

RFR-Genotoxic Studies

(July 2006)

Aitken RJ, Bennetts LE, Sawyer D, Wiklendt AM, King BV. Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline. *International Journal of Andrology*, 28:171-179, 2005.

Concern has arisen over human exposures to radio frequency electromagnetic radiation (RFEMR), including a recent report indicating that regular mobile phone use can negatively impact upon human semen quality. These effects would be particularly serious if the biological effects of RFEMR included the induction of DNA damage in male germ cells. In this study, mice were exposed to 900 MHz RFEMR at a specific absorption rate of approximately 90 mW/kg inside a waveguide for 7 days at 12 h per day. Following exposure, DNA damage to caudal epididymal spermatozoa was assessed by quantitative PCR (QPCR) as well as alkaline and pulsed-field gel electrophoresis. The treated mice were overtly normal and all assessment criteria, including sperm number, morphology and vitality were not significantly affected. Gel electrophoresis revealed no gross evidence of increased single- or double-DNA strand breakage in spermatozoa taken from treated animals. However, a detailed analysis of DNA integrity using QPCR revealed statistically significant damage to both the mitochondrial genome ($p < 0.05$) and the nuclear β -globin locus ($p < 0.01$). This study suggests that while RFEMR does not have a dramatic impact on male germ cell development, a significant genotoxic effect on epididymal spermatozoa is evident and deserves further investigation.

Antonopoulos A, Eisenbrandt H, Obe G. Effects of high-frequency electromagnetic fields on human lymphocytes in vitro. *Mutation Research*, 395(2-3):209-214, 1997.

Human peripheral lymphocytes were incubated in the presence of high-frequency electromagnetic fields of 380, 900 and 1800 MHz. The measured endpoints were cell cycle progression and the frequencies of sister-chromatid exchanges. No differences between treated and control cultures could be found.

Balode, Z. Assessment of radio-frequency electromagnetic radiation by the micronucleus test in bovine peripheral erythrocytes. *Science of the Total Environment*, 180(1):81-85, 1996.

Previous bioindicative studies in the Skrunda Radio Location Station area have focused on the somatic influence of electromagnetic radiation on plants, but it is also important to study genetic effects. We have chosen cows as test animals for cytogenetical evaluation because they live in the same general exposure area as humans, are confined to specific locations and are chronically exposed to radiation. Blood samples were obtained from female Latvian Brown cows from a farm close to and in front of the Skrunda Radar and from cows in a

control area. A simplified alternative to the Schiff method of DNA staining for identification of micronuclei in peripheral erythrocytes was applied. Microscopically, micronuclei in peripheral blood erythrocytes were round in shape and exhibited a strong red colour. They are easily detectable as the only coloured bodies in the uncoloured erythrocytes. From each individual animal 2000 erythrocytes were examined at a magnification of x 1000 for the presence of micronuclei. The counting of micronuclei in peripheral erythrocytes gave low average incidences, 0.6 per 1000 in the exposed group and 0.1 per 1000 in the control, but statistically significant ($P < 0.01$) differences were found in the frequency distribution between the control and exposed groups.

Baohong W, 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. *Mutation Research*, 578(1-2):149-57, 2005.

The aim of this investigation was to study the synergistic DNA damage effects in human lymphocytes induced by 1.8 GHz radiofrequency field radiation (RFR, SAR of 3 W/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 times (0 or 21 h) 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 21 h incubation after exposure ($P > 0.05$). There were significant differences of DNA damage indexes between MMC group and RFR+MMC co-exposure group at 0 and 21 h 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 21 h 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 1.8 GHz RFR (SAR, 3 W/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 1.8 GHz RFR with BLM or MMS were not obvious.

Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. *Bioelectromagnetics*, 26(3):173-184, 2005.

We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power

frequency magnetic field (50 Hz, 15 μ T peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock.

Belyaev IY, Koch CB, Terenius O, Roxstrom-Lindquist K, Malmgren LO, H Sommer W, Salford LG, Persson BR. Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. *Bioelectromagnetics*, 27(4):295-306, 2006.

We investigated whether exposure of rat brain to microwaves (MWs) of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression. An exposure installation was used based on a test mobile phone employing a GSM signal at 915 MHz, all standard modulations included, output power level in pulses 2 W, specific absorption rate (SAR) 0.4 mW/g. Rats were exposed or sham exposed to MWs during 2 h. After exposure, cell suspensions were prepared from brain samples, as well as from spleen and thymus. For analysis of gene expression patterns, total RNA was extracted from cerebellum. Changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependencies (AVTD). DNA double strand breaks (DSBs) were analyzed by pulsed-field gel electrophoresis (PFGE). Effects of MW exposure were observed on neither conformation of chromatin nor DNA DSBs. Gene expression profiles were obtained by Affymetrix U34 GeneChips representing 8800 rat genes and analyzed with the Affymetrix Microarray Suite (MAS) 5.0 software. In cerebellum from all exposed animals, 11 genes were upregulated in a range of 1.34-2.74 fold and one gene was downregulated 0.48-fold ($P < .0025$). The induced genes encode proteins with diverse functions including neurotransmitter

regulation, blood-brain barrier (BBB), and melatonin production. The data shows that GSM MWs at 915 MHz did not induce PFGE-detectable DNA double stranded breaks or changes in chromatin conformation, but affected expression of genes in rat brain cells

Bisht KS, Moros EG, Straube WL, Baty JD, Roti Roti JL. The Effect of 835.62 MHz FDMA or 847.74 MHz CDMA Modulated Radiofrequency Radiation on the Induction of Micronuclei in C3H 10T1/2 Cells. *Radiation Research*, 157(5):506–515, 2002.

To determine if radiofrequency (RF) radiation induces the formation of micronuclei, C3H 10T_{1/2} cells were exposed to 835.62 MHz frequency division multiple access (FDMA) or 847.74 MHz code division multiple access (CDMA) modulated RF radiation. After the exposure to RF radiation, the micronucleus assay was performed by the cytokinesis block method using cytochalasin B treatment. The micronuclei appearing after mitosis were scored in binucleated cells using acridine orange staining. The frequency of micronuclei was scored both as the percentage of binucleated cells with micronuclei and as the number of micronuclei per 100 binucleated cells. Treatment of cells with cytochalasin B at a concentration of 2 µg/ml for 22 h was found to yield the maximum number of binucleated cells in C3H 10T_{1/2} cells. The method used for the micronucleus assay in the present study detected a highly significant dose response for both indices of micronucleus production in the dose range of 0.1–1.2 Gy and it was sensitive enough to detect a significant ($P > 0.05$) increase in micronuclei after doses of 0.3 Gy in exponentially growing cells and after 0.9 Gy in plateau-phase cells. Exponentially growing cells or plateau-phase cells were exposed to CDMA (3.2 or 4.8 W/kg) or FDMA (3.2 or 5.1 W/kg) RF radiation for 3, 8, 16 or 24 h. In three repeat experiments, no exposure condition was found by analysis of variance to result in a significant increase relative to sham-exposed cells either in the percentage of binucleated cells with micronuclei or in the number of micronuclei per 100 binucleated cells. In this study, data from cells exposed to different RF signals at two SARs were compared to a common sham-exposed sample. We used the Dunnett's test, which is specifically designed for this purpose, and found no significant exposure-related differences for either plateau-phase cells or exponentially growing cells. Thus the results of this study are not consistent with the possibility that these RF radiations induce micronuclei.

Busljeta I, Trosic I, Milkovic-Kraus S. Erythropoietic changes in rats after 2.45 GJz nonthermal irradiation. *International Journal of Hygiene and Environmental Health*, 207(6):549-554, 2004.

The purpose of this study was to observe the erythropoietic changes in rats subchronically exposed to radiofrequency microwave (RF/MW) irradiation at nonthermal level. Adult male Wistar rats (N=40) were exposed to 2.45 GHz continuous RF/MW fields for 2 hours daily, 7 days a week, at 5-10 mW/cm². Exposed animals were divided

into four subgroups (n=10 animals in each subgroup) in order to be irradiated for 2, 8, 15 and 30 days. Animals were sacrificed on the final irradiation day of each treated subgroup. Unexposed rats were used as control (N=24). Six animals were included into the each control subgroup. Bone marrow smears were examined to determine absolute counts of anuclear cells and erythropoietic precursor cells. The absolute erythrocyte count, haemoglobin and haematocrit values were observed in the peripheral blood by an automatic cell counter. The bone marrow cytogenetic analysis was accomplished by micronucleus (MN) tests. In the exposed animals erythrocyte count, haemoglobin and haematocrit were increased in peripheral blood on irradiation days 8 and 15. Concurrently, anuclear cells and erythropoietic precursor cells were significantly decreased ($p < 0.05$) in the bone marrow on day 15, but micronucleated cells' frequency was increased. In the applied experimental condition, RF/MW radiation might cause disturbance in red cell maturation and proliferation, and induce micronucleus formation in erythropoietic cells.

Chang SK, Choi JS, Gil HW, Yang JO, Lee EY, Jeon YS, Lee ZW, Lee M, Hong MY, Ho Son T, Hong SY. Genotoxicity evaluation of electromagnetic fields generated by 835-MHz mobile phone frequency band. *European Journal of Cancer Prevention*, 14(2):175-179, 2005.

It is still unclear whether the exposure to electromagnetic fields (EMFs) generated by mobile phone radiation is directly linked to cancer. We examined the biological effects of an EMF at 835 MHz, the most widely used communication frequency band in Korean CDMA mobile phone networks, on bacterial reverse mutation (Ames assay) and DNA stability (in vitro DNA degradation). In the Ames assay, tester strains alone or combined with positive mutagen were applied in an artificial mobile phone frequency EMF generator with continuous waveform at a specific absorption rate (SAR) of 4 W/kg for 48 h. In the presence of the 835-MHz EMF radiation, incubation with positive mutagen 4-nitroquinoline-1-oxide and cumene hydroxide further increased the mutation rate in *Escherichia coli* WP2 and TA102, respectively, while the contrary results in *Salmonella typhimurium* TA98 and TA1535 treated with 4-nitroquinoline-1-oxide and sodium azide, respectively, were shown as antimutagenic. However, these mutagenic or co-mutagenic effects of 835-MHz radiation were not significantly repeated in other relevant strains with same mutation type. In the DNA degradation test, the exposure to 835-MHz EMF did not change the rate of degradation observed using plasmid pBluescript SK(+) as an indicator. Thus, we suggest that 835-MHz EMF under the conditions of our study neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro.

Ciaravino V, Meltz ML, Erwin DN. Absence of a synergistic effect between moderate-power radio-frequency electromagnetic radiation and adriamycin on cell-cycle progression and sister-chromatid exchange. *Bioelectromagnetics*, 12(5):289-298, 1991.

In our laboratories we are conducting investigations of potential interactions between radio-frequency electromagnetic radiation (RFR) and chemicals that are toxic by different mechanisms to mammalian cells. The RFR is being tested at frequencies in the microwave range and at different power levels. We report here on the 1) ability of simultaneous RFR exposures to alter the distribution of cells in first and second mitoses from that after treatment by adriamycin alone, and 2) on the ability of simultaneous RFR exposure to alter the extent of sister chromatid exchanges (SCEs) induced by adriamycin alone. This chemical was selected because of its reported mechanism of action and because it is of interest in the treatment of cancer. In our studies, Chinese hamster ovary (CHO) cells were exposed for 2 h simultaneously to adriamycin and pulsed RFR at a frequency of 2,450 MHz and a specific absorption rate of 33.8 W/Kg. The maximal temperature (in the tissue-culture medium) was 39.7 +/- 0.2 degrees C. The experiments were controlled for chemical and RFR exposures, as well as for temperature. Verified statistically, the data indicate that the RFR did not affect changes in cell progression caused by adriamycin, and the RFR did not change the number of SCEs that were induced by the adriamycin, which adriamycin is known to affect cells by damaging their membranes and DNA.

d'Ambrosio G, Massa R, Scarfi MR, Zeni O. Cytogenetic damage in human lymphocytes following GSMK phase modulated microwave exposure. *Bioelectromagnetics*, 23:7-13, 2002.

The present study investigated, using in vitro experiments on human lymphocytes, whether exposure to a microwave frequency used for mobile communication, either unmodulated or in presence of phase only modulation, can cause modification of cell proliferation kinetics and/or genotoxic effects, by evaluating the cytokinesis block proliferation index and the micronucleus frequency. In the GSM 1800 mobile communication systems the field is both phase (Gaussian minimum shift keying, GSMK) and amplitude (time domain multiple access, TDMA) modulated. The present study investigated only the effects of phase modulation, and no amplitude modulation was applied. Human peripheral blood cultures were exposed to 1.748 GHz, either continuous wave (CW) or phase only modulated wave (GSMK), for 15 min. The maximum specific absorption rate (~5 W/kg) was higher than that occurring in the head of mobile phone users; however, no changes were found in cell proliferation kinetics after exposure to either CW or GSMK fields. As far as genotoxicity is concerned, the micronucleus frequency result was not affected by CW exposure; however, a statistically significant micronucleus effect was found following exposure to phase modulated field. These results would suggest a genotoxic power of the phase modulation per se.

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. *Mutation Research*, 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.

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

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

Gadhia PK, Shah T, Mistry A, Pithawala M, Tamakuwala D. A preliminary study to assess possible chromosomal damage among users of digital mobile phones. *Electromagnetic Biology and Medicine*, 22(2-3):149-159, 2003.

In a preliminary study to examine possible lymphocyte chromosomal damage, we have tested two cytogenetic endpoints, namely, chromosomal aberrations (CA) and sister chromatid exchange frequencies (SCE), in 24 mobile phone users (12 nonsmoker–nonalcoholic subjects and 12 smoker–alcoholics), who used digital mobile phones for at least 2 years, employing Gaussian Minimum Shift Keying modulations with uplink frequencies at 935–960 MHz. and downlinks at 890–915 MHz. For comparison, the control study group included another 24 individuals, matched according to their age, sex, drinking and smoking habits, as well as similar health status, working habits, and professional careers; but did not use mobile phones. Blood samples of 12 mobile users (6 smoker–alcoholic and 6 nonsmoker–nonalcoholic) and 12 controls (identical to mobile users in every respect) were further treated with a known mutagen Mitomycin-C (MMC) to find out comutagenic/synergistic effect. A complete blood picture for each individual

was assessed with an automatic particle cell counter. There was a significant increase ($P < 0.05$) in dicentric chromosomes among mobile users who were smoker–alcoholic as compared to nonsmoker–nonalcoholic; the same held true for controls of both types. After MMC treatment, there was a significant increase in dicentrics ($P < 0.05$) and ring chromosomes ($P < 0.001$) in both smoker–alcoholic and nonsmoker–nonalcoholic mobile users when compared with the controls. Although SCEs showed a significant increase among mobile users, no change in cell cycle progression was noted. The hematological picture showed only minor variations between mobile users and controls.

Gandhi G, Anita. Genetic damage in mobile phone users: some preliminary findings. *Indian Journal of Human Genetics*, 11(2): 99-104, 2005 [a].

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.

Gandhi G, Singh P. Cytogenetic damage in mobile phone users: preliminary data. *International Journal of Human Genetics*, 5(4):259-265, 2005 [b].

Mobile telephones, sometimes called cellular (cell) phones or handies, are now an integral part of modern life. The mobile phone handsets are low-powered radiofrequency transmitters, emitting maximum powers in the range of 0.2 to 0.6 watts. Scientific concerns have increased sufficiently over the possible hazard to health from using cell phones. The reported adverse health effects include physiological, behavioural and cognitive changes as well as tumour formation and genetic damage. However findings are controversial and no consensus exists. Genotoxicity has been observed either in lower organisms or in vitro studies. The aim of the present study hence was to detect any

cytogenetic damage in mobile phone users by analysing short term peripheral lymphocyte cultures for chromosomal aberrations and the buccal mucosal cells for micronuclei (aneugenicity and clastogenicity). The results revealed increased number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes indicating the genotoxic response from mobile phone use.

Garaj-Vrhovac V, Horvat D, Koren Z. The effect of microwave radiation on the cell genome. *Mutation Research*, 243(2):87-93, 1990.

Cultured V79 Chinese hamster cells were exposed to continuous radiation, frequency 7.7 GHz, power density 30 mW/cm² for 15, 30, and 60 min. The parameters investigated were the incorporation of [3H]thymidine and the frequency of chromosome aberrations. Data obtained by 2 methods (the incorporation of [3H]thymidine into DNA and autoradiography) showed that the inhibition of [3H]thymidine incorporation took place by complete prevention of DNA from entering into the S phase. The normal rate of incorporation of [3H]thymidine was recovered within 1 generation cycle of V79 cells. Mutagenic tests performed concurrently showed that even DNA macromolecules were involved in the process. In comparison with the control samples there was a higher frequency of specific chromosome lesions in cells that had been irradiated. Results discussed in this study suggest that microwave radiation causes changes in the synthesis as well as in the structure of DNA molecules.

Garaj-Vrhovac V, Horvat D, Koren Z. The relationship between colony-forming ability, chromosome aberrations and incidence of micronuclei in V79 Chinese hamster cells exposed to microwave radiation. *Mutation Research*, 263(3):143-149, 1991.

Cultured V79 Chinese hamster fibroblast cells were exposed to continuous radiation, frequency 7.7 GHz, power density 0.5 mW/cm² for 15, 30 and 60 min. The effect of microwave radiation on cell survival and on the incidence and frequency of micronuclei and structural chromosome aberrations was investigated. The decrease in the number of irradiated V79 cell colonies was related to the power density applied and to the time of exposure. In comparison with the control samples there was a significantly higher frequency of specific chromosome aberrations such as dicentric and ring chromosomes in irradiated cells. The presence of micronuclei in irradiated cells confirmed the changes that had occurred in chromosome structure. These results suggest that microwave radiation can induce damage in the structure of chromosomal DNA.

Garaj-Vrhovac V, Fucic A, Horvat D. The correlation between the frequency of micronuclei and specific chromosome aberrations in human lymphocytes exposed to microwave radiation in vitro. *Mutation Research*, 281(3):181-186, 1992.

Human whole-blood samples were exposed to continuous microwave radiation, frequency 7.7 GHz, power density 0.5, 10 and 30 mW/cm² for 10, 30 and 60 min. A correlation between specific chromosomal aberrations and the incidence of micronuclei after in vitro exposure was observed. In all experimental conditions, the frequency of all types of chromosomal aberrations was significantly higher than in the control samples. In the irradiated samples the presence of dicentric and ring chromosomes was established. The incidence of micronuclei was also higher in the exposed samples. The results of the structural chromosome aberration test and of the micronucleus test were comparatively analyzed. The values obtained showed a positive correlation between micronuclei and specific chromosomal aberrations (acentric fragments and dicentric chromosomes). The results of the study indicate that microwave radiation causes changes in the genome of somatic human cells and that the applied tests are equally sensitive for the detection of the genotoxicity of microwaves.

Garaj-Vrhovac, V. Micronucleus assay and lymphocyte mitotic activity in risk assessment of occupational exposure to microwave radiation. *Chemosphere*, 39(13):2301-2312, 1999.

The effects of radiofrequency electromagnetic radiation (RFR) on the cell kinetics and genome damages in peripheral blood lymphocytes were determined in lymphocytes of 12 subjects occupationally exposed to microwave radiation. Results showed an increase in frequency of micronuclei (MN) as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls. According to previous reports micronucleus assay can serve as a suitable indicator for the assessment of exposure to genotoxic agents (such as RFR) and the analysis of mitotic activity as an additional parameter for the efficient biomonitoring.

Garson OM, McRobert TL, Campbell LJ, Hocking BA, Gordon I. A chromosomal study of workers with long-term exposure to radio-frequency radiation. *Medical Journal of Australia*, 155(5):289-292, 1991.

OBJECTIVE: To examine whether an increased level of chromosome damage occurs in the stimulated lymphocytes of radio-linemen after long-term but intermittent exposure to radio-frequency radiation (RFR) during the course of their work. **DESIGN AND PARTICIPANTS:** Chromosome studies were performed on blood samples from 38 radio-linemen matched by age with 38 controls, all of whom were employed by Telecom Australia. The radio-linemen had all worked with RFR in the range 400 kHz-20 GHz with exposures at or below the Australian occupational limits, and the controls were members of the clerical staff who had no exposure to RFR. Two hundred metaphases from each subject were studied and chromosome damage was scored by an observer who was blind to the status of the subjects. **RESULTS:** The ratio of the rate of aberrant cells in the radio-linemen group to that in the control group was 1.0 (95% confidence interval, 0.8-1.3). There were no statistically significant differences in the types of aberrations that

were scored. CONCLUSION: Exposure to RFR at or below the described limits did not appear to cause any increase in chromosomal damage in circulating lymphocytes.

Gorlitz BD, Muller M, Ebert S, Hecker H, Kuster N, Dasenbrock C. Effects of 1-week and 6-week exposure to GSM/DCS radiofrequency radiation on micronucleus formation in B6C3F1 mice. *Radiation Research*, 164(4 Pt 1):431-439, 2005..

The aim of this study was to examine the possible induction of micronuclei in erythrocytes of the peripheral blood and bone marrow and in keratinocytes and spleen lymphocytes of mice exposed to radiofrequency (RF) radiation for 2 h per day over periods of 1 and 6 weeks, respectively. The applied signal simulated the exposure from GSM900 and DCS1800 handsets, including the low-frequency amplitude-modulation components as they occur during speaking (GSM Basic), listening (DTX) and moving within the environment (handovers, power control). The carrier frequency was set to the center of the system's uplink band, i.e., 902 MHz for GSM and 1747 MHz for DCS. Uniform whole-body exposure was achieved by restraining the mice in tubes at fixed positions in the exposure setup. Mice were exposed to slot-averaged whole-body SARs of 33.2, 11.0, 3.7 and 0 mW/g during the 1-week study and 24.9, 8.3, 2.8 and 0 mW/g during the 6-week study. Exposure levels for the 1- and 6-week studies were determined in a pretest to confirm that no thermal effect was present that could influence the genotoxic end points. During both experiments and for both frequencies, no clinical abnormalities were detected in the animals. Cells of the bone marrow from the femur (1-week study), erythrocytes of the peripheral blood (6-week study), keratinocytes from the tail root, and lymphocytes from the spleen (both studies) were isolated on slides and stained for micronucleus analysis. Two thousand cells per animal were scored in erythrocyte and keratinocyte samples. In spleen lymphocytes, 1000 binucleated lymphocytes were scored for each animal. The RF-field exposure had no influence on the formation of red blood cells. After 1 week of exposure, the ratio of polychromatic to normochromatic erythrocytes was unchanged in the treated groups compared to the sham-exposed groups. Furthermore, the RF-field exposure of mice did not induce an increase in the number of micronuclei in erythrocytes of the bone marrow or peripheral blood, in keratinocytes, or in spleen lymphocytes compared to the sham-treated control.

Gos P, Eicher B, Kohli J, Heyer WD. No mutagenic or recombinogenic effects of mobile phone fields at 900 MHz detected in the yeast *saccharomyces cerevisiae*. *Bioelectromagnetics*, 21(7):515-523, 2000.

Both actively growing and resting cells of the yeast *Saccharomyces cerevisiae* were exposed to 900-MHz fields that closely matched the Global System for Mobile Communication (GSM) pulsed modulation format signals for mobile phones at specific absorption rates (SAR) of 0.13 and 1.3 W/kg. Two identical anechoic test chambers were constructed to perform concurrent control and test experiments under well-controlled exposure conditions. Using specific test strains, we examined the genotoxic potential of mobile phone fields, alone and in combination, with a known genotoxic compound, the alkylating agent methyl

methansulfonate. Mutation rates were monitored by two test systems, a widely used gene-specific forward mutation assay at CAN1 and a wide-range assay measuring the induction of respiration-deficient (petite) clones that have lost their mitochondrial function. In addition, two further assays measured the recombinogenic effect of mobile phone fields to detect possible effects on genomic stability: First, an intrachromosomal, deletion-formation assay previously developed for genotoxic screening; and second, an intragenic recombination assay in the ADE2 gene. Fluctuation tests failed to detect any significant effect of mobile phone fields on forward mutation rates at CAN1, on the frequency of petite formation, on rates of intrachromosomal deletion formation, or on rates of intragenic recombination in the absence or presence of the genotoxic agent methyl methansulfonate.

Haider T, Knasmueller S, Kundi M, Haider M. Clastogenic effects of radiofrequency radiations on chromosomes of Tradescantia. *Mutation Research*, 324(1-2):65-68, 1994.

The clastogenicity of electromagnetic fields (EMF) has so far been studied only under laboratory conditions. We used the Tradescantia-micronucleus (Trad-MCN) bioassay in an in situ experiment to find out whether short-wave electromagnetic fields used for broadcasting (10-21 MHz) may show genotoxic effects. Plant cuttings bearing young flower buds were exposed (30 h) on both sides of a slewable curtain antenna (300/500 kW, 40-170 V/m) and 15 m (90 V/m) and 30 m (70 V/m) distant from a vertical cage antenna (100 kW) as well as at the neighbors living near the broadcasting station (200 m, 1-3 V/m). The exposure at both sides of the slewable curtain antenna was performed simultaneously within cages, one of the Faraday type shielding the field and one non-shielding mesh cage. Laboratory controls were maintained for comparison. Higher MCN frequencies than in laboratory controls were found for all exposure sites in the immediate vicinity of the antennae, where the exposure standards of the electric field strength of the International Radiation Protection Association (IRPA) were exceeded. The results at all exposure sites except one were statistically significant. Since the parallel exposure in a non-shielding and a shielding cage also revealed significant differences in MCN frequencies (the latter showing no significant differences from laboratory controls), the clastogenic effects are clearly attributable to the short-wave radiation from the antennae.

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. *Radiation Research*, 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.

Kerbacher JJ, Meltz ML, Erwin DN. Influence of radiofrequency radiation on chromosome aberrations in CHO cells and its interaction with DNA-damaging agents. *Radiation Research*, 123(3):311-319, 1990.

A limited number of contradictory reports have appeared in the literature about the ability of radiofrequency (rf) radiation to induce chromosome aberrations in different biological systems. The technical documentation associated with such reports is often absent or deficient. In addition, no information is available as to whether any additional genotoxic hazard would result from a simultaneous exposure of mammalian cells to rf radiation and a chemical which (by itself) induces chromosome aberrations. In the work described, we have therefore tested two hypotheses. The first is that rf radiation by itself, at power densities and exposure conditions which are higher than is consistent with accepted safety guidelines, can induce chromosome aberrations in mammalian cells. The second is that, during a simultaneous exposure to a chemical known to be genotoxic, rf radiation can affect molecules, biochemical processes, or cellular organelles, and thus result in an increase or decrease in chromosome aberrations. Mitomycin C (MMC) and Adriamycin (ADR) were selected because they act by different mechanisms, and because they might put normal cells at risk during combined-modality rf radiation (hyperthermia)-chemotherapy treatment of cancer. The studies were performed with suitable 37 degrees C and equivalent convection heating-temperature controls in a manner designed to discriminate between any thermal and possible nonthermal action. Radiofrequency exposures were conducted for 2 h under conditions resulting in measurable heating (a maximum increase of 3.2 degrees C), with pulsed-wave rf radiation at a frequency of 2450 MHz and an average net forward power of 600 W, resulting in an SAR of 33.8 W/kg. Treatments with MMC or ADR were for a total of 2.5 h and encompassed the 2-h rf radiation exposure period. The CHO cells from each of the conditions were subsequently analyzed for chromosome aberrations. In cells exposed to rf radiation alone, and where a maximum temperature of approximately 40 degrees C was achieved in the tissue culture medium, no alteration in the frequency from 37 degrees C control levels was observed. Relative to the chemical treatment with MMC alone at 37 degrees C, for two different concentrations, no alteration was observed in the extent of chromosome aberrations induced by either simultaneous rf radiation exposure or convection heating to equivalent temperatures. At the ADR concentration that was used, most of the indices of chromosome aberrations which were scored indicated a similar result.

Komatsubara Y, Hirose H, Sakurai T, Koyama S, Suzuki Y, Taki M, Miyakoshi J. Effect of high-frequency electromagnetic fields with a wide range of SARs on chromosomal aberrations in murine m5S cells. *Mutation Research*, 587(1-2):114-119, 2005.

To investigate the induction of chromosomal aberrations in mouse m5S cells after exposure to high-frequency electromagnetic fields (HFEMFs) at 2.45GHz, cells were exposed for 2h at average specific absorption rates (SARs) of 5, 10, 20, 50 and 100W/kg with continuous wave-form (CW), or at a mean SAR of 100W/kg (with a maximum of 900W/kg) with pulse wave-form (PW). The effects of HFEMF exposure were compared with those in sham-exposed controls and with mitomycin C (MMC) or X-ray treatment as positive controls. We examined all structural, chromatid-type and chromosome-type changes after HFEMF exposures and treatments with MMC and X-rays. No significant differences were observed following exposure to HFEMFs at SARs from 5 to 100W/kg CW and at a mean SAR of 100W/kg PW (a maximum SAR of 900W/kg) compared with sham-exposed controls, whereas treatments with MMC and X-rays increased the frequency of chromatid-type and chromosome-type aberrations. In summary, HFEMF exposures at 2.45GHz for 2h with up to 100W/kg SAR CW and an average 100W/kg PW (a maximum SAR of 900W/kg) do not induce chromosomal aberrations in m5S cells. Furthermore, there was no difference between exposures to CW and PW HFEMFs.

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

There has been considerable discussion about the influence of high-frequency electromagnetic fields (HFEMF) on the human body. In particular, HFEMF used for mobile phones may be of great concern for human health. In order to investigate the properties of HFEMF, we have examined the effects of 2.45-GHz EMF on micronucleus (MN) formation in Chinese hamster ovary (CHO)-K1 cells. MN formation is induced by chromosomal breakage or inhibition of spindles during cell division and leads to cell damage. We also examined the influence of heat on MN formation, since HFEMF exposure causes a rise in temperature. CHO-K1 cells were exposed to HFEMF for 2 h at average specific absorption rates (SARs) of 5, 10, 20, 50, 100, and 200 W/kg, and the effects on these cells were compared with those in sham-exposed control cells. The cells were also treated with bleomycin alone as a positive control or with combined treatment of HFEMF exposure and bleomycin. Heat treatment was performed at temperatures of 37, 38, 39, 40, 41, and 42 degrees C. The MN frequency in cells exposed to HFEMF at a SAR of lower than 50 W/kg did not differ from the sham-exposed controls, while those at SARs of 100 and 200 W/kg were significantly higher when compared with the sham-exposed controls. There was no apparent combined effect of HFEMF exposure and bleomycin treatment. On heat treatment at temperatures from 38-42 degrees C, the MN frequency increased in a temperature-dependent manner. We also showed that an increase

in SAR causes a rise in temperature and this may be connected to the increase in MN formation generated by exposure to HFEMF.

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. *International Journal of Radiation Biology*, 80(1):11-20, 2004 [a].

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 (¹³⁷Cs) 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.

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. *Radiation Research*, 161(2): 201-214, 2004 [b].

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 cisplatin (CDDP, a positive control for DNA crosslinks), C3H 10T(1/2) cells were irradiated with 4 Gy of gamma rays (¹³⁷Cs). 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.

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.

Lai H, Singh NP. Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. *International Journal of Radiation Biology*, 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.

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 [a].

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-phenylnitron (PBN) (100 mg/kg/injection, i.p.) blocks this effects of RFR. Since both melatonin and PBN are efficient free radical scavengers it is hypothesized that free radicals are involved in RFR-induced DNA damage in the brain cells of rats. Since cumulated DNA strand breaks in brain cells can lead to neurodegenerative diseases and cancer and an excess of free radicals in cells has been suggested to be the cause of various human diseases, data from this study could have important implications for the health effects of RFR exposure.

Lai H, Singh NP. Interaction of microwaves and a temporally incoherent magnetic field on single and double DNA strand breaks in rat brain cells. *Electromagnetic Biology and Medicine* 24(1):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/cm², 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.

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

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 ms 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.

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. *Radiation Research*, 156(3):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.

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

Cytogenetic analyses were performed on human peripheral blood lymphocytes exposed to 2450 MHz microwaves during 30 and 120 min at a constant temperature of 36.1 degrees C (body temperature). The temperature was kept constant by means of a temperature probe put in the blood sample which gives feedback to a microcomputer that controls the microwave supply. We found a marked increase in the frequency of chromosome aberrations (including dicentric chromosomes and acentric fragments) and

micronuclei. On the other hand the microwave exposure did not influence the cell kinetics nor the sister chromatid exchange (SCE) frequency.

Maes A, Collier M, Slaets D, Verschaeve L. Cytogenic effects of microwaves from mobile communication frequencies (954 MHz), *Electro- and Magnetobiology*, 14(2): 91-98, 1995.

Whole blood samples were exposed to a 945 MHz emitting antenna from a GSM (Global System for Mobile Communication) base station and cultivated for analysis of chromosome aberrations. A limited number of blood samples from maintenance workers being professionally exposed to microwaves of this and other frequencies was also investigated. Although some cytogenetic damage was obtained in vitro when blood samples were very close to the antenna, we may, according to our results, consider that microwaves emitted by a GSM base station are not able to induce genetic defects in the general population.

Maes A, Collier M, Slaets D, Verschaeve L. 954 MHz microwaves enhance the mutagenic properties of mitomycin C. *Environmental and Molecular Mutagenesis*, 28(1):26-30, 1996.

This paper focuses on the combined effects of microwaves from mobile communication frequencies and a chemical DNA damaging agent mitomycin C (MMC). The investigation was performed in vitro by exposing whole blood samples to a 954 MHz emitting antenna from a GSM (Global System for Mobile Communication) base station, followed by lymphocyte cultivation in the presence of MMC. A highly reproducible synergistic effect was observed as based on the frequencies of sister chromatid exchanges in metaphase figures.

Maes A, Collier M, Van Gorp U, Vandoninck S, Verschaeve L. Cytogenetic effects of 935.2-MHz (GSM) microwaves alone and in combination with mitomycin C. *Mutation Research*, 393(1-2):151-156, 1997.

This paper focuses on the genetic effects of microwaves from mobile communication frequencies (935.2 MHz) alone and in combination with a chemical DNA-damaging agent (mitomycin C). Three cytogenetic endpoints were investigated after in vitro exposure of human whole blood cells. These endpoints were the 'classical' chromosome aberration test, the sister chromatid exchange test and the alkaline comet assay. No direct cytogenetic effect was found. The combined exposure of the cells to the radiofrequency fields followed by their cultivation in the presence of mitomycin C revealed a very weak effect when compared to cells exposed to mitomycin C alone.

Maes A, Collier M, Verschaeve L. Cytogenetic investigations on microwaves emitted by a 455.7 MHz car phone. *Folia biologica (Praha)* 46(5):175-180, 2000.

The chromosome aberration or sister chromatid exchange frequency was determined in 455.7 MHz microwave-exposed human lymphocytes and in lymphocytes that were subsequently exposed to MMC or X-rays. The exposure was performed by placing the cells at 5 cm from the antenna of a car phone. In this way the specific absorption ratio was approximately 6.5 W/kg. The temperature and humidity was kept constant during the experiments. No statistically significant difference was found between microwave-exposed and unexposed control samples. When the microwave exposure was followed by exposure to MMC, some differences were found between the combined treatments and the MMC treatments alone. However, there was no consistency in the results. Combined treatments with X-rays did not provide any indication of a synergistic action between the RF fields and X-rays, either. Our data therefore do not support the hypothesis that RF fields act synergistically with chemical or physical mutagens.

Maes A, Collier M, Verschaeve L. Cytogenetic effects of 900 MHz (GSM) microwaves on human lymphocytes. *Bioelectromagnetics*, 22(2):91-96, 2001

The cytogenetic effects of 900 MHz radiofrequency fields were investigated with the chromosome aberration and sister chromatid exchange frequency methods. Three different modes of exposure (continuous, pseudo-random and dummy burst) were studied for different power outputs (0, 2, 8, 15, 25, 50 W). The specific absorption rates varied between 0 and 10 W/kg. We investigated the possible effects of the 900 MHz radiation alone as well as of combined exposure to the chemical or physical mutagens mitomycin C and X-rays. Overall, no indication was found of a mutagenic, and/or co-mutagenic/synergistic effect of this kind of nonionizing radiation.

Maes A, Van Gorp U, Verschaeve L. Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation. *Mutagenesis*, 21(2):139-142, 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.

Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL. Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation. *Radiation Research*, 148(6):608-617, 1997 [a].

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.

Malyapa RS, Ahern EW, Bi C, Straube WL, LaRegina M, Pickard WF, Roti Roti JL. DNA damage in rat brain cells after in vivo exposure to 2450 MHz electromagnetic radiation and various methods of euthanasia. *Radiation Research*, 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 MHz continuous-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.

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). *Radiation Research*, 148(6):618-627, 1997 [b].

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.

Markova E, Hillert L, Malmgren L, Persson BR, Belyaev IY. Microwaves from GSM mobile telephones affect 53BP1 and gamma-H2AX foci in human lymphocytes from hypersensitive and healthy persons. *Environmental Health Perspectives*, 113(9):1172-1177, 2005.

The data on biologic effects of nonthermal microwaves (MWs) from mobile telephones are diverse, and these effects are presently ignored by safety standards of the International Commission for Non-Ionizing Radiation Protection (ICNIRP). In the

present study, we investigated effects of MWs of Global System for Mobile Communication (GSM) at different carrier frequencies on human lymphocytes from healthy persons and from persons reporting hypersensitivity to electromagnetic fields (EMFs). We measured the changes in chromatin conformation, which are indicative of stress response and genotoxic effects, by the method of anomalous viscosity time dependence, and we analyzed tumor suppressor p53-binding protein 1 (53BP1) and phosphorylated histone H2AX (gamma-H2AX), which have been shown to colocalize in distinct foci with DNA double-strand breaks (DSBs), using immunofluorescence confocal laser microscopy. We found that MWs from GSM mobile telephones affect chromatin conformation and 53BP1/gamma-H2AX foci similar to heat shock. For the first time, we report here that effects of MWs from mobile telephones on human lymphocytes are dependent on carrier frequency. On average, the same response was observed in lymphocytes from hypersensitive and healthy subjects.

Mashevich M, Folkman D, Kesar A, Barbul A, Korenstein R, Jerby E, Avivi L. Exposure of human peripheral blood lymphocytes to electromagnetic fields associated with cellular phones leads to chromosomal instability. *Bioelectromagnetics*, 24(2):82-90, 2003.

Whether exposure to radiation emitted from cellular phones poses a health hazard is at the focus of current debate. We have examined whether in vitro exposure of human peripheral blood lymphocytes (PBL) to continuous 830 MHz electromagnetic fields causes losses and gains of chromosomes (aneuploidy), a major “somatic mutation” leading to genomic instability and thereby to cancer. PBL were irradiated at different average absorption rates (SAR) in the range of 1.6-8.8 W/kg for 72 hr in an exposure system based on a parallel plate resonator at temperatures ranging from 34.5-37.5 °C. The averaged SAR and its distribution in the exposed tissue culture flask were determined by combining measurements and numerical analysis based on a finite element simulation code. A linear increase in chromosome 17 aneuploidy was observed as a function of the SAR value, demonstrating that this radiation has a genotoxic effect. The SAR dependent aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in the SAR dependent genetic toxicity. Control experiments (i.e., without any RF radiation) carried out in the temperature range of 34.5-38.5 °C showed that elevated temperature is not associated with either the genetic or epigenetic alterations observed following RF radiation - the increased levels of aneuploidy and the modification in replication of the centromeric DNA arrays. These findings indicate that the genotoxic effect of the electromagnetic radiation is elicited via a non-thermal pathway. Moreover, the fact that aneuploidy is a phenomenon known to increase the risk for cancer, should be taken into consideration in future evaluation of exposure guidelines.

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. *Radiation Research*, 158(4):523-533, 2002 [a].

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.

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. *Radiation Research*, 158(4):534-537, 2002 [b].

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.

McNamee JP, Bellier PV, Gajda GB, Lavallee BF, Marro L, Lemay E, Thansandote A. No evidence for genotoxic effects from 24 h exposure of human leukocytes to 1.9 GHz radiofrequency fields. *Radiation Research*, 159(5):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.

Meltz ML, Eagan P, Erwin DN. Proflavin and microwave radiation: absence of a mutagenic interaction. *Bioelectromagnetics*, 11(2):149-157, 1990.

The potential ability of radiofrequency electromagnetic radiation (RFR) in the microwave range to induce mutagenesis, chromosomal aberrations, and sister chromatid exchanges in mammalian cells is being explored in our laboratories. In addition, we have also been examining the ability of simultaneous exposure to RFR and chemical mutagens to alter the genotoxic damage induced by chemical mutagens acting alone. We have performed experiments to determine whether there is an interaction between 2.45-GHz, pulsed-wave, RFR and proflavin, a DNA-intercalating drug. The endpoint studied was forward mutation at the thymidine kinase locus in L5178Y mouse leukemic cells. Any effect on the size distribution of the resulting colonies of mutated cells was also examined. The exposures were performed at net forward powers of 500 or 600 W, resulting in a specific absorption rate (SAR) of approximately 40 W/kg. The culture-medium temperature reached a 3 degrees C maximal increase during the 4-h exposure; appropriate 37 degrees C and convection-heating temperature controls (TC) were performed. In no case was there any indication of a statistically significant increase in the induced mutant frequency due to the simultaneous exposure to RFR and proflavin, as compared with the proflavin exposures alone. There was also no indication of any change in the colony-size distribution of the resulting mutant colonies, neither, and there was no evidence in these experiments of any mutagenic action by the RFR exposure alone.

Narasimhan V, Huh WK. Altered restriction patterns of microwave irradiated lambdaphage DNA. *Biochemistry International*, 25(2):363-370, 1991.

Samples of lambdaphage DNA exposed to short pulses of microwave irradiation were subjected to restriction fragmentation by Eco RI and Bam HI. Eco RI digests of microwaved DNA samples yielded three additional fragments ranging in base pair

lengths between 24,226 and 7,421 besides the six expected fragments. While Bam HI digests of the microwaved samples did not yield any additional fragments, mobilities of the Bam HI fragments from the microwaved DNA samples were slower and the bands were broader in comparison to those from native samples. We attribute these altered restriction patterns to the conformational anomalies in DNA resulting from single strand breaks and localized strand separations induced by microwave irradiation.

Ono T, Saito Y, Komura J, Ikehata H, Tarusawa Y, Nojima T, Goukon K, Ohba Y, Wang J, Fujiwara O, Sato R. Absence of mutagenic effects of 2.45 GHz radiofrequency exposure in spleen, liver, brain, and testis of lacZ-transgenic mouse exposed in utero. *Tohoku Journal of Experimental Medicine*, 202(2):93-103, 2004.

A possible mutagenic effect of 2.45 GHz radiofrequency exposure was examined using lacZ-transgenic Muta mice. Pregnant animals were exposed intermittently at a whole-body averaged specific absorption rate of 0.71 W/kg (10 seconds on, 50 seconds off which is 4.3 W/kg during the 10 seconds exposure). Offspring that were exposed in utero for 16 hours a day, from the embryonic age of 0 to 15 days, were examined at 10 weeks of age. To minimize thermal effects, the exposure was given in repeated bursts of 10 seconds of exposure followed by 50 seconds of no exposure. Mutation frequencies at the lacZ gene in spleen, liver, brain, and testis were similar to those observed in non-exposed mice. Quality of mutation assessed by sequencing the nucleotides of mutant DNAs revealed no appreciable difference between exposed and non-exposed samples. The data suggest that the level of radiofrequency exposure studied is not mutagenic when administered in utero in short repeated bursts.

Paulraj R, Behari J. Single strand DNA breaks in rat brain cells exposed to microwave radiation. *Mutation Research*, 596(1-2):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.

Phillips, JL, Ivaschuk O, Ishida-Jones T, Jones RA, Campbell-Beachler M, Haggren, W. DNA damage in Molt-4 T- lymphoblastoid cells exposed to cellular

telephone radiofrequency fields in vitro. *Bioelectrochemistry and Bioenergetics*, 45(1):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 Kolmogorov-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.

Roti Roti JL, Malyapa RS, Bisht KS, Ahern EW, Moros EG, Pickard WF, Straube WL. Neoplastic transformation in C3H 10T(1/2) Cells after exposure to 835.62 MHz FDMA and 847.74 MHz CDMA radiations. *Radiation Research*, 155(1):239-247, 2001.

The effect of radiofrequency (RF) radiation in the cellular phone communication range (835.62 MHz frequency division multiple access, FDMA; 847.74 MHz code division multiple access, CDMA) on neoplastic transformation frequency was measured using the in vitro C3H 10T(1/2) cell transformation assay system. To determine if 835.62 MHz FDMA or 847.74 MHz CDMA radiations have any genotoxic effects that induce neoplastic transformation, C3H 10T(1/2) cells were exposed at 37 degrees C to either of the above radiations [each at a specific absorption rate (SAR) of 0.6 W/kg] or sham-exposed at the same time for 7 days. After the culture medium was changed, the cultures were transferred to incubators and refed with fresh growth medium every 7 days. After 42 days, the cells were fixed and stained with Giemsa, and transformed foci were scored. To determine if exposure to 835.62 MHz FDMA or 847.74 MHz CDMA radiation has any epigenetic effects that can promote neoplastic transformation, cells were first exposed to 4.5 Gy of X rays to induce the transformation process and then exposed to the above radiations (SAR = 0.6 W/kg) in temperature-controlled irradiators with weekly refeeding for 42 days. After both the 7-day RF exposure and the 42-day RF exposure after X irradiation, no statistically significant differences in the transformation frequencies were observed between incubator controls, the sham-exposed (maintained in

irradiators without power to the antenna), and the 835.62 MHz FDMA or 847.74 MHz CDMA-exposed groups.

Sarimov R, Malmgren LOG, Markova, E, Persson, BRR, Belyaev, IY, Nonthermal GSM microwaves affect chromatin conformation in human lymphocytes similar to heat shock. *IEEE Transactions on Plasma Science*, 32:1600-1608, 2004.

Here we investigated whether microwaves (MWs) of Global System for Mobile Communication (GSM) induce changes in chromatin conformation in human lymphocytes. Effects of MWs were studied at different frequencies in the range of 895-915 MHz in experiments with lymphocytes from seven healthy persons. Exposure was performed in transverse electromagnetic transmission line cell (TEM-cell) using a GSM test-mobile phone. All standard modulations included 2 W output power in the pulses, specific absorbed rate (SAR) being 5.4 mW/kg. Changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependencies (AVTD). Heat shock and treatment with the genotoxic agent camptothecin, were used as positive controls. 30-min exposure to MWs at 900 and 905 MHz resulted in statistically significant condensation of chromatin in lymphocytes from 1 of 3 tested donors. This condensation was similar to effects of heat shock within the temperature window of 40/spl deg/C-44/spl deg/C. Analysis of pooled data from all donors showed statistically significant effect of 30-min exposure to MWs. Stronger effects of MWs was found following 1-h exposure. In replicated experiments, cells from four out of five donors responded to 905 MHz. Responses to 915 MHz were observed in cells from 1 out of 5 donors, $p < 0.002$. Dependent on donor, condensation, 3 donors, or decondensation, 1 donor, of chromatin was found in response to 1-h exposure. Analysis of pooled data from all donors showed statistically significant effect of 1-h exposure to MWs. In cells from one donor, this effect was frequency-dependent ($p < 0.01$). Effects of MWs correlated statistically significantly with effects of heat shock and initial state of chromatin before exposure. MWs at 895 and 915 MHz affected chromatin conformation in transformed lymphocytes. The conclusion- GSM microwaves under specific conditions of exposure affected human lymphocytes similar to stress response. The data suggested that the MW effects differ at various GSM frequencies and vary between donors.

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(1):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.

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

The potential mutagenic effect of low power microwave at the DNA sequence level in the mouse genome was evaluated by direct DNA analysis. Animals were exposed to microwave at a power density of 1 mW/cm² for 2 h/day at a frequency of 2.45 GHz over a period of 120, 150 and 200 days. HinfI digested DNA samples from testis and brain of control and exposed animals were hybridized with a synthetic oligo probe (OAT 36) comprising nine repeats of 5'-GACA-3'. As compared to control animals, band patterns in exposed animals were found to be distinctly altered in the range of 7-8 kb which was also substantiated by densitometric analysis. Though the mechanism of this rearrangement is not yet clear, the results obtained at the present dose are of significance. This dose, which has been set as the safe limit for general public exposure by the Non-Ionizing Radiation Committee of the International Radiation Protection Association, may imply a need for (re)evaluation of the mutagenic potential of microwaves at the prescribed safe limit for the personnel and people who are being exposed.

Scarfi, MR, Fresegna AM, Villani P, Pinto R, Marino C, Sarti M, Altavista P, Sannino A, Lovisolo GA. Exposure to radiofrequency radiation (900 MHz, GSM signal) does not affect micronucleus frequency and cell proliferation in human peripheral blood lymphocytes: an interlaboratory study. *Radiation Research*, 165(6):655-663, 2006

The objective of this study was to investigate whether 24 h exposure to radiofrequency electromagnetic fields similar to those emitted by mobile phones induces genotoxic effects and/or effects on cell cycle kinetics in cultured human peripheral blood lymphocytes. The effect of 900 MHz exposure (GSM signal) was evaluated at four

specific absorption rates (SARs, 0, 1, 5 and 10 W/kg peak values). The exposures were carried out in wire patch cells under strictly controlled conditions of both temperature and dosimetry, and the induction of genotoxic effects was evaluated in lymphocyte cultures from 10 healthy donors by applying the cytokinesis-block micronucleus assay. Positive controls were provided by using mitomycin C. Two research groups were involved in the study, one at ENEA, Rome, and the other at CNR-IREA, Naples. Each laboratory tested five donors, and the resulting slides were scored by both laboratories. Following this experimental scheme, it was also possible to compare the results obtained by cross-scoring of slides. The results obtained provided no evidence for the existence of genotoxic or cytotoxic effects in the range of SARs investigated. These findings were confirmed in the two groups of five donors examined in the two laboratories and when the same slides were scored by two operators.

Semin IuA, Shvartsburg LK, Dubovik BV. [Changes in the secondary structure of DNA under the influence of external low-intensity electromagnetic field.] *Radiatsionnaia Biologiia, Radioecologia* 35(1):36-41, 1995 [in Russian].

The effect of weak RF on the stability of DNA secondary structure was studied in vitro. DNA was exposed in the presence of glycine and formaldehyde. Aminomethynol compounds, which form in this medium, react with DNA bases at single-strand sites, which prevents recovery from damage to the DNA secondary structure. The damage accumulates during the incubation, and its amount can be estimated from the dynamics of thermal DNA denaturalization after RF or sha exposure. Samples were exposed in an anechoic chamber at 18°C at 10 different microwave frequencies simultaneously (4- to 8 GHz, 25 ms pulses, 0.4 to 0.7 mW/cm² peak power, 1- to 6-Hz repetition rate, no heating). Parallel control samples were sham exposed in a shielded area in the same chamber. The experiments established that irradiation at 3 or 4 Hz and 0.6 mW/cm² peak power clearly increased the accumulated damage to the DNA secondary structure (P < .00001). However, changing the pulse repetition rate to 1, 5, 6 Hz, as well as changing the peak power to 0.4 or 0.7 mW/cm², eliminated the effect entirely. Thus, the effect occurred only within narrow 'windows' of the peak intensities and modulation frequencies.

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. *International Journal of Radiation Biology*, 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.

Sykes PJ, McCallum BD, Bangay MJ, Hooker AM, Morley AA. Effect of exposure to 900 MHz radiofrequency radiation on intrachromosomal recombination in pKZ1 mice. *Radiation Research*, 156(5):495-502, 2001.

Radiofrequency (RF) radiation emitted from mobile phones is not considered to be directly genotoxic, but it may have downstream effects on cellular DNA. We studied the effect of 4 W/kg pulsed 900 MHz RF radiation on somatic intrachromosomal recombination in the spleen in the pKZ1 recombination mutagenesis model. Somatic intrachromosomal recombination inversion events were detected in spleen tissue of pKZ1 mice by histochemical staining for E. coli beta-galactosidase protein in cells in which the lacZ transgene has undergone an inversion event. pKZ1 mice were exposed daily for 30 min to plane-wave fields of 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms for 1, 5 or 25 days. Three days after the last exposure, spleen sections were screened for DNA inversion events. There was no significant difference between the control and treated groups in the 1- and 5-day exposure groups, but there was a significant reduction in inversions below the spontaneous frequency in the 25-day exposure group. This observation suggests that exposure to RF radiation can lead to a perturbation in recombination frequency which may have implications for recombination repair of DNA. The biological significance of a reduction below the spontaneous frequency is not known. The number of mice in each treatment group in this study was small (n = 10 or n = 20). Therefore, repetition of this study with a larger number of animals is required to confirm these observations.

Takahashi S, Inaguma S, Cho Y-M, Imaida K, Wang J, Fujiwara O, Shirai T. Lack of mutation induction with exposure to 1.5 GHz electromagnetic near fields used for cellular phones in brains of big blue mice. *Cancer Research*, 62(7):1956-1960, 2002.

The possible mutagenic potential of exposure to 1.5 GHz electromagnetic near field (EMF) was investigated using brain tissues of Big Blue mice (BBM). Male BBM were locally exposed to EMF in the head region at 2.0, 0.67, and 0 W/kg specific absorption rate for 90 min/day, 5 days/week, for 4 weeks. No gliosis or degenerative lesions were histopathologically noted in brain tissues, and no obvious differences in Ki-67 labeling and apoptotic indices of glial cells were evident among the groups. There was no significant variation in the frequency of independent mutations of the *lacI* transgene in the brains. G:C to A:T transitions at CpG sites constituted the most prevalent mutations in all groups and at all time points. Deletion mutations were slightly increased in both the high and low EMF exposure groups as compared with the sham-exposed group, but the differences were not statistically significant. These findings suggest that exposure to 1.5 GHz EMF is not mutagenic to mouse brain cells and does not create any increased hazard with regard to brain tumor development.

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(2):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.

Trosic I. Multinucleated giant cell appearance after whole body microwave irradiation of rats. *International Journal of Hygiene and Environmental Health*, 204(2-3):133-138, 2001.

Multinucleated giant cells are common for some chronic inflammatory processes in the lung. These cells are formed by fusion of macrophages, but how the process relates to the kinetics of alveolar macrophage generation is not clear. This study investigated the influence of 2450 MHz microwave irradiation on alveolar macrophage kinetics and formation of multinucleated giant cells after whole body irradiation of rats. The range of electromagnetic radiation was selected as 2450 MHz microwaves at a power density of 5-15 mW/cm². A group of experimental animals was divided in four subgroups that received 2, 8, 13 and 22 irradiation treatments of two hours each. The animals were killed on experimental days 1, 8, 16, and 30. Free lung cell population was obtained by bronchoalveolar lavage. Cell response to the selected irradiation level was followed quantitatively, qualitatively and morphologically using standard laboratory methods. Total cell number retrieved by lavage slightly decreased in treated animals showing time- and dose-dependence. Cell viability did not significantly change in the irradiated animal group (G2) as compared with the control group (G1). Multinucleated cells significantly increased ($p < 0.01$) in treated animals. The elevation of the number of nuclei per cell was time- and dose-dependent. Macrophages with two nucleoli were more common in animals treated twice or eight times. Polynucleation, that is three and more nucleoli in a single cell, was frequently observed after 13 or 22 treatments. Binucleation and multinucleation of alveolar macrophages were sensitive time- and dose-dependent morphological indicators of pulmonary stress.

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

Adult male Wistar rats were exposed for 2h a day, 7 days a week for up to 30 days to continuous 2450MHz radiofrequency microwave (rf/MW) radiation at a power density of 5-10mW/cm². Sham-exposed rats were used as controls. After ether anesthesia, experimental animals were euthanized on the final irradiation day for each treated group. Peripheral blood smears were examined for the extent of genotoxicity, as indicated by the presence of micronuclei in polychromatic erythrocytes (PCEs). The results for the time-course of PCEs indicated significant differences ($P < 0.05$) for the 2nd, the 8th and the 15th day between control and treated subgroups of animals. Increased influx of immature erythrocytes into the peripheral circulation at the beginning of the experiment revealed that the proliferation and maturation of nucleated erythropoietic cells were affected by exposure to the 2450MHz radiofrequency radiation. Such findings are indicators of radiation effects on bone-marrow erythropoiesis and their subsequent effects in circulating red cells. The incidence of micronuclei/1000 PCEs in peripheral blood was significantly increased ($P < 0.05$) in the subgroup exposed to rf/MW radiation after eight irradiation treatments of 2h each in comparison with the sham-exposed control group. It is likely that an adaptive mechanism, both in erythrocytopoiesis and genotoxicity appeared in the rat experimental model during the subchronic irradiation treatment.

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

An in vivo mammalian cytogenetic test (the erythrocyte micronucleus assay) was used to investigate the extent of genetic damage in bone marrow red cells of rats exposed to radiofrequency/microwave (RF/MW) radiation. Wistar rats (n = 40) were exposed to a 2.45 GHz continuous RF/MW field for 2 h daily, 7 days a week, at a power density of 5-10 mW/cm². The whole body average specific absorption rate (SARs) was calculated to be 1.25 +/- 0.36 (SE) W/kg. Four subgroups were irradiated for 4, 16, 30 and 60 h. Sham-exposed controls (n = 24) were included in the study. The animals of each treated subgroup were killed on the final day of irradiation. Bone marrow smears were examined to determine the extent of genotoxicity after particular treatment times. The results were statistically evaluated using non-parametric Mann-Whitney and Kruskal-Wallis tests. In comparison with the sham-exposed subgroups, the findings of polychromatic erythrocytes (PCE) revealed significant differences (P < 0.05) for experimental days 8 and 15. The frequency of micronucleated PCEs was also significantly increased on experimental day 15 (P < 0.05). Pair-wise comparison of data obtained after 2, 8 and 30 irradiation treatments did not reveal statistically significant differences between sham-exposed and treated subgroups. Under the applied experimental conditions the findings revealed a transient effect on proliferation and maturation of erythropoietic cells in the rat bone marrow and the sporadic appearance of micronucleated immature bone marrow red cells.

Trosic I, Busljeta I. Erythropoietic dynamic equilibrium in rats maintained after microwave irradiation. *Experimental and Toxicologic Pathology*, 57(3):247-251, 2006.

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

haemopoietic equilibrium in rats by the end of experiment indicates possibility of activation/adaptation process in rats to the selected experimental conditions of subchronic RF/MW exposure.

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. *Radiation Research*, 165(5):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 µg/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.

Vijayalaxmi, Mohan, N, Meltz, ML, Wittler, MA. Proliferation and cytogenetic studies in human blood lymphocytes exposed in vitro to 2450 MHz radiofrequency radiation. *International Journal of Radiation Biology*, 72(6):751-757, 1997 [a].

Aliquots of human peripheral blood collected from two healthy human volunteers were exposed in vitro to continuous wave 2450 MHz radiofrequency radiation (RFR), either continuously for a period of 90 min or intermittently for a total exposure period of 90 min (30 min on and 30 min off, repeated three times). Blood aliquots which were sham-exposed or exposed in vitro to 150 cGy gamma radiation served as controls. The continuous wave 2450 MHz RFR was generated with a net forward power of 34.5 W and transmitted from a standard gain rectangular antenna horn in a vertically downward direction. The mean power density at the position of the cells was 5.0 mW/cm². The mean specific absorption rate calculated by Finite Difference Time Domain analysis was 12.46 W/kg. Immediately after exposure, lymphocytes were cultured for 48 and 72 h to determine the incidence of chromosomal aberrations and micronuclei, respectively. Proliferation indices were also recorded. There were no significant differences between RFR-exposed and sham-exposed lymphocytes with respect to; (a) mitotic indices; (b) incidence of cells showing chromosome damage; (c) exchange aberrations; (d) acentric fragments; (e) binucleate lymphocytes, and (f) micronuclei, for either the continuous or intermittent RFR exposures. In contrast, the response of positive control

cells exposed to 150 cGy gamma radiation was significantly different from RFR-exposed and sham-exposed lymphocytes. Thus, there is no evidence for an effect on mitogen-stimulated proliferation kinetics or for excess genotoxicity within 72 h in human blood lymphocytes exposed in vitro to 2450 MHz RFR.

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

C3H/HeJ mice, which are prone to mammary tumors, were exposed for 20 h/day, 7 days/week, over 18 months to continuous-wave 2450 MHz radiofrequency (RF) radiation in circularly polarized wave guides at a whole-body average specific absorption rate of 1.0 W/kg. Sham-exposed mice were used as controls. The positive controls were the sentinel mice treated with mitomycin C during the last 24 h before necropsy. At the end of the 18 months, all mice were necropsied. Peripheral blood and bone marrow smears were examined for the extent of genotoxicity as indicated by the presence of micronuclei in polychromatic erythrocytes (PCEs). The results indicate that the incidence of micronuclei/1,000 PCEs was not significantly different between groups exposed to RF radiation (62 mice) and sham-exposed groups (58 mice), and the mean frequencies were 4.5 +/- 1.23 and 4.0 +/- 1.12 in peripheral blood and 6.1 +/- 1.78 and 5.7 +/- 1.60 in bone marrow, respectively. In contrast, the positive controls (7 mice) showed a significantly elevated incidence of micronuclei/1,000 PCEs in peripheral blood and bone marrow, and the mean frequencies were 50.9 +/- 6.18 and 55.2 +/- 4.65, respectively. When the animals with mammary tumors were considered separately, there were no significant differences in the incidence of micronuclei/1,000 PCEs between the group exposed to RF radiation (12 mice) and the sham-exposed group (8 mice), and the mean frequencies were 4.6 +/- 1.03 and 4.1 +/- 0.89 in peripheral blood and 6.1 +/- 1.76 and 5.5 +/- 1.51 in bone marrow, respectively. Thus there was no evidence for genotoxicity in mice prone to mammary tumors that were exposed chronically to 2450 MHz RF radiation compared with sham-exposed controls.

A correction was published in a subsequent issue of the journal, stating that there was actually a significant increase in micronucleus formation in peripheral blood and bone marrow cells after chronic exposure to the radiofrequency radiation. "Vijayalaxmi, Frei, MR, Dusch, SJ, Guel, V, Meltz, ML, Jauchem, JR, Correction of an error in calculation in the article "Frequency of micronuclei in the peripheral blood and bone marrow of cancer-prone mice chronically exposed to 2450 MHz radiofrequency radiation" (Radiation Research, 147(4):495-500, 1997). Radiation Research, 149(3):308, 1998 "

Vijayalaxmi, Seaman RL, Belt ML, Doyle JM, Mathur SP, Prihoda TJ., Frequency of micronuclei in the blood and bone marrow cells of mice exposed to ultra-wideband electromagnetic radiation. *International Journal of Radiation Biology*, 75(1):115-120, 1999.

PURPOSE: To investigate the extent of genetic damage in the peripheral blood and bone marrow cells of mice exposed to ultra-wideband electromagnetic radiation (UWBR). **MATERIALS AND METHODS:** CF-1 male mice were exposed to UWBR for 15 min at an estimated whole-body average specific absorption rate of 37 mW x kg(-1). Groups of untreated control and positive control mice injected with mitomycin C were also included in the study. After various treatments, half of the mice were killed at 18 h, and the other half at 24 h. Peripheral blood and bone marrow smears were examined to determine the extent of genotoxicity, as assessed by the presence of micronuclei (MN) in polychromatic erythrocytes (PCE). **RESULTS:** The percentages of PCE and the incidence of MN per 2000 PCE in both tissues in mice killed at 18 h were similar to the frequencies observed in mice terminated at 24 h. There were no significant differences in the percentage of PCE between control and the mice with or without UWBR exposure; the group mean values (+/- standard deviation) were in the range of 3.1+/-0.14 to 3.2+/-0.23 in peripheral blood, and 49.0+/-3.56 to 52.3+/-4.02 in bone marrow. The mean incidence of MN per 2000 PCE in control and in mice with or without UWBR exposure ranged from 7.7+/-2.00 to 9.7+/-2.54 in peripheral blood and 7.4+/-2.32 to 10.0+/-3.27 in bone marrow. Pairwise comparison of the data did not reveal statistically significant differences between the control and mice with or without UWBR exposure groups (excluding positive controls). **CONCLUSION:** Under the experimental conditions tested, there was no evidence for excess genotoxicity in peripheral blood or bone marrow cells of mice exposed to UWBR.

Vijayalaxmi, Leal BZ, Szilagyi M, Prihoda TJ, Meltz ML. Primary DNA damage in human blood lymphocytes exposed in vitro to 2450 MHz radiofrequency radiation. *Radiation Research*, 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.

Vijayalaxmi, Pickard WF, Bisht KS, Prihoda TJ, Meltz ML, LaRegina MC, Roti Roti JL, Straube WL, Moros EG. Micronuclei in the peripheral blood and bone marrow cells of rats exposed to 2450 MHz radiofrequency radiation. *International Journal of Radiation Biology*, 77(11):1109-1115, 2001 [a].

PURPOSE: To determine the incidence of micronuclei in peripheral blood and bone marrow cells of rats exposed continuously for 24h to 2450 MHz continuous wave radiofrequency radiation (RFR) at an average whole-body specific absorption rate (SAR) of 12W/kg. **MATERIALS AND METHODS:** Eight adult male Sprague-Dawley rats were exposed to 2450 MHz RFR in circularly polarized waveguides. Eight sham-exposed rats were kept in similar waveguides without the transmission of RFR. Four rats were treated with mitomycin-C (MMC) and used as positive controls. All rats were necropsied 24h after the end of RFR and sham exposures, and after the 24h treatment with MMC. Peripheral blood and bone marrow smears were examined to determine the frequency of micronuclei (MN) in polychromatic erythrocytes (PCE). **RESULTS:** The results indicated that the incidence of MN/2000 PCE were not significantly different between RFR- and sham-exposed rats. The group mean frequencies of MN in the peripheral blood were 2.3+/-0.7 in RFR-exposed rats and 2.1+/-0.6 in sham-exposed rats. In bone marrow cells, the average MN incidence was 3.8+/-1.0 in RFR-exposed rats and 3.4+/-0.7 in sham-exposed rats. The corresponding values in positive control rats treated with MMC were 23.5+/-4.7 in the peripheral blood and 33.8+/-7.4 in bone marrow cells. **CONCLUSION:** There was no evidence for the induction of MN in peripheral blood and bone marrow cells of rats exposed for 24h to 2450 MHz continuous wave RFR at a whole body average SAR of 12 W/kg.

Vijayalaxmi , Leal BZ, Meltz ML, Pickard WF, Bisht KS, Roti Roti JL, Straube WL, Moros EG. Cytogenetic studies in human Bblood lymphocytes exposed in vitro to radiofrequency radiation at a cellular telephone frequency (835.62 MHz, FDMA). *Radiation Research*, 155(1):113-121, 2001 [b].

Freshly collected peripheral blood samples from four healthy human volunteers were diluted with RPMI 1640 tissue culture medium and exposed in sterile T-75 tissue culture flasks in vitro for 24 h to 835.62 MHz radiofrequency (RF) radiation, a frequency employed for customer-to-base station transmission of cellular telephone communications. An analog signal was used, and the access technology was frequency division multiple access (FDMA, continuous wave). A nominal net forward power of 68 W was used, and the nominal power density at the center of the exposure flask was 860 W/m². The mean specific absorption rate in the exposure flask was 4.4 or 5.0 W/kg. Aliquots of diluted blood that were sham-exposed or exposed in vitro to an acute dose of 1.50 Gy of gamma radiation were used as negative or positive controls. Immediately after

the exposures, the lymphocytes were stimulated with a mitogen, phytohemagglutinin, and cultured for 48 or 72 h to determine the extent of genetic damage, as assessed from the frequencies of chromosomal aberrations and micronuclei. The extent of alteration in the kinetics of cell proliferation was determined from the mitotic indices in 48-h cultures and from the incidence of binucleate cells in 72-h cultures. The data indicated no significant differences between RF-radiation- and sham-exposed lymphocytes with respect to mitotic indices, incidence of exchange aberrations, excess fragments, binucleate cells, and micronuclei. In contrast, the response of the lymphocytes exposed to gamma radiation was significantly different from both RF-radiation- and sham-exposed cells for all of these indices. Thus, under the experimental conditions tested, there is no evidence for the induction of chromosomal aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 835.62 MHz RF radiation at SARs of 4.4 or 5.0 W/kg.

Vijayalaxmi, Bisht KS, Pickard WF, Meltz ML, Roti Roti JL, Moros EG. Chromosome damage and micronucleus formation in human blood lymphocytes exposed in vitro to radiofrequency radiation at a cellular telephone frequency (847.74 MHz, CDMA). *Radiation Research*, 156(4):430-432, 2001 [c].

Peripheral blood samples collected from four healthy nonsmoking human volunteers were diluted with tissue culture medium and exposed in vitro for 24 h to 847.74 MHz radiofrequency (RF) radiation (continuous wave), a frequency employed for cellular telephone communications. A code division multiple access (CDMA) technology was used with a nominal net forward power of 75 W and a nominal power density of 950 W/m² (95 mW/cm²). The mean specific absorption rate (SAR) was 4.9 or 5.5 W/kg. Blood aliquots that were sham-exposed or exposed in vitro to an acute dose of 1.5 Gy of gamma radiation were included in the study as controls. The temperatures of the medium during RF-radiation and sham exposures in the Radial Transmission Line facility were controlled at 37 +/- 0.3 degrees C. Immediately after the exposures, lymphocytes were cultured at 37 +/- 1 degrees C for 48 or 72 h. The extent of genetic damage was assessed from the incidence of chromosome aberrations and micronuclei. The kinetics of cell proliferation was determined from the mitotic indices in 48-h cultures and from the incidence of binucleate cells in 72-h cultures. The data indicated no significant differences between RF-radiation-exposed and sham-exposed lymphocytes with respect to mitotic indices, frequencies of exchange aberrations, excess fragments, binucleate cells, and micronuclei. The response of gamma-irradiated lymphocytes was significantly different from that of both RF-radiation-exposed and sham-exposed cells for all of these indices. Thus there was no evidence for induction of chromosome aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 847.74 MHz RF radiation (CDMA) at SARs of 4.9 or 5.5 W/kg.

Vijayalaxmi, Sasser LB, Morris JE, Wilson BW, Anderson LE. Genotoxic potential of 1.6 GHz wireless communication signal: in vivo two-year bioassay. *Radiation Research*, 159(4):558-564, 2003.

Timed-pregnant Fischer 344 rats (from nineteenth day of gestation) and their nursing offspring (until weaning) were exposed to a far-field 1.6 GHz Iridium wireless communication signal for 2 h/day, 7 days/week. Far-field whole-body exposures were conducted with a field intensity of 0.43 mW/cm² and whole-body average specific absorption rate (SAR) of 0.036 to 0.077 W/kg (0.10 to 0.22 W/kg in the brain). This was followed by chronic, head-only exposures of male and female offspring to a near-field 1.6 GHz signal for 2 h/day, 5 days/week, over 2 years. Near-field exposures were conducted at an SAR of 0.16 or 1.6 W/kg in the brain. Concurrent sham-exposed and cage control rats were also included in the study. At the end of 2 years, all rats were necropsied. Bone marrow smears were examined for the extent of genotoxicity, assessed from the presence of micronuclei in polychromatic erythrocytes. The results indicated that the incidence of micronuclei/2000 polychromatic erythrocytes were not significantly different between 1.6 GHz-exposed, sham-exposed and cage control rats. The group mean frequencies were 5.6 +/- 1.8 (130 rats exposed to 1.6 GHz at 0.16 W/kg SAR), 5.4 +/- 1.5 (135 rats exposed to 1.6 GHz at 1.6 W/kg SAR), 5.6 +/- 1.7 (119 sham-exposed rats), and 5.8 +/- 1.8 (100 cage control rats). In contrast, positive control rats treated with mitomycin C exhibited significantly elevated incidence of micronuclei/2000 polychromatic erythrocytes in bone marrow cells; the mean frequency was 38.2 +/- 7.0 (five rats). Thus there was no evidence for excess genotoxicity in rats that were chronically exposed to 1.6 GHz compared to sham-exposed and cage controls.

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):258-65, 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.

Zeni O, Schiavoni AS, Sannino A, Antolini A, Forigo D, Bersani F, Scarfi MR. Lack of genotoxic effects (micronucleus induction) in human lymphocytes exposed in vitro to 900 MHz electromagnetic fields. *Radiation Research*, 160(2):152-158, 2003.

In the present study, we investigated the induction of genotoxic effects in human peripheral blood lymphocytes after exposure to electromagnetic fields used in mobile communication systems (frequency 900 MHz). For this purpose, the incidence of micronuclei was evaluated by applying the cytokinesis-block micronucleus assay. Cytotoxicity was also investigated using the cytokinesis-block proliferation index. The experiments were performed on peripheral blood from 20 healthy donors, and several conditions were tested by varying the duration of exposure, the specific absorption rate (SAR), and the signal [continuous-wave (CW) or GSM (Global System of Mobile Communication) modulated signal]. The following exposures were carried out: (1) CW intermittent exposure (SAR = 1.6 W/kg) for 6 min followed by a 3-h pause (14 on/off cycles); (2) GSM signal, intermittent exposure as described in (1); (3) GSM signal, intermittent exposure as described in (1) 24 h before stimulation with phytohemagglutinin (8 on/off cycles); (4) GSM signal, intermittent exposure (SAR = 0.2 W/kg) 1 h per day for 3 days. The SARs were estimated numerically. No statistically significant differences were detected in any case in terms of either micronucleus frequency or cell cycle kinetics.

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 [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.

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. *Biomedical and Environmental Sciences* 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.

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

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

Zotti-Martelli L, Peccatori M, Maggini V, Ballardin M, Barale R. Individual responsiveness to induction of micronuclei in human lymphocytes after exposure in vitro to 1800-MHz microwave radiation. *Mutation Research*, 582(1-2):42-52, 2005.

The widespread application of microwaves is of great concern in view of possible consequences for human health. Many in vitro studies have been carried out to detect possible effects on DNA and chromatin structure following exposure to microwave radiation. The aim of this study is to assess the capability of microwaves, at different power densities and exposure times, to induce genotoxic effects as evaluated by the in vitro micronucleus (MN) assay on peripheral blood lymphocytes from nine different healthy donors, and to investigate also the possible inter-individual response variability. Whole blood samples were exposed for 60, 120 and 180min to continuous microwave radiation with a frequency of 1800MHz and power densities of 5, 10 and 20mW/cm(2). Reproducibility was tested by repeating the experiment 3 months later. Multivariate analysis showed that lymphocyte proliferation indices were significantly different among donors ($p < 0.004$) and between experiments ($p < 0.01$), whereas the applied power density and the exposure time did not have any effect on them. Both spontaneous and induced MN frequencies varied in a highly significant way among donors ($p < 0.009$) and between experiments ($p < 0.002$), and a statistically significant increase of MN, although rather low, was observed dependent on exposure time ($p = 0.0004$) and applied power density ($p = 0.0166$). A considerable decrease in spontaneous and induced MN frequencies was measured in the second experiment. The results show that microwaves are able to induce MN in short-time exposures to medium power density fields. Our data analysis highlights a wide inter-individual variability in the response, which was confirmed to be a characteristic reproducible trait by means of the second experiment.