

Abstracts on research into bio-effects of RF at low levels of exposure

The Working Group considered the following studies in investigating research into bio-effects at low levels of exposure.

IN VITRO

Albee LD, Goswami PC, Spitz DR, Ridnour LA. “A polymerase chain reaction assay for simultaneous detection and quantitation of proto-oncogene and GAPD mRNAs”. *Cell Proliferation* 30:271-282, 1998.

This study used an RT-PCR assay to explore the possible effects of analog (835.62 MHz FMCW) or digital (847.74 MHz CDMA) cellular telephone signals on proto-oncogene expression. It examined possible effects on the transit of quiescent cells into the proliferation cycle, and the transit of log phase cells into the plateau growth phase. There was no effect of RF exposure on the expression of *c-jun* or *c-myc* proto-oncogenes. While very small increases of *c-fos* expression were observed during one specific phase of the cell cycle, the researchers concluded that due to the small magnitude of the observed increase it was not likely to be biologically relevant.

Cain CD, Thomas DL, Adey WR. “Focus formation of C3H/10T1/2 cells and exposure to a 836.55 MHz modulated radiofrequency field”. *Bioelectromagnetics* 18(3):237-243, 1997.

Disruption of communication between transformed cells and normal cells is involved in tumor promotion. We have tested the hypothesis that exposures to radiofrequency (RF) fields using a form of digital modulation (TDMA) and a chemical tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), are copromoters that enhance focus formation of transformed cells in coculture with parental C3H/10T1/2 murine fibroblasts. RF field exposures did not influence TPA's dose-dependent promotion of focus formation in coculture. Cell cultures were exposed to an 836.55 MHz TDMA-modulated field in TEM transmission line chambers, with incident energies that simulated field intensities at a user's head. Specific absorption rates (SARs) of 0.15, 1.5, and 15 $\mu\text{W/g}$ were used during each digital packet, and the packet frequency was 50/s. The TEM chambers were placed in a commercial incubator at 37 degrees C and 95% humidity/5% CO₂. The RF field exposures were in a repeating cycle, 20 min on, 20 min off, 24 h/day for 28 days. At 1.5 $\mu\text{W/g}$, TPA-induced focus formation (at 10, 30, and 50 ng/ml) was not significantly different in RF-exposed cultures compared to parallel sham-exposed cultures in ten independent experiments in terms of the number, density, and area of foci. Similarly, at 0.15 and 15.0 $\mu\text{W/g}$, in two and four experiments, respectively, RF exposure did not alter TPA-induced focus formation. The findings support a conclusion that repeated exposures to this RF field do not influence tumor promotion in vitro, based on the RF field's inability to enhance TPA-induced focus formation.

Cleary SF, Cao G, Liu LM, Egle PM, Shelton KR. “Stress proteins are not induced in mammalian cells exposed to radiofrequency or microwave radiation”. *Bioelectromagnetics* 18(7):499-505, 1997.

The induction of stress proteins in HeLa and CHO cells was investigated following a 2 h exposure to radiofrequency (RF) or microwave radiation. Cells were exposed or sham

exposed in vitro under isothermal (37 +/- 0.2 degrees C) conditions. HeLa cells were exposed to 27- or 2450 MHz continuous wave (CW) radiation at a specific absorption rate (SAR) of 25 W/kg. CHO cells were exposed to CW 27 MHz radiation at a SAR of 100 W/kg. Parallel positive control studies included 2 h exposure of HeLa or CHO cells to 40 degrees C or to 45 microM cadmium sulfate. Stress protein induction was assayed 24 h after treatment by electrophoresis of whole-cell extracted protein labeled with [35S]-methionine. Both cell types exhibited well-characterized responses to the positive control stresses. Under these exposure conditions, neither microwave nor RF radiation had a detectable effect on stress protein induction as determined by either comparison of RF-exposed cells with sham-exposed cells or comparison with heat-stressed or Cd⁺⁺ positive control cells.

Donnellan M, McKenzie DR, French PW. "Effects of exposure to electromagnetic radiation at 835 MHz on growth, morphology and secretory characteristics of a mast cell analogue, RBL-2H3". *Cell Biol Int* 21:427-439, 1997.

A mast cell line, RBL-2H3, was exposed to 835 MHz for 20 minutes, three times per day for 7 days at a power density of 8.1 +/- 3 mW/cm². From day 4 onwards, it was observed that the rate of DNA synthesis and cell replication increased, that actin distribution and cell morphology became altered, and the amount of beta-hexosaminidase (a marker of granule secretion) released in response to a calcium ionophore was significantly enhanced, in comparison to unexposed cultures. There were no effects seen on levels of cytoskeletal protein synthesis or of beta-actin mRNA. Morphological changes persisted following subculture for at least 7 days in the absence of further exposure. It is hypothesized that effects of exposure to an electromagnetic field at 835 MHz may be mediated via a signal transduction pathway.

Fesenko EE, Geletyuk VI, Kazachenko VN, Chemeris NK. "Preliminary microwave irradiation of water solutions changes their channel-modifying activity". *FEBS Lett* 366(1):49-52, 1995.

Earlier we have shown that millimetre microwaves (42.25 GHz) of non-thermal power, upon direct admittance into an experiment bath, greatly influence activation characteristics of single Ca(2+)-dependent K⁺ channels (in particular, the channel open state probability, P_o). Here we present new data showing that similar changes in P_o arise due to the substitution of a control bath solution for a preliminary microwave irradiated one of the same composition (100 mmol/l KCl with Ca²⁺ added), with irradiation time being 20-30 min. Therefore, due to the exposure to the field the solution acquires some new properties that are important for the channel activity. The irradiation terminated, the solution retains a new state for at least 10-20 min (solution memory). The data suggest that the effects of the field on the channels are mediated, at least partially, by changes in the solution properties.

French PW, Donnellan M, McKenzie DR. "Electromagnetic radiation at 835 MHz changes the morphology and inhibits proliferation of a human astrocytoma cell line". *Bioelectrochem Bioenerg* 43:13-18, 1997.

A human astrocytoma cell line, U-87 MG, was exposed to 835 MHz electromagnetic radiation for 20 min, 3 times per day for 7 days, at a power density of either 40+15 mW/cm² or 8.1 + 3 mW/cm². At the low power density, it was observed that the rate of DNA synthesis decreased, and that the cells flattened and spread out in comparison to unexposed

culture. At 40 mW/cm², there were no effects seen on cell proliferation, but alteration in cell morphology included increased cell spreading and also the appearance of actin-containing blebs at localized sites on the membrane. It is hypothesised that 835 MHz radiation at low power density may be affecting a signal transduction pathway involved in cell proliferation.

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.

Geletyuk VI, Kazachenko VN, Chemeris NK, Fesenko EE. “Dual effects of microwaves on single Ca(2+)-activated K⁺ channels in cultured kidney cells” *FEBS Lett* 359(1):85-88, 1995.

Using the patch voltage-clamp method, possible effects of millimetre microwaves (42.25 GHz) on single Ca(2+)-activated K⁺ channels in cultured kidney cells (Vero) were investigated. It was found that exposure to the field of non-thermal power (about 100 microW/cm²) for 20-30 min greatly modifies both the Hill coefficient and an apparent affinity of the channels for Ca²⁺(i). The data suggest that the field alters both cooperativity and binding characteristics of the channel activation by internal Ca²⁺. The effects depend on initial sensitivity of the channels to Ca²⁺ and the Ca²⁺ concentration applied.

Goswami PC, Albee LD, Parsian AJ, Baty JD, Moros EG, Pickard WF, Roti-Roti JL, Hunt CR. “Expression of proto-oncogene and activities of multiple transcription factors in RF exposed cells, using C3H10T1/2 mouse embryo fibroblast cells exposed to 835.62 and 847.74 MHz cellphone radiations”. *Radiat Res* 151(3):300-309, 1999.

This study was designed to determine whether two differently modulated radiofrequencies of the type generally used in cellular phone communications could elicit a general stress response in a biological system. The two modulations and frequencies studied were a frequency-modulated continuous wave (FMCW) with a carrier frequency of 835.62 MHz and a code division multiple-access (CDMA) modulation centered on 847.74 MHz. Changes in proto-oncogene expression, determined by measuring Fos, Jun, and Myc mRNA levels as well as by the DNA-binding activity of the AP1, AP2 and NF-kappaB transcription factors, were used as indicators of a general stress response. The effect of radiofrequency exposure on proto-oncogene expression was assessed:

1. in exponentially growing C3H 10T 1/2 mouse embryo fibroblasts during their transition to plateau phase and
2. during transition of serum-deprived cells to the proliferation cycle after serum stimulation.

Exposure of serum-deprived cells to 835.62 MHz FMCW or 847.74 MHz CDMA microwaves (at an average specific absorption rate, SAR, of 0.6 W/kg) did not significantly

change the kinetics of proto-oncogene expression after serum stimulation. Similarly, these exposures did not affect either the Jun and Myc mRNA levels or the DNA-binding activity of AP1, AP2 and NF-kappaB in exponential cells during transit to plateau-phase growth. Therefore, these results suggest that the radiofrequency exposure is unlikely to elicit a general stress response in cells of this cell line under these conditions. However, statistically significant increases (approximately 2-fold, $P = 0.001$) in Fos mRNA levels were detected in exponential cells in transit to the plateau phase and in plateau-phase cells exposed to 835.62 MHz FMCW microwaves. For 847.74 MHz CDMA exposure, the increase was 1.4-fold ($P = 0.04$). This increase in Fos expression suggests that expression of specific genes could be affected by radiofrequency exposure.

Harvey C, French PW. “Effects on protein kinase C and gene expression in a human mast cell line, HMC-1, following microwave exposure”. *Cell Biol Int* 23(11):739-748, 2000.

We used a resonant cavity which delivered a continuous wave exposure at 864.3 MHz at an average specific absorption rate (SAR) of 7 W/kg to determine non-thermal biological effects of microwave exposure. A human mast cell line, HMC-1, was used as the biological target. Cells were given three exposures each of 20-min duration daily for 7 days. The temperature of the cell culture medium during the exposure fell to 26.5 degrees C. Effects were seen on localization of protein kinase C, and expression of three genes of 588 screened. The affected genes included the proto-oncogene *c-kit*, the transcription factor Nucleoside diphosphate kinase B and the apoptosis-associated gene DAD-1. Stress response genes were variably upregulated. No significant effect on morphology or on F-actin distribution was detected. We conclude that low-power microwave exposure may act on HMC-1 cells by altering gene expression via a mechanism involving activation of protein kinase C, and at temperatures well below those known to induce a heat shock response.

Ivaschuk OI, Ishida-Jones T, Adey WR, Phillips JL. “Exposure of nerve growth factor treated PC12 rat pheochromocytoma cells to a modulated RF field at 836.55 MHz”. *Bioelectromagnetics* 18: 223-229, 1997.

This study used nerve growth factor (NGF)-treated PC12 pheochromocytoma cells to study the possible effects of RF exposure (836.55 MHz TDMA) on the expression of the immediate early genes *c-fos* and *c-jun*. Cells were exposed for 20, 40 or 60 minutes to an RF signal in a repeating cycle (20 minutes on/20 minutes off) at 0.26, 2.6 and 26 $\mu\text{W/g}$. The researchers found no change in *c-fos* expression at any time point at any of these three power levels. A decrease in the expression of *c-jun* (average 39 percent) was observed after 20 minutes of exposure at the highest power level (9 mW/cm^2), which is in excess of exposure from cellular phones.

Kwee S and Raskmark P. “Changes in cell proliferation due to environmental non-ionizing radiation 2. Microwave radiation”. *Bioelectrochem Bioenerg* 44: 251, 1998.

Due to the use of mobile telephones, there is an increased exposure of the environment to weak radiofrequency (RF) electromagnetic fields, emitted by these devices. This study was undertaken to investigate if the microwave radiation from these fields will have a similar effect on cell proliferation as weak electromagnetic (ELF) fields. The field was generated by signal simulation of the Global System for Mobile communications (GSM) of 960 MHz. Cell cultures, growing in microtiter plates, were exposed in a specially constructed chamber, a

Transverse Electromagnetic (TEM) cell. The Specific Absorption Rate (SAR) values for each cell well were calculated for this exposure system. Experiments were performed on cell cultures of transformed human epithelial amnion cells (AMA), which were exposed to 960 MHz microwave fields at three different power levels and three different exposure times, respectively. It was found that cell growth in the exposed cells was decreased in comparison to that in the control and sham exposed cells. Cell proliferation during the period following exposure varied not only with the various SAR levels, but also with the length of exposure time. On the other hand, repeated periods of exposure did not seem to change the effects. There was a general linear correlation between power level and growth change. However, the exposure time required to obtain the maximum effect was not the same for the various power levels. It turned out that at low power level, a maximum effect was first reached after a longer exposure time than at higher power level. A similar phenomenon was registered in the studies on ELF electromagnetic fields. Here, it was found that there was a linear correlation between the length of exposure time to obtain maximum effect and field strength.

Litovitz T A, Penafiel L M, Farrel J M, Krause D, Meister R, Mullins J M. "Bioeffects induced by exposure to microwaves are mitigated by superposition of ELF noise." *Bioelectromagnetics* 18(6): 422-30, 1997.

We have previously demonstrated that microwave fields, amplitude modulated (AM) by an extremely low-frequency (ELF) sine wave, can induce a nearly twofold enhancement in the activity of ornithine decarboxylase (ODC) in L929 cells at SAR levels of the order of 2.5 W/kg. Similar, although less pronounced, effects were also observed from exposure to a typical digital cellular phone test signal of the same power level, burst modulated at 50 Hz. We have also shown that ODC enhancement in L929 cells produced by exposure to ELF fields can be inhibited by superposition of ELF noise. In the present study, we explore the possibility that similar inhibition techniques can be used to suppress the microwave response. We concurrently exposed L929 cells to 60 Hz AM microwave fields or a 50 Hz burst-modulated DAMPS (Digital Advanced Mobile Phone System) digital cellular phone field at levels known to produce ODC enhancement, together with band-limited 30-100 Hz ELF noise with root mean square amplitude of up to 10 microT. All exposures were carried out for 8 h, which was previously found to yield the peak microwave response. In both cases, the ODC enhancement was found to decrease exponentially as a function of the noise root mean square amplitude. With 60 Hz AM microwaves, complete inhibition was obtained with noise levels at or above 2 microT. With the DAMPS digital cellular phone signal, complete inhibition occurred with noise levels at or above 5 microT. These results suggest a possible practical means to inhibit biological effects from exposure to both ELF and microwave fields.

Malyapa, R S, Ahern EW, Straube W L, Moros E G, Pickard W F, and Roti Roti J L. "Measurement of DNA damage after exposure to electromagnetic radiation in the cellular phone communication frequency band (835.62 and 847.74 MHz)." *Radiat Res* 148(6): 618-27, 1997.

Mouse C3H 10T1/2 fibroblasts and human glioblastoma U87MG cells were exposed to cellular phone communication frequency radiations to investigate whether such exposure produces DNA damage in in vitro cultures. Two types of frequency modulations were studied: frequency-modulated continuous-wave (FMCW), with a carrier frequency of 835.62 MHz, and code-division multiple-access (CDMA) centered on 847.74 MHz. Exponentially growing (U87MG and C3H 10T1/2 cells) and plateau-phase (C3H 10T1/2 cells) cultures were exposed to either FMCW or CDMA radiation for varying periods up to 24 h in specially

designed radial transmission lines (RTLs) that provided relatively uniform exposure with a specific absorption rate (SAR) of 0.6 W/kg. Temperatures in the RTLs were monitored continuously and maintained at 37 +/- 0.3 degrees C. Sham exposure of cultures in an RTL (negative control) and ¹³⁷Cs gamma-irradiated samples (positive control) were included with every experiment. The alkaline comet assay as described by Olive et al. (Exp. Cell Res. 198, 259-269, 1992) was used to measure DNA damage. No significant differences were observed between the test group exposed to FMCW or CDMA radiation and the sham-treated negative controls. Our results indicate that exposure of cultured mammalian cells to cellular phone communication frequencies under these conditions at an SAR of 0.6 W/kg does not cause DNA damage as measured by the alkaline comet assay.

Penafiel L M, Litovitz T, Krause D, Desta A, Mullins J M. "Role of modulation on the effect of microwaves on ornithine decarboxylase activity in L929 cells." *Bioelectromagnetics* 18(2): 132-41, 1997.

The effect of 835 MHz microwaves on the activity of ornithine decarboxylase (ODC) in L929 murine cell was investigated at an SAR of approximately 2.5 W/kg. The results depended upon the type of modulation employed. AM frequencies of 16 Hz and 60 Hz produced a transient increase in ODC activity that reached a peak at 8 h of exposure and returned to control levels after 24 h of exposure. In this case, ODC was increased by a maximum of 90% relative to control levels. A 40% increase in ODC activity was also observed after 8 h of exposure with a typical signal from a TDMA digital cellular telephone operating in the middle of its transmission frequency range (approximately 840 MHz). This signal was burst modulated at 50 Hz, with approximately 30% duty cycle. By contrast, 8 h exposure with 835 MHz microwaves amplitude modulated with speech produced no significant change in ODC activity. Further investigations, with 8 h of exposure to AM microwaves, as a function of modulation frequency, revealed that the response is frequency dependent, decreasing sharply at 6 Hz and 600 Hz. Exposure with 835 MHz microwaves, frequency modulated with a 60 Hz sinusoid, yielded no significant enhancement in ODC activity for exposure times ranging between 2 and 24 h. Similarly, exposure with a typical signal from an AMPS analog cellular telephone, which uses a form of frequency modulation, produced no significant enhancement in ODC activity. Exposure with 835 MHz continuous wave microwaves produced no effects for exposure times between 2 and 24 h, except for a small but statistically significant enhancement in ODC activity after 6 h of exposure. Comparison of these results suggests that effects are much more robust when the modulation causes low-frequency periodic changes in the amplitude of the microwave carrier.

Phillips JL, Ivaschuk O, Oshida-Jones T, Jones RA, Campbell-Beaclar M, Haggren W. "DNA damage in Molt-4 T-lymphoblastoid cells exposed to cellular telephone RFs In Vitro". *Bioelectrochemistry and Bioenergetics* 45:103-110 1998.

This study explored possible DNA damage (single-strand breaks) in Molt-4 T-lymphoblastoid cells exposed to pulsed 813.5625 MHz (iDEN) or 836.35 MHz (TDMA) cellular telephone signals. Tail moment and comet extent were measured as indicators of DNA damage. Increases as well as decreases in DNA damage were reported, depending on exposure duration and signal type. The researchers concluded that further studies were warranted to distinguish possible direct RF effects on DNA damage from effects on DNA repair.

Romano-Spica V, Mucci N, Ursini CL, Ianni A, Bhat NK. “Ets1 oncogene induction by ELF-modulated 50 MHz RF electromagnetic field”. *Bioelectromagnetics* 21(1):8-18, 2000.

We have analyzed gene expression in hemopoietic and testicular cell types after their exposure to 50 MHz radiofrequency (RF) non-ionizing radiation modulated (80%) with a 16 Hz frequency. The exposure system generates a 0.2 microT magnetic field parallel to the ground and a 60 V/m electric field orthogonal to the earth's magnetic field. Exposure conditions were selected so as to interfere with the calcium ion flow. Under these electromagnetic field (EMF) conditions, we observed an overexpression of the ets1 mRNA in Jurkat T-lymphoblastoid and Leydig TM3 cell lines. This effect was observed only in the presence of the 16 Hz modulation, corresponding to the resonance frequency for calcium ion with a DC magnetic field of 45.7 microT. We have also identified a putative candidate gene repressed after EMF exposure. The experimental model described in this paper may contribute to the understanding of the biological mechanisms involved in EMF effects.

Schirmacher A, Winters S, Fischer S, Goeke J, Galla H J, Kullnick U, Ringelstein E B and Stögbauer F. “Electromagnetic fields (1.8 GHz) increase the permeability to sucrose of the blood-brain barrier in vitro”. *Bioelectromagnetics* 21:338-345, 2000.

We report an investigation on the influence of high frequency electromagnetic fields (EMF) on the permeability of an in vitro model of the blood-brain barrier (BBB). Our model was a co-culture consisting of rat astrocytes and porcine brain capillary endothelial cells (BCEC). Samples were characterized morphologically by scanning electron microscopy and immunocytochemistry. The BBB phenotype of the BCEC was shown by the presence of zona occludens protein (ZO-1) as a marker for tight junctions and the close contact of the cells together with the absence of intercellular clefts. Permeability measurements using ¹⁴C-sucrose indicated a physiological tightness which correlated with the morphological findings and verified the usefulness of our in vitro model. Samples were exposed to EMF conforming to the GSM1800-standard used in mobile telephones (1.8 GHz). The permeability of the samples was monitored over four days and compared with results of samples that were cultured identically but not exposed to EMF. Exposure to EMF increased permeability for ¹⁴C-sucrose significantly compared to unexposed samples. The underlying pathophysiological mechanism remains to be investigated.

Velizarov S, Raskmark P, Kwee S. “The effects of radiofrequency fields on cell proliferation are non-thermal”. *Bioelectrochem Bioenerg* 48(1):177-180, 1999.

The number of reports on the effects induced by radiofrequency (RF) electromagnetic fields and microwave (MW) radiation in various cellular systems is still increasing. Until now no satisfactory mechanism has been proposed to explain the biological effects of these fields. One of the current theories is that heat generation by RF/MW is the cause, in spite of the fact that a great number of studies under isothermal conditions have reported significant cellular changes after exposure to RF/MW. Therefore, this study was undertaken to investigate which effect MW radiation from these fields in combination with a significant change of temperature could have on cell proliferation. The experiments were performed on the same cell line, and with the same exposure system as in a previous work [S. Kwee, P. Raskmark, Changes in cell proliferation due to environmental non-ionizing radiation: 2. Microwave radiation, *Bioelectrochem. Bioenerg.*, 44 (1998), pp. 251-255]. The field was generated by signal simulation of the Global System for Mobile communications (GSM) of 960 MHz. Cell cultures, growing in microtiter plates, were exposed in a specially constructed chamber, a

Transverse Electromagnetic (TEM) cell. The Specific Absorption Rate (SAR) value for each cell well was calculated for this exposure system. However, in this study the cells were exposed to the field at a higher or lower temperature than the temperature in the field-free incubator i.e., the temperature in the TEM cell was either 39 or 35 +/- 0.1 degrees C. The corresponding sham experiments were performed under exactly the same experimental conditions. The results showed that there was a significant change in cell proliferation in the exposed cells in comparison to the non-exposed (control) cells at both temperatures. On the other hand, no significant change in proliferation rate was found in the sham-exposed cells at both temperatures. This shows that biological effects due to RF/MW cannot be attributed only to a change of temperature. Since the RF/MW induced changes were of the same order of magnitude at both temperatures and also comparable to our previous results under isothermal conditions at 37 degrees C, cellular stress caused by electromagnetic fields could initiate the changes in cell cycle reaction rates. It is widely accepted that certain classes of heat-shock proteins are involved in these stress reactions.

Wolke S, Neibig U, Elsner R, Gollnick F, Meyer R. "Calcium homeostasis of isolated heart muscle cells exposed to pulsed high-frequency electromagnetic fields". *Bioelectromagnetics* 17:144-53, 1996

The intracellular calcium concentration ($[Ca^{2+}]_i$) of isolated ventricular cardiac myocytes of the guinea pig was measured during the application of pulsed high-frequency electromagnetic fields. The high-frequency fields were applied in a transverse electromagnetic cell designed to allow microscopic observation of the myocytes during the presence of the high-frequency fields. The $[Ca^{2+}]_i$ was measured as fura-2 fluorescence by means of digital image analysis. Both the carrier frequency and the square-wave pulse-modulation pattern were varied during the experiments (carrier frequencies: 900, 1,300, and 1,800 MHz pulse modulated at 217 Hz with 14% duty cycle; pulsation pattern at 900 MHz: continuous wave, 16 Hz, and 50 Hz modulation with 50% duty cycle and 30 kHz modulation with 80% duty cycle). The mean specific absorption rate (SAR) values in the solution were within one order of magnitude of 1 mW/kg. They varied depending on the applied carrier frequency and pulse pattern. The experiments were designed in three phases: 500 s of sham exposure, followed by 500 s of field exposure, then chemical stimulation without field. The chemical stimulation (K^+ -depolarization) indicated the viability of the cells. The K^+ depolarization yielded a significant increase in $[Ca^{2+}]_i$. Significant differences between sham exposure and high-frequency field exposure were not found except when a very small but statistically significant difference was detected in the case of 900 MHz/50 Hz. However, this small difference was not regarded as a relevant effect of the exposure.

IN VIVO

Adey WR, Byus CV, Cain CD, Higgins RJ, Jones RA, Kean CJ, Kuster N, MacMurray A, Stagg RB, Zimmerman G, Phillips JL, Haggren W. "Spontaneous and nitrosourea-induced primary tumors of the CNS in rats chronically exposed to 836 MHz modulated microwaves". *Radiat Res* 152(3):293-302, 1999.

We have tested an 836.55 MHz field with North American Digital Cellular (NADC) modulation in a 2-year animal bioassay that included fetal exposure. In offspring of pregnant Fischer 344 rats, we tested both spontaneous tumorigenicity and the incidence of induced central nervous system (CNS) tumors after a single dose of the carcinogen ethylnitrosourea (ENU) in utero, followed by intermittent digital-phone field exposure for 24 months. Far-field exposures began on gestational day 19 and continued until weaning at age 21 days. Near-field exposures began at 35 days and continued for the next 22 months, 4 consecutive days weekly, 2 h/day. SAR levels simulated localized peak brain exposures of a cell phone user. Of the 236 original rats, 182 (77%) survived to the termination of the whole experiment and were sacrificed at age 709-712 days. The 54 rats (23%) that died during the study ("preterm rats") formed a separate group for some statistical analyses. There was no evidence of tumorigenic effects in the CNS from exposure to the TDMA field. However, some evidence of tumor-inhibiting effects of TDMA exposure was apparent. Overall, the TDMA field-exposed animals exhibited trends toward a reduced incidence of spontaneous CNS tumors ($P < 0.16$, two-tailed) and ENU-induced CNS tumors ($P < 0.16$, two-tailed). In preterm rats, where primary neural tumors were determined to be the cause of death, fields decreased the incidence of ENU-induced tumors ($P < 0.03$, two-tailed). We discuss a possible approach to evaluating with greater certainty the possible inhibitory effects of TDMA-field exposure on tumorigenesis in the CNS.

Asanami S, Shimono K. "High body temperature induces micronuclei in mouse bone marrow". *Mutation Research* 390(1-2):79-83, 1997.

The effect of body temperature on micronucleus formation was examined in mice in order to shed light on possible mechanisms of micronuclei induction by heat. Male ddY-mice were exposed to 30 degrees-C for 1 to 6 hours, 37 degrees for 0.5 to 4 hours, and 40 degrees for 1 to 2 hours. Controls were exposed to room temperature. Relative humidity was maintained at about 30% for all conditions. A thermistor thermometer was used to measure rectal temperature. About 24 hours after exposure, bone marrow cells were isolated and analyzed microscopically for micronucleated polychromatic erythrocytes (MNPCEs). Prior to heat exposure, the mean rectal temperature equaled 37.6+/-0.5 degrees. The mean rectal temperature of mice exposed to 37 and 40 degrees increased to about 40.5 degrees. The MNPCE frequency was significantly increased in mice exposed to 37 degrees for 3 or 4 hours or 40 degrees for 1 or 2 hours, compared to controls. The frequency of MNPCEs increased at rectal temperatures equal to or greater than 39.5 degrees. In mice exposed to 37 or 40 degrees, the frequency of large micronuclei was significantly increased, with about 25% of all MNPCEs containing large micronuclei. In controls, only 5% of the MNPCEs present contained large micronuclei. The authors conclude that high body temperatures induce micronuclei formation in mouse bone marrow cells which is one possible mechanism of mitotic disruption by heat.

Brown-Woodman P D, Hadley J A, Richardson L, Bright D, Porter D. "Evaluation of reproductive function of female rats exposed to radiofrequency fields (27.12 MHz) near a shortwave diathermy device." *Health Phys* 56(4):521-5, 1989.

In recent years, there has been increased concern regarding effects of operator exposure to the electromagnetic (EM) field associated with shortwave diathermy devices. The present study was designed to investigate the effects, on rats, of repeated exposure to such an EM field. Following repeated exposure for 5 wk, a reduction in fertility occurred as indicated by a reduced number of matings in exposed rats compared to sham-irradiated rats and a reduction in the number of rats that conceived after mating. The data suggest that female operators could experience reduced fertility, if they remained close to the console for prolonged periods. This has particular significance for the physiotherapy profession.

Chagnaud JL, Moreau JM, Veyret B. "No effect of short-term exposure to GSM-modulated low-power microwaves on benzo(a)pyrene-induced tumours in rat". *Int J Radiat Biol* 75(10):1251-1256, 1999.

PURPOSE: In view of current interest in the biological effects of amplitude-modulated microwaves arising from the rapid development of mobile communications, the effects of low-level microwaves on cancer development were investigated using a rat sarcoma model.

MATERIALS AND METHODS: Two-month-old female Sprague-Dawley rats were treated by injection of benzo(a)pyrene and irradiated with GSM (Global System for Mobile)-modulated 900-MHz microwaves in an anechoic chamber at 55 or 200 microW cm⁻² (75 and 270 mW kg⁻¹) average whole-body SAR, 2h daily for 2 weeks). Rats were exposed from day 20, 40 or 75 after carcinogen injection. Additional groups of rats were sham-exposed in a second anechoic chamber. Anti-phosphatidylinositol autoantibody levels were evaluated in sera to monitor malignant transformation.

RESULTS: Microwave exposure had no effect on the development of tumours. No acceleration or delays in tumour onset were observed. Animal survival was not modified and serum autoantibody levels were similar in exposed and sham-exposed groups.

CONCLUSION: Low-level GSM microwave exposure of rat bearing benzo(a)pyrene-induced tumours had no effect on auto-antibody levels, tumour appearance and survival. The low exposure levels used here correspond to exposure limits for whole-body exposure of humans.

Daniells C, Duce I, Thomas D, Sewell P, Tattersall J, de Pomerai D. "Transgenic nematodes as biomonitors of microwave-induced stress". *Mutat Res* 399:55-64, 1998.

Transgenic nematodes (*Caenorhabditis elegans* strain PC72), carrying a stress-inducible reporter gene (*Escherichia coli* beta-galactosidase) under the control of a *C. elegans* hsp16 heat-shock promoter, have been used to monitor toxicant responses both in water and soil. Because these transgenic nematodes respond both to heat and toxic chemicals by synthesising an easily detectable reporter product, they afford a useful preliminary screen for stress responses (whether thermal or non-thermal) induced by microwave radiation or other electromagnetic fields. We have used a transverse electromagnetic (TEM) cell fed from one end by a source and terminated at the other end by a matched load. Most studies were conducted using a frequency of 750 MHz, at a nominal power setting of 27 dBm. The TEM cell was held in an incubator at 25 degrees C inside a shielded room; corresponding controls were shielded and placed in the same 25 degrees C incubator; additional baseline controls were held at 15 degrees C (worm growth temperature). Stress responses were measured in

terms of beta-galactosidase (reporter) induction above control levels. The time-course of response to continuous microwave radiation showed significant differences from 25 degrees C controls both at 2 and 16 h, but not at 4 or 8 h. Using a 5 x 5 multiwell plate array exposed for 2 h, the 25 microwaved samples showed highly significant responses compared with a similar control array. The wells most strongly affected were those in the rows closest to the source, whereas the most distant row did not rise above control levels, suggesting a shadow effect. These differential responses are difficult to reconcile with general heating effects, although localised power absorption affords a possible explanation. Experiments in which the frequency and/or power settings were varied suggested a greater response at 21 than at 27 dBm, both at 750 and 300 MHz, although extremely variable responses were observed at 24 dBm and 750 MHz. Thus, lower power levels tended, if anything, to induce larger responses (with the above-mentioned exception), which is opposite to the trend anticipated for any simple heating effect. These results are reproducible and data acquisition is both rapid and simple. The evidence accrued to date suggests that microwave radiation causes measurable stress to transgenic nematodes, presumably reflecting increased levels of protein damage within cells (the common signal thought to trigger hsp gene induction). The response levels observed are comparable to those observed with moderate concentrations (ppm) of metal ions such as Zn₂₊ and Cu₂₊. We conclude that this approach deserves further and more detailed investigation, but that it has already demonstrated clear biological effects of microwave radiation in terms of the activation of cellular stress responses (hsp gene induction).

De Pomerai D, Daniells C, David H, Allan J, Duce I, Mutwakil M, Thomas D, Sewell P, Tattersall J, Jones D, Candido P. “Non-thermal heat-shock response to microwaves”. *Nature* 405:417-418, 2000.

Nematode worms (*C. elegans*) exposed overnight to 750-MHz microwaves at a SAR of 0.001 W/kg showed an increased in heat shock proteins (HSPs). (Heat shock proteins are induced in most organisms by adverse conditions (such as heat or toxins) that cause damage to cellular proteins, acting as molecular chaperones to rescue damaged proteins). The authors give several arguments that the microwave-induced effect on HSPs is non-thermal and suggest that 'current exposure limits for microwave equipment may need to be reconsidered.

Detlavs I, Dombrovska L, Turauska A, Shkirmante B, Slutskii L. “Experimental study of the effects of radiofrequency electromagnetic fields on animals with soft tissue wounds”. *Sci Total Environ* 180(1):35-42, 1996.

The effect of radio frequency electromagnetic fields (RF EMF) was studied on Wistar rats with excised full-thickness dermal wounds in the interscapular region. The wounded regions of experimental animals were subjected to EMF for 30 min daily during the first 5 days after wound infliction. Control animals received no treatment. We used RF EMF with (1) frequency 53.53 GHz without modulation; (2) frequency 42.19 GHz without modulation; (3) frequency 42.19 GHz, but with a frequency modulation band 200-MHz wide. On the 7th day the animals were terminated and the granulation-fibrous tissue (GFT) developed in the wounds was subjected to complex quantitative biochemical analysis. RF EMF without frequency modulation decreased the amounts of glycoprotein macromolecules, diminishing the inflammatory exudation. In striking contrast, under the influence of RF EMF with frequency modulation, hexoses and especially sialic acid concentrations were significantly elevated ($P < 0.001$). This indicated intensification of exudative phenomena. As a consequence of inflammation inhibition in the treatment without frequency modulation, the total collagen accumulation was lowered. However, when frequency was modulated, the

inflammatory phenomena were intensified, and pronounced accumulation of collagenous proteins was noted. Thus, our experiments confirm the effects of non-thermal EMF on the reparative-proliferative processes of animals with soft tissue wounds.

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

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

Fesenko EE, Makar VR, Novoselova EG, Sadovnikov VB. “Microwaves and cellular immunity. I. Effect of whole body microwave irradiation on tumor necrosis factor production in mouse cells”. *Bioelectrochem Bioenerg* 49(1):29-35, 1999.

Whole body microwave sinusoidal irradiation of male NMRI mice with 8.15-18 GHz (1 Hz within) at a power density of 1 microW/cm² caused a significant enhancement of TNF production in peritoneal macrophages and splenic T lymphocytes. Microwave radiation affected T cells, facilitating their capacity to proliferate in response to mitogenic stimulation. The exposure duration necessary for the stimulation of cellular immunity ranged from 5 h to 3 days. Chronic irradiation of mice for 7 days produced the decreasing of TNF production in peritoneal macrophages. The exposure of mice for 24 h increased the TNF production and immune proliferative response, and these stimulatory effects persisted over 3 days after the termination of exposure. Microwave treatment increased the endogenously produced TNF more effectively than did lipopolysaccharide, one of the most potential stimuli of synthesis of this cytokine. The role of microwaves as a factor interfering with the process of cell immunity is discussed.

Frei, M R, Berger R E, Dusch S J, Guel V, Jauchem J R, Merritt J H and Stedham M A. “Chronic exposure of cancer-prone mice to low-level 2450 MHz radiofrequency radiation.” *Bioelectromagnetics* 19(1): 20-31, 1998a.

The purpose of this study was to determine whether chronic, low-level exposure of mammary-tumor-prone mice to 2450 MHz radiofrequency radiation (RFR) promotes an earlier onset (decreased latency), a greater total incidence, or a faster growth rate of mammary tumors. One hundred C3H/ HeJ mice were exposed in circularly polarized waveguides (CWG) for 18 months (20 h/day, 7 days/wk) to continuous-wave, 2450 MHz RFR at a whole body average specific absorption rate (SAR) of 0.3 W/kg; 100 mice were sham exposed. Before exposure, SARs were determined calorimetrically; during experimentation, SARs were monitored by differential power measurement. All animals were visually inspected twice daily and were removed from the CWG cages for a weekly inspection, palpation, and weighing. From the time of detection, tumor size was measured weekly. Animals that died spontaneously, became moribund, or were killed after 18 months of exposure were completely necropsied; tissues were fixed and subjected to

histopathological evaluations. Results showed no significant difference in weight profiles between sham-irradiated and irradiated mice. Concerning mammary carcinomas, there was no significant difference between groups with respect to palpated tumor incidence (sham = 52%; irradiated = 44%), latency to tumor onset (sham = 62.3 +/- 1.2 wk; irradiated = 64.0 +/- 1.6 wk), and rate of tumor growth. In general, histopathological examination revealed no significant differences in numbers of malignant, metastatic, or benign neoplasms between the two groups; a significantly greater incidence of alveolar-bronchiolar adenoma in the sham-irradiated mice was the only exception. In addition, survival analysis showed no significant difference in cumulative percent survival between sham and irradiated animals. Thus, results indicate that under the conditions of this study, long-term, low-level exposure of mammary-tumor-prone mice to 2450 MHz RFR did not affect mammary tumor incidence, latency to tumor onset, tumor growth rate, or animal longevity when compared with sham-irradiated controls.

Frei MR, Jauchem JR, Dusch SJ, Merritt JH, Berger RE, Stedham MA. "Chronic, low-level (1.0 W/kg) exposure of mice prone to mammary cancer to 2450 MHz microwaves". *Radiat Res* 150(5):568-576, 1998b.

In a previous study (Frei et al., *Bioelectromagnetics* 19, 20-31, 1998), we showed that low-level (0.3 W/kg), long-term exposure of mice prone to mammary tumors to 2450 MHz radiofrequency (RF) radiation did not affect the incidence of mammary tumors, latency to tumor onset, tumor growth rate or animal survival when compared to sham-irradiated animals. In the current study, the specific absorption rate (SAR) was increased from 0.3 W/kg to 1.0 W/kg. The same biological end points were used. One hundred C3H/HeJ mice were exposed in circularly polarized waveguides for 78 weeks (20 h/day, 7 days/week) to continuous-wave, 2450 MHz RF radiation; 100 mice were sham-exposed. There was no significant difference between exposed and sham-exposed groups with respect to the incidence of palpated mammary tumors (sham-exposed = 30%; irradiated = 38%), latency to tumor onset (sham-exposed = 62.0 +/- 2.3 weeks; irradiated = 62.5 +/- 2.2 weeks) and rate of tumor growth. Histopathological evaluations revealed no significant difference in numbers of malignant, metastatic or benign neoplasms between the two groups. Thus long-term exposures of mice prone to mammary tumors to 2450 MHz RF radiation at SARs of 0.3 and 1.0 W/kg had no significant effects when compared to sham-irradiated animals.

Fritze K, Wiessner C, Kuster N, Sommer C, Gass P, Hermann DM, Kiessling M, Hossmann KA. "Effect of global system for mobile communication microwave exposure on the genomic response of the rat brain". *Neuroscience* 81(3):627-639, 1997a.

The acute effect of global system for mobile communication (GSM) microwave exposure on the genomic response of the central nervous system was studied in rats by measuring changes in the messenger RNAs of hsp70, the transcription factor genes c-fos and c-jun and the glial structural gene GFAP using in situ hybridization histochemistry. Protein products of transcription factors, stress proteins and marker proteins of astroglial and microglial activation were assessed by immunocytochemistry. Cell proliferation was evaluated by bromodeoxyuridine incorporation. A special GSM radiofrequency test set, connected to a commercial cellular phone operating in the discontinuous transmission mode, was used to simulate GSM exposure. The study was conducted at time averaged and brain averaged specific absorption rates of 0.3 W/kg (GSM exposure), 1.5 W/kg (GSM exposure) and 7.5 W/kg (continuous wave exposure), respectively. Immediately after exposure, in situ

hybridization revealed slight induction of hsp70 messenger RNA in the cerebellum and hippocampus after 7.5 W/kg exposure, but not at lower intensities. A slightly increased expression of c-fos messenger RNA was observed in the cerebellum, neocortex and piriform cortex of all groups subjected to immobilization, but no differences were found amongst different exposure conditions. C-jun and GFAP messenger RNAs did not increase in any of the experimental groups. 24 h after exposure, immunocytochemical analysis of FOS and JUN proteins (c-FOS, FOS B, c-JUN JUN B, JUN D), of HSP70 or of KROX-20 and -24 did not reveal any alterations. Seven days after exposure, neither increased cell proliferation nor altered expression of astroglial and microglial marker proteins were observed. In conclusion, acute high intensity microwave exposure of immobilized rats may induce some minor stress response but does not result in lasting adaptive or reactive changes of the brain.

Fritze K, Sommer C, Schmitz B, Mies G, Hossmann KA, Kiessling M, Wiessner C. "Effect of global system for mobile communication (GSM) microwave exposure on blood-brain barrier permeability in rat". *Acta Neuropathol* 94(5):465-470, 1997b.

We investigated the effects of global system for mobile communication (GSM) microwave exposure on the permeability of the blood-brain barrier using a calibrated microwave exposure system in the 900 MHz band. Rats were restrained in a carousel of circularly arranged plastic tubes and sham-exposed or microwave irradiated for a duration of 4 h at specific brain absorption rates (SAR) ranging from 0.3 to 7.5 W/kg. The extravasation of proteins was assessed either at the end of exposure or 7 days later in three to five coronal brain slices by immunohistochemical staining of serum albumin. As a positive control two rats were subjected to cold injury. In the brains of freely moving control rats (n = 20) only one spot of extravasated serum albumin could be detected in one animal. In the sham-exposed control group (n = 20) three animals exhibited a total of 4 extravasations. In animals irradiated for 4 h at SAR of 0.3, 1.5 and 7.5 W/kg (n = 20 in each group) five out of the ten animals of each group killed at the end of the exposure showed 7, 6 and 14 extravasations, respectively. In the ten animals of each group killed 7 days after exposure, the total number of extravasations was 2, 0 and 1, respectively. The increase in serum albumin extravasations after microwave exposure reached significance only in the group exposed to the highest SAR of 7.5 W/kg but not at the lower intensities. Histological injury was not observed in any of the examined brains. Compared to other pathological conditions with increased blood-brain barrier permeability such as cold injury, the here observed serum albumin extravasations are very modest and, moreover, reversible. Microwave exposure in the frequency and intensity range of mobile telephony is unlikely to produce pathologically significant changes of the blood-brain barrier permeability.

Higashikubo R, Culbreth VO, Spitz DR, LaRegina MC, Pickard WF, Straube WL, Moros EG, Roti Roti JL. "Radiofrequency electromagnetic fields have no effect on the in vivo proliferation of the 9L brain tumor". *Radiat Res* 152(6):665-671, 1999.

The intracranial 9L tumor model was used to determine if exposure to a radiofrequency (RF) electromagnetic field similar to those used in cellular telephone has any effects on the growth of a central nervous system tumor. Fischer 344 rats implanted with different numbers of 9L gliosarcoma cells were exposed to 835.62 MHz frequency-modulated continuous wave (FMCW) or 847.74 MHz code division multiple access (CDMA) RF field with nominal slot-average specific absorption rates in the brain of 0.75 +/- 0.25 W/kg. The animals were

exposed to the RF field for 4 h a day, 5 days a week starting 4 weeks prior to and up to 150 days after the implantation of tumor cells. Among sham-exposed animals injected with 2 to 10 viable cells (group 1), the median survival was 70 days, with 27% of the animals surviving at 150 days. The median survival length and final survival fraction for animals injected with 11 to 36 viable cells (group 2) were 52 days and 14%, respectively, while the values for those injected with 37 to 100 cells (group 3) were 45 days and 0%. The animals exposed to CDMA or FMCW had similar survival parameters, and the statistical comparison of the survival curves for each of the groups 1, 2 and 3 showed no significant differences compared to sham-exposed controls.

Imaida, K., M. Taki, Yamaguchi T, Ito T, Watanabe S, Wake K, Aimoto A, Kamimura Y, Ito N and Shirai T. "Lack of promoting effects of the electromagnetic near-field used for cellular phones (929.2 MHz) on rat liver carcinogenesis in a medium- term liver bioassay." *Carcinogenesis* 19(2): 311-4, 1998a.

The possible cancer promotion potential of local exposure to a pulse modulated 929.2 MHz electromagnetic near-field on chemically-initiated rat liver carcinogenesis was investigated employing a medium-term bioassay. A 929.2-MHz electromagnetic near-field of time division multiple access (TDMA) signal for PDC (Personal Digital Cellular, Japanese cellular telephone standard) system was directed to rats through a quarter-wavelength monopole antenna. Maximum local specific absorption rates (SARs) on temporal average were 7.2-6.6 W/kg within the whole body and 2.0-1.7 W/kg within the liver, which was the target organ. The whole-body average SARs on temporal average were 0.80-0.58 W/kg. Temporal peak SARs had three times these values due to the duty ratio of the PDC signal. Exposure was for 90 min a day, 5 days a week, over 6 weeks. The exposure apparatus was specially designed for this experiment, to allow exposure of the lateral mid-section of the rat body to the electromagnetic near-field. Male F344 rats, 6 week-old, were initially (at week 0) given a single dose of diethylnitrosamine (DEN, 200 mg/kg body wt, i.p.). At 2 weeks later, exposure (48 rats) or sham-exposure (48 rats) was started. The exposure of electromagnetic near-fields was performed using the exposure apparatus mentioned above. At week 3, all rats were subjected to a 2/3 partial hepatectomy. At week 8 (i.e. after 6 weeks exposure or sham-exposure), the experiment was terminated and all rats were killed. Carcinogenic potential was scored by comparing the numbers and areas of the induced glutathione S- transferase placental form (GST-P) positive foci in the livers of the exposed and sham-exposed rats. A further group of 24 animals, given only DEN and partial hepatectomy, served as the controls. The numbers (no./cm²) of GST-P positive foci were 4.61 +/- 1.77, 5.21 +/- 1.92 (P 0.05, versus control) and 4.09 +/- 1.47 and the areas (mm²/cm²) were 0.30 +/- 0.16, 0.36 +/- 0.21 and 0.28 +/- 0.15, for the exposed, sham-exposed and control groups, respectively. There were no significant differences between the exposed and sham-exposed groups. These findings clearly indicated that local body exposure to a 929.2-MHz field, modulated in a PDC waveform, has no significant effect on rat liver carcinogenesis under the experimental conditions employed.

Imaida K, Taki M, Watanabe S, Wake K, Kamimura Y, Ito T, Yamaguchi T, Ito N, Shirai T. "The 1.5 GHz electromagnetic near-field used for cellular phones does not promote rat liver carcinogenesis in a medium-term liver bioassay". *Jpn J Cancer Res* 89(10):995-1002, 1998b.

We have recently established that local exposure to a 929.2 MHz electromagnetic near-field, used for cellular phones, does not promote rat liver carcinogenesis in a medium-term

bioassay system. In the present study, a 1.439 GHz electromagnetic near-field (EMF), another microwave band employed for cellular phones in Japan, was similarly investigated. Time division multiple access (TDMA) signals for the Personal Digital Cellular (PDC) Japanese cellular telephone standard system were directed to rats through a quarter-wavelength monopole antenna. Numerical dosimetry showed that the peak SARs within the liver were 1.91-0.937 W/kg, while the whole-body average specific absorption rates (SARs) were 0.680-0.453 W/kg, when the time-averaged antenna radiation power was 0.33 W. Exposure was for 90 min a day, 5 days a week, over 6 weeks, to male F344 rats given a single dose of diethylnitrosamine (200 mg/kg, i.p.) 2 weeks previously. At week 3, all rats were subjected to a two-thirds partial hepatectomy. At week 8, the experiment was terminated and the animals were killed. Carcinogenic potential was scored by comparing the numbers and areas of the induced glutathione S-transferase placental form (GST-P)-positive foci in the livers of exposed (48) and sham-exposed rats (48). Despite increased serum levels of corticosterone, adrenocorticotrophic hormone (ACTH) and melatonin, the numbers and the areas of GST-P-positive foci were not significantly altered by the exposure. These findings clearly indicated that local body exposure to a 1.439 GHz EMF, as in the case of a 929.2 MHz field, has no promoting effect on rat liver carcinogenesis in the present model.

Jauchem JR, Seaman RL, Lehnert HM, Mathur SP, Ryan KL, Frei MR, Hurt WD. "Ultra-wideband electromagnetic pulses: Lack of effects on heart rate and blood pressure during two-minute exposures of rats". *Bioelectromagnetics* 19:330-333, 1998.

Exposure to fast-rise-time ultra-wideband (UWB) electromagnetic pulses has been postulated to result in effects on biological tissue (including the cardiovascular system). In the current study, 10 anesthetized Sprague-Dawley rats were exposed to pulses produced by a Sandia UWB pulse generator (average values of exposures over three different pulse repetition rates: rise time, 174-218 ps; peak E field, 87-104 kV/m; pulse duration, 0.97-0.99 ns). Exposures to 50, 500 and 1000 pulses/s resulted in no significant changes in heart rate or mean arterial blood pressure measured every 30 s during 2 min of exposure and for 2 min after the exposure. The results suggest that acute UWB whole-body exposure under these conditions does not have an immediate detrimental effect on these cardiovascular system variables in anesthetized rats.

Jauchem JR, Frei MR, Ryan KL, Merritt JH, Murphy MR. "Lack of effects on heart rate and blood pressure in ketamine-anesthetized rats briefly exposed to ultra-wideband electromagnetic pulses". *IEEE Trans Biomed Eng* 46(1):117-120, 1999.

Fourteen Sprague-Dawley rats were exposed to pulses produced by a Bournlea ultra-wideband (UWB) pulse generator (rise time, 318-337 ps; maximum E field, 19-21 kV/m). Exposures at a repetition frequency of 1 kHz for 0.5 s or to repetitive pulse trains (2-s exposure periods alternating with 2 s of no exposure, for a total of 2 min) resulted in no significant changes in heart rate or mean arterial blood pressure. These results suggest that acute whole-body exposure to UWB pulses does not have a detrimental effect on the cardiovascular system.

Jensh RP. “Behavioral teratologic studies using microwave radiation: is there an increased risk from exposure to cellular phones and microwave ovens?”. *Reprod Toxicol* 11(4):601-11 1997.

The objective of the investigations presented in this review was to determine if there are adverse effects due to chronic prenatal microwave exposure in rats at term and/or alterations in neonatal and adult offspring psychophysiological development and growth. Following the establishment of a nonhyperthermal power density level of microwave radiation, pregnant rats were exposed throughout pregnancy to continuous wave 915 MHz, 2450 MHz, or 6000 MHz radiation at power density levels of 10, 20, or 35 mW/cm², respectively. Teratologic evaluation included the following parameters: maternal weight and weight gain; mean litter size; maternal organ weight and organ weight/body weight ratios; body weight ratios of brain, liver, kidneys, and ovaries; maternal peripheral blood parameters including hematocrit, hemoglobin, and white cell counts; number of resorptions and resorption rate; number of abnormalities and abnormality rate; mean term fetal weight. Mothers were rebred, and the second, nonexposed litters were evaluated for teratogenic effects. Exposed offspring were evaluated using the following perinatal and adult tests: eye opening, surface righting, negative geotaxis, auditory startle, air righting, open field, activity wheel, swimming, and forelimb hanging. Offspring were also monitored for weekly weight and weight gain. Animals exposed to 915 MHz did not exhibit any consistent significant alterations in any of the above parameters. Exposure to 2450 MHz resulted only in a significantly increased adult offspring activity level compared to nonexposed offspring. Offspring exposed to 6000 MHz radiation exhibited an initial slight, but significant, retardation in term weight, while mothers had a significantly reduced monocyte count. No changes in any of the other term parameters were observed. A few postnatal parameters were affected in offspring exposed to 6000 MHz. Weekly weights were lower in the exposed offspring, but they recovered by the fifth week. Eye opening was delayed, and there were changes in the water T-maze and open field performance levels. Several organ/body weight ratios differed from those of the control offspring. These results indicate that exposure to 6000 MHz radiation at this power density level may result in subtle long-term neurophysiologic alterations. However, in the absence of a hyperthermic state, the microwave frequencies tested, which included frequencies used in cellular phones and microwave ovens, do not induce a consistent, significant increase in reproductive risk as assessed by classical morphologic and postnatal psychophysiological parameters.

Juutilainen J, Heikinen P, Kosma V-M, Hongisto T, Huuskonen H, Hyysalo P, Komulainen H, Kumlin T, Lahtinen T, Land S, Penttila I, Puranen L and Väänänen A. “Do pulse-modulated or continuous 900 MHz RF fields enhance the carcinogenic effect of ionising radiation in mice?”. IN *Proceedings 1 st International Medical Scientific Congress “Non-Ionizing High-frequency EM Radiations: Researching the Epidemiology and Clinical Evidence”*, Rome, November 1999, *in press*.

Abstract not available

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

Effects of in vivo microwave exposure on DNA strand breaks (a form of DNA damage) were investigated in rat brain cells. In previous research, we have found that acute (2 hours) exposure to pulsed (2 microseconds pulses, 500 pps) 2450-MHz radiofrequency

electromagnetic radiation (RFR) (power density 2 mW/cm², average whole body specific absorption rate 1.2 W/kg) caused an increase in DNA single- and double-strand breaks in brain cells of the rat when assayed 4 hours post exposure using a microgel electrophoresis assay. In the present study, we found that treatment of rats immediately before and after RFR exposure with either melatonin (1 mg/kg/injection, SC) or the spin-trap compound N-tert-butyl-alpha-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, Carino MA, Singh NP. “Naltrexone blocks RFR-induced DNA double strand breaks in rat brain cells”. *Wireless Networks* 3:471-476, 1997.

Previous research in our laboratory has shown that various effects of radiofrequency electromagnetic radiation (RFR) exposure on the nervous system are mediated by endogenous opioids in the brain. We have also found that acute exposure to RFR induced DNA strand breaks in brain cells of the rat. The present experiment was carried out to investigate whether endogenous opioids are also involved in RFR-induced DNA strand breaks. Rats were treated with the opioid antagonist naltrexone (1 mg/kg, IP) immediately before and after exposure to 2450-MHz pulses (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.

Lu ST, Mathur SP, Akyel Y, Lee JC. “Ultrawide-band electromagnetic pulses induced hypotension in rats”. *Physiol Behav* Jan 1-15;65(4-5):753-761, 1999; Corrected and republished in *Physiol Behav* 67(3):753-761, 1999.

The ultrawide-band (UWB) electromagnetic pulses are used as a new modality in radar technology. Biological effects of extremely high peak E-field, fast rise time, ultrashort pulse width, and ultrawide band have not been investigated heretofore due to the lack of animal exposure facilities. A new biological effects database is needed to establish personnel protection guidelines for these new type of radiofrequency radiation. Functional indices of the cardiovascular system (heart rate, systolic, mean, and diastolic pressures) were selected to represent biological end points that may be susceptible to the UWB radiation. A noninvasive tail-cuff photoelectric sensor sphygmomanometer was used. Male Wistar-Kyoto rats were subjected to sham exposure, 0.5-kHz (93 kV/m, 180 ps rise time, 1.00 ns pulse width, whole-body averaged specific absorption rate, SAR = 70 mW/kg) or a 1-kHz (85 kV/m, 200 ps rise time, 1.03 ns pulse width, SAR = 121 mW/kg) UWB fields in a tapered parallel plate GTEM cell for 6 min. Cardiovascular functions were evaluated from 45 min to 4 weeks after exposures. Significant decrease in arterial blood pressures (hypotension) was found. In contrast, heart rate was not altered by these exposures. The UWB radiation-induced hypotension was a robust, consistent, and persistent effect.

Magras IN, Xenos TD. "RF radiation-induced changes in the prenatal development of mice". *Bioelectromagnetics* 18(6):455-461, 1997.

The possible effects of radiofrequency (RF) radiation on prenatal development has been investigated in mice. This study consisted of RF level measurements and in vivo experiments at several places around an "antenna park." At these locations RF power densities between 168 nW/cm² and 1053 nW/cm² were measured. Twelve pairs of mice, divided in two groups, were placed in locations of different power densities and were repeatedly mated five times. One hundred eighteen newborns were collected. They were measured, weighed, and examined macro- and microscopically. A progressive decrease in the number of newborns per dam was observed, which ended in irreversible infertility. The prenatal development of the newborns, however, evaluated by the crown-rump length, the body weight, and the number of the lumbar, sacral, and coccygeal vertebrae, was improved.

Malyapa, R. S., Ahern E W, Straube W L, Moros E G, Pickard W F, and Roti Roti J L. "Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation". *Radiat Res* 148(6): 608-17, 1997a.

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, Starube WL, Moros EG, Pickard WR, Roti Roti JL. "DNA damage in rat brain cells following in vivo exposure to 2450 MHz EMR and methods of euthanasia". *Radiation Research* 149: 637-646 1998.

This study was a further follow-up investigation of possible RF-induced DNA (strand breaks) as reported by Drs Lai and Singh at the University of Washington in 1995. It simulated the 2450 MHz continuous wave (CW) exposures used by Drs Lai and Singh and found no evidence of RF-induced DNA damage in the exposed vs. unexposed animals. This finding was consistent with the results of earlier in vitro studies conducted and researched by the same research team.

Morrissey JJ, Raney S, Heasley E, Rathinavelu P, Dauphinee M, Fallon JH. “IRIDIUM exposure increases c-fos expression in the mouse brain only at levels which likely result in tissue heating”. *Neuroscience* 92(4):1539-1546, 1999.

With the rapid development of wireless communication technology over the last 20 years, there has been some public concern over possible health effects of long-term, low-level radiofrequency exposure from cellular telephones. As an initial step in compiling a database for risk analysis by government agencies, the effects of 1-h exposure of mice to a 1.6-GHz radiofrequency signal, given as either a continuous wave or pulse modulated at 11 Hz with a duty cycle of 4:1 and a pulse duration of 9.2 ms (IRIDIUM), on c-fos gene expression in the brain was investigated. The IRIDIUM signal is the operating frequency for a ground-to-satellite-to-ground cellular communications web which has recently become fully operational, and was named as such due to the original designed employment of the same number of low orbiting satellites as there are electrons orbiting the nucleus of an iridium atom. The expression of c-fos was not significantly elevated in the brains of mice until exposure levels exceeded six times the peak dose and 30 times the whole body average dose as maximal cellular telephone exposure limits in humans. Higher level exposure using either continuous wave (analog) or IRIDIUM signals elevated c-fos to a similar extent, suggesting no obvious pulsed modulation-specific effects. The pattern of c-fos elevation in limbic cortex and subcortex areas at higher exposure levels is most consistent with a stress response due to thermal perception coupled with restraint and/or neuron activity near thermoregulatory regions, and not consistent with any direct interaction of IRIDIUM energy with brain tissue.

Novoselova EG, Fesenko EE, Makar VR, Sadovnikov VB. “Microwaves and cellular immunity. II. Immunostimulating effects of microwaves and naturally occurring antioxidant nutrients”. *Bioelectrochem Bioenerg* 49(1):37-41, 1999.

The effect of 8.15-18 GHz (1 Hz within) microwave radiation at a power density of 1 microW/cm² on the tumor necrosis factor (TNF) production and immune response was tested. A single 5 h whole-body exposure induced a significant increase in TNF production in peritoneal macrophages and splenic T cells. The mitogenic response in T lymphocytes increased after microwave exposure. The activation of cellular immunity was observed within 3 days after exposure. The diet containing lipid-soluble nutrients (beta-carotene, alpha-tocopherol and ubiquinone Q9) increased the activity of macrophages and T cells from irradiated mice. These results demonstrate that irradiation with low-power density microwaves stimulates the immune potential of macrophages and T cells, and the antioxidant treatment enhances the effect of microwaves, in particular at later terms, when the effect of irradiation is reduced.

Persson BRR, Salford LG, Brun A. “Blood-brain barrier permeability in rats exposed to electromagnetic fields used in wireless communication”. *Wireless Networks* 3:455-461, 1997.

Biological effects of radio frequency electromagnetic fields (EMF) on the blood-brain barrier (BBB) have been studied in Fischer 344 rats of both sexes. The rats were not anesthetised during the exposure. The brains were perfused with saline for 3-4 minutes, and thereafter perfusion fixed with 4% formaldehyde for 5-6 minutes. Whole coronal sections of the brains were dehydrated and embedded in paraffin and sectioned at 5 micrometers. Albumin and fibrinogen were demonstrated immunochemically and classified as normal versus pathological leakage. In the present investigation we exposed male and female Fischer 344 rats in a

Transverse Electromagnetic Transmission line chamber to microwaves of 915 MHz as continuous wave (CW) and pulse-modulated with different pulse power and at various time intervals. The CW-pulse power varied from 0.001 W to 10 W and the exposure time from 2 min to 960 min. In each experiment we exposed 4-6 rats with 2-4 controls randomly placed in excited and non-excited TEM cells, respectively. We have in total investigated 630 exposed rats at various modulation frequencies and 372 controls. The frequency of pathological rats is significantly increased ($P < 0.0001$) from 62/372 (ratio 0.17 + 0.02) for control rats to 244/630 (ratio: 0.39 + 0.043) in all exposed rats. Grouping the exposed animals according to the level or specific absorption energy (J/kg) give significant difference in all levels above 1.5 J/kg. The exposure was 915 MHz microwaves either pulse modulated (PW) at 217 Hz with 0.57 ms pulse width, at 50 Hz with 6.6 ms pulse width or continuous wave (CW). The frequency of pathological rats (0.17) among controls in the various groups is not significantly different. The frequency of pathological rats was 170/480 (0.35 + 0.03) among rats exposed to pulse modulated (PW) and 74/149 (0.50 + 0.07) among rats exposed to continuous wave exposure (CW). These results are both highly significantly different to their corresponding controls ($p < 0.0001$) and the frequency of pathological rats after exposure to pulsed radiation (PW) is significantly less ($p < 0.002$) than after exposure to continuous wave radiation (CW).

Stark KD, Krebs T, Altpeter E, Manz B, Griot C, Abelin T. “Absence of chronic effect of exposure to short-wave radio broadcast signal on salivary melatonin concentrations in dairy cattle”. *J Pineal Res* 22(4):171-176, 1997.

A pilot study was conducted to investigate the influence of electromagnetic fields in the short-wave range (3-30 MHz) radio transmitter signals on salivary melatonin concentration in dairy cattle. The hypothesis to be tested was whether EMF exposure would lower salivary melatonin concentrations, and whether removal of the EMF source would be followed by higher concentration levels. For this pilot study, a controlled intervention trial was designed. Two commercial dairy herds at two farms were compared, one located at a distance of 500 m (exposed), the other at a distance of 4,000 m (unexposed) from the transmitter. At each farm, five cows were monitored with respect to their salivary melatonin concentrations over a period of ten consecutive days. Saliva samples were collected at two-hour intervals during the dark phase of the night. As an additional intervention, the short-wave transmitter was switched off during three of the ten days (off phase). The samples were analyzed using a radioimmunoassay. The average nightly field strength readings were 21-fold greater on the exposed farm (1.59 mA/m) than on the control farm (0.076 mA/m). The mean values of the two initial nights did not show a statistically significant difference between exposed and unexposed cows. Therefore, a chronic melatonin reduction effect seemed unlikely. However, on the first night of re-exposure after the transmitter had been off for three days, the difference in salivary melatonin concentration between the two farms (3.89 pg/ml, CI: 2.04, 7.41) was statistically significant, indicating a two- to seven-fold increase of melatonin concentration. Thus, a delayed acute effect of EMF on melatonin concentration cannot completely be excluded. However, results should be interpreted with caution and further trials are required in order to confirm the results.

Toler, J C, Shelton W W, Frei M R, Merrit J H, and Stedham M A. “Long-term, low-level exposure of mice prone to mammary tumors to 435 MHz radiofrequency radiation”. *Radiat Res* 148(3): 227-34, 1997.

The purpose of this study was to determine if chronic, low-level exposure of mice prone to mammary tumors to 435 MHz radiofrequency (RF) radiation promotes an earlier onset, a

faster growth rate or a greater total incidence of mammary tumors than in sham-exposed controls. Two hundred female C3H/HeJ mice were exposed for 21 months (22 h/day, 7 days/week) to a horizontally polarized 435 MHz pulse-wave (1.0 micro pulse width, 1.0 kHz pulse rate) RF radiation environment with an incident power density of 1.0 mW/cm² (SAR = 0.32 W/kg). An additional 200 mice were sham-exposed. Animals that died spontaneously, became moribund or were euthanized after 21 months of exposure were completely necropsied; tissues were subjected to histopathological examinations. Concerning mammary carcinomas, there were no significant differences between the two groups with respect to latency to tumor onset, tumor growth rate and overall tumor incidence. Histopathological examination revealed no significant differences in numbers of malignant, metastatic or benign neoplasms between groups. Survival probability was estimated by the Kaplan-Meier method; no significant difference between groups was noted (Cox's test). Under the conditions of this long-term study, low-level exposure of mice prone to mammary tumors to 435 MHz RF radiation did not affect the incidence of mammary tumors, tumor growth rate, latency to tumor onset or animal longevity when compared to sham- exposed controls.

Tsurita G, Nagawa H, Ueno S, Watanabe S, Taki M. “Biological and morphological effects on the brain after exposure of rats to a 1439 MHz TDMA field”. *Bioelectromagnetics* 21:364-371, 2000.

We investigated the effects of exposure to a 1439 MHz TDMA (Time Division Multiple Access) field, as used in cellular phones, on the permeability of the blood-brain barrier (BBB), on the morphological changes of the brain, and on body-mass fluctuations. Male Sprague-Dawley (SD) rats were divided into three groups of eight rats each. The rats in the EM(+) group, which had their heads arrayed in a circle near the central antenna of an exposure system, were exposed to a 1439 MHz field for one hour a day. The rats in EM(-) group were also in the exposure system, however, without high-frequency electromagnetic wave (HF-EMW) exposure. The animals in the control group were neither placed in the system nor exposed to HF-EMWs. The exposure period was two or four weeks. The energy dose rate peaked at 2 W/kg in the brain; the average over the whole body was 0.25 W/kg. The changes in the permeability of BBB were investigated by Evans blue injection method and by immunostaining of serum albumin. HF-EMWs had no effect on the permeability of BBB. The morphological changes in the cerebellum were investigated by assessing the degeneration of Purkinje cells and the cell concentration in the granular layer. No significant changes were observed in the groups of rats exposed to HF-EMWs for two or four weeks. Averaged body masses were not affected by HF-EMWs exposure. In conclusion, a 1439 MHz TDMA field did not induce observable changes in the permeability of the BBB, morphological changes in the cerebellums, or body mass changes in rats, as evaluated by the conventional methods.

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

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. 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. 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. A correction was published in a subsequent issue of the journal, stating that there was actually a significant increase in micronucleus formation in peripheral blood and bone marrow cells after chronic exposure to the radiofrequency radiation.

(Vijayalaxmi, Frei, MR, Dusch, SJ, Guel, V, Meltz, ML, Jauchem, JR, Correction of an error in calculation in the article "Frequency of micronuclei in the peripheral blood and bone marrow of cancer-prone mice chronically exposed to 2450 MHz radiofrequency radiation" (Radiat. Res. 147, 495-500, 1997). Radiat Res 149(3):308, 1998)

Vollrath L, Spessert R, Kratzsch T, Keiner M, Hollmann H. "No short-term effects of high-frequency electromagnetic fields on the mammalian pineal gland". *Bioelectromagnetics* 18:293-298, 1997.

There is ample experimental evidence that changes of earth-strength static magnetic fields, pulsed magnetic fields, or alternating electric fields (60 Hz) depress the nocturnally enhanced melatonin synthesis of the pineal gland of certain mammals. No data on the effects of high-frequency electromagnetic fields on melatonin synthesis is available. In the present study, exposure to 900 MHz electromagnetic fields [0.1 to 0.6 mW/cm², approximately 0.06 to 0.36 W/kg specific absorption rate (SAR) in rats and 0.04 W/kg in Djungarian hamsters; both continuous and/or pulsed at 217 Hz, for 15 min to 6 h] at day or night had no notable short-term effect on pineal melatonin synthesis in male and female Sprague-Dawley rats and Djungarian hamsters. Pineal synaptic ribbon profile numbers (studied in rats only) were likewise not affected. The 900 MHz electromagnetic fields, unpulsed or pulsed at 217 Hz, as applied in the present study, have no short-term effect on the mammalian pineal gland.

Vorobyov VV, Galchenko AA, Kukushkin NI, Akoev IG. "Effects of weak microwave fields amplitude modulated at ELF on EEG of symmetric brain areas in rats". *Bioelectromagnetics* 18(4):293-298, 1997.

Averaged electroencephalogram (EEG) frequency spectra were studied in eight unanesthetized and unmyorelaxed adult male rats with chronically implanted carbon electrodes in symmetrical somesthetic areas when a weak (0.1-0.2 mW/cm) microwave (MW, 945 MHz) field, amplitude-modulated at extremely low frequency (ELF) (4 Hz), was applied. Intermittent (1 min "On," 1 min "Off") field exposure (10-min duration) was used. Hemispheric asymmetry in frequency spectra (averaged data for 10 or 1 min) of an ongoing EEG was characterized by a power decrease in the 1.5-3 Hz range on the left hemisphere and by a power decrease in the 10-14 and 20-30 Hz ranges on the right hemisphere. No

differences between control and exposure experiments were shown under these routines of data averaging. Significant elevations of EEG asymmetry in 10-14 Hz range were observed during the first 20 s after four from five onsets of the MW field, when averaged spectra were obtained for every 10 s. Under neither control nor pre- and postexposure conditions was this effect observed. These results are discussed with respect to interaction of MW fields with the EEG generators.

HUMAN STUDIES

Borbely AA, Huber R, Graf T, Fuchs B, Gallmann E, Achermann P. "Pulsed high-frequency electromagnetic field affects human sleep and sleep electroencephalogram". *Neurosci Lett* 275(3):207-210, 1999.

To investigate whether the electromagnetic field (EMF) emitted by digital radiotelephone handsets affects the brain, healthy, young subjects were exposed during an entire night-time sleep episode to an intermittent radiation schedule (900 MHz; maximum specific absorption rate (SAR) 1 W/kg) consisting of alternating 15-min on-15-min off intervals. Compared with a control night with sham exposure, the amount of waking after sleep onset was reduced from 18 to 12 min. Spectral power of the electroencephalogram in non-rapid eye movement sleep was increased. The maximum rise occurred in the 10-11 Hz and 13.5-14 Hz bands during the initial part of sleep and then subsided. The results demonstrate that pulsed high-frequency EMF in the range of radiotelephones may promote sleep and modify the sleep EEG.

Braune S, Wrocklage C, Raczek J, Gailus T, Lucking CH. "Resting blood pressure increase during exposure to a radio-frequency electromagnetic field". *Lancet* 351(9119):1857-1858, 1998a.

A single-blind placebo-controlled study conducted on seven healthy men and three women volunteers aged between 26 and 36 years continuously measured blood pressure, heart rate, capillary perfusion, as well as subjective well-being in relation to mobile telephone electromagnetic fields (EMFs) (GSM 900 MHz, 2 watt, 217 Hz frame repetition rate). The study subjects were unaware of when they were being exposed because the telephone was fixed in a typical position on the right-hand side of the head and operated by remote control with no sound. The exposure protocol involved phases with placebo and with EMF exposure of 35 minutes each with a fixed sequence to allow for effects that might persist after exposure. The researchers found that even though the people subjectively noticed no differences in well-being, their resting blood pressure increased during exposure to the EMF: "We conclude that exposure of the right hemisphere to a radiofrequency EMF for 35 minutes causes an increase in sympathetic efferent activity with increases in resting blood pressure between 5 and 10 mm Hg, most likely due to more pronounced vasoconstriction."

CONCLUSION: Exposure of the right hemisphere to a radiofrequency EMF for 35 min causes in human subjects an increase in sympathetic efferent activity with increases the resting blood pressure between 5-10 mm Hg. The effect is likely caused by vasoconstriction.

Braune S, Wrocklage C Raczek J, Gailus T and Lucking C H. "Correspondence (Stuart W J Reid, George Gettinby): Radiofrequency electromagnetic field from mobile phones". *Lancet* 352:576, 1998b.

Sir-In an age when journalism is a powerful route for the dissemination of scientific findings to the lay public, it is incumbent upon the research community to be transparent and explicit in the communication of new findings and key advances. Add to this media-driven environment, the heady mix of a widespread new technology and potential health scare exemplified by the advent of mobile phones, our responsibility, as scientists, is even more evident. The report by S Braune and co-workers on mobile phones (June 20, p 1857) raises many questions that cannot be answered from the published description of their study.

Experiments on a small number of participants are prone to constraints, particularly in terms of type II errors and statistical power. One of the options available to the researcher faced with small sample sizes is to increase the number of observations per participant, and from the information published this would seem to be the course of action adopted by the investigators. Such a design requires appropriate analysis, but this detail is missing from the paper. The use of placebo and experimental groups is to be commended, but the argument set out by Braune for the use of a fixed order of placebo or electromagnetic fields exposure is flawed. Experimental designs such as randomised block or crossover trials would overcome the concerns of the research team. The results reported by Braune and colleagues as significant are reflected on the figures ($p < 0.0001$), and in order for the reader to interpret data on these types, the design, and particularly the analytical techniques, are essential pieces of information. Certainly, with small group sizes and the increased likelihood of type II errors, observed differences may well be real ones, but with a topic of such sensitivity and public interest, it is crucial that both lay readers and the researchers should be able to understand and, if necessary, repeat the experiments described. Furthermore, two types of data are reported: absolute values and differences. When the different components of the study show significance in one and not the other, the requirement for a statement of the statistical methods adopted is obvious. Our concerns may have arisen because of deficiencies in communication rather than scientific practice. However, when the findings of studies of this type appear in the popular press, our communication or journalistic skills may be as important as our scientific prowess. For the record, in our experience, mobile phones used in a public area are likely to raise the blood pressure of non-users; on occasion, so too, does reading the literature!

De Seze R, Fabbro-Peray P, Miro L. "GSM radiocellular telephones do not disturb the secretion of antepituitary hormones in humans". *Bioelectromagnetics* 19(5):271-278, 1998.

It is known that the endocrine system of experimental animals is susceptible to perturbation by radiofrequency (RF) radiation. Because of the recent interest in health and safety issues of cellular telephones, an experiment was designed to evaluate the effect of a 900 MHz RF radiation emitted by a Global System for Mobile radiotelephone (217 Hz impulses, one-eighth duty cycle, 2 W peak power) on human endocrine functions. Twenty healthy male volunteers aged from 19 to 40 were inducted in the present experiment. Each subject was exposed to RF radiation through the use of a cellular phone 2 h/day, 5 days/wk, for 1 month. Subjects were their own control. End points were serum adrenocorticotropin, thyrotropin, growth hormone, prolactin, luteinizing hormone, and follicle stimulating hormone concentrations. These end points were determined in nine weekly blood samples obtained starting 3 weeks before the commencement of the exposure and ending 2 weeks after exposures. All but one blood sample was drawn 48 h after each weekly session. The seventh drawing was performed the morning after the last weekly exposure. Within each individual, the preexposure hormone concentration was used as a control. Results indicated that all hormone concentrations remained within normal physiologic ranges. A difference was not noted among the nine weekly samples in five of six hormones studied. There was a significant change only in thyrotropin concentration, showing a 21% decrease on the seventh sampling. Because this change recovered fully during the postexposure period, it is concluded that 1 month of intermittent exposures to RF radiation from a cellular telephone does not induce a long-lasting or cumulative effect on the hormone secretion rate of the anterior pituitary gland in humans

De Seze R, Ayoub J, Peray P, Miro L, Touitou Y. “The effects of radiocellular telephones on the circadian patterns of melatonin secretion, a chronobiological rhythm marker”. *J Pineal Res* 27(4):237-242, 1999.

A decrease in melatonin secretion has been observed in small mammals under exposure to extremely low frequency electromagnetic fields. As there is some concern about possible health effects of the increasing use of radiocellular telephones emitting radiofrequency electromagnetic fields, we examined whether such fields would alter melatonin levels in the human. Volunteers were two groups totalling 38 men, 20-32 yr old. Exposures were to commercially available cellular telephones of the GSM 900 type (Global System for Mobile communication at 900 MHz) or DCS 1800 type (Digital Communication System at 1800 MHz), for 2 hr/day, 5 days/wk, for 4 wk, at their maximum power. Attention of the volunteers was sustained by TV projection of movies. Blood samples were collected hourly during the night and every 3 hr in the daytime. Four sampling sessions were performed at 15-day intervals: before the beginning of the exposure period, at the middle and the end of the exposure period, and 15 days later to evaluate the persistence or late appearance of potential effects. Evaluated parameters were the maximum serum concentration, the time of this maximum, and the area under the curve of the hormone profile. Melatonin circadian profile was not disrupted in 37 young male volunteers submitted to a typical pattern of exposure to the electromagnetic fields generated by two common types of cell phones.

Eulitz C, Ullsperger P, Freude G, Elbert T. “Mobile phones modulate response patterns of human brain activity”. *Neuroreport* 9(14):3229-3232, 1998.

Mobile phones emit a pulsed high-frequency electromagnetic field (PEMF) which may penetrate the scalp and the skull. Increasingly, there is an interest in the interaction of this pulsed microwave radiation with the human brain. Our investigations show that these electromagnetic fields alter distinct aspects of the brain's electrical response to acoustic stimuli. More precisely, our results demonstrate that aspects of the induced but not the evoked brain activity during PEMF exposure can be different from those not influenced by PEMF radiation. This effect appears in higher frequency bands when subjects process task-relevant target stimuli but was not present for irrelevant standard stimuli. As the induced brain activity in higher frequency bands has been proposed to be a correlate of coherent high-frequency neuronal activity, PEMF exposure may provide means to systematically alter the pattern fluctuations in neural mass activity.

Freude G, Ullsperger P, Eggert S, Ruppe I. “Effects of microwaves emitted by cellular phones on human slow brain potentials”. *Bioelectromagnetics* 19(6):384-387, 1998.

The influence of electromagnetic fields (EMF) emitted by cellular phones on preparatory slow brain potentials (SP) was studied in two different experimental tasks: In the first, healthy male human subjects had to perform simple self-paced finger movements to elicit a Bereitschaftspotential; in the second, they performed a complex and cognitive demanding visual monitoring task (VMT). Both tasks were performed with and without EMF exposure in counterbalanced order. Whereas subjects' performance did not differ between the EMF exposure conditions, SP parameters were influenced by EMF in the VMT: EMF exposure effected a significant decrease of SPs at central and temporo-parieto-occipital brain regions, but not at the frontal one. In the simple finger movement task, EMF did not affect the Bereitschaftspotential.

Freude G, Ullsperger P, Eggert S, Ruppe I. “Microwaves emitted by cellular telephones affect human slow brain potentials”. *Eur J Appl Physiol* 81(1-2):18-27, 2000.

The influence of electromagnetic fields (EMF) emitted by cellular telephones on preparatory slow brain potentials (SP) was studied in two experiments, about 6 months apart. In the first experiment, a significant decrease of SP was found during exposure to EMF in a complex visual monitoring task (VMT). This effect was replicated in the second experiment. In addition to the VMT, EMF effects on SP were analysed in two further, less demanding tasks: in a simple finger movement task to elicit a Bereitschaftspotential (BP) and in a two-stimulus task to elicit a contingent negative variation (CNV). In comparison to the VMT, no significant main EMF effects were found in BP and CNV tasks. The results accounted for a selective EMF effect on particular aspects of human information processing, but did not indicate any influence on human performance, well-being and health.

Hladky A, Musil J, Roth Z, Urban P, Blazkova V. “Acute effects of using a mobile phone on CNS functions”. *Cent Eur J Public Health* 7(4):165-167. 1999.

Twenty volunteers participated in two experiments exploring the acute effects of using the mobile phone Motorola GSM 8700 on the functions of the CNS. When speaking (5 minutes reading a text from daily newspapers) the electromagnetic fields from the mobile apparatus did not affect the visual evoked potentials. Also a 6-min exposure did not reveal any effect of electromagnetic fields on the results in two tests (memory and attention) performed while speaking into the mobile. On the other hand the phone call itself strongly influenced the performance in a secondary task applying a test of switching attention which is a good model for driving a car. The response and decision speed were significantly worse. This is a proof that even a slight psychological stress involved in calling while driving can be a great risk.

Kellenyi L, Thuroczy G, Faludy B, Lenard L. “Effects of mobile GSM radiotelephone exposure on the auditory brainstem response (ABR)”. *Neurobiology* 7:79-81, 1999.

A 15-min exposure to GSM phone radiation caused an increase in auditory brainstem response in the exposed side of human subjects. Subjects also showed a hearing deficiency in the high frequency range (20 dB hearing deficiency from 2 KHz to 10 KHz).

Koivisto M, Revonsuo A, Krause C, Haarala C, Sillanmaki L, Laine M, Hamalainen H. “Effects of 902 MHz electromagnetic field emitted by cellular telephones on response times in humans”. *Neuroreport* 11(2):413-415, 2000.

The present study examined possible influences of a 902 MHz electromagnetic field emitted by cellular telephones on cognitive functioning in 48 healthy humans. A battery of 12 reaction time tasks was performed twice by each participant in a counterbalanced order: once with and once without the exposure to the field. The results showed that the exposure to the electromagnetic field speeded up response times in simple reaction time and vigilance tasks and that the cognitive time needed in a mental arithmetics task was decreased. The results suggest that exposure to the electromagnetic field emitted by cellular telephones may have a facilitatory effect on brain functioning, especially in tasks requiring attention and manipulation of information in working memory.

Krause CM, Sillanmaki L, Koivisto M, Haggqvist A, Saarela C, Revonsuo A, Laine M, Hamalainen H. “Effects of electromagnetic field emitted by cellular phones on the EEG during a memory task”. *Neuroreport* 11(4):761-764, 2000.

The effects of electromagnetic fields (EMF) emitted by cellular phones on the ERD/ERS of the 4-6 Hz, 6-8 Hz, 8-10 Hz and 10-12 Hz EEG frequency bands were studied in 16 normal subjects performing an auditory memory task. All subjects performed the memory task both with and without exposure to a digital 902 MHz EMF in counterbalanced order. The exposure to EMF significantly increased EEG power in the 8-10 Hz frequency band only. Nonetheless, the presence of EMF altered the ERD/ERS responses in all studied frequency bands as a function of time and memory task (encoding vs retrieval). Our results suggest that the exposure to EMF does not alter the resting EEG per se but modifies the brain responses significantly during a memory task.

Mann K, Roschke J. “Effects of pulsed high-frequency electromagnetic fields on human sleep”. *Neuropsychobiology* 33(1):41-47, 1996.

In the present study we investigated the influence of pulsed high-frequency electromagnetic fields of digital mobile radio telephones on sleep in healthy humans. Besides a hypnotic effect with shortening of sleep onset latency, a REM suppressive effect with reduction of duration and percentage of REM sleep was found. Moreover, spectral analysis revealed qualitative alterations of the EEG signal during REM sleep with an increased spectral power density. Knowing the relevance of REM sleep for adequate information processing in the brain, especially concerning mnemonic functions and learning processes, the results emphasize the necessity to carry out further investigations on the interaction of this type of electromagnetic fields and the human organism.

Mann K, Roschke J, Connemann B, Beta H. “No effects of pulsed high-frequency electromagnetic fields on heart rate variability during human sleep”. *Neuropsychobiology* 38(4):251-256, 1998.

The influence of pulsed high-frequency electromagnetic fields emitted by digital mobile radio telephones on heart rate during sleep in healthy humans was investigated. Beside mean RR interval and total variability of RR intervals based on calculation of the standard deviation, heart rate variability was assessed in the frequency domain by spectral power analysis providing information about the balance between the two branches of the autonomic nervous system. For most parameters, significant differences between different sleep stages were found. In particular, slow-wave sleep was characterized by a low ratio of low- and high-frequency components, indicating a predominance of the parasympathetic over the sympathetic tone. In contrast, during REM sleep the autonomic balance was shifted in favor of the sympathetic activity. For all heart rate parameters, no significant effects were detected under exposure to the field compared to placebo condition. Thus, under the given experimental conditions, autonomic control of heart rate was not affected by weak-pulsed high-frequency electromagnetic fields.

Mann K, Wagner P, Brunn G, Hassan F, Hiemke C, Roschke J. “Effects of pulsed high-frequency electromagnetic fields on the neuroendocrine system”. *Neuroendocrinology* 67(2):139-144, 1998.

The influence of pulsed high-frequency electromagnetic fields emitted from a circularly polarized antenna on the neuroendocrine system in healthy humans was investigated (900

MHz electromagnetic field, pulsed with 217 Hz, average power density 0.02 mW/cm²). Nocturnal hormone profiles of growth hormone (GH), cortisol, luteinizing hormone (LH) and melatonin were determined under polysomnographic control. An alteration in the hypothalamo-pituitary-adrenal axis activity was found with a slight, transient elevation in the cortisol serum level immediately after onset of field exposure which persisted for 1 h. For GH, LH and melatonin, no significant effects were found under exposure to the field compared to the placebo condition, regarding both total hormone production during the entire night and dynamic characteristics of the secretion pattern. Also the evaluation of the sleep EEG data revealed no significant alterations under field exposure, although there was a trend to an REM suppressive effect. The results indicate that weak high-frequency electromagnetic fields have no effects on nocturnal hormone secretion except for a slight elevation in cortisol production which is transient, pointing to an adaptation of the organism to the stimulus.

Preece AW, Iwi G, Davies-Smith A, Wesnes K, Butler S, Lim E, Varey A. “Effect of a 915-MHz simulated mobile phone signal on cognitive function in man”. *Int J Radiat Biol* 75(4):447-456, 1999.

PURPOSE: To examine whether a simulated mobile telephone transmission at 915 MHz has an effect on cognitive function in man.

MATERIALS AND METHODS: Thirty-six subjects in two groups were each given two training sessions and then three test sessions in a randomized three-way cross-over design. About 1 W mean power at 915 MHz from a quarter-wave antenna mounted on a physical copy of an analogue phone, as a sine wave, or modulated at 217 Hz with 12.5 percent duty cycle, or no power, was applied to the left squamous temple region of the subjects while they undertook a series of cognitive function tests lasting approximately 25-30 min. The second group was investigated for sleep, consumption of alcohol and beverages, and any other substances that might affect performance.

RESULTS: In both groups, the only test affected was the choice reaction time and this showed as an increase in speed (a decrease in reaction time). There were no changes in word, number or picture recall, or in spatial memory. While an effect of visit-order was evident suggesting a learning effect of repeat tests, the design of the study allowed for this. Additionally, there was no systematic error introduced as a result of consumption of substances or sleep time.

CONCLUSIONS: There was evidence of an increase in responsiveness, strongly in the analogue and less in the digital simulation, in choice reaction time. This could be associated with an effect on the angular gyrus that acts as an interface between the visual and speech centres and which lies directly under and on the same side as the antenna. Such an effect could be consistent with mild localized heating, or possibly a non-thermal response, which is nevertheless power-dependent.

Szmigielski S, Bortkiewicz A, Gadzicka E, Zmyslony M, Kubacki R. “Alteration of diurnal rhythms of blood pressure and heart rate to workers exposed to radiofrequency electromagnetic fields”. *Blood Press Monit* 3(6):323-330, 1998.

BACKGROUND: In previous studies we found measurable effects on variability of heart rate and on blood-pressure parameters of workers exposed to radiofrequency electromagnetic fields (EMF) compared with a control population, but none of the effects could be assigned clinical significance. In general, the obtained results strongly suggested that deregulation of the autonomic control of the circulatory system was occurring. Therefore, it seemed logical

that analysis of diurnal rhythms of blood pressure and heart rate, on the basis of data from 24 h recordings, might further support the above hypothesis.

OBJECTIVE: The aim of this study was to determine the course of diurnal rhythms of blood pressure and heart rate in a group of workers exposed to various intensities of radiofrequency electromagnetic fields.

METHODS: In the study we used 61 healthy workers (aged 30-50 years) who had been exposed to radiofrequency EMF of 0.738-1.503 Mhz and 42 healthy workers at radio-line stations (aged 28-49 years), who had not been exposed to EMF occupationally. The work patterns of these two groups were identical (12 h day working shift, 24 h interval, 12 h night shift and then 48 h rest). During the second day of the rest period 24 h ambulatory blood pressure (ABP) was recorded. For analysis of diurnal rhythms the group of exposed workers was divided into two subgroups: group A of 38 subjects exposed to low intensities of radiofrequency EMF (20-180 V/m) and group B of 23 subjects exposed to high intensities of radiofrequency EMF (200-550 V/m). Parameters of diurnal rhythms of blood pressure and heart rate (acrophase, amplitude and mean) were calculated by performing a least-square fit of a 24 h cosinor (single cosinor analysis) at $P < 0.05$.

RESULTS: Healthy men aged 28-49 years, working on a pattern of 12-24-12-48 h, exhibited typical, well-preserved diurnal rhythms of blood pressure and heart rate with two maxima (at about 1400 and 1700-1800 h) and one minimum (at about 0200-0400 h). For workers exposed to radiofrequency EMF we noted a significant lowering of the amplitudes of rhythms of blood pressure and heart rate ($P < 0.01$) and a shift of the acrophase to an earlier time (1100-1200 h; $P < 0.05$). These changes were more pronounced among workers exposed to high intensities of radiofrequency EMF.

CONCLUSIONS: Occupational exposure to radiofrequency EMF can result in changes of the diurnal rhythms of blood pressure and heart rate with lowering of their amplitudes and a shift of the acrophase. The clinical relevance of the present finding needs to be investigated in further studies.

Urban P, Lukas E, Roth Z. "Does acute exposure to the electromagnetic field emitted by a mobile phone influence visual evoked potentials?". *Cent Eur J Public Health* 6(4):288-290, 1998.

To search for a potential negative influence on the central nervous system (CNS) of the electromagnetic field emitted by a mobile phone, the authors performed a pilot experimental study of the influence of a single short acute exposure to the GSM mobile phone Motorola 8700, using visual evoked potentials (VEP) examination as an electrophysiological marker of CNS dysfunction. The study group consisted of 20 healthy volunteers. The duration of exposure was 5 minutes. The output power of the device was 1.5 W when the antenna was pulled up. Five parameters of VEP were evaluated by means of multifactorial ANOVA. Confounding effects of age, sex, and of the call in itself were taken into consideration. No statistically significant influence of the above-described exposure to the electromagnetic field emitted by the mobile phone on latencies or amplitudes of VEP was observed.

Wagner P, Roschke J, Mann K, Hiller W, Frank C. "Human sleep under the influence of pulsed radiofrequency electromagnetic fields: a polysomnographic study using standardized conditions". *Bioelectromagnetics* 19(3):199-202, 1998.

To investigate the influence of radiofrequency electromagnetic fields (EMFs) of cellular phone GSM signals on human sleep electroencephalographic (EEG) pattern, all-night polysomnographies of 24 healthy male subjects were recorded, both with and without

exposure to a circular polarized EMF (900 MHz, pulsed with a frequency of 217 Hz, pulse width 577 micros, power flux density 0.2 W/m^2). Suppression of rapid eye movement (REM) sleep as well as a sleep-inducing effect under field exposure did not reach statistical significance, so that previous results indicating alterations of these sleep parameters could not be replicated. Spectral power analysis also did not reveal any alterations of the EEG rhythms during EMF exposure. The failure to confirm our previous results might be due to dose-dependent effects of the EMF on the human sleep profile.

EPIDEMIOLOGICAL STUDIES

Hansson Mild K, Oftedal G, Sandström M, Wilén J, Tynes T, Haugsdal B and Hauger E. "Comparison of symptoms experienced by users of analogue and digital mobile phones. A Swedish–Norwegian epidemiological study". *Arbetslivsrapport* 1998:23. Solna, Sweden, Arbetslivsinstitutet, 1998.

Questionnaires were used to register exposure factors, symptoms (both in general and symptoms related to the use of the mobile phone), and possible confounding factors such as gender, age, VDT work and psychosocial factors for which the calculated odds ratios were adjusted. Symptoms versus exposure factors Our main hypothesis was that the users of GSM phones experienced more symptoms than NMT users. Symptoms experienced in connection with mobile phone calls In Sweden 13% and in Norway 30% of the respondents had experienced at least one symptom in connection with MP calls. In addition to asking for symptoms experienced in connection with MP use, we asked whether symptoms occurred or were aggravated in connection with the use of ordinary phone and VDT. More people attributed their symptoms to MP use than to the use of ordinary phone. In fact, GSM users reported warmth sensation on the ear and behind or around the ear less frequently than NMT users. A: Norway OR (95% C.I.) NMT GSM Symptoms 2-15 min/d 15-60 min/d > 60 min/d 2-15 min/d 15-60 min/d > 60 min/d In bold $p < 0.05$ 7 B: Sweden OR (95% C.I.) NMT GSM Symptoms 2-15 min/d 15-60 min/d > 60 min/d 2-15 min/d 15-60 min/d > 60 min/d

Hardell L, Nasman A, Pahlson A, Hallquist A, Mild KH. "Use of cellular telephones and the risk for brain tumours: A case-control study". *Int J Oncol* 15(1):113-116, 1999.

The use of cellular telephones has increased dramatically during the 1990's in the world. In the 1980's the analogue NMT system was used whereas the digital GSM system was introduced in early 1990's and is now the preferred system. Case reports of brain tumours in users initiated this case-control study on brain tumours and use of cellular telephones. Also other exposures were assessed. All cases, both males and females, with histopathologically verified brain tumour living in Uppsala-Orebro region (1994-96) and Stockholm region (1995-96) aged 20-80 at the time of diagnosis and alive at start of the study were included, 233 in total. Two controls to each case were selected from the Swedish Population Register matched for sex, age and study region. Exposure was assessed by questionnaires supplemented over the phone. The analyses were based on answers from 209 (90%) cases and 425 (91%) controls. Use of cellular telephone gave odds ratio (OR) = 0.98 with 95% confidence interval (CI) = 0.69-1.41. For the digital GSM system OR = 0.97, CI = 0.61-1.56 and for the analogue NMT system OR = 0.94, CI = 0.62-1.44 were calculated. Dose-response analysis and using different tumour induction periods gave similar results. Non-significantly increased risk was found for tumour in the temporal or occipital lobe on the same side as a cellular phone had been used, right side OR = 2.45, CI = 0.78-7.76, left side OR = 2.40, CI = 0.52-10.9 Increased risk was found only for use of the NMT system. For GSM use the observation time is still too short for definite conclusions. An increased risk for brain tumour in the anatomical area close to the use of a cellular telephone should be especially studied in the future.

Hardell L, Nasman A, Pahlson A, Hallquist A. "Case-control study on radiology work, medical X-ray investigations, and use of cellular telephones as risk factors for brain tumors". *Medscape General Medicine* May 4:19, 2000.

Context. Ionizing radiation is a well-established risk factor for brain tumors. During recent years, microwave exposure from the use of cellular telephones has been discussed as a potential risk factor.

Objective. To determine risk factors for brain tumors.

Design. A case-control study, with exposure assessed by questionnaires.

Participants. A total of 233 currently living men and women, aged 20 to 80 years, were included. The case patients had histopathologically verified brain tumors and lived in the Uppsala-Orebro region (1994-1996) or the Stockholm region (1995-1996). Two matched controls to each case were selected from the Swedish Population Register.

Main Outcome Measures. Ionizing radiation and use of cellular telephones as risk factors for brain tumors.

Results. A total of 209 cases (90%) and 425 controls (91%) answered the questionnaire.

- Work as a physician yielded an odds ratio (OR) of 6.00, with a 95% confidence interval (CI) of 0.62 to 57.7. All three case patients had worked with fluoroscopy.
- Radiotherapy of the head and neck region yielded an OR of 3.61 (95% CI, 0.65-19.9).
- Medical diagnostic x-ray examination of the same area yielded an OR of 2.10 (95% CI, 1.25-3.53), with a tumor induction period of 5 years or more.
- Chemical industry work yielded an OR of 4.10 (95% CI, 1.25-13.4), and laboratory work yielded an OR of 3.21 (95% CI, 1.16-8.85).
- Ipsilateral use of cellular telephones increased the risk for tumors in the temporal, temporoparietal, and occipital lobes (OR, 2.42; 95% CI, 0.97-6.05), ie, the anatomic areas with highest exposure to microwaves from a mobile telephone.

The result was further strengthened (OR, 2.62; 95% CI, 1.02-6.71) in a multivariate analysis that included laboratory work and medical diagnostic x-ray investigations of the head and neck.

Conclusion. Exposure to ionizing radiation, work in laboratories, and work in the chemical industry increased the risk of brain tumors. Use of a cellular telephone was associated with an increased risk in the anatomic area with highest exposure.

Hocking B. "Preliminary report: symptoms associated with mobile phone use". *Occup Med (Lond)* 48(6):357-360, 1998.

Mobile phone use is ubiquitous, although the alleged health effects of low level radio-frequency radiation (RFR) used in transmission are contentious. Following isolated reports of headache-like symptoms arising in some users, a survey has been conducted to characterize the symptoms sometimes associated with mobile phone usage. A notice of interest in cases was placed in a major medical journal and this was publicized by the media. Respondents were interviewed by telephone using a structured questionnaire. Forty respondents from diverse occupations described unpleasant sensations such as a burning feeling or a dull ache mainly occurring in the temporal, occipital or auricular areas. The symptoms often began minutes after beginning a call, but could come on later during the day. The symptoms usually ceased within an hour after the call, but could last until evening. Symptoms did not occur when using an ordinary handset, and were different from ordinary headaches. There were several reports suggestive of intra-cranial effects. Three respondents reported local symptoms associated with wearing their mobile phone on their belts. There was one cluster of cases in a workplace. Seventy-five per cent of cases were associated with digital mobile phones. Most

of the respondents obtained relief by altering their patterns of telephone usage or type of phone. Cranial and other diverse symptoms may arise associated with mobile phone usage. Physicians and users alike should be alert to this. Further work is needed to determine the range of effects, their mechanism and the possible implications for safety limits of RFR.

Kolmodin-Hedman B, Hansson Mild K, Hagberg M, Jonsson E, Andersson MC, Eriksson A. "Health problems among operators of plastic welding machines and exposure to radiofrequency electromagnetic fields." *Int Arch Occup Environ Health* 60(4): 243-7, 1988.

To study possible medical effects of high radiofrequency radiation (RF), 113 Swedish men and women were studied by means of a structured interview and rating of subjective symptoms. A test session was included in order to examine coordination and muscular function of the hands. A neurological test concerning two-point discrimination (2-PD) was also done. As referents, 23 women, sewing machine operators and assembly workers, were chosen, interviewed and likewise tested. Exposure measurements were taken of the RF fields around the welding machines. The present Swedish ceiling value of 250 W/m² for the equivalent power density was exceeded in more than 50% of the machines. The highest leakage fields, both for electric and magnetic fields, were found near machines used in factories for ready-made clothing, which gave a high exposure to the hands. Irritative eye symptoms were reported by 23% of the men and 40% of the women. A group of 27 persons was selected for a clinical eye examination and checked by photographs, and nine persons had modest conjunctivitis. A high prevalence of numbness in hands, especially among women, was found. A significantly impaired 2-PD was found in the exposed women as compared to the referent group. The pregnancy outcome for 305 female plastic welders during 1974-1984 did not show any significant differences with the Swedish average concerning malformation or prenatal mortality.

Lagorio S, Rossi P. "Mortality of plastic-ware workers exposed to radiofrequencies." *Bioelectromagnetics* 18(6): 418-21, 1997.

The mortality experience of a cohort of Italian plastic-ware workers exposed to radiofrequency (RF)-electromagnetic fields generated by dielectric heat sealers was investigated. Follow-up extended from 1962 to 1992. The standardised mortality ratio (SMR) analysis was restricted to 481 women workers, representing 78% of the total person-years at risk. Mortality from malignant neoplasms was slightly elevated, and increased risks of leukemia and accidents were detected. The all-cancer SMR was higher among women employed in the sealing department, where exposure to RF occurred, than in the whole cohort. This study raises interest in a possible association between exposure to RF radiation and cancer risk. However, the study power was very small, and the possible confounding effects of exposure to solvents and vinyl chloride monomer (VCM) could not be ruled out. The hypothesis of an increased risk of cancer after radiofrequency exposure should be further explored by means of analytical studies characterised by adequate power and more accurate exposure assessment.

Reeves G I. "Review of extensive workups of 34 patients overexposed to radiofrequency radiation." *Aviat Space Environ Med* 71(3): 206-15, 2000.

BACKGROUND: The medical records of 34 patients seen at the Aerospace Medicine Directorate, U.S. Air Force Research Laboratory for confirmed exposure to radiofrequency

radiation (RFR) exceeding the permitted exposure limits were reviewed to see if RFR overexposure created any detectable clinical or laboratory alterations that could be correlated with power density or the product of power density and time exposed. The goal of this study was to determine which physiological and laboratory parameters required closest attention on work up of future patients with RFR exposure.

METHODS: All 34 patients received an extensive history and physical examination, and a large battery of laboratory studies. Clinical findings were also compared with laboratory results.

RESULTS: A sensation of warmth was positively associated with power density. A negative correlation was observed between an abnormal tissue destruction screen and power density. Sophisticated neurological tests in 23 patients and extensive psychometric and psychological exams in 30 patients revealed no neurological or ophthalmologic findings attributable to RFR. A few patients reported burning pain that resolved over several weeks; neurological findings were minimal or absent. **CONCLUSIONS:** Patients with suspected RFR overexposures need to be seen promptly at the nearest medical facility. Based on this study, an extensive evaluation of persons overexposed to non-ionizing radiation should not be routinely performed. However, a careful history and physical examination with laboratory studies as indicated should be performed and the patient's concerns about RFR effects addressed fully.

INTERACTION OF THERMAL LEVELS OF RF WITH OTHER AGENTS

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

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

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

Radiofrequency (RF) radiation is used in a variety of workplaces. In addition to RF radiation, many workers are concurrently exposed to numerous chemicals; exposed workers include those involved with the microelectronics industry, plastic sealers, and electrosurgical units. The developmental toxicity of RF radiation is associated with the degree and duration of hyperthermia induced by the exposure. Previous animal research indicates that hyperthermia induced by an elevation in ambient temperature can potentiate the toxicity and teratogenicity of some chemical agents. We previously demonstrated that combined exposure to RF radiation (10 MHz) and the industrial solvent, 2-methoxyethanol (2ME), produces enhanced teratogenicity in rats. The purpose of the present research is to determine the effects of varying the degree and duration of hyperthermia induced by RF radiation (sufficient to maintain colonic temperatures at control [38.5], 39.0, 40.0, or 41.0 degrees C for up to 6 h) and 2ME (100 mg/kg) administered on gestation day 13 of rats. Focusing on characterizing the dose-response pattern of interactions, this research seeks to determine the lowest interactive effect level. Day 20 fetuses were examined for external and skeletal malformations. The results are consistent with previous observations. Significant interactions were observed between 2ME and RF radiation sufficient to maintain colonic

temperatures at 41 degrees C for 1 h, but no consistent interactions were seen at lower temperatures even with longer durations. These data indicate that combined exposure effects should be considered when developing both RF radiation and chemical exposure guidelines and intervention strategies.

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

OBJECTIVE: This research was conducted to determine if altered environmental temperatures would affect the interactive developmental toxicity of radiofrequency (RF) radiation and the industrial solvent, 2-methoxyethanol (2ME). This is important because RF radiation is used in a variety of workplaces that have poorly controlled environmental temperatures, and many workers are concurrently exposed to various chemicals. Furthermore, we have previously demonstrated that combined exposure to RF radiation (10 MHz) and 2ME produces enhanced teratogenicity in rats.

METHODS: RF radiation sufficient to maintain colonic temperatures at the control value (38degrees), 39.0 degrees or 40.0 degrees C for 2 or 4 h combined with either 0 or 100 mg/kg 2ME at environmental temperatures of 18 degrees , 24 degrees and 30 degrees C (65 degrees , 75 degrees , and 85 degrees F) were given on gestation day 13 to Sprague-Dawley rats. Dams were killed on gestation day 20, and the fetuses were examined for external malformations.

RESULTS AND CONCLUSIONS: Environmental temperature does affect the specific absorption rate (SAR) necessary to maintain a specific colonic temperature but does not affect the interactive developmental toxicity of RF radiation and 2ME in rats. These results, consistent with the literature, add to the evidence that the developmental toxicity of RF radiation (combined or alone) is associated with colonic temperature, not with SAR.

Nelson BK, Snyder DL, Shaw PB. “Developmental toxicity interactions of salicylic acid and radiofrequency radiation or 2-methoxyethanol in rats”. *Reprod Toxicol* 13(2):137-145, 1999.

Radiofrequency (RF) radiation is used in a variety of workplaces where workers are concurrently exposed to chemicals. Combined exposure to RF radiation (10 MHz) and the industrial solvent, 2-methoxyethanol (2ME), produces enhanced teratogenicity in rats. The purpose of the present research was to determine if the synergistic effects noted for RF radiation and 2ME are generalizable to other chemicals. Since salicylic acid (SA) is widely used as an analgesic and is teratogenic in animals, SA was selected to address generalizability. Based on the literature and our pilot studies, 0, 250, or 350 mg/kg SA were administered by gavage on gestation Day 9 or 13 to rats. Concurrently rats given SA on Day 9 were exposed to RF radiation sufficient to maintain colonic temperature at 41 degrees C for 60 min (or sham). Those given SA on Day 13 were also given 0 or 100 mg/kg 2ME (gavage). Dams were sacrificed on gestation Day 20, and the fetuses were examined for external malformations. The data provide no evidence of synergistic interactions between RF radiation and salicylic acid (resorptions and malformations). Limited evidence of antagonism was observed between 2ME and salicylic acid (fetal weights). This investigation highlights the importance of additional research on interactions in developmental toxicology, and emphasizes the need to consider combined exposure effects when developing both physical agent and chemical agent exposure guidelines and intervention strategies.

DOSIMETRY

Chou CK, Chan KW, McDougall JA, Guy AW. “Development of rat head exposure system for simulating human exposure to RF fields from wireless telephone telephones”. *Bioelectromagnetics* 20(suppl 4):75-92, 1999.

The aim of this project was to develop an animal exposure system for the biological effect studies of radio frequency fields from handheld wireless telephones, with energy deposition in animal brains comparable to those in humans. The finite-difference time-domain (FDTD) method was initially used to compute specific absorption rate (SAR) in an ellipsoidal rat model exposed with various size loop antennas at different distances from the model. A 3×1 cm rectangular loop produced acceptable SAR patterns. A numerical rat model based on CT images was developed by curve-fitting Hounsfield Units of CT image pixels to tissue dielectric properties and densities. To design a loop for operating at high power levels, energy coupling and impedance matching were optimized using capacitively coupled feed lines embedded in a Teflon rod. Sprague Dawley rats were exposed with the 3×1 cm loop antennas, tuned to 837 or 1957 MHz for thermographically determined SAR distributions. Point SARs in brains of restrained rats were also determined thermometrically using fiberoptic probes. Calculated and measured SAR patterns and results from the various exposure configurations are in general agreement. The FDTD computed average brain SAR and ratio of head to whole body absorption were 23.8 W/kg/W and 62% at 837 MHz, and 22.6 W/kg/W and 89% at 1957 MHz. The average brain to whole body SAR ratio was 20 to 1 for both frequencies. At 837 MHz, the maximum measured SAR in the restrained rat brains was 51 W/kg/W in the cerebellum and 40 W/kg/W at the top of the cerebrum. An exposure system operating at 837 MHz is ready for in vivo biological effect studies of radio frequency fields from portable cellular telephones. Two-tenths of a watt input power to the loop antenna will produce 10 W/kg maximum SAR, and an estimated 4.8 W/kg average brain SAR in a 300 g medium size rat.

Guy AW, Chou CK, McDougall JA. “A quarter century of in vitro research: a new look at exposure methods”. *Bioelectromagnetics* 20(Suppl 4):21-39, 1999.

The specific absorption rate (SAR) distributions in radio frequency-exposed solutions containing suspended or plated cells in vessels used for in vitro research were calculated by the finite-difference-time-domain method, graphed in color, and statistically analyzed in terms of uniformity for application to research on safety of wireless devices. The uniformity of SAR was quantified by visual inspection of colored plots, histograms, means, standard deviations, and maximums for the cell suspensions exposed in test tubes, Petri dishes, and rectangular flasks. Exposure sources included plane waves, transverse electromagnetic (TEM) cells, and striplines used at frequencies of 837, 2450, or 3,000 MHz. The results demonstrated that the most nonuniform SARs for plated or suspended cells in solution occurred for exposures of test tubes and rectangular flasks with plane waves, polarized for maximal absorption. The most uniform SARs for a layer of cells occurred for exposure of Petri dishes oriented for weakest coupling to the fields in a TEM cell. Additional improvement in uniformity was found to be possible by restricting the edge of the layer of cells from being too near the edges of the dish. It was not possible to achieve satisfactory uniformity in the SAR in cell suspensions exposed in standard vessels to any of the sources. The best but not satisfactory SAR uniformity was observed for cells suspended in the lowest 1-ml volume of the liquid contained in a test tube exposed at the bottom in a TEM cell. Experimental measurements of average SAR by temperature change for this case varied from

18% higher to 26% lower than finite difference time domain-derived values. The most uniform SAR distribution for cell suspensions in nonstandard containers was found for a rectangular slab configuration exposed in a stripline with the plates separated from the media by a thin layer of insulation. It is possible to experimentally implement this model by placing a fluid-filled thin-wall rectangular container tightly between the plates of a stripline.

Rowley J, Anderson V. "A critical review of resonant cavities for experimental in vitro exposure to radiofrequency fields". *Proc. Inaugural Conference IEEE EMBS (Vic) Melbourne, Australia, February 1998:150-153, 1998.*

In this paper the use of a resonant cavity for radiofrequency (RF) exposure of biological materials is reviewed. In particular the paper draws on a detailed examination of a 1.1m × 1.1m × 1.1m cubic resonant exposure facility that has been used in a number of scientific studies reporting in vitro effects following RF exposures. We conclude that this type of exposure system has a number of inherent deficiencies that make it unsuitable for its intended application. The exposure field inside the cavity is highly non-uniform and extremely difficult to determine experimentally or theoretically without making gross simplifications. The field structure is sensitive to small perturbations of geometry and frequency with consequent effects on repeatability. Potential confounders such as solvent outgassing from support structures inside the chamber and lack of temperature control have also been identified.

REVIEWS

International Commission of Non-Ionising Radiation Protection. “Guidelines for limiting exposure to time varying electric, magnetic, and electromagnetic fields (up to 300 GHz)”. *Health Physics* 74:494-522, 1998.

(www.icnirp.de/)

Independent Expert Group on Mobile Phones. “Mobile phones and health”. NRPB, Didcot, UK, 2000.

(www.iegmp.org.uk)

Jauchem JR. “Health effects of microwave exposures: a review of the recent (1995-1998) literature”. *J Microw Power Electromagn Energy* 33(4):263-74, 1998.

Occupational or residential exposures to radio frequency radiation, including microwaves, have been alleged to result in health problems, including leukemia, other cancers, and reproductive mishaps. This review covers the recent literature (from 1995 to 1998) dealing with possible health effects, including original studies, reviews, commentaries, and editorials. A number of these articles have presented misconceptions, errors in citation, and inaccuracies regarding the alleged effects. Epidemiological reviews and studies dealing with exposures to radio frequency radiation from television transmitters, cellular telephones and towers, magnetic resonance imaging, phased array radar systems, and other occupational exposures are analyzed. On the basis of studies reported in the past several years, one can conclude that the evidence for any proven health effects of low-level microwave exposure is minimal to non-existent.

Royal Society of Canada Expert Panel Report. “A review of the potential risks of radiofrequency fields from wireless telecommunication devices”. Royal Society of Canada, Ottawa, RSC.EPR 99-1, 1999.

(www.rsc.ca/english/RFreport.pdf)

Verschaeve L and Maes A. “Genetic, carcinogenic and teratogenic effects of radiofrequency fields.” *Mutat Res* 410(2): 141-65, 1998.

This paper reviews the literature data on the genetic toxicology of radiofrequency (RF) radiation. Whereas in the past most studies were devoted to microwave ovens and radar equipment, it is now mobile telecommunication that attracts most attention. Therefore we focus on mobile telephone frequencies where possible. According to a great majority of the papers, radiofrequency fields, and mobile telephone frequencies in particular, are not genotoxic: they do not induce genetic effects in vitro and in vivo, at least under non-thermal exposure conditions, and do not seem to be teratogenic or to induce cancer. Yet, some investigations gave rather alarming results that should be confirmed and completed by further experiments. Among them the investigation of synergistic effects and of possible mechanisms of action should be emphasised.

(*Ken Karipidis, November 2000*)