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.