE/FCC public safety limits remain unchanged and are still inadequate and obsolete with respect to prolonged, low-intensity NIER exposures.

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Exhibit A

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure (Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities (Pages 1 – 11)

http://www.bioinitiative.org/rf-color-charts/ DOWNLOAD RF Color Charts

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

Power Density (Microwatts/centimeter2 - uW/cm2)		Reference
As low as (10^{-13}) or 100 femtowatts/cm2	Super-low intensity RFR effects at MW reasonant frequencies resulted in changes in genes; problems with chromatin conformation (DNA)	Belyaev, 1997
5 picowatts/cm2 (10-^{12})	Changed growth rates in yeast cells	Grundler, 1992
0.1 nanowatt/cm2 (10-^{10}) or 100 picowatts/cm2	Super-low intensity RFR effects at MW reasonant frequencies resulted in changes in genes; problems with chromatin condensation (DNA) intensities comparable to base stations	Belyaev, 1997
0.00034 uW/cm2	Chronic exposure to mobile phone pulsed RF significantly reduced sperm count,	Behari, 2006
0.0005 uW/cm2	RFR decreased cell proliferation at 960 MHz GSM 217 Hz for 30-min exposure	Velizarov, 1999
0.0006 - 0.0128 uW/cm2	Fatigue, depressive tendency, sleeping disorders, concentration difficulties, cardio- vascular problems reported with exposure to GSM 900/1800 MHz cell phone signal at base station level exposures.	Oberfeld, 2004
0.0009 uW/cm2	RFR induced 10%-40% increase in DNA synthesis in glioma cells (brain)	Stagg, 1997
0.003 - 0.02 uW/cm2	In children and adolescents (8-17 yrs) short-term exposure caused headache, irritation, concentration difficulties in school.	Heinrich, 2010
0.003 to 0.05 uW/cm2	In children and adolescents (8-17 yrs) short-term exposure caused conduct problems in school (behavioral problems)	Thomas, 2010
0.005 uW/cm2	In adults (30-60 yrs) chronic exposure caused sleep disturbances, (but not significantly increased across the entire population)	Mohler, 2010
0.005 - 0.04 uW/cm2	Adults exposed to short-term cell phone radiation reported headaches, concentration difficulties (differences not significant, but elevated)	Thomas, 2008
0.006 - 0.01 uW/cm2	Chronic exposure to base station RF (whole-body) in humans showed increased stress hormones; dopamine levels substantially decreased; higher levels of adrenaline and nor-adrenaline; dose-response seen; produced chronic physiological stress in cells even after 1.5 years.	Buchner, 2012
0.01 - 0.11 uW/cm2	RFR from cell towers caused fatigue, headaches, sleeping problems	Navarro, 2003

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effeccts	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

Power Density (Microwatts/centimeter2 - uW/cm2)		Reference
0.01 - 0.05 uW/cm2	Adults (18-91 yrs) with short-term exposure to GSM cell phone radiation reported headache, neurological problems, sleep and concentration problems.	Hutter, 2006
0.005 - 0.04 uW/cm2	Adults exposed to short-term cell phone radiation reported headaches, concentration difficulties (differences not significant, but elevated)	Thomas, 2008
0.015 - 0.21 uW/cm2	Adults exposed to short-term GSM 900 radiation reported changes in mental state (e.g., calmness) but limitations of study on language descriptors prevented refined word choices (stupified, zoned-out)	Augner, 2009
0.05 - 0.1 uW/cm2	RFR linked to adverse neurological, cardio symptoms and cancer risk	Khurana, 2010
0.05 - 0.1 uW/cm2	RFR related to headache, concentration and sleeping problems, fatigue	Kundi, 2009
0.07 - 0.1 uW/cm2	Sperm head abnormalities in mice exposed for 6-months to base station level RF/MW. Sperm head abnormalities occurred in 39% to 46% exposed mice (only 2% in controls) abnormalities was also found to be dose dependent. The implications of the pin-head and banana-shaped sperm head. The occurrence of sperm head observed increase occurrence of sperm head abnormalities on the reproductive health of humans living in close proximity to GSM base stations were discussed."	Otitoloju, 2010
0.38 uW/cm2	RFR affected calcium metabolism in heart cells	Schwartz, 1990
0.8 - 10 uW/cm2	RFR caused emotional behavior changes, free-radical damage by super-weak MWs	Akoev, 2002
0.13 uW/cm2	RFR from 3G cell towers decreased cognition, well-being	Zwamborn, 2003
0.16 uW/cm2	Motor function, memory and attention of school children affected (Latvia)	Kolodynski, 1996
0.168 - 1.053 uW/cm2	Irreversible infertility in mice after 5 generations of exposure to RFR from an 'antenna park'	Magras & Zenos, 1997
0.2 - 8 uW/cm2	RFR caused a two-fold increase in leukemia in children	Hocking, 1996
0.2 - 8 uW/cm2	RFR decreased survival in children with leukemia	Hocking, 2000
0.21 - 1.28 uW/cm2	Adolescents and adults exposed only 45 min to UMTS cell phone radiation reported increases In headaches.	Riddervold, 2008

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effeccts	Sleep, neuron firing rate, EEG, memory, learning, behavior
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Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

Power Density (Microwatts/centimeter2 - uW/cm2)		Reference
0.5 uW/cm2	Significant degeneration of seminiferous epithelium in mice at 2.45 GHz, 30-40 min.	Saunders, 1981
0.5 - 1.0 uW/cm2	Wi-FI level laptop exposure for 4-hr resulted in decrease in sperm viability, DNA fragmentation with sperm samples placed in petri dishes under a laptop connected via WI-FI to the internet.	Avendano, 2012
1.0 uW/cm2	RFR induced pathological leakage of the blood-brain barrier	Persson, 1997
1.0 uW/cm2	RFR caused significant effect on immune function in mice	Fesenko, 1999
1.0 uW/cm2	RFR affected function of the immune system	Novoselova, 1999
1.0 uW/cm2	Short-term (50 min) exposure in electrosensitive patients, caused loss of well-being after GSM and especially UMTS cell phone radiation exposure	Eltiti, 2007
1.3 - 5.7 uW/cm2	RFR associated with a doubling of leukemia in adults	Dolk, 1997
1.25 uW/cm2	RFR exposure affected kidney development in rats (in-utero exposure)	Pyrpasopoulou, 2004
1.5 uW/cm2	RFR reduced memory function in rats	Nittby, 2007
2 uW/cm2	RFR induced double-strand DNA damage in rat brain cells	Kesari, 2008
2.5 uW/cm2	RFR affected calcium concentrations in heart muscle cells	Wolke, 1996
2 - 4 uW/cm2	Altered cell membranes; acetycholine-induced ion channel disruption	D'Inzeo, 1988
4 uW/cm2	RFR caused changes in hippocampus (brain memory and learning)	Tattersall, 2001
4 - 15 uW/cm2	Memory impairment, slowed motor skills and retarded learning in children	Chiang, 1989
5 uW/cm2	RFR caused drop in NK lymphocytes (immune function decreased)	Boscolo, 2001
5.25 uW/cm2	20 minutes of RFR at cell tower frequencies induced cell stress response	Kwee, 2001
5 - 10 uW/cm2	RFR caused impaired nervous system activity	Dumansky, 1974
6 uW/cm2	RFR induced DNA damage in cells	Phillips, 1998

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effeccts	Sleep, neuron firing rate, EEG, memory, learning, behavior
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Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

Power Density (Microwatts/centimeter2 - uW/cm2)		Reference
8.75 uW/cm2	RFR at 900 MHz for 2-12 hours caused DNA breaks in leukemia cells	Marinelli, 2004
10 uW/cm2	Changes in behavior (avoidance) after 0.5 hour exposure to pulsed RFR	Navakatikian, 1994
10 - 100 uW/cm2	Increased risk in radar operators of cancer; very short latency period; dose response to exposure level of RFR reported.	Richter, 2000
12.5 uW/cm2	RFR caused calcium efflux in cells - can affect many critical cell functions	Dutta, 1989
13.5 uW/cm2	RFR affected human lymphocytes - induced stress response in cells	Sarimov, 2004
14.75 uW/cm2	RFR increased biomarker for cell division in glioma brain tumor cells	Stagg, 1997
20 uW/cm2	Increase in serum cortisol (a stress hormone)	Mann, 1998
28.2 uW/cm2	RFR increased free radical production in rat cells	Yurekli, 2006
37.5 uW/cm2	Immune system effects - elevation of PFC count (antibody producing cells	Veyret, 1991
45 uW/cm2	Pulsed RFR affected serum testosterone levels in mice	Forgacs, 2006
50 uW/cm2	Cell phone RFR caused a pathological leakage of the blood-brain barrier in 1 hour	Salford, 2003
50 uW/cm2	An 18% reduction in REM sleep (important to memory and learning functions)	Mann, 1996
60 uW/cm2	RFR caused structural changes in cells of mouse embryos	Somozy, 1991
60 uW/cm2	Pulsed RFR affected immune function in white blood cells	Stankiewicz, 2006
60 uW/cm2	Cortex of the brain was activated by 15 minutes of 902 MHz cell phone	Lebedeva, 2000
65 uW/cm2	RFR affected genes related to cancer	Ivaschuk, 1999
92.5 uW/cm2	RFR caused genetic changes in human white blood cells	Belyaev, 2005
100 uW/cm2	Changes in immune function	Elekes, 1996
100 uW/cm2	A 24.3% drop in testosterone after 6 hours of CW RFR exposure	Navakatikian, 1994

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effeccts	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

Power Density (Microwatts/centimeter2 - uW/cm2)		Reference
120 uW/cm2	A pathological leakage in the blood-brain barrier with 915 MHz cell RF	Salford, 1994
500 uW/cm2	Intestinal epithelial cells exposed to 2.45 GHz pulsed at 16 Hz showed changes in intercellular calcium.	Somozy, 1993
500 uW/cm2	A 24.6% drop in testosterone and 23.2% drop in insulin after 12 hrs of pulsed RFR exposure.	Navakatikian, 1994

STANDARDS		
530 - 600 uW/cm2	Limit for uncontrolled public exposure to 800-900 MHz	ANSI/IEEE and FCC
1000 uW/cm2	PCS STANDARD for public exposure (as of September 1,1997)	FCC, 1996
5000 uW/cm2	PCS STANDARD for occupational exposure (as of September 1, 1997)	FCC, 1996
BACKGROUND LEVELS		
0.003 uW/cm2	Background RF levels in US cities and suburbs in the 1990s	Mantiply, 1997
0.05 uW/cm2	Median ambient power density in cities in Sweden (30-2000 MHz)	Hamnierius, 2000
0.1 - 10 uW/cm2	Ambient power density within 100-200' of cell site in US (data from 2000)	Sage, 2000

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effeccts	Sleep, neuron firing rate, EEG, memory, learning, behavior
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Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		Reference
0.000064 - 0.000078 W/Kg	Well-being and cognitive function affected in humans exposed to GSM-UMTS cell phone frequencies; RF levels similar near cell sites	TNO Physics and
0.00015 - 0.003 W/Kg	Calcium ion movement in isolated frog heart tissue is increased 18% (P<.01) and by 21% (P<.05) by weak RF field modulated at 16 Hz	Schwartz, 1990
0.000021 - 0.0021 W/Kg	Changes in cell cycle; cell proliferation (960 MHz GSM mobile phone)	Kwee, 1997
0.0003 - 0.06 W/Kg	Neurobehavioral disorders in offspring of pregnant mice exposed in utero to cell phones - dose-response impaired glutamatergic synaptic transmission onto layer V pyramidal neurons of the prefrontal cortex. Hyperactivity and impaired memory function in offspring. Altered brain development.	Aldad, 2012
0.0009 W/Kg	Changes in brain glial cells with TDMA 836.55 MHz frequency	Stagg, 1997
0.0016 - 0.0044 W/Kg	Very low power 700 MHz CW affects excitability of hippocampus tissue, consistent with reported behavioral changes.	Tattersall, 2001
0.0021 W/Kg	Heat shock protein HSP 70 is activated by very low intensity microwave exposure in human epithelial amnion cells	Kwee, 2001
0.0024 - 0.024 W/Kg	Digital cell phone RFR at very low intensities causes DNA damage in human cells; both DNA damage and impairment of DNA is reported	Phillips, 1998
0.0027 W/Kg	Changes in active avoidance conditioned behavioral effect is seen after one-half hour of pulsed radiofrequency radiation	Navakatikian, 1994
0.0035 W/Kg	900 MHz cell phone signal induces DNA breaks and early activation of p53 gene; short exposure of 2-12 hours leads cells to acquire greater survival chance - linked to tumor agressiveness.	Marinelli, 2004
0.0095 W/Kg	MW modulated at 7 Hz produces more errors in short-term memory functioin on complex tasks (can affect cognitive processes such as attention and memory)	Lass, 2002
0.001 W/Kg	750 MHz continuous wave (CW) RFR exposure caused increase in heat shock protein (stress proteins). Equivalent to what would be induced by 3 degree C. heating of tissue (but no heating occurred)	De Pomerai, 2000

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effeccts	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		Reference
0.001 W/Kg	Statistically significant change in intracellular calcium concentration in heart muscle cells exposed to RFR (900 MHz/50 Hz modulation)	Wolke, 1996
0.0021 W/Kg	A significant change in cell proliferation not attributable to thermal heating. RFR induces non-thermal stress proteins (960 MHz GSM)	Velizarov, 1999
0.004 - 0.008 W/Kg	915 MHz cell phone RFR caused pathological leakage of blood-brain barrier. Worst at lower SAR levels and worse with CW compared to Frequency of pathological changes was 35% in rats exposed to pulsed radiation at 50% to continuous wave RFR. Effects observed at a specific absorption (SA) of > 1.5 joules/Kg in human tissues	Persson, 1997
0.0059 W/Kg	Cell phone RFR induces glioma (brain cancer) cells to significantly increase thymidine uptake, which may be indication of more cell division	Stagg, 1997
0.014 W/Kg	Sperm damage from oxidative stress and lowered melatonin levels resulted from 2-hr per day/45 days exposure to 10 GHz.	Kumar, 2012
0.015 W/Kg	Immune system effects - elevation of PFC count (antibody-producing cells)	Veyret, 1991
0.02 W/Kg	A single, 2-hr exposure to GSM cell phone radiation results in serious neuron damage (brain cell damage) and death in cortex, hippocampus, and basal ganglia of brain- even 50+ days later blood-brain barrier is still leaking albumin (P<.002) following only one cell phone exposure	Salford, 2003
0.026 W/Kg	Activity of c-jun (oncogene or cancer gene) was altered in cells after 20 minutes exposure to cell phone digital TDMA signal	Ivaschuk, 1997
0.0317 W/Kg	Decrease in eating and drinking behavior	Ray, 1990
0.037 W/Kg	Hyperactivity caused by nitric oxide synthase inhibitor is countered by exposure to ultra-wide band pulses (600/sec) for 30 min	Seaman, 1999
0.037 - 0.040 W/Kg	A 1-hr cell phone exposure causes chromatin condensation; impaired DNA repair mechanisms; last 3 days (longer than stress response) the effect reaches saturation in only one hour of exposure; electro- sensitive (ES) people have different response in formation of DNA repair foci, compared to healthy individuals; effects depend on carrier frequency (915 MHz = 0.037 W/Kg but 1947 MHz = 0.040 W/Kg)	Belyaev, 2008

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
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Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
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SAR (Watts/Kilogram)		Reference
0.05 W/Kg	Significant increase in firing rate of neurons (350%) with pulsed 900 MHz cell phone radiation exposure (but not with CW) in avian brain cells	Beason, 2002
0.09 W/Kg	900 MHz study of mice for 7 days, 12-hr per day (whole-body) resulted in significant effect on mitochondria and genome stability	Aitken, 2005
0.091 W/Kg	Wireless internet 2400 MHz, 24-hrs per day/20 weeks increased DNA damage and reduced DNA repair; levels below 802.11 g Authors say "findings raise questions about safety of radiofrequency exposure from Wi-Fi internet access devices for growing organisms of reproductive age, with a potential effect on fertility and integrity of germ cells" (male germ cells are the reproductive cells=sperm)	Atasoy, 2012
0.11 W/Kg	Increased cell death (apoptosis) and DNA fragmentation at 2.45 GHz for 35 days exposure (chronic exposure study)	Kesari, 2010
0.121 W/Kg	Cardiovascular system shows significant decrease in arterial blood pressure (hypotension) after exposure to ultra-wide band pulses	Lu, 1999
0.13 - 1.4 W/Kg	Lymphoma cancer rate doubled with two 1/2-hr exposures per day of cell phone radiation for 18 months (pulsed 900 MHz cell signal)	Repacholi, 1997
0.14 W/Kg	Elevation of immune response to RFR exposure	Elekes, 1996
0.141 W/Kg	Structural changes in testes - smaller diameter of seminiferous	Dasdag, 1999
0.15 - 0.4 W/Kg	Statistically significant increase in malignant tumors in rats chronically exposed to RFR	Chou, 1992
0.26 W/Kg	Harmful effects to the eye/certain drugs sensitize the eye to RFR	Kues, 1992
0.28 - 1.33 W/Kg	Significant increase in reported headaches with increasing use of hand-held cell phone use (maximum tested was 60 min per day)	Chia, 2000
0.3 - 0.44 W/Kg	Cell phone use results in changes in cognitive thinking/mental tasks related to memory retrieval	Krause, 2000
0.3 - 0.44 W/Kg	Attention function of brain and brain responses are speeded up	Preece, 1999
0.3 - 0.46 W/Kg	Cell phone RFR doubles pathological leakage of blood-brain barrier permeability at two days (P=.002) and triples permeability at four days (P=.001) at 1800 MHz GSM cell phone radiation	Schirmacher, 2000

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
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Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		Reference
0.43 W/Kg	Significant decrease in sperm mobility; drop in sperm concentration; and decrease in seminiferous tubules at 800 MHz, 8-hr/day, 12 weeks, with mobile phone radiation level on STANDBY ONLY (in rabbits)	Salama, 2008
0.5 W/Kg	900 MHz pulsed RF affects firing rate of neurons (Lymnea stagnalis) but continuous wave had no effect	Bolshakov, 1992
0.58 - 0.75 W/Kg	Decrease in brain tumors after chronic exposure to RFR at 836 MHz	Adey, 1999
0.6 - 0.9 W/Kg	Mouse embryos develop fragile cranial bones from in utero 900 MHz The authors say "(O)ur results clearly show that even modest exposure (e.g., 6 min daily for 21 days" is sufficient to interfere with the normal mouse developmental process"	Fragopoulou, 2009
0.6 and 1.2 W/Kg	Increase in DNA single and double-strand DNA breaks in rat brain cells with exposure to 2450 MHz RFR	Lai & Singh, 1996
0.795 W/Kg	GSM 900 MHz, 217 Hz significantly decreases ovarian development and size of ovaries, due to DNA damage and premature cell death of nurse cells and follicles in ovaries (that nourish egg cells)	Panagopoulous, 2012
0.87 W/Kg	Altered human mental performance after exposure to GSM cell phone radiation (900 MHz TDMA digital cell phone signal)	Hamblin, 2004
0.87 W/Kg	Change in human brainwaves; decrease in EEG potential and statistically significant change in alpha (8-13 Hz) and beta (13-22 Hz) brainwave activity in humans at 900 MHz; exposures 6/min per day for 21 days (chronic exposure)	D'Costa, 2003
0.9 W/Kg	Decreased sperm count and more sperm cell death (apoptosis) after 35 days exposure, 2-hr per day	Kesari, 2012
< 1.0 W/Kg	Rats exposed to mobile phone radiation on STANDBY ONLY for 11-hr 45-min plus 15-min TRANSMIT mode; 2 times per day for 21 days showed decreased number of ovarian follicles in pups born to these pregnant rats. The authors conclude "the decreased number of follicles in pups exposed to mobile phone microwaves suggest that intrauterine exposure has toxic effects on ovaries."	Gul, 2009
0.4 - 1.0 W/Kg	One 6-hr exposure to 1800 MHz cell phone radiation in human sperm cells caused a significant dose response and reduced sperm motility and viability; reactive oxygen species levels were significantly increased after exposure to 1.0 W/Kg; study confirms detrimental effects of RF/MW to human sperm. The authors conclude "(T)hese findings have clear implicatiions for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring."	De Iuliis, 2009

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effeccts	Sleep, neuron firing rate, EEG, memory, learning, behavior
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Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		Reference
1.0 W/Kg	Human semen degraded by exposure to cell phone frequency RF increased free-radical damage.	De Iuliis, 2009
1.0 W/Kg	Motility, sperm count, sperm morphology, and viability reduced in active cell phone users (human males) in dose-dependent manner.	Agarwal, 2008
1.0 W/Kg	GSM cell phone use modulates brain wave oscillations and sleep EEG	Huber, 2002
1.0 W/Kg	Cell phone RFR during waking hours affects brain wave activity. (EEG patterns) during subsequent sleep	Achermann, 2000
1.0 W/Kg	Cell phone use causes nitric oxide (NO) nasal vasodilation (swelling inside nasal passage) on side of head phone use	Paredi, 2001
1.0 W/Kg	Four-fold increase in eye cancer (uveal melanoma) in cell phone users	Stang, 2001
1.0 W/Kg	Increase in headache, fatigue and heating behind ear in cell phone users	Sandstrom, 2001
1.0 W/Kg	Significant increase in concentration difficulties using 1800 MHz cell phone compared to 900 MHz cell phone	Santini, 2001
1.0 W/Kg	Sleep patterns and brain wave activity are changed with 900 MHz cell phone radiation exposure during sleep	Borbely, 1999
1.4 W/Kg	GSM cell phone exposure induced heat shock protein HSP 70 by 360% (stress response) and phosphorylation of ELK-1 by 390%	Weisbrot, 2003
1.46 W/Kg	850 MHz cell phone radiation decreases sperm motility, viability is significantly decreased; increased oxidative damage (free-radicals) significantly decreased; increased oxidative damage (free-radicals)	Agarwal, 2009
1.48 W/Kg	A significant decrease in protein kinase C activity at 112 MHz with 2-hr per day for 35 days; hippocampus is site, consistent with reports that RFR negatively affects learning and memory functions	Paulraj, 2004
1.0 - 2.0 W/Kg	Significant elevation in micronuclei in peripheral blood cells at 2450 MHz (8 treatments of 2-hr each)	Trosic, 2002
1.5 W/Kg	GSM cell phone exposure affected gene expression levels in tumor suppressor p53-deficient embryonic stem cells; and significantly increased HSP 70 heat shock protein production	Czyz, 2004

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effeccts	Sleep, neuron firing rate, EEG, memory, learning, behavior
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Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		Reference
1.8 W/Kg	Whole-body exposure to RF cell phone radiation of 900-1800 MHz 1 cm from head of rats caused high incidence of sperm cell death; deformation of sperm cells; prominent clumping together of sperm cells into "grass bundle shapes" that are unable to separate/swim. Sperm cells unable to swim and fertilize in normal manner.	Yan, 2007
2.0 W/Kg	GSM cell phone exposure of 1-hr activated heat shock protein HSP 27 (stress response) and P38 MAPK (mutagen-activated protein kinase) that authors say facilitates brain cancer and increased blood-brain barrier permeability, allowing toxins to cross BBB into brain	Leszczynski, 2002
2 W/Kg	900 MHz cell phone exposure caused brain cell oxidative damage by increasing levels of NO, MDA, XO and ADA in brain cells; caused statistically significant increase in 'dark neurons' or damaged brain cells in cortex, hippocampus and basal ganglia with a 1-hr exposure for 7 consecutive days	Ilhan, 2004
2.6 W/Kg	900 MHz cell phone exposure for 1-hr significantly altered protein expression levels in 38 proteins following irradiation; activates P38 MAP kinase stress signalling pathway and leads to changes in cell sie and shape (shrinking and rounding up) and to activation of HSP 27, a stress protein (heat shock protein)	Leszczynski, 2004
2.0 - 3.0 W/Kg	RFR accelerated development of both skin and breast tumors	Szmigielski, 1982
2 W/Kg	Pulse-modulated RFR and MF affect brain physiology (sleep study)	Schmidt, 2012

STANDARDS		
0.08 W/Kg	IEEE Standard uncontrolled public environment (whole body)	IEEE
0.4 W/Kg	IEEE Standard controlled occupational environment (whole body)	IEEE
1.6 W/Kg	FCC (IEEE) SAR limit for 1 gram of tissue in a partial body exposure	FCC, 1996
2 W/Kg	ICNIRP SAR limit for 10 grams of tissue	ICNIRP, 1996

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effeccts	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Exhibit B

Reference List
Reported Biological Effects from Radiofrequency Radiation (RFR) at Low-Intensity Exposure Levels

(Cell Tower, WI-FI, Wireless Laptop, Wireless Utility Meters 'smart meters')

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BioInitiatiive Author; Prof. Henry Lai PhD. and Blake Levitt
Comments, Aug. 26, 2013

FCC 13-39

Before the
Federal Communications Commission
Washington, D.C. 20554

In the Matter of	)	
	)	
Reassessment of Federal Communications Commission Radiofrequency Exposure Limits and Policies	)	ET Docket No. 13-84
	)	
Proposed Changes in the Commission's Rules Regarding Human Exposure to Radiofrequency Electromagnetic Fields	)	ET Docket No. 03-137

To: Office of the Secretary
Federal Communications Commission
Washington, DC 20554

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State of Connecticut, Litchfield County, USA

I, B. Blake Levitt, attest that my statements are true to the best of my knowledge.

I, Henry C. Lai, attest that my statements are true to the best of my knowledge.

Comments for FCC ET Docket No. 013-84 and ET Docket No. 03-137

1. My name is B. Blake Levitt. My address is 355 Lake Road, Warren. Connecticut 06777, USA.

2. I am a medical/science journalist, former *New York Times* contributor, and author of *Electromagnetic Fields, a Consumer's Guide to the Issues and How to Protect Ourselves* (Harcourt Brace, First Edition; iUniverse Back-In –Print Edition, 2007) which won a chapter Award of Excellence from the American Medical Writers Association; and Editor of *Cell Towers, Wireless Convenience? or Environmental Hazard? Proceedings of the "Cell Towers Forum," State of the Science, State of the Law* (Safe Goods/New Century Publishing, 2001). I am also the co-author, with Dr. Henry C. Lai, of *Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays,* published in Environmental Reviews/NRC Research Press, 2010 (Environ Rev: 18: 369-295 doi:10.1139/A10-018).[1] I have published widely on the health and environmental effects of low-level nonionizing radiation for over 20 years for both the lay and professional reader. I have also consulted nationally for municipalities struggling with safer cell tower and infrastructure siting after passage of The Telecommunications Act of 1996 (TCA) which restricted local and state rights regarding the ability to take radiofrequency radiation (RF) into consideration in telecommunications tower/antenna array siting. I am also on the Executive Committee of The Berkshire-Litchfield Environmental Council (BLEC), a 501 (3)(c) non-profit organization that focuses on environmental issues affecting the Northwest corner of Connecticut and the Berkshires region of Massachusetts. BLEC has sponsored

[1]Levitt, B.B., Lai, H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays, Enviro. Rev. 369-395 (2010), doi:10.1139/A 10-018 http://www.nrcresearchpress.com/doi/pdf/10.1139/A10-018

several educational forums on safe infrastructure siting and has researched in depth the environmental effects of low-level ambient RF exposures to myriad species.

3. My name is Henry C. Lai. My address is 5557, 35th Ave., NE, Seattle, Washington 98105, USA

4. I am a Research Professor Emeritus of Bioengineering in the University of Washington, Seattle, WA, USA. I have carried out research on the biological effects of nonionizing electromagnetic fields for more than 30 years and published numerous papers on the topic. I am a co-editor of the journal Electromagnetic Biology and Medicine. I was one of the "subject matter experts" interviewed for the July 2012 report "Telecommunications: Exposure and Testing Requirements for Mobile Phones Should be Reassessed" by the Government Accountability Office (GAO).

5. Background:

On March 29, 2013, the FCC issued an Order, Notice of Proposed Rulemaking (NPRM) and a Notice of Inquiry (NOI) as a single document (13-39) in response to the July 2012 report from the Government Accountability Office GAO which recommended, among other things, that the Commission:[2]

• Reassess the current FCC radiofrequency radiation (RFR) exposure limits, including its effects on human health; the costs/benefits in keeping the current limits; to seek the opinions of relevant health/safety agencies; and to change the limits if determined necessary.

• Reassess whether the current mobile phone testing requirements, given new technologies and different use patterns, do in fact result in the identification of maximum RF energy exposures, especially when mobile phones are held against the body – the head in particular -- and to update testing requirements if determined necessary.

The NPRM proposes to standardize all criteria for frequency, power density, and antenna separation in order to determine whether a facility or device should be exempt from routine evaluation for harm to the human body. This would do away with the current categorical exclusions. The NPRM also discusses distinctions between general population and occupational RFR exposure and proposes new requirements for signs and barriers at transmitter sites.

[2] "Telecommunications: Exposure and Testing Requirements for Mobile Phones Should be Reassessed." http://www.gao.gov/assets/600/592901.pdf

The NOI addresses three areas: the propriety of existing standards and policies; possible options for precautionary exposure reduction; and possible improvements to the equipment authorization process and policies as they relate to RF exposure.

The first two points address whether thermal damage (tissue-overheating), which is the current focus of FCC standards, is the only RFR risk, or whether other human health damage can be caused by chronic exposures with cumulative effects over longer periods of time.

This is the first time in 17 years that the FCC has looked at the adequacy of its thermal-based RFR-exposure standards to protect human health. The FCC is admittedly not expert in the subject and defers to other agencies and professional organizations. Nevertheless, FCC is charged by law with adopting and enforcing RF exposure safeguards. The rationale and overall model is therefore critical for biological accuracy. Toward that end, FCC has called upon better-informed agencies such as the Environmental Protection Agency (EPA) for opinions on ambient exposures, and the Food and Drug Administration (FDA) for opinions on consumer products, as well as industry groups like the International Electrical and Electronics Engineers (IEEE) which has a financial stake in relaxed regulation, as well as the knowledgeable public for input on key areas of concern. Unfortunately, programs within EPA for this kind of research and policy-making have been almost completely defunded, leaving few there to render a considered opinion; and FDA's funding has also been reduced. This, in effect, leaves industry groups with the most clout.

The FCC expresses confidence in the current thermal-only basis, but acknowledges that with the rapid proliferation of wireless devices over the years, as well as the ubiquity of antennas needed for supportive infrastructure, and the new technological designs that allow much closer-to-the-body operation and medical implantation, that a new review is in order. The GAO report expressed similar confidence in the current methodology. This is in stark opposition to the most current data, and the direction that many other countries are taking regarding precautionary approaches.

Neither these authors, nor many expert members of the international research community, harbor the same confidence in such narrowly defined standards, which are premised upon understanding underlying biological mechanisms. Many now think that, given the peer-reviewed literature published since 1997 that setting an exposure threshold should based mainly on the knowledge at which level biological/health effects are observed, and not on the mechanism of the effects. Most of that research has come from outside of the U.S, including the recent classification of RF fields as a 2B (possible) carcinogen by the International Agency for Research on Cancer (IARC) at the World Health Organization

(WHO).[3] Indeed hundreds of studies have found biological/health effects at orders of magnitude below the current FCC thresholds. The changes regarding SAR allowances for the pinna (ear), as well as possible new setbacks from products and infrastructure, and potential new classifications that would supplant categorical exclusions, go nowhere near far enough in protecting public health and, in some areas, may serve to increase exposures to the general population.

6. FCC Comments from NPRM and NOI:

> ¶16. In this *Order*, we adopt rules explicitly permitting licensees and grantees to demonstrate that they comply with the Commission's RF exposure rules based on specific absorption rate (SAR) in lieu of maximum permissible exposure (MPE) for fixed and mobile transmitters. Providing an additional option for parties to demonstrate that they comply with the RF exposure limits could reduce those parties' expenses in some cases. Additionally, in the *Order*, we classify the outer ear as an extremity based on similarities to other parts of the body such as the hands and feet, which are already classified as extremities. This reclassification of the outer ear as an extremity is consistent with health agency comment and industry standards and should eliminate unnecessary compliance costs that could occur under alternative evaluation schemes.
>
> Accordingly, in the *Notice*, we requested comment on classifying the pinna (outer ear) as an extremity, to which less stringent exposure criteria would apply. While we received comments both for and against this classification, we amend section 1.1310 of our rules to subject the pinna to the same RF exposure limit currently applicable to hands, wrists, feet, and ankles.
>
> ¶44.
> *Background*. Our localized SAR limit for the general population is 1.6 W/kg as averaged over any one gram cube of tissue, except for extremities, explicitly defined in our existing rules as the hands, wrists, feet, and ankles, where the limit is 4 W/kg as averaged over any ten gram cube of tissue.78
> (For occupational exposure, the localized SAR limit is 8 W/kg as averaged over any one gram cube of tissue, except for within the extremities where it is limited to 20 W/kg as averaged over any ten gram cube of tissue.) In the *Notice*, 79 we referred to deliberations by the

[3] http://www.iarc.fr/en/media-centre/pr/2011/pdfs/pr208_E.pdf

> IEEE of a standard revision that would treat the pinna of the human ear also as an extremity for the purpose of SAR evaluation.80 We invited comment on whether we should consider adopting such a revision once approved by the IEEE .In the meantime, IEEE revisions characterizing the pinna as an extremity have been issued in IEEE Standards C95.1b-2004 and C95.1-2005. We note that classification of the pinna is only relevant to evaluation of localized SAR and not MPE. The MPE limits were derived under the assumption of whole body exposure, and control of localized SAR is implicit in their derivation.
>
> ¶45.
> *Comments*. Ericsson and Motorola both supported those revisions, and Motorola recommended that the Commission adopt it by reference in a separate rulemaking…. This revision has now been adopted by the IEEE as Amendment 2 to IEEE Std. C95.1 (IEEE Std. C95.1b-2004).
> The pinna is the external part of the ear that extends away from the skull, consisting primarily of cartilage.

7. Author Comments:

Our comments are mainly on the validity and adequacy of the current guidelines in the protection of the general public exposed to radiofrequency radiation (RFR). Reclassification of the pinna as an extremity is of secondary importance. However, we do not agree with such a reclassification.

There are two major situations of radiofrequency field exposure: 1) near-field exposure, in which the source is close to the body and only part of the body is exposed and the pattern of energy absorption is relatively stationary; and 2) far-field exposure in which the source is away at a distance greater than two wavelengths of the radiation, the whole body is exposed and the pattern of energy absorption is more variable as the object moves in the field. The main cause of near-field exposure is in the use of cell phones or other wireless communication devices when the radiation is concentrated to the head of the user. In the far-field situation, the main sources are RF-transmission towers in the vicinity, e.g., radio and TV towers, cell phone base stations, and wireless emitters and radars. The FCC regulates both near- and far-field exposures.

The current RF exposure guidelines need a major overhaul but under no circumstance should be made more lenient. The guidelines are based on limited and obsolete scientific data and illogical rationale. It can be misleading to discuss the exposure standards based on thermal v. non-thermal effects. It is very difficult to scientifically differentiate between RF-

induced thermal and non-thermal biological effects. An increase in tissue temperature does not necessarily imply that an effect being observed is thermal in nature only. Guidelines should be based mainly on the exposure levels (SAR or power density) at which biological effects have been consistently observed, not the mechanism of the effects.

While expanding the Commission's RF exposure rules to be based on specific absorption rate (SAR) is broadening toward a more biologically based standard rather than a doseimetry based model such as the maximum permissible exposure (MPE), SAR should not be used in lieu of MPE for fixed and mobile transmitters. Because of complex numerous variables, SAR is almost impossible to determine in the field and should not be used for ambient exposures.

Computational models for SAR calculation can be quit reliable, however. [4] Because of this, we recommend that FCC require manufacturers to provide state-of-the-art data on their phones, posted both on the FCC's website and made available to consumers at point-of-sale. Although SAR is the most biologically relevant, MPE has been used as a surrogate to determine SAR. The main emphasis for far-field exposures should remain on an MPE model, simply because it is easier to measure, control and mitigate when necessary. SAR is far too complex for a field model for infrastructure exposures and is therefore unreliable. It could actually make the standards less clear and enforceable as industry could easily hide behind specious SAR models and increase the power output of transmitters.

8. Specific Absorption Rate (SAR):

When a cell phone is held close to the head, the radiofrequency energy penetrates the head and is absorbed by body tissues. Depending on various physical factors, energy absorption is not uniform. High and low energy deposition areas are formed. The amount of energy absorption is measured by the specific absorption rate (SAR) which is the rate of energy absorbed by a unit mass of tissue, generally expressed in W/kg. Energy distribution can be calculated using computer simulation. Guidelines are set by limiting the peak SAR. In order to do that, the amount of tissue for peak SAR consideration has to be defined. In the present standards, the limiting SAR is defined by 1 or 10 gm of tissue, i.e., the SAR within 1 or 10 gm of brain/head tissue should not exceed a certain value. In the IEEE guideline, the peak SAR in the head is not to exceed 1.6 W/kg averaged over 10 g of tissue. The proposed FCC guideline of SAR of 1.6 W/kg over 1 gm of tissue is the near-field (partial body) exposure situation. This was derived from an erroneous rationale. The rationale was that the

[4] In fact, it was just such computerized SAR calculation that caused concern for the reliability of cell phone industry claims about power density and which lead to the reclassification of the pinna as an 'extremity.' Om Ghandi's and Neils Kuster's calculations showed that some cell phones exceeded the 4W/kg limit. That was why cell phone manufacturers came to recommend holding the phone a few inches away from the head. Without reclassification, some cell phone manufacturers would have had to pull their models from the market.

0.4 W/kg guideline was a whole body exposure situation (i.e., an animal's behavior was disrupted when it's whole body was exposed to 4 W/kg), and when part of the body is exposed, as in the case of cell phone use, that part of the body should be able to take more radiation. Thus, the guideline for partial body exposure was increased 4 times to 1.6 W/kg. There is no evidence that the partial body can tolerate more energy deposition than the whole body. The opposite may be true. Up to 300,000 brain cells (neurons and glial cells) can be contained within 1 cu mm of brain tissue. Genetic damage in one single cell, as caused by exposure to RFR from a cell phone, is enough to lead to cancer.

The current FCC guidelines for the SAR are based on narrow data from one set of experiments carried out in the 1980's[5] [6] which showed behavioral disruption in animals after exposure to RFR at a whole body SAR of 4 W/kg. These studies have not been independently replicated, yet are enshrined in the standards. Many other experiments since then have shown behavioral and other physiological effects in animals and humans at a SAR lower than 4 W/kg but no changes to the guidelines have been made.[7] This point ties directly into the reclassification of the pinna (ear) as an extremity, which would allow cell handset exposures to increase to 4W/kg from the current 1.6 W/kg averaged over one gram of tissue.

As examples, Table I below lists a group of low-intensity *in vitro* studies reported in the literature. 'Low intensity' is defined as a SAR less than 0.1 W/kg, which is 1/40 of the biologically effective SAR used in the setting of present RF guidelines. In addition, since cell phones are the major source of near-field exposure and modulations may have a significant role in eliciting biological effects, only studies using cell phone frequencies and modulations were considered. (It should be noted that signals used in these studies could only partially match the modulations of cell phone signals.) An additional criterion is that SARs are provided in these studies. There are 17 papers that satisfied these criteria listed in Table 1. The biological effects reported by these studies included: genetic effects, cell proliferation, membrane chemistry, protein damage, calcium metabolism, stress protein production, immunological changes, and DNA damage. The average SAR of these 17 studies is 0.029 W/kg (range 0.07 – 0.000021 W/kg). The duration of exposure ranged from 15 min to 72 hours.

Table 2 is a list of in vivo animal studies. There are 12 studies that fit the criteria. Animal species of these studies included mouse, rat and hamster. Endpoints studied included

[5] de Lorge, J., and Ezell, C.S. 1980. Observing-responses of rats exposed to 1.28- and 5.62-GHz microwaves. Bioelectromagnetics, 1(2): 183–198, 1980.

[6] de Lorge, J.O. 1984. Operant behavior and colonic temperature of Macaca mulatta exposed to radiofrequency fields at and above resonant frequencies. Bioelectromagnetics, 5(2): 233–246, 1984.

[7] Lai, H. Biological effects of radiofrequency radiation from wireless transmission towers. In *"Cell Towers: Wireless Convenience? OrEnvironmental Hazard?"* Levitt, B.B. (ed.), New Century Publishing, East Canaan, CT, 2001, pp. 65-74, 2001.

effects on: testosterone and insulin levels, DNA double strand breaks, reproductive system, metabolism, memory functions, blood-brain barrier, embryonic kidney development, immune system, and free radical formation. The average SAR of these 13 studies is 0.015 W/kg.

It is very obvious from the data presented in the tables that recent studies do not support the use of 4 W/kg SAR as the basis of exposure limits.

Table 1. In vitro studies (800-2000 MHz) (n = 17); Average = 0.029 W/kg (range 0.07 – 0.000021 W/kg, median 0.025 W/kg)

		SAR (W/kg)	Effect reported
Belyaev et al.(2005)	915 MHz, GSM, 24 & 48 hr	0.037	Genetic changes in human white blood cells
Belyaev et al.(2009)	915 MHz, 1947 MHz; GSM, UMTS 24 & 72 hr	0.037	DNA repair mechanism in human white blood cells
Capri et al.(2004)	900 MHz, GSM 1 hr/day, 3 days	0.07	Cell proliferation and membrane chemistry
De Pomerai et al. (2003)	1 GHz 24 & 48 hr	0.015	Protein damages
Dutta et al. (1984)	915 MHz, sinusoidal AM at 16 Hz	0.05	Increase in calcium efflux in human neuroblastoma cells
Ivaschuk et al. (1997)	836.55 MHz, TDMA 20 min	0.026	Transcript levels for c-jun were altered in nerve growth factor-treated PC12 rat pheochromocytoma cells
Kwee et al. (2001)	960 MHz, GSM 20 min	0.0021	Hsp-70 stress protein increased in transformed human epithelial amnion cells
Makova et al. (2005)	915 and 905 MHz, GSM 1 hr	0.037	chromatin conformation in human white blood cells affected
Marinelli et al. (2004)	900 MHz CW 2 - 48 hr	0.0035	Cell's self-defense responses triggered by DNA damage.
Pavicic et al. (2008)	864 and 935 MHz, CW, 1-3 hrs	0.08	Growth affected in Chinese hamster V79 cells.
Phillips et al. (1998)	813.5625 MHz	0.0024	DNA damage in human

	(iDEN); 836.55 MHz (TDMA) 2 hr and 21 hr		leukemia cells.
Sarimov et al. (04)	895-915 MHz GSM 30 min	0.0054	Human lymphocyte chromatin affected similar to stress response.
Schwarz et al. (2008)	1950 MHz UMTS 24 hr	0.05	Genes in human fibroblasts.
Stagg et al. (1997)	836.55 MHz TDMA duty cycle 33% 24 hr	0.0059	Glioma cells showed significant increases in thymidine incorporation, which may be an indication of an increase in cell division.
Stankiewicz et al. (2006)	900 MHz GSM 217 Hz pulses-.577 ms width 15 min	0.024	Immune activities of human white blood cells affected.
Velizarov et al. (1999)	960 MHz GSM 217 Hz square-pulse, duty cycle 12% 30 min	0.000021	Decrease in proliferation of human epithelial amnion cells.
Wolke et al. (1996)	900, 1300, 1800 MHz, square-wave modulated at 217 Hz; Also 900 MHz with CW, 16 Hz, 50 Hz and 30 KHz modulations	0.001	Calcium concentration in heart muscle cells of guinea pig.

Table 2: Non-human in vivo studies with SAR N=14, mean = 0.015 W/kg (range: 0.004 – 0.02 W/kg), median = 0.014 W/kg

		SAR (W/kg)	Effects reported
Forgacs et al. (2006)	1800 MHz, GSM- 217 Hz pulses, 576 µs pulse width; 2 hr/day, 10 days	0.018	Increase in serum testosterone.
Kesari and Behari (2009)	50 GHz; 2hr/day, 45 days	0.0008	Double strand DNA breaks observed in brain cells

Kesari and Behari (2010)	50 GHz; 2 hr/day, 45 days	0.0008	Reproductive system of male rats
Kesari et al. (2010)	2450 MHz, 50-Hz modulation, 2 hr/day, 35 days	0.11	DNA double strand breaks in brain cells.
Kumar et al. (2010a)	10 GHz, 2h/day 45 days	0.014	Cellular changes and increase in reactive oxygen species in testes
Kumar et al. (2010b)	10 GHz, 2 h/day, 45 days 50 GHz, 2h/day, 45 days	0.014 0.0008	Genetic damages in blood cells
Lerchl et al. (2008)	383 MHz (TETRA), 900 and 1800 MHz (GSM) 24 hr/day, 60 days	0.08	Metabolic changes.
Navakatikian and Tomashevskaya (1994)	2450 MHz CW and 3000 MHz pulse-modulated 2 μs pulses at 400 Hz Single (0.5-12 hr) or repeated (15-60 days, 7-12 hr/day) exposure, CW-no effect	0.0027	Behavioral and endocrine changes, and decreases in blood concentrations of testosterone and insulin.
Nittby et al. (2007)	900 MHz GSM 2hr/wk, 55wk	0.0006	Reduced memory functions.
Perssson et al. (1997)	915 MHz-CW and pulse-modulated (217-Hz, 0.57 ms; 50-Hz, 6.6 ms) 2-960 min; CW more potent	0.0004	Increase in permeability of the blood-brain barrier.
Pyrpasopoulou et al. (2004)	9.4 GHz GSM (50 Hz pulses, 20 μs pulse length) 1-7 days postcoitum	0.0005	Exposure during early gestation affected kidney development.
Salford et al. (2003)	915 MHz GSM 2 hr	0.02	Nerve cell damage in brain.
Veyret et al. (1991)	9.4 GHz 1 μs pulses at 1000 pps, also with or without sinusoidal AM between 14 and 41 MHz, response only with AM modulation, direction	0.015	Functions of the immune system.

	of response depended on AM frequency		
Yurekli et al. (2006)	945 MHz GSM, 217 Hz pulse-modulation 7 hr/day, 8 days	0.0113	Free radical chemistry.

9. Pinna (Ear) as 'Extremity':

The current FCC standards are 1.6 W/kg as averaged over any one gram cube of tissue, except for extremities, specifically defined by FCC as the hands, wrists, feet, and ankles, where the limit is 4 W/kg as averaged over any ten gram cube of tissue. For occupational exposure, the localized SAR limit is 8 W/kg as averaged over any one gram cube of tissue, except for within the extremities where it is limited to 20 W/kg as averaged over any ten gram cube of tissue. (The FCC notes that classification of the pinna is only relevant to evaluation of localized SAR and not MPE. The MPE limits were derived under the assumption of whole body exposure, and control of localized SAR, is implicit in their derivation.)

We think the rationale for considering the external ear (pinna or auricle) as an extremity should be re-examined more carefully. The auricle is simply not an 'extremity.' Just a casual look at the Medline comes up with some alarming information. First, it is very obvious that the auricle is histologically different from the arms and legs. There are no bone, tendon, and skeletal muscle.

Let us first consider the possible thermal effect on the auricle while using a cell phone. The 'rationale document' states very well that the auricle can probably handle the heat load. But, it fails to consider individuals who cannot thermo-regulate very well. This is not uncommon. For example, the micro-circulation of the auricle is controlled by, among other neurotransmitter systems, the adrenergic and serotonergic systems [Li et al, 1998, 2000; White et al., 1985; see references below]. People who take alpha-2 agonists for hypertension, beta-agonists for asthma, and serotonin-agonists for psychiatric depression would be vulnerable to thermal damage to the auricle when using a cell phone. Should customers who use these therapeutic drugs have additional warnings when using cell phones?

In addition, Oftedal et al (2000) recently reported that "…sensations of warmth on the ear and behind/around the ear, burning sensations in the facial skin and headaches were most commonly reported by cell phone users."

Cancer of the auricle is not uncommon [e.g., Hayter et al., 1996; Moriyama et al., 2000; Silva et al., 2000; Worley et al., 1999], because the auricle does not consist mainly of

post-mitotic cells like the arms and legs. And that the question of whether RFR can cause genetic damage is far from settled.

Thirdly, the auricle, different from the arms and legs, is innervated by the vagus nerve. The vagus also innervates many other vital organs in the body, including, for example, the heart, GI-tract, and reproductive organs. Vagus reflexes are well known [Engel, 1979; Gupta et al., 1986]. Stimulating the auricle can affect these organs. Two important case reports include stoppage of the heart [Prasad et al., 1984] and epilepsy [Santanelli et al., 1985] triggered by stimulation of the auricle in humans.

Reclassification of the pinna as an extremity was a mistake. Such reclassification now allows the SAR to increase from 1.6 W/kg (averaged over 1 gm of tissue) to 4 W/kg (averaged over 10 gm of tissue) which will allow the emission power density of cell phone handsets to increase. Also, this reclassification does not take into consideration that many people – especially the young – now text rather than put a cell phone directly to the head. An increase to the higher SAR with the accompanying allowable increase in cell phone emissions, will create much stronger RFR exposures to the eyes since screens are small and now typically held close to the face for viewing purposes. The eye is a highly conductive aqueous saline organ – the exact opposite of cartilage. One study reported an increased risk of melanoma of the eye[8] with cell phone exposures but the same authors were not able to replicate their own work.[9] This area warrants close follow-up. The reclassification is inviting adverse effects to the ear, the brain, the eyes, and potentially other systems in the body.

There has been no clear rationale by FDA or FCC or IEEE for treating the ear as an extremity. Other than facilitating higher power output for potentially better operation of the handsets which is only in industry's favor, there is no real public advantage and possible public health endangerment. It is obvious why the IEEE, as an industry group with no medical training, would push for this reclassification but a complete mystery why the FDA went along with it.

10. Blanket Exemptions -- Cumulative Effects Not Considered, Smart Grid/Metering Case in Point:

FCC proposes to standardize compliance via adopting thresholds of power, distance and frequency for routine environmental evaluation. Below the threshold of one milliwatt (1

[8] Stang A, Anastassiou G, Ahrens W, Bromen K, Bornfeld N, Jöckel KH. The possible role of radiofrequency radiation in the development of uveal melanoma. Epidemiology. 12(1):7-12, 2001.
[9] Stang A, Schmidt-Pokrzywniak A, Lash TL, Lommatzsch PK, Taubert G, Bornfeld N, Jöckel KH. Mobile phone use and risk of uveal melanoma: results of the risk factors for uveal melanoma case-control study. J Natl Cancer Inst. 101(2):120-123, 2009.

mW) of power or less, services or devices would be exempt, continuing the blanket exemption for the most popular and ubiquitous consumer products today, as well as those to be developed in the future. Yet no cumulative exposure criterion is set for radiating sources for myriad products operating simultaneously. Exemptions are taken one product or service at a time and with this ruling, FCC will continue that policy without setting levels for the sum of effects from different sources and cumulative effects over time, such as DNA damage in the genome that become larger with repeated exposure.

There has been an exponential increase for both low-level RFR fixed transmitters like wifi, and voluntary personal portable/mobile devices. This is in addition to involuntary exposures from accompanying infrastructure like cell towers with multiple providers, antennas mounted on/in existing structures, and DAS systems which bring RFR much closer to the population. There is an increasing new layer of RF with smart grid/metering -- an involuntary direct RF delivery system into homes and businesses. In addition, there has been a large increase in the use of implantable medical devices such as cardiac pacemakers, insulin pumps and deep brain stimulators for Parkinsons Disease, among others, that are susceptible to interference from near-and-far field RFR. And there are increasing uses of implantable RFID devices, too. Both personal environments and large ambient environmental RFR levels have risen dramatically in the last 20 years, and continue to do so.

In the 2010 paper that we published in *Environmental Reviews* [10] -- one of the peer-reviewed publications of Canada's privately owned National Research Council Press -- we included a chart of 59 peer-reviewed studies showing various biological effects at low intensity RFR exposures far below current FCC standards (see Table 3 below). This was the first paper to specifically explore the data on biological effects now common in most urban and suburban settings. All of the works cited apply to what FCC now categorically excludes. Works cited, for instance, would apply to smart grid/metering technology and wifi routers placed on desk tops near a user's head. Such devices therefore cannot be considered benign, despite adherence to FCC guidelines for exemption. In the case of smart meters, RF couples with domestic wiring and travels throughout a building. Because of such coupling with conductive material, no distance from the transmitting source would be effective regulation here**.** And peak exposures during the device's duty cycle, which is the most pertinent exposure parameter, is time-averaged away. This is not protective of public health.

The listed exposure levels at which biological/health effects have been observed are much lower than the FCC's SAR of 4 W/kg, and actually include levels that one would

[10] Levitt, B. B., Lai, H., Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays, Enviro. Rev. 369-395 (2010), doi:10.1139/A 10-018 http://www.nrcresearchpress.com/doi/pdf/10.1139/A10-018

encounter in modern urban/suburban environments today. Furthermore, exposure to smart meter RF, for example, is chronic and unavoidable.

In the very least, FCC should call for a thorough assessment of the smart meter buildout until the emission levels from access points are known, setbacks for access points are recommended from nearby residences/businesses, and a better assessment of cumulative exposures from meters, access points, and wireless components placed on or in appliances themselves -- both singly and in multiples working simultaneously -- can be determined.

We recommend that FCC also advise EPA, FCC, DOE and the legislature that more extensive assessment of smart-grid/metering is needed before this buildout proceeds further. Some of the studies in the chart below are comparable to such exposures.

Table 3. A list of studies reporting biological effects at low intensities of RFR. These papers gave either SAR (W/kg) or power density ($\mu W/cm^2$) of exposure.

		SAR (W/kg)	Power density ($\mu W/cm^2$)	Effects reported
Belyaev et al. (2005) (in vitro)	915 MHz, GSM 24 & 48 hr	0.037		Genetic changes in human white blood cells
Belyaev et al. (2009) (in vitro)	915 MHz, 1947 MHz GSM, UMTS 24 & 72 hr	0.037		DNA repair mechanism in human white blood cells
Blackman et al. (1980) (in vitro)	50 MHz, AM at 16 Hz	0.0014		Calcium in forebrain of chickens
Boscol et al. (2001) (in vivo) (human whole body)	500 KHz-3 GHz, TV broadcast		0.5	Immunological system in women
Campisi et al. (2010) (in vitro)	900 MHz, CW or 50-Hz AM, 14 days, 5, 10, 20 min per day, CW- no effect		26	DNA damage in human glial cells
Capri et al. (2004) (in vitro)	900 MHz, GSM 1 hr/day, 3 days	0.07		A slight decrease in cell proliferation when human immune cells were stimulated with mitogen and a slight increase in the number of cells with altered distribution of phosphatidylserine across the membrane.
Chiang et al. (1989) (in vivo) (human whole body)	People lived close to AM radio and radar installations for more than one year		10	People lived and worked near AM radio antennae and radar installations showed deficits in psychological and short-term memory tests.
De Pomerai et al. (2003) (in vitro)	1 GHz 24 & 48 hr	0.015		Protein damages
D'Inzeo et al.	10.75 GHz CW	0.008		Operation of acetylcholine-related ion-channels in

(1988) (in vitro)	30-120 sec			cells. These channels play important roles in physiological and behavioral functions.
Dutta et al. (1984) (in vitro)	915 MHz, sinusoidal AM at 16 Hz	0.05		Increase in calcium efflux in brain cancer cells.
Dutta et al. (1989) (in vitro)	147 MHz, sinusoidal AM at 16 Hz 30 min	0.005		Increase in calcium efflux in brain cancer cells.
Fesenko et al. (1999) (in vivo) (mouse-wavelength in mm range)	From 8.15 - 18 GHz 5 hr to 7 days direction of response depended on exposure duration		1	Change in immunological functions.
Forgacs et al. (2006) (in vivo) (mouse whole body)	1800 MHz, GSM-217 Hz pulses, 576 μs pulse width; 2hr/day, 10 days	0.018		Increase in serum testosterone.
Guler et al. (2010) (In vivo) (rabbit whole body)	1800 MHz AM at 217 Hz, 15 min/day, 7 days		52	Oxidative lipid and DNA damages in the brain of pregnant rabbits
Hjollund et al. (1997) (in vivo) (human partial or whole body)	Military radars		10	Sperm counts of Danish military personnel, who operated mobile ground-to-air missile units that use several RFR emitting radar systems, were significantly lower compared to references.
Ivaschuk et al. (1999) (in vitro)	836.55 MHz, TDMA 20 min	0.026		A gene related to cancer.
Jech et al. (2001) (in vivo) (human partial body exposure- not included)	900 MHz, GSM-217 Hz pulses, 577 μs pulse width; 45 min; narcoleptic patients	0.06		Improved cognitive functions.
Kesari and Behari (2009a) (in vivo) (rat whole body)	50 GHz; 2hr/day, 45 days	0.0008		Double strand DNA breaks observed in brain cells
Kesari and Behari (2009b) (in vivo) (rat whole body)	50 GHz; 2hr/day, 45 days	0.0008		Reproductive system of male rats
Kesari et al. (2010) (in vivo) (rat whole body)	2450 MHz, 50-Hz modulation, 2 h/day, 35 days	0.11		DNA double strand breaks in brain cells.
Kwee et al. (2001) (in vitro)	960 MHz, GSM 20 min	0.0021		Increased stress protein in human epithelial amnion cells.
Lebedeva et al. (2000) (in vivo) (human partial body)	902.4 MHz, GSM 20 min		60	Brain wave activation.
Lerchl et al. (2008)	383 MHz (TETRA),	0.08		Metabolic changes.

(in vivo) (hamster whole body)	900 and 1800 MHz (GSM) 24 hr/day, 60 days			
Magras and Xenos (1999) (in vivo) (mouse whole body)	'Antenna park'-TV and FM-radio, Exposure over several generations		0.168	Decrease in reproductive function.
Makova et al. (2005) (in vitro)	915 and 905 MHz, GSM 1 hr	0.037		Chromatin conformation in human white blood cells.
Mann et al. (1998) (in vivo) (human whole body)	900 MHz GSM pulse-modulated at 217 Hz, 577 μs width, 8 hr		20	A transient increase in blood cortisol.
Marinelli et al. (2004) (in vitro)	900 MHz CW 2 - 48 hr	0.0035		Cell's self-defense responses triggered by DNA damage.
Navakatikian and Tomashevskaya (1994) (in vivo) (rat whole body)	2450 MHz CW and 3000 MHz pulse-modulated 2 μs pulses at 400 Hz Single (0.5-12hr) or repeated (15-60 days, 7-12 hr/day) exppsure, CW-no effect	0.0027		Behavioral and endocrine changes, and decreases in blood concentrations of testosterone and insulin.
Nittby et al. (2007) (in vivo) (rat whole body)	900 MHz GSM 2hr/wk, 55wk	0.0006		Reduced memory functions.
Novoselova et al. (1999) (in vivo) (mouse whole body- wavelength in mm range)	From 8.15 -18 GHz, 1 sec sweep time-16 ms reverse, 5 hr		1	Functions of the immune system.
Novoselova et al. (2004) (in vivo) (mouse whole body- wavelength in mm range)	From 8.15 -18 GHz, 1 sec sweep time-16 ms reverse, 1. 5 hr/day, 30 days		1	Decreased tumor growth rate and enhanced survival.
Pavicic et al. (2008) (in vitro)	864 and 935 MHz, CW, 1-3 hrs	0.08		Growth affected in Chinese hamster V79 cells.
Panagopoulos et al. (2010) (in vivo) (fly whole body)	GSM 900 and 1800 6 min/day, 5 days		1 - 10	Reproductive capacity and induced cell death.
Panagopoulos and Margaritis (2010a) (in vivo) (fly whole body)	GSM 900 and 1800 6 min/day, 5 days		10	'Window' effect of GSM radiation on reproductive capacity and cell death.
Panagopoulos and Margaritis (2010b) (in vivo) (fly whole body)	GSM 900 and 1800 1- 21 min/day, 5 days		10	Reproductive capacity of the fly decreased linearly with increased duration of exposure.
Pérez-Castejón et al. (2009) (in vitro)	9.6 GHz , 90% AM, 24 hrs	0.0004		Increased proliferation rate in human astrocytoma cancer cells.

Perssson et al. (1997) (in vivo) (mouse whole body)	915 MHz-CW and pulse-modulated (217-Hz, 0.57 ms; 50-Hz, 6.6 ms) 2-960 min; CW more potent	0.0004		Increase in permeability of the blood-brain barrier.
Phillips et al. (1998) (in vitro)	813.5625 MHz (iDEN); 836.55 MHz (TDMA) 2 hr and 21 hr	0.0024		DNA damage in human leukemia cells.
Polonga-Moraru et al. (2002) (in vitro)	2.45 GHz 1hr		15	Change in membrane of cells in the retina.
Pyrpasopoulou et al. (2004) (in vivo) (rat whole body)	9.4 GHz GSM (50 Hz pulses, 20 μs pulse length) 1-7 days postcoitum	0.0005		Exposure during early gestation affected kidney development.
Roux et al. (2008a) (in vivo) (tomato whole body)	900 MHz		7	Gene expression and energy metabolism.
Roux et al. (2008b) (in vivo) (plant whole body)	900 MHz		7	Energy metabolism.
Salford et al. (2003) (in vivo) (rat whole body)	915 MHz GSM 2 hr	0.02		Nerve cell damage in brain.
Sarimov et al. (2004) (in vitro)	895-915 MHz GSM 30 min	0.0054		Human lymphocyte chromatin affected similar to stress response.
Schwartz et al. (1990) (in vitro)	240 MHz-CW and sinusoidal modulation at 0.5 and 16 Hz, 30 min, effect only observed at 16-Hz modulation	0.00015		Calcium movement in the heart.
Schwarz et al. (2008) (in vitro)	1950 MHz UMTS 24 hr	0.05		Genes in human fibroblasts.
Somosy et al. (1991) (in vitro)	2.45 GHz, CW and 16 Hz square-modulation, modulated field more potent than CW	0.024		Molecular and structural changes in cells of mouse embryos.
Stagg et al. (1997) (in vitro)	836.55 MHz TDMA duty cycle 33% 24 hr	0.0059		Glioma cells showed significant increases in thymidine incorporation, which may be an indication of an increase in cell division.
Stankiewicz et al. (2006) (in vitro)	900 MHz GSM 217 Hz pulses-.577 ms width 15 min	0.024		Immune activities of human white blood cells.
Tattersall et al. (2001) (in vitro)	700 MHz CW, 5-15 min	0.0016		Function of the hippocampus.
Velizarov et al. (1999) (in vitro)	960 MHz GSM 217 Hz square-	0.000021		Decrease in proliferation of human epithelial amnion cells.

	pulse, duty cycle 12% 30 min			
Veyret et al. (1991) (in vivo) (mouse whole body)	9.4 GHz 1 □s pulses at 1000 pps, also with or without sinusoidal AM between 14 and 41 MHz, response only with AM modulation, direction of response depended on AM frequency	0.015		Functions of the immune system.
Vian et al. (2006) (in vivo) plant	900 MHz		7	Stress gene expression.
Wolke et al. (1996) (in vitro)	900, 1300, 1800 MHz, square-wave modulated at 217 Hz; Also 900 MHz with CW, 16 Hz, 50 Hz and 30 KHz modulations	0.001		Calcium concentration in heart muscle cells of guinea pig.
Yurekli et al. (2006) (in vivo) (rat whole body)	945 MHz GSM, 217 Hz pulse-modulation 7 hr/day, 8 days	0.0113		Free radical chemistry.

11. Chronic Exposures, Cumulative Effects, Different Waveforms:

Another important consideration in the setting of RFR exposure guidelines is the effect of **chronic/repeated exposure**. There is not much data on the biological effects of chronic RFR exposure, although some exist. (A list of chronic exposure studies can be found in sections 6 and 9 of http://www.bioinitiative.org/table-of-contents/). There are research data showing that the effects of chronic low level exposures are different than those of acute short-term thermal exposures. A set of similar experiments[11],[12] to those of de Lodge et al[3,4] was carried out in the 1980's to study the effects of repeated RFR exposures. The researchers concluded:

[11] D'Andrea, J.A., DeWitt, J.R., Emmerson, R.Y., Bailey, C., Stensaas, S., and Gandhi, O. P.,Intermittent exposure of rat to 2450-MHz microwaves at 2.5 mW/cm^2: behavioral and physiological effects, Bioelectromagnetics 7:315-328, 1986.

[12] D'Andrea, J.A., DeWitt, J.R., Gandhi, O. P., Stensaas, S., Lords, J.L., and Nielson, H.C.,Behavioral and physiological effects of chronic 2450-MHz microwave irradiation of the rat at 0.5 mW/cm^2, Bioelectromagnetics 7:45-56, 1986.

> "…the threshold for behavioral and physiological effects of chronic *(long-term)* RFR exposure in the rat occurs between 0.5 mW/cm^2 **(0.14 W/kg)** and 2.5 mW/cm^2 **(0.7 W/kg)**."

It appears that chronic exposure sensitized the animals to RFR. Therefore, it is insufficient to apply a guideline based on acute exposure (i.e., the data of de Lodge et al.) to a chronic exposure situation such as would be experienced with smart grid/metering technology and most others that are now categorically excluded.

An important question is whether RFR's biological effects are cumulative? There are studies that indicate RFR effects can accumulate with repeated exposures[13]. This is an important consideration in light of so many wireless devices in our midst today. No agency takes chronic exposure or cumulative effects into consideration. Each device or new technology is considered a stand-alone. Therefore, today's true exposures are unknown. This is especially troubling with smart grid/metering's peak exposures during the duty cycle and RFR emissions from 'access points' in the larger grid network. These points have significantly higher duty cycles in order to co-ordinate the signals from thousands of meters.

Another important consideration in the setting of guidelines for RFR exposure is the **waveform characteristics of the field**. There are many reports indicating that the waveform of RFR significantly alters its effectiveness in causing biological effects. Wave characteristics should be factored into the setting of new RFR exposure guidelines, since RFRs in the human environment today are of many different waveforms and characteristics.

And another important consideration is waveforms' specific effects. The following are some examples of reports regarding waveform specificity. (A more extensive list of studies showing waveform-specific biological effects can be found in sections 6 and 9 of http://www.bioinitiative.org/table-of-contents/).

- Campisi et al. (2010) reported increases in free radical activity and DNA fragmentation in brain cells after acute exposure to a 50-Hz amplitude-modulated 900-MHz RFR, whereas a continuous-wave 9000-MHz field produced no effect.
- Franzellitti et al. (2010) showed increased DNA strand breaks in trophoblasts after exposure to a 217-Hz modulated 1.8 GHz-RFR, but a continuous-wave field of the same carrier frequency was without effect.
- Tkalec et al (2013) reported that AM-modulated (1 KHz sinusoidal) 900-MHz RFR is more potent than non-modulated field in causing DNA damage in coelomocytes of exposed earthworms.

[13] Lai, H. Biological effects of radiofrequency radiation from wireless transmission towers. In *"Cell Towers: Wireless Convenience? Or Environmental Hazard?"* Levitt, B.B. (ed.), New Century Publishing, East Canaan, CT, 2001, pp. 65-74, 2001.

- Luukkonen et al. (2009) reported a continuous-wave 872-MHz RFR increased chemically-induced DNA strand breaks and free radicals in human neuroblastoma cells, whereas a GSM-modulated 872-MHz field had no significant effect.
- Zhang et al. (2008) found that gene expression in rat neurons is more sensitive to intermittent than continuous exposure to a 1.8 GHz-RFR.
- López-Martín et al. (2009) found that GSM and unmodulated RFR caused different effects on c-Fos gene expression in the rat brain.
- Croft et al. (2010) reported that 2G, but not 3G, cell phone radiation affected resting EEG.
- Hung et al. (2007) showed that 2, 8, 217 Hz-modulated RFR differentially affected sleep.
- Lopez-Martin et al. (2009) reported that modulated and non-modulated RFR had different effects on gene expression in the brain.
- Nylund et al. (2010) found that different carrier-frequencies (900 MHz verses 1800 MHz) had different effects on protein expression.
- Schmid et al. (2012) concluded that "modulation frequency components (of a RFR) within a physiological range may be sufficient to induce changes in sleep EEG".

Clearly there are more complex factors affecting biological processes with RFR exposures than just SAR and MPE. FCC needs to take waveforms and other transmission factors such as modulation into consideration when setting standards, especially in light of newer systems with far more complicated signaling characteristics.

11. Increasing Ambient Exposures: Humans and Wildlife

Today's wireless applications are raising ambient background levels with no FCC, EPA or other regulatory oversight. New additions to the mix include smart grid/metering creating low-level blanket exposures at ground level, and 3G/4G networks offering endless "apps," TV/music/video downloads, e-books, photos, voice, WiMax Internet connectivity and texting, all via cell phones and tablets. Then there are universal GPS systems close to a user's head (on a close lateral level with the eye) when mounted on a car dashboard. GPS works off of distant satellites and requires stronger signal emission. There is also a host of RF/radar devices built into automobiles today to detect animals on the road or park a vehicle without engaging the driver.

WiMax, already being build out, is ubiquitous wireless internet connectivity intended especially for rural communities that are now low RF areas. WiMax alone will introduce a new blanket of RFR with some systems capable of transmitting in a 12,000 square mile radius with a 62-mile reach from one antenna. The military and Homeland Security has also exponentially increased their use of wireless technology. All of these technologies use extremely complex signals that carry a lot of information. Given the data cited above in Table 3 regarding biological effects at very low intensities, we can no longer afford a presumption of safety with ever-increasing background levels.

RF is a form of energetic air pollution that requires far more regulation by FCC and other agencies, particularly the EPA and the U.S. Fish and Wildlife Service (USFWS). But there is no funding available to study, much less regulate RF at these agencies.

Prior to the telecom buildout in the early 1990's, baseline ambient RFR data was gathered in 1980 by the EPA in the largest multi-region survey ever performed. This data can be used to compare with today's rising exposures, yet no agency has continued to gather information, nor has this early study been updated in the U.S. EPA researchers, Richard Tell and Edwin Mantiply (1980)[14], assessed background levels of broadcast signal field intensity RFR for three years and obtained data at 486 locations distributed throughout 15 large cities. The data collectively represented 14,000 measurements of very high frequency (VHF) and ultra high frequency (UHF) radiation used in TV broadcast in ambient environments and they estimated exposure at 47,000 census districts within the metropolitan boundaries of those cities. At the time, ground-based broadcast signals from TV, AM radio and the then-increasing FM radio transmissions were the only exposures. There were no cellular services and very little satellite transmission at that time.

The study found that 20 percent of the total U.S. population was exposed to time-averaged VHF and UHF broadcast radiation at a median level of 0.0005 microwatts per centimeter squared (μW/cm2). The data suggested that only 1 percent of the population, or about 441,000 people, were potentially exposed to levels greater than 1μW/cm2 – the safety limit recommended by the USSR which was 1000 times more stringent than the U.S. safety guidelines back then. The data seemed reassuring for the general population at that time. Much has changed since then.

One European survey was reported on in Microwave News in 2000.[15] It found that background RFR levels in several cities had increased 10 times over the previous two decades. Changes in U.S. cities were thought to be comparable. In the European report, the primary cause was mobile phone technology. The short piece read:

> **Urban Electrosmog Increasing**
>
> RF/MW radiation levels in urban areas are approximately ten times higher than they were 20 years ago—and most of the increase is due to wireless communications, according to Dr. Yngve Hamnerius of Chalmers University of Technology in Göteborg, Sweden.

[14] Tell, R. A., Mantiply, E. D., Population Exposure to VHF and UHF Broadcast Radiation in the United States, Proceedings of the IEEE, Vol. 68, NO 1, January 1980.
[15] Urban Electrosmog Increasing, Microwave News, Vol. XX No.4, July/august 2000, p. 3. http://microwavenews.com/news/backissues/j-a00issue.pdf

> Hamnerius measured radiation levels in the 30 MHz-2 GHz frequency range at 26 sites across Sweden with varying levels of urbanization. In cities, the median power density was 0.05 μW/cm2, with a 61% average contribution from GSM base stations. In rural environments, the radiation levels were about 1,000 times lower with the largest contribution coming from television broadcasters, which account for 48% of the total.
>
> Hamnerius contrasted his results with those of Richard Tell and Edwin Mantiply in the late 1970s, when both were at the U.S. Environmental Protection Agency in Las Vegas. Their survey of 12 large American cities showed that the median exposure of the population was 0.005 W/cm2 (see *Radio Science, 17*, pp.39S-47S, 1982).

The following is a list of RF-levels measured in other countries.

- Amoako et al. (2009)- Ghana- 900-1800 MHz- 0.001 $\mu W/cm^2$
- Dode et al. (2011)- Brazil- cell tower- 0.04 - 40.78 $\mu W/cm^2$
- Dhami (2011)-India-10 MHz-8 GHz- 1.148 $\mu W/cm^2$
- Firlarer et al. (2003)- Turkey- GSM900 MHz - 3 $\mu W/cm^2$
- Frei et al. (2009)- Switzerland- 12 different bands from FM (88 MHz- 108 MHz) to W-LAN (2.4-2.5 GHz) - 0.013 $\mu W/cm^2$
- Henderson et al. (2006)- Australia- 870-1200 MHz- 0.8 $\mu W/cm^2$
- Joseph et al. (2008)- Belgium – FM, GSM900, GSM1800 and UMTS- 0.07 $\mu W/cm^2$
- Kim et al. (2010)- Korea- CDMA800 and CDMA1800- 0.6 $\mu W/cm^2$
- Thuroczy et al. (2006)- Hungary- 9 bands between 80-2200 MHz- 0.025 $\mu W/cm^2$
- Viel et al. (2009) - France- 12 bands: FM to mobile phone- 0.6 $\mu W/cm^2$

Although cellular service did not exist when the EPA survey was done, cell service now functions in the UHF bands and higher frequencies. So today's exposures are broadly comparable to background levels noted in that EPA review, which can be used as a baseline. When the U.S. switched to digital TV in 2008, it freed up spectrum "white space" previously used for analog TV transmission. That spectrum space is now allocated for 4G wireless Internet and both the VHF and UHF bands will be used in the upcoming ubiquitous WiMax service in rural areas.

The advent of digital technology, which simulates pulsed waves, significantly changed communications signaling characteristics, allowing for a second universal transmission system to be built on top of the old analog signals. This not only doubled

overall environmental RFR exposures, it introduced a completely new kind. It was the introduction of digital technology that facilitated the reshuffling of various RF bands in the 'limited real estate' of the electromagnetic spectrum. This reshuffling continues at FCC today with new upcoming airwave auctions. There is never enough spectrum to satisfy society's desire to use it. As a consequence, we have now filled in most of the lower nonionizing bands with commercial, private, and military use; split the signals; digitized them; and are now branching into higher frequencies such as infrared to be used in communications.

There is virtually no research to indicate that this is safe for either humans or wildlife but other species are highly sensitive in ways that humans are not. Some infrared frequencies are visible to other species. For instance, birds see the color red in ways that we do not and steady red lights atop towers are attractants at night. Red steady lighted towers are known to kill many more birds that white flashing lights.[16]

Birds' feathers are also known to have piezoelectric properties and are capable of conducting EMF/RF deep within bird body cavities. And birds are known to be sensitive to RFR. [17],[18]

According to Albert M. Manville, II, Ph.D., Senior Wildlife Biologist, Division of Migratory Bird Management at the U.S. Fish and Wildlife Service[19]:

> " The effects of radiation from communication towers on nesting and roosting wild birds are yet unstudied in U.S., although in Europe, Balmori (2005) found strong negative correlations between levels of tower-emitted microwave radiation and bird breeding, nesting, and roosting in the vicinity of electromagnetic fields in Spain. He documented nest and site abandonment, plumage deterioration, locomotion problems, and death in House Sparrows, White Storks, Rock Doves, Magpies, Collared Doves, and other species. While these species had historically been documented to roost and nest in these areas, Balmori (2005) did not observe these symptoms prior to construction of the cellular phone towers. Balmori and Hallberg (2007) and Everaert and Bauwens (2007) found similar strong negative correlations among male House Sparrows. Under laboratory conditions, T. Litovitz (pers. comm.) and De Carlo *et al.* (2002) raised troubling concerns about impacts of low-level, non-thermal radiation from the standard 915 MHz cell phone frequency

[16] Manville, A.M., Anthropogenic-related Bird Mortality Focusing on Steps to Address Human-caused Problems – a White Paper for the Anthropogenic Panel, 5th International Partners in Flight Conference, August 27, 2013, Snowbird, Utah
[17] Tanner, J.A. Effect of Microwave Radiation on Birds, *Nature* 210, 636 (07 May 1966);doi:10.1038/210636a0
[18] Tanner, J.A., Romero-Sierra, C., Davie, S.J. Non-thermal Effects of Microwave Radiation on Birds, *Nature* 216, 1139 (16 December 1967); doi:10.1038/2161139a0
[19] Albert M. Manville, II, Ph.D., Senior Wildlife Biologist, Division of Migratory Bird Management (DMBM), U.S. Fish and Wildlife Service, 4401 N. Fairfax Dr.–MBSP 4107 Arlington, VA 22203; 703/358-1963; albert_manville@fws.gov

> on domestic chicken embryos – with lethal results (Manville 2009). Given the findings of the studies mentioned above, field studies should be conducted in North America to validate potential impacts of communication tower radiation – both direct and indirect – to birds and potentially other animals. However, these have yet to be performed." (See References section for Manville citations.)

Dr. Manville is also on the Radio Frequency Inter-Agency Work Group (RFIAWG) and has worked closely with the FCC on towers and bird-death mitigation.

Birds are not the only species of fauna and flora affected. RFR can induce electric and magnetic fields in living tissue. While a complete literature review is beyond the scope of these comments, a selected sampling of both ELF and RFR exposures noted in wildlife includes:

- Alfonso Balmori[20] found that sparrows and other bird species abandoned areas where RF backgrounds were highest due to the presence of cell phone base stations. Other species affected included bats, invertebrates, insects, domestic animals, trees and bushes.
- Ioannis Magras and Thomas Zenos,[21] found increased rates of infertility and growth abnormalities in test animals at some distance from antenna parks where exposure levels were well below standards. By the fifth generation, test animals were permanently infertile.
- Andrea De Carlo, Nicole White, Fuling Guo, Peter Garret, and Theodore Litovitz[22] found decreases in the production of heat shock proteins in chick embryos. Heat shock proteins help maintain the conformation of cellular proteins during periods of stress. A decrease in their production diminishes cellular protection in a way that could lead to cancer and other diseases.
- Atsuko Kobayashi and Joseph Kirchvink[23] found myriad species contain the magnetic crystal magnetite and rely on it for critical activities in mating, direction-finding, and migratory patterns, among other things. Magnetite couples with external EMF/RF couples a million times more efficiently than any other known biological material**.**

[20] Balmori, A.M., The Effects of Microwave Radiation on the Wildlife, Preliminary Results, Valloid, Spain, 2003.
[21] Magras, I., Zenos, T., RF Radiation-Induced Changes in the Prenatal Development of Mice, Bioelectromagnetics 18:455-461, 1997).
[22] DeCarlo, A., White, N., Guo, F., Garret,G. Litivitz, T., Chronic Electromagnetic Field Exposure Decreases HSP70 Levels and Lowers Cytoprotection, Journal of Cellular Biochemistry 84:447-454, 2002.
[23] Kobayashi, A., Kirchvink, J.,Magnetoreception and Electromagnetic Field Effects: Sensory Perception of the Geomagnetic Field in Animals and Humans," Electromagnetic Fields, Biological Interactions and Mechanisms, Ed: Martin Blank, Advances in Chemistry Series 250, 1995, p 367-394.

- W. Loscher and G. Kas,[24] found severe behavioral anomalies in dairy cows near TV and RF-transmitting towers. Effects included lower milk production, excitability, birth defects, mastitis and others.
- A. Belyavskaya[25] found that plant roots exposed to extremely low magnetic fields exhibited a strong cytochemical reaction in root cells after exposure.

Other species are affected by increasing ambient backgrounds, perhaps even more so than humans due to their different physiologies. Effects seen in the literature for both *in vitro* and *in vivo* research include habitat loss and abandonment, infertility, adverse reproductive outcomes, cellular stress, and chemical changes, among others. And there are plausible mechanisms for biological action with the presence of magnetite in all species studied. Yet there are no guidelines at any regulatory agency to protect the environment, even though the FCC standards are considered – erroneously in our opinion – to include "environmental" exposures. There are glaring holes in this presumption.

Cellular communication infrastructure, though orders of magnitude lower in power density than broadcast facilities, are vastly more ubiquitous and placed much closer to the human population and wildlife in both urban and rural areas. The increasing advent of technologies like WiMax now affects formerly low RFR environments. Broadband-over-Powerlines will add to the rural exposures. We are doing this with no understanding of the broader consequences.

The rise in ambient RF levels is the single biggest environmental alteration within the last 20 years. Follow-up of the Tell and Mantiply/EPA study and the Hamnerius survey are imperative given today's increasing ambient RF levels.

12: Assessing Outdoor Far-Field Exposures:

Assessing outdoor exposures can be particularly difficult for a variety of factors. One question involves how best to capture field exposure data, e.g. through computer estimates or actual dosimetry measurements? Distance from a generating source has traditionally been used as a surrogate for probable power density but that is imperfect at best, given how RF energy couples with the environment once transmitted. Complicated factors and numerous variables come into play, such as orientation toward the transmitting source, species, size,

[24] Loscher, W., Kas G;, Conspicuous behavioral abnormalities in a dairy cow herd near TV and Radio transmitting antenna, Prakt. Tierarzt [Practical Veterinary Surgeon], 79:5, 437-444, 1998.
[25] Belyavskaya, N.A., Ultrastructure and Calcium Balance in Meristem Cells of Pea Roots Exposed to Extremely Low Magnetic Fields, Elsevier Sciences, Ltd. Pergamon, Adv. Space Res. Vol. 28, No. 4, pp. 645-450, 2001.

physical composition, genetics, presence of metal objects and topography, to name a few.[26] In human populations, the wearing of personal dosimetry devices appears promising for capturing cumulative exposure data.[27] But attaching RF devices to wildlife is ill-advised despite the frequent use of radio collars and RFID chips by biologists to study wildlife. Deadly sarcomas have been observed in tissue around RFID chips imbedded in domestic pets, for instance.[28] While RFID chips are supposed to be passive until called upon to give up information by a device, these sarcomas are an alarm signal that RFID's are: 1) malfunctioning; and 2) the low-level fields caused by the batteries may be affecting tissue. Radio collars attached typically at the head to wildlife transmit constantly and work off of satellites, thus requiring stronger emissions.

One study that indicates the increasing background levels of mobile phone infrastructure was done on humans in 2009 using personal dosimetry devices to examine the total exposure levels of RFR in the Swiss urban population[29]. What they found was startling. Nearly a third of the test subjects' cumulative exposures were from cell tower base stations. Prior to this study, exposure from base stations was thought to be insignificant due to their low-power densities and to affect only those living or working in close proximity to such infrastructure. But this study showed that the general population moves in and out of these particular fields with more regularity than previously thought. That assessment would apply to wildlife, too.

In the study, a sample of 166 volunteers from Basel, Switzerland, agreed to wear personal exposure meters (called exposimeters). Frei et al found that nearly one third of total exposures came from cell phone base stations. Participants carried an exposimeter for 1 week and also completed an activity diary. Results found a mean weekly exposure to all RF and/or EMF sources was 0.013 milliwatts per square centimeter (mW/cm2). Exposure was mainly from mobile phone base stations (32.0%); mobile phone handsets (29.1%); and domestic digital enhanced cordless telecommunications (DECT) phones (22.7%). Mean values were highest in trains (0.116 mW/cm2), airports (0.074 mW/cm2), and tramways or buses (0.036 mW/cm2) and were higher during the daytime (0.016 mW/cm2) than the nighttime (0.008 mW/cm2).

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[26] Levitt, B. B., Lai, H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays, Enviro. Rev. 369-395 (2010), doi:10.1139/A 10-018 http://www.nrcresearchpress.com/doi/pdf/10.1139/A10-018

[27] Radon, K., Spegel, H., Meyer, N., Klein, J., Brix, J., Wiedenhofer,A., Eder, H., Praml, G., Schulze, A., Ehrenstein, V., von Kries,R., and Nowak, D. 2006. Personal dosimetry of exposure to mobiletelephone base stations? An epidemiological feasibilitystudy comparing the Maschek dosimeter prototype and Antennessa SP-090 system. Bioelectromagnetics, **27**(1): 77–81.doi:10.1002/bem.20175.

[28] Lewan, Todd. Chip Implants Linked to Animal Tumors, The Associated Press Saturday, September 8, 2007; 2:04 PM

[29] Frei, P., Mohler, E., Neubauer, G., Theis, G., Burgi, A., Frohlich, J., Braun-Fahrlander, C., Bolte, J., Egger, M., and Roosli, M. Temporal and spatial variability of personal exposure to radio frequency electromagnetic fields. Environ. Res 109(6):779–785. doi:10.1016/j.envres.2009.04.015.

Another surprising finding of this study implied that at the belt, backpack, or in close vicinity to the body in test subjects, the mean base station contribution corresponded to about 7 min of mobile phone use. In other words, ambient exposure from infrastructure was a significant contributor beyond one's personal choice to use individual devices.

RF field strength falls off rapidly with distance from the transmitting source, but predicting actual exposures based on simple distance from antennas using standardized computer formulas is inadequate. Actual exposure metrics can be far more complex in both urban and rural areas, to humans and wildlife alike. Contributing to the complexity is the fact that the narrow vertical spread of the beam creates a low RF field strength at the ground directly below the antenna. As a person or wildlife species moves away or within a particular field, exposures can become complicated, creating peaks and valleys in field strength. Scattering and attenuation alter field strength in relation to building placement, architectural composition, the presence of trees, soil type, and topographical features such as mountains and rock formations.[30] Power density levels can be 1-to-100 times lower inside a building, for instance, depending on construction materials. Exposures can differ greatly depending on numerous factors, such as orientation toward the generating source, as well as the presence of conductive mediums like water, or minerals in soil containing salt, iron and copper. Exposures can be twice as high in upper floors as in lower floors, as found by Anglesio et al.[31] This would apply to birds/bats/bees and other insects receiving higher exposures when flying at a lateral plane with transmitting antennas atop a tower or mounted on other structures.

Although distance from a transmitting source has been shown to be an unreliable determinant for accurate exposure predictions, it is nevertheless useful in general ways. For instance, it has been shown that radiation levels from a tower with 15 non-broadcast radio systems will fall off to natural background levels at approximately 1500 feet, or approximately 500 meters.[32] This would be in general agreement with the lessening of symptoms in human populations living near cell towers at a distance over 1000 ft (300

[30] Kasevich, R.S., Brief Overview of the Effects of Electromagnetic Fields on the Environment; Cell Towers, Wireless Convenience? or Environmental Hazard? Proceedings of the "Cell Towers Forum," State of the Science, State of the Law, Safe Goods/New Century Publishing, 2001, pp.170-175.

[31] Anglesio, L., Benedetto, A., Bonino, A., Colla, D., Martire, F., Saudino Fusette, S., and d'Amore, G. 2001. Population exposureto electromagnetic fields generated by radio base stations: evaluation of the urban background by using provisional model andinstrumental measurements. Radiat. Prot. Dosimetry, **97**: 355–358. PMID:11878419. 2001.

[32] Rinebold, J.M., Centralized Siting of Telecommunications Faciulities: Cell Towers, Wireless Convenience? or Environmental Hazard? Proceedings of the "Cell Towers Forum," State of the Science, State of the Law Safe Goods/New Century Publishing, 2001, pp. 133.

meters) found by Santini et al[33], Abdel-Rassoul et al, [34]Hutter etc al ,[35] Navarro et al,[36] and Oberfield et al.[37]

Unfortunately, there is very little far-field distance-to-safety ratios research for wildlife as this has not been studied with that focus in mind. What little EMF/RF field research on wildlife has been conducted, has been focused on behavior, mortality and reproductive outcomes.

13. Conclusion: The following are suggestions to FCC in updating the RFR exposure standards:

- Use both SAR and MPE but not interchangeably.
- Post SAR's on the FCC's website, on products, and at point-of-sale.
- Take waveform specifics and modulation into consideration.
- Increase tower/antenna array monitoring for compliance with FCC standards.
- Institute large setbacks from tower installations, 1500' minimum for cell towers at 150' in height. Lower height DAS systems should be discouraged unless large setbacks from dwellings/business can be attained.
- Tell Congress that the EPA should be refunded for EMF/RF research and standards setting/review; and that USFWS should have research appropriations to specifically study RFR effects on wildlife.
- Decrease MPE's – FCC is supposed to regulate the airwaves and enforce safety. Assisting industry is secondary.
- Reduce categorical exclusions based solely on power density. Ubiquity of exposures, such as from smart grid/metering, also count.
- Set limits for chronic exposures from multiple sources and cumulative effects.
- Make clear that FCC standards as currently written are for human exposures only and do not include wildlife or protect the environment.
- Take a Precautionary Approach
- Institute more field measurement and less computation.

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Appendix A Attached:

Levitt, B.B., Lai, H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays, Enviro. Rev. 369-395 (2010), doi:10.1139/A 10-018 http://www.nrcresearchpress.com/doi/pdf/10.1139/A10-018

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