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PLENARY SESSION I
TRANSCRANIAL MAGNETIC STIMULATION (TMS)
Chairs: Dr. Shoogo Ueno and Dr. Frank Prato

PRINCIPLES AND APPLICATIONS OF TRANSCRANIAL MAGNETIC STIMULATION. Shoogo Ueno. Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan.

INTRODUCTION: Transcranial magnetic stimulation (TMS) has become an important tool for the study of the functional organization of the human brain as well as for the potential treatment of central nervous system diseases such as depression. The principles of localized magnetic stimulation, field calculations and functional brain mappings obtained by TMS, nerve excitation models, and applications for cognitive neuroscience are reviewed. In addition, the effects of repetitive TMS (rTMS) on the protection or recovery of injured hippocampal neurons in rats are discussed.

Localized Magnetic Stimulation: TMS is a technique to stimulate the brain by magnetically induced eddy currents through a coil positioned on the surface of the head [Barker et al., 1985]. Mapping studies are carried out by a method of localized and vectorial magnetic stimulation using a figure eight coil [Ueno et al., 1988]. The basic principle is to concentrate induced eddy currents locally near a target by a pair of opposing pulsed magnetic fields produced by a figure-eight coil. When a transient current flows through a coil exterior to the head, time-varying magnetic fields are generated in the brain. The time-varying magnetic fields induce eddy currents that stimulate nerve fibers. When a single coil is used, the induced eddy currents flow in a concentrated manner. The maximum current density is attained beneath the coil and, in principle, the current density is zero at the center. When a figure-eight coil is used, flow patterns of induced currents create two vortices that merge at the point beneath the intersection of the figure-eight. A computer simulation shows that the current density at the merging point is three times greater than that in the surrounding areas. Hence, localized stimulation is attained. For TMS of the human brain, transient magnetic fields in the order of 1 T and a duration of 0.1-0.2 ms are generally used. These transient magnetic fields contribute to the depolarization of nerve cells in the brain. This method facilitates stimulation of the motor cortex of the human brain within a 5 mm resolution [Ueno et al., 1989; Ueno et al., 1990]. Vectorial stimulation can be attained because the concentrated eddy currents at the target under the intersection of the figure eight coil flow parallel to the tangent of the two circular coils. Based upon this principle, functional maps of the human motor cortex related to the hand, arm, and foot areas were obtained by measuring the motor evoked potentials (MEPs) of the peripheral muscle responses to the TMS. The functional maps showed that an optimal direction of stimulating induced currents for neuronal excitation exists in each functional area of the cortex. These vectorial characteristics in TMS reflect, in part, anatomical and functional organization of the neurons and neuronal fibers of the brain [Ueno et al., 1991].

Nerve Excitation Models: The introduction of nerve excitation models has widened our understanding of the mechanisms of nerve excitation elicited by magnetic stimulation [Basser and Roth, 1991; Esselle and Stuchly, 1992; Nagarajan and Durand, 1993; Hyodo and Ueno, 1996]. The theoretical nerve excitation models have shown that for neuronal excitation, a negative peak of the spatial gradient of induced electric fields, the activating function, contributes to the depolarization of the membrane.

The site of neuronal excitation corresponds to the site of the maximal value of the activating function. Therefore, the relationship between the coil position and the target depends on several factors such as spatial distribution of the activating function and anatomical structures of neuronal fibers. The spatial distribution of the activating function is determined by the geometry of the volume and electrical inhomogeneities [Liu and Ueno, 2000]. However, specific neurons cannot be selected because the region of excitation in magnetic stimulation cannot be selectively restricted.

TMS and Associative Memory Tasks: Working memory is dependent on the prefrontal granular cortex and associative memory is dependent on the hippocampus and temporal lobe. We used TMS to investigate memory encoding and retrieval, particularly the role of the dorsolateral prefrontal cortex in associative memory for visual patterns [Epstein et al., 2002]. Our associative memory task involved pairs of Kanji characters and abstract patterns. Ten healthy right-handed native Japanese speakers were presented three sets of six paired stimuli in the order Kanji-pattern, Kanji-pattern, Kanji pattern. Each item was displayed for 500 msec with a 500 msec interval between each Kanji presentation and pattern presentation. There was a 2000 msec interval between each set. Subjects were instructed to remember all three pairs of word-pattern associations. Novel abstract patterns were selected to impede verbal identification and to force encoding on the basis of internal spatial organization. Memorizing such unfamiliar patterns quickly in pairs is challenging.

TMS pulses were delivered from a magnetic stimulator using a figure-eight-coil. Paired TMS pulses were applied during the blanking intervals at 120 % of the motor threshold to the left dorsolateral prefrontal cortex, the right dorsolateral cortex or the vertex. No coil was placed on the head for the control. The overall percentage of correct responses was reduced with TMS over the right dorsolateral prefrontal cortex compared to the left. Two out of 10 subjects reported spontaneously that the memory task seemed more difficult during right prefrontal TMS. There were no significant differences among vertex stimulation, left frontal stimulation, and inactive controls.

These results indicate that TMS disrupts associative learning for abstract patterns over the right dorsolateral prefrontal cortex, and suggest that the participating cortical networks may be lateralized in accordance with classic concepts of hemispheric specialization.

Repetitive TMS and Hippocampal Neurons in Rats: rTMS has a potential therapeutic application for the treatment of central nervous system diseases such as depression and Parkinson's disease. This study focused on whether rTMS aids in the protection or recovery of injured rat hippocampal neurons that were damaged after MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) injections [Funamizu et al., 2002]. Male Wistar rats received four MPTP injections per day to induce the degeneration of neurons prior to stimulation. MPTP, a neurotoxin, causes lesions of the basal ganglia, which induces Parkinson-like symptoms in mammals. RTMS was administered for 48 hours with the following stimulation parameters, 1.2 T, biphasic pulses 365 μsec, 25 pulses/sec ×8 sec×10 trains = 2000 pulses. After 72 hours of observation, the rats were sacrificed, and tissues were collected and prepared for nissl staining. The control group did not receive rTMS. Histochemical analyses were performed to determine the effect of rTMS on rat hippocampal CA3 cells.

The hippocampal CA3 cells of the control group remained damaged without rTMS, whereas the rat hippocampal CA3 cells of the stimulated group appeared to be repaired or normal. These results indicate that rTMS can protect or repair the injured cells of the rat hippocampal CA3 cells. We hypothesize that rTMS stimulates BDNF (brain-derived neurotrophic factor) expression, which may have contributed to the recovery process.

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The author wishes to thank M. Sekino, A. Hyodo, and R. Liu for their cooperative work on the field calculations and nerve excitation models, Dr. C.M. Epstein of Emory University for his cooperative work on the associative memory tasks, and H. Funamizu and M. Ogiue-Ikeda for their work on rat hippocampal neurons. The work was supported in part by a Grant-in-Aid for Scientific Research, Ministry of Education, Science and Culture, Japan.

IMAGING THE ACTION OF TMS ON THE HUMAN BRAIN. J.C. Rothwell, Sobell Department, Institute of Neurology, Queen Square, London WC1N 3BG, UK

INTRODUCTION: At the present time, transcranial magnetic stimulation (TMS) is the method of choice for non-invasive stimulation of neurones within the brain of healthy conscious subjects. Although successful, it has been difficult in the past to measure in any detail the effects it has ongoing neural activity. Much of what we know comes from indirect behavioural measures. For example, the twitch of muscles produced by a single brief stimulus over the motor cortex can be analysed to show that the stimulus sets up a brief (10-15 ms) burst of repetitive activity in the motor cortex followed by an active process of inhibition. Rare opportunities to record from patients who have electrodes implanted into CNS structures have confirmed this. In the present talk I will show how further information about the action of TMS can be obtained non-invasively by coupling the technique with other functional imaging modalities (PET, fMRI, EEG).
OBJECTIVES: (1) to analyse the effect of TMS at the site of stimulation. (2) to measure the activation this produces in distant structures that are known to receive anatomical connections from the stimulated area. The latter is of particular interest since TMS preferentially activates pathways that are active at the time the stimulus is applied (because they are more excitable to external stimulation). Thus TMS may give a picture of functional as well as anatomical connectivity.
RESULTS: EEG, fMRI and PET can all be used to observe activity in areas distant from the site of stimulation. EEG successfully gives an indication of the time course of these effects, whereas PET and fMRI give higher spatial resolution as well as the ability to examine effects in structures deep in the brain (basal ganglia, thalamus and cerebellum). It is more difficult to image activity at the site of stimulation. An electrical artefact obscures the EEG for several milliseconds, and a “shadow” of the coil interferes with fMRI measures. Ongoing studies in several laboratories are investigating how TMS stimulation interferes with the normal pattern of brain activity when subjects are performing particular tasks. They can also indicate how the brain adapts to such disturbances to try to maintain optimal performance. Studies in patients with neurological disease have shown how the excitability of pathways is changed by disease. It may even be possible to restore a more normal pattern of brain activation by using TMS selectively to reinforce excitability in certain patient groups.

CONCLUSIONS: TMS is the best tool we have for directly influencing the activity of the human brain. Studies that combine the technique with other imaging methodologies can provide details of how stimulation affects function both at the site of stimulation and at a distance.

TRANSCRANIAL MAGNETIC STIMULATION AND FUNCTIONAL IMAGING. E. Wassermann, National Institute of Neurological Disorders and Stroke National Institute of Health, Bethesda, MD, USA

Transcranial magnetic stimulation (TMS) of the brain has been combined with various means of measuring brain activity as a means of characterizing the local mechanism of various TMS effects and in order to expose connections between the stimulated area and other brain regions. These recording modalities include measures of cerebral blood flow (BOLD MRI and H215O PET) a measure of cerebral metabolism (18fluorodeoxyglucose (FDG) PET) and EEG. Each has particular advantages and drawbacks. BOLD MRI has been used primarily as a means of detecting the local effects of TMS. Studies to date have been essentially limited to demonstrations of feasibility. H2150 PET has been used to demonstrate connections between cortical areas as well as local effects. This technique has the ability to examine the whole brain for areas where changes in blood flow are caused by the stimulation. "Parametric" designs, where a stimulation parameter is varied and a statistical measure of correlation between this parameter and regional blood flow is derived, are particularly appropriate to this technique. H2~50 studies are beginning to provide new and scientifically interesting data. FDG PET is unique in that uptake occurs over many minutes and the radiopharmaceutical remains localized in active areas for hours after administration. This has made it possible to examine the cumulative effect of longer periods of stimulation and stimulation delivered outside the scanner using image subtraction methods. EEG has been combined with TMS in studies aimed at measuring and mapping the local and distant responses to TMS. This approach is potentially useful, particularly for measuring the excitability of the cortex by TMS in areas outside of the motor cortex. However, while considerably simpler than functional imaging, the technique is somewhat demanding, prone to artifact, and has not been adopted widely to date. As TMS is refined and new applications developed, combinations with measurement techniques will become increasingly important.

SESSION 1: IN VITRO STUDIES I
Chairs: Gabi Nindl and Robert Goldberg

1-1

EFFECTS OF THERAPEUTIC PULSED ELECTROMAGNETIC FIELDS ON GROWTH, APOPTOTIC DEATH AND INTERLEUKIN-2 PRODUCTION OF A NORMAL AND INFLAMMATORY T LYMPHOCYTE CELL MODEL. G. Nindl[1], E.F. Hughes[1], M.S. Markov[2], W.X. Balcavage[1], M.T. Johnson[1]. [1]Terre Haute Center for Medical Education, Indiana University School of Medicine, Terre Haute, Indiana, 47809 USA. [2]EMF Therapeutics, Inc., Chattanooga, Tennessee 37405, USA.

OBJECTIVE: This study was designed to determine the effects of a therapeutic electromagnetic field (TEMF) on normal and inflammatory T lymphocytes and to explore the potential use of TEMFs for treatment of inflammatory diseases.
BACKGROUND: It has been shown in a clinical trial that the TEMF signal significantly reduces lower back pain while animal and cell culture studies suggested an anti-angiogenic effect and a stimulation of sensory neuron outgrowth. The latter results indicate that TEMF might be useful in treating illnesses such as cancer and neuronal disorders. Since many modern therapies have objectionable side effects, such as inflammation, the studies reported here involve a determination of how TEMFs might affect immune cells and if TEMFs might alter the course of inflammation. The beneficial effect of TEMF therapy on pain

initially led us to hypothesize that TEMFs reduce inflammation. A second goal of this study is to investigate a potential anti-inflammatory effect of TEMFs in a clinical setting.
METHODS: Human Jurkat cells (E6-1, ATCC) were grown to mid log phase culture in RPMI 1640 containing fetal bovine serum, and replated at 10^6 cells/ml in 6-well or 96-well culture dishes before use in experiments. Cells were exposed to TEMFs in three different states: without activation, with partial activation by plate-bound anti-CD3 (10 μg/ml) and with full activation by a combination of plate-bound anti-CD3 and phorbol myristate acetate (PMA, 50 ng/ml). The three cell states were used as models for normal proliferating, apoptotic, and inflammatory T lymphocytes, respectively. Cell cultures were exposed to 120 pps semi-sine wave pulsating magnetic fields at 5 – 20 mT amplitude range for 10 minutes at 37 °C. Control cultures were placed at areas with DC fields similar to those measured inside the TEMF exposure system, or they were placed inside unpowered coils before or after the experimental samples. Cell proliferation was assayed by 3 hour pulsed [^{3}H]thymidine incorporation into DNA, hemocytometer cell counts and Coulter Counter Model ZM cell counts. Apoptotic cell death was evaluated by measuring caspase-3 activity using a fluorogenic caspase-3 substrate and a Perkin Elmer spectrofluorometer. As an indicator of fully activated, inflammatory T cells, interleukin-2 (IL-2) in cell culture supernatants was quantified by ELISA (Bioscience Inc.).
RESULTS: Cell Proliferation, caspase-3 activity and IL-2 production of normal inactivated Jurkat cells was unaffected by TEMFs of various intensities. TEMFs of 15 and 20 mT slightly increased caspase-3 activity and decreased cell proliferation of partially activated/ apoptotic Jurkat cells. In fully activated/ inflammatory Jurkat cells the same TEMFs significantly increased IL-2 production up to 2-fold and decreased cell proliferation up to 30 %. The TEMF effects were dependent on the cell cycle of the exposed cells.
CONCLUSION: TEMFs do not activate or otherwise alter the activity of normal, proliferating T lymphocytes and only weakly affect partially activated/ apoptotic T lymphocytes. These data indicate that TEMF therapy is not likely to cause inflammation as a side effect. In addition, the results indicate that TEMF therapy would not prevent the natural apoptotic elimination of inappropriately activated T lymphocytes such as might be induced by autoimmune signals. It even appears that TEMFs have the potential to slightly promote this negative selection. On the other hand, TEMFs strongly affect IL-2 production of inflammatory lymphocytes in the Jurkat cell model. This result indicates that TEMFs can be used to modulate inflammation, in accord with our original hypothesis.
This study was supported by EMF Therapeutics, Inc. (Chattanooga, TN).

1-2

PHOSPHORYLATION OF HSP27 - THE MOLECULAR MECHANISM FOR MOBILE PHONE RADIATION-INDUCED INCREASE IN BLOOD-BRAIN BARRIER PERMEABILITY. D. Leszczynski. Bio-NIR Research Group, Radiobiology Laboratory, STUK - Radiation and Nuclear Safety Authority, Helsinki, Finland.

BACKGROUND: The question of whether mobile phone radiation (RF-EMF) is hazardous to health remains unanswered. In earlier study (1) has been demonstrated that the 1-hour non-thermal exposure of human endothelial cell line EA.hy926 to SAR of 2W/kg (900MHz GSM signal) leads, among others, to: (i) transient increase in phosphorylation of hsp27 stress response protein, which was prevented by SB203580, a specific inhibitor of p38 mitogen-activated protein kinase (p38MAPK), and (ii) transient changes in protein expression levels of hsp27 and p38MAPK. Phosphorylated hsp27 has been shown to regulate, among others, cell apoptosis - due to inhibition of the proteolytic activation of pro-caspase-9, and stability of stress fibers - due to increasing polymerization of actin. The latter, when occurring in endothelial cells lining brain's capillary blood vessels, might be of critical importance for the functioning of blood-brain barrier because stabilization of stress fibers was shown to cause: (i) cell shrinkage, leading to opening of spaces between cells, (ii) increase in the permeability and pinocytosis of endothelial monolayer, (iii) increase in

formation of apoptosis-unrelated blebs on the surface of endothelial cells, which may obstruct blood flow through capillary blood vessels, (iv) stronger responsiveness of endothelial cells to estrogen and, when stimulated by this hormone, to secrete larger than normally amounts of basic fibroblast growth factor (bFGF) which could, in endocrine manner, stimulate de-differentiation and proliferation of endothelial cells leading to the associated with proliferative state - cell shrinkage and unveiling of basal membrane.
HYPOTHESIS: Based on the above, has been proposed (1) that: the activation (phosphorylation) of hsp27 by mobile phone radiation might be the molecular mechanism regulating (i) caspase-9-dependent apoptotic pathway and (ii) increase in blood-brain barrier permeability, which has been observed in some animal experiments.
OBJECTIVE: To determine whether physiological responses of endothelial cells, which are associated with the hsp27 phosphorylation and might affect permeability of blood-brain barrier (stability of stress fibers, cell size/shape), occur in the mobile phone radiation exposed cultures of human endothelial cells.
METHODS: Human endothelial cell line EA.hy926 cells, grown on microscope cover slides, were exposed for 1h to 900MHz GSM signal at an average SAR of 2W/kg. Temperature of cell cultures remained throughout irradiation period at 37 ± 0.3^{o}C thus the effects reported here are of non-thermal nature. Cells on cover slides were fixed either immediately or 1h after the end of irradiation. The expression of hsp27 was determined by indirect immunohistochemistry in order to confirm that the cells respond to irradiation in the same way as in the previous study (1). The appearance of cells (size, shape) and stabilization of stress fibers was determined by staining of the cells with phalloidin that was labeled with fluorescent-dye (AlexiaFluor).
RESULTS AND DISCUSSION: As expected, 1h exposure of cells to mobile phone radiation increased expression of hsp27. However, in order to increase hsp27 expression by heat shock was required 3h incubation of cells at 43^{o}C (1h exposure had no effect). This observation, together with the measurements showing that temperature of medium was throughout RF-EMF exposure period at 37 ± 0.3^{o}C, suggest that the observed here effects are of non-thermal nature. The stability of stress fibers, as determined by the pattern of staining with phalloidin-AlexiaFluor, increased after 1h irradiation and did not decline during the 1h of post-irradiation incubation (Figure 1 A,B,C). Induction of the stability of stress fibers caused cells to shrink. As seen in Figure 1 A,B,C - brightly stained cells with stabilized stress fibers rounded-up and appeared as smaller than the adjacent non-stained cells. These rounded-up, stress fibers expressing, cells contacted in-between only through thin pseudopods (Figure 1D,E,F). The observed hsp27-related increase in the stability of stress fibers and caused by it changes in cell shape and size of EA.hy926 cells support the proposed above hypothesis that the hsp27/p38MAPK stress signaling pathway might be the molecular mechanism regulating mobile phone radiation-induced permeability of blood-brain barrier. Furthermore, the possible RF-EMF-induced breakage of the blood-brain barrier, if occurring repeatedly over a long period of time might become a health hazard because of the possible extra-capillary accumulation of molecules that might cause brain tissue damage.
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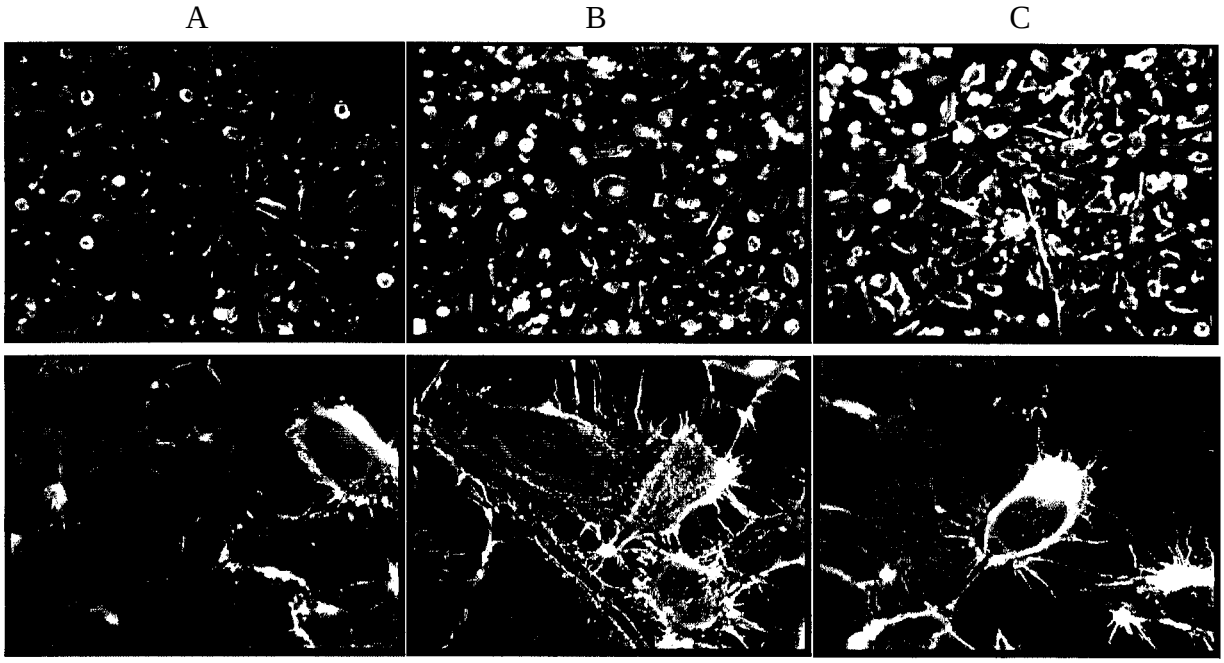

Figure 1.

Expression of stress fibers in EA.hy926 cells detected using phalloidin-AlexiaFluor staining.
Upper panel: (A) sham, (B) immediately after 1h irradiation, (C) 1h irradiation + 1h incubation, Lower panel: (D) sham, (E) immediately after 1h irradiation, (F) 1h irradiation + 1h incubation; using higher microscope magnification shows that cells with stabilized stress fibers were shrinking and connected with each-other with thin pseudopods.

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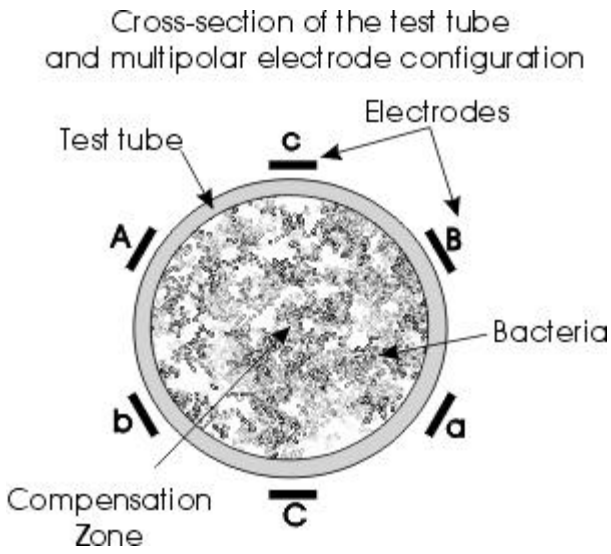
1-3

A COMPENSATION ZONE OF MULTIPOLAR SYSTEM OF EM FIELDS STIMULATES BACTERIAL GROWTH. A. Zavalin, E. Collins, S. Morgan. Fisk University, Nashville, Tennessee 37208, USA.

OBJECTIVE: The goal of the work is to conduct a new system approach – using an assembly of interdependent emitters and generators to produce a multipolar system of EM fields or currents, applied to media in a bioreactor. It is possible to say now – application of multipolar systems of EM fields and electric currents promise to be the part of new direction – “multipolar technology”.



METHODS: Recent work in our lab has demonstrated experimentally significant advantage of multipolar systems of AC EM fields, having a geometrically symmetrical "star" configuration. The parameters of the electrical signals on each electrode of the multipolar systems were as follows: synchronous sine AC voltage with amplitudes 0.1-15 V and frequencies 0.5-10 kHz. The AC voltages were produced by the symmetrical system of interdependent transformers, powered by amplified function generator. Number of electrodes, used in the experiment, was 2, 3, 5 or 6. Output parameters were not optimized for the best growth stimulation and are the subject for the future research. Net electric signal distribution in the central zone among the electrodes, as measured by oscilloscope, shows zero value within certain area, approximately corresponding to the center of symmetry. This zone, produced by compensation of the voltages from each electrode, we call a "compensation zone". In the current work detailed results only for E.coli cultures are presented. The standard HMS174 strains were chosen as a well-known object for experiments and industry applications. The bacterial cultures were grown in the standard glass 13x100 mm test tubes and on the petri dishes. In these particular experiments the electrodes were located outside of the test tube to avoid possible contaminations. The 4 mL of LB-media in the test tube were inoculated by 40 uL of the mother culture. Test tubes were placed in the compensation zone among the electrodes. All procedures were made in sterile conditions. Bacteria concentration was measured under the applied voltages by diode laser @ 635 nm every 2 s. The measuring system, electrodes and test tube holder were assembled on the dielectric platform and placed in the shaking incubator @ 37°C and 300 rpm. The computer system collected data automatically without involving the operator. Results obtained were also verified for selected samples by measuring them in the spectrophotometer outside of the incubator @ 600 and 635 nm every 2 h. The control test tubes were placed in the system without voltages applied. Results were averaged for 3-5 test tubes. In addition, the cultures on the petri dishes were used to determine the possible difference in the colony growing patterns. The dishes were placed on the top of the flat electrode system and patterns were scanned every 2 h.

CONCLUSIONS: Preliminary experiments show considerable increase of the growth rate or gas yield (up to 200% for bacteria, yeast and protozoa) inside the compensation zone of the 5- and 6-polar systems. The growth of microorganisms was depressed in 2-polar systems, which are more similar to the widely used experimental configurations.

The detailed statistical data, based on growth curves for standard E.coli cultures in the test tubes are presented. Stimulation effect is mostly obtained on the lag and log phases of the curves.

The results obtained and possible mechanisms of the stimulation are discussed.

The effect of the system of EMF to living organisms could be significant in the locations of the several emitters, working at the same frequency, which is typical in the industrial areas. In this situation, ecological monitoring measurements of the net field in the compensation zone will show low values, but the biological effect need to be tested directly in addition.

1-4

EFFECTS OF ELF AND MICROWAVES ON HUMAN LYMPHOCYTES FROM HYPERSENSITIVE PERSONS. I. Belyaev[1], L. Hillert[2], C. Tamm[1*], M. Harms-Ringdahl[1*], L. Malmgren[3*], B. Persson[3*]. [1]Department of Genetic and Cellular Toxicology, Stockholm University, Stockholm, Sweden; [2]Department of Environmental Health, Karolinska Hospital, Stockholm, Sweden; [3]Department of Radiation Physics, Lund University, Lund, Sweden.

INTRODUCTION: Hypersensitivity to electricity (electromagnetic fields, EMF) is a fairly new phenomenon and etiology of the EMF hypersensitivity is not yet known. There are several symptoms that these people experience in the proximity to different sources of EMF. The symptoms are not specific to this illness and there is no known pathophysiological marker or diagnostic test [1-2]. No causal relationship between EMF and symptoms has yet been proven, but sensitivity to specific frequencies has been suggested [1]. The frequency dependent non-thermal effects of ELF magnetic fields and microwaves on the conformation of chromatin in lymphocytes have been described and individual variability has been observed [3, 4].

OBJECTIVE: Here, we used specific conditions of exposure to ELF to investigate if the response of lymphocytes from hypersensitive persons is different as compared to healthy subjects. We also used GSM modulated microwaves, which have been previously shown to affect brain blood barrier in rats [5].

MATERIALS AND METHODS: Fresh blood samples from two groups of donors, 7 persons reporting electrosensitivity and 7 healthy controls matched by gender, age and smoking habits were coded and all data were analysed in blind. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). Apoptosis was determined up to 72 h by morphological changes. Apoptotic fragmentation of DNA was analyzed by pulsed-field gel electrophoresis (PFGE). Sinusoidal magnetic field (8 Hz, 30 μT amplitude or 50 Hz, 15 μT amplitude) was applied using Helmholtz coils. Installation employing GSM signal, 915 MHz, all modulations included, SAR=1-2 mW/g in the TEM-cell was used. All exposures were 2 h. The data were analyzed with the t-test.

RESULTS: Exposure to ELF at 8 Hz and specific static magnetic field as described in [3] resulted in statistically significant changes of chromatin conformation, which disappeared 19 h after exposure. This ELF exposure resulted in apoptotic DNA fragmentation, which was comparable with the response induced by 2 cGy of γ-rays. No significant differences in effects were seen between groups of control and hypersensitive donors. However, a trend to a prolonged state of relaxed chromatin was observed in lymphocytes from hypersensitive persons. No effects of 8 Hz exposure on apoptosis or AVTD were observed when static magnetic field was changed by 10 μT. Exposure either to 50 Hz or microwaves resulted in significant condensation of chromatin which was comparable with heat shock at 41^{o}C. This condensation disappeared 2 h after exposure and no apoptosis was observed during 72 h. Comparison of pooled data obtained with 50 Hz and 915 MHz did not show significant differences in effects between 4 control and 4 sensitive subjects. However, in 3 of 4 matched pairs, both 50 Hz and 915 MHz stronger affected cells from hypersensitive persons.

CONCLUSIONS: The data suggested that ELF magnetic fields and microwaves under specific conditions of exposure affect lymphocytes from healthy and electrosensitive donors. ELF under specific conditions of exposure resulted in apoptotic DNA fragmentation. These effects differ at different frequencies and vary between donors. In some cases, cells from electrosensitive donors responded stronger than cells from gender- and age-matched control subjects, but the results need to be confirmed in a larger study group.

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1-5

EXTREMELY LOW FREQUENCY MAGNETIC FIELDS INDUCE HEAT SHOCK PROTEINS IN HL-60 CELLS. W. Sontag. Forschungszentrum Karlsruhe, Institute for Biomedical Engineering and Biophysics, POB 3640, D-76021 Karlsruhe, Germany.

OBJECTIVE: Conditions of moderate stress induce, in mammalian cells, a variety of proteins, called heat shock proteins or chaperons, which are known to protect the cells against a second stronger stress. It has been reported that treatment with low frequency magnetic fields can also induce heat shock proteins.
METHODS: Human promyelocytic leukaemia HL-60 cells were grown in flasks with RPMI 1640 medium supplemented with 15% fetal calf serum, L-glutamine, MEM, sodium pyruvate and antibiotics. For use in the experiments, the cells were held on 35 mm petri dishes at a density of 500,000/ml. The cells were treated with sinusoidal magnetic fields of various frequencies at a flux density of 1 mT within Helmholtz coils. Simultaneously, control experiments were run with Helmholtz coils in antiparallel wiring, resulting in a zero magnet field. Both Helmholtz devices were placed in the same incubator (37° C, 5% CO_2) in a humidified atmosphere. After exposure, the cells were washed with cold PBS by centrifugation and lysed afterwards. Quantitation of hsp72 protein was performed using enzyme immunoassay according to the instructions of the manufacturer (EKS-700, StressGen).
RESULTS AND DISCUSSION: Treatment of cells with thermal stress (30 min at 43° C) resulted in a 2.5 fold induction of hsp72 with a maximum at 2 hours after treatment. A similar kinetics was obtained after a 15 min treatment with a 50 Hz magnetic field: the hsp72 level increased to a maximum at 2 hours after treatment and decreased below the control values afterwards. Therefore, for these exposure conditions (15 min treatment with 1 mT and hsp72 determination 2 hours after exposure) the influence of frequency was examined. In the range from 0 to 60 Hz, the hsp72 protein level was found to be statistically different from the controls only at 20 (decrease) and 50 Hz (increase) [$p < 0.05$, t-test]. Thus, exposure to magnetic fields modulates expression of hsp72 protein in a frequency dependent way, even though the field treatment under the conditions investigated (15 min at 1 mT) is less efficient than thermal induction.

1-6

EMF ENHANCED NEURITE LENGTH IN A CHICK DRG MODEL IS SIGNAL DEPENDENT. E. Herbst[1], P. Resig[2], S. Ranney[2*], B.F. Sisken[2]. [1]Herbst Research, Inc., Edgewater, New Jersey 07020-0589, USA. [2]Center for Biomedical Engineering, University of Kentucky, Lexington, Kentucky 40506-0070, USA.

OBJECTIVES: To investigate effects of three different electromagnetic fields (EMFs) in a chick embryo dorsal root ganglia (DRG) model *in vitro* for preliminary information on the effects of dB/dt (time rate change of the magnetic flux density) and B on neurite outgrowth.

METHODS: All three EMFs tested had the same peak magnetic flux density B of 0.3 mT and the same repetition rate of 2 Hz but their shapes were different. The EMFs were induced in 30cm x 30cm square horizontal Helmholtz coil powered by a current-output power amplifier with a bandwidth of 50 kHz, max available current of 10A and a voltage compliance of 50V. The current-output amplifier was designed because the magnetic flux density is directly proportional to the current and can easily be controlled by setting appropriate electrical parameters of a current signal. Magnetic fields corresponding to the field induced by one of the signals (20 ms pulse) had previously shown effects on nerve regeneration *in vivo* and a similar field with a lower maximum flux density of 0.05 mT had previously shown effects in a chick DRG model *in vitro* [1]. We used this pulsed magnetic field as the basis for our investigation and added two other signals. Signal 2 had the same rise and fall time as the original signal but did not have a 20 ms long DC portion and as a result, no DC magnetic field. Signal 3 had higher dB/dt.
Dorsal root ganglia (DRG) dissected from 8 1/2 to 9 days old chick embryos were explanted to 60 mm culture dishes coated with rat tail collagen and cultured [1]. Each experiment consisted of 6 dishes in the EMF treated group and 6 dishes in a sham control group, with 12 DRGs per dish. Four DRGs were placed on a circle 5 mm from the center of the dish and the other eight 20 mm from the center. Both the EMF treated and the sham control group had 3 concentrations of NGF (0ng/ml, 2ng/ml, and 50 ng/ml [2]), with 2 dishes per concentration. The cultures were fed with neurobasal and N2 supplement (Gibco Go., New York) and cultured for 48 hours at 37°C and 95% air, 5%CO_2, before fixation with phosphate-buffered formalin. EMF was applied for 2 hours/day for 2 days. Each experiment was repeated 7 to 8 times. Assessment of neurite outgrowth was performed by manual measurement [3} of DRG neurite length and number of neurites on pictures taken of each DRG. Mean neurite length and mean number of neurites were calculated for each location (inner and outer DRGs) in each dish. Statistical analysis was performed by fitting a series of linear mixed models to this data and p-value was set to 0.05 for all main effects and all interaction effects. For statistically significant effects, a post-hoc comparison of mean response was based on Fisher's least significant difference procedure.
RESULTS: The mean neurite length response for signal 3 at the NGF concentration of 2 ng/ml was significantly larger than the mean response for control ($p=0.0002$). There was no effect on the neurite length for the same signal at the NGF concentration of 0ng/ml nor at 50 ng/ml ($p=0.47$ and $p=0.61$, respectively). There were no effects for the other two signals at the NGF concentration of 2 ng/ml. There was no effect on the neurite number for any of the signals.
CONCLUSIONS: This study was designed to systematically test specific electrical parameters of EMFs, such as magnetic field's rise and fall times and dB/dt, and their effects on neurite outgrowth. The highly significant effect on the neurite length for signal 3 and no effect for signal 2 would indicate the importance of dB/dt and of the corresponding electric field.
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We thank Dr. Richard Kryscio, Dept of Statistics, Univ. of KY, for help with statistical analysis. Supported in part by the NIH grant: IR43 NS33033-01 to Herbst Research, Inc.

SESSION 2: SPECIAL SYMPOSIUM I: RADIOFREQUENCY FIELDS AND COGNITION

Chair: Alan Preece

2-1

EMF EFFECTS ON HUMAN COGNITIVE PROCESSES AND THE EEG. C.M. Krause. Laboratory of Computational Engineering, Helsinki University of Technology, PB 9400, 02015 HUT, Finland.

INTRODUCTION: Several studies have reported effects of RF fields on cognition and others have suggested effects also on the EEG, especially during the performance of cognitive/memory tasks. However, at present, the mechanisms underlying such effects remain unexplained.
OBJECTIVES: To review and discuss recent findings on the effects of RF fields on human cognitive processes and on the EEG with special emphasis on the results of the Turku research group. The Turku research group has studied the effects of RF fields on human cognition utilizing several behavioural tests (e.g., reaction times, short-term memory tasks). Additionally, the effects of RF fields on the EEG have been explored during cognitive tasks (auditory memory task, visual n-back task).
RESULTS: On the behavioural level the exposure to RF fields speeds up reaction times and decreases the time needed in mental arithmetic and in working memory tasks. The exposure to EMF alters lower frequency EEG responses (~8 Hz), especially during retrieval from memory.
DISCUSSION: The choice of appropriate behavioural measures, or tests, to study EMF effects on cognition is still largely a matter of debate and controversy. How are the terms "cognition" and "cognitive processes" defined and how are these capacities measured? What conclusions can be drawn from statistically significant effects of EMF on behavioural measures and on the EEG?

2-2

EFFECTS OF MOBILE PHONE EMISSIONS ON HUMAN COGNITION. A.W. Wood, D.L. Hamblin*, Con K.K. Stough*. School of Biophysical Sciences & Electrical Engineering, Swinburne University of Technology, Hawthorn, Victoria 3122, Australia

A review was undertaken to compare and contrast the outcomes of published reports on effects of mobile phone-type emissions on human brain electrical activity or sleep variables.
Eighteen such studies were identified in which spontaneous or evoked EEG was measured or site of putative effects inferred. Of these, nine report measurements of alpha band power. Although, in general, outcomes have been inconsistent and comparison between individual studies is difficult, enhanced EEG alpha band power has been noted in several studies. This phenomenon has also been observed in some animal studies. Enhanced alpha band power is also consistent with the performance decrements observed in some recent Extremely Low Frequency (ELF) studies, which highlights the possible role of ELF fields associated with battery current in mobile phone handsets. On the other hand, improved performance has been observed with more complex cognitive tasks in relation to mobile phone exposure. Significant cognitive effects have been reported using both modulated and unmodulated RF carrier implying that the possibility of putative effects being due to ELF demodulation is unlikely. There are no obvious associations between site of exposure and regions of brain from which effects are reported or implied. Lastly, effects have been reported to occur both during exposure and up to an hour or so after cessation of exposure.
The majority of sample types in these studies have involved young, healthy males with studies lasting for no more than a few hours (except for sleep studies). Sample sizes have been 48 or less limiting the statistical power of these studies. Inconsistent methodologies rule out detailed meta-analyses.

The issues of combined exposure with ELF, long-term effects and the inclusion of a wider representation of mobile phone users all need to be addressed.

2-3

A REVIEW OF THERMAL COGNITIVE EFFECTS OF RADIOFREQUENCY RADIATION ABSORBTION. J.A. D'Andrea. Naval Health Research Center Detachment, Brooks Air Force Base, Texas 78235 USA.

During the past 3 decades scientific studies have sought to understand health effects of nonionizing radiation in the radiofrequency (RFR) portion of the spectrum. Public interest in these health studies has grown as the number and kind of devices emitting RFR have led to greater exposures of the population. Safe exposure standards have been recommended by many organizations. Many of these standards have used behavioral performance as the assay for harmful effects of RFR exposure. For example, DeLorge (1976) used rhesus monkeys performing an observing task and documented the threshold for behavioral change during RFR exposure at a specific absorption rate (SAR) of 4 W/kg. The task performance measures were observing responses on one lever and detection responses on a second lever. Monkeys were taught to detect the frequency of two different auditory signals in a simple reaction time paradigm to earn food reward. Exposures at 2450 MHz of 4 W/kg (61 mW/cm^2) produced some effects on latency but exposures at 5 W/kg (72 mW/cm^2) had marked effects. The changes were a 50% decrease in the number of observing responses and a 100% increase in latency to respond to the correct auditory signal. Stern [1980] has suggested that the reduction of responding on a learned task during exposure may solely reflect the animals' attempts to engage in other thermoregulatory behaviors (i.e., escape) that are not compatible with behaviors such as lever pressing required by the behavioral task.

D'Andrea (1999) reviewed behavioral effects of RFR exposures and hypothesized that it is likely that effects on cognitive performance may occur at lower SARs than those that totally disrupt ongoing behaviors. Several studies have shown changes in reaction time or other cognitive tasks, but only at SARs that produce body temperature increases. Mickley et al. [1994] investigated memory deficits in rats exposed to microwaves at 600 MHz and determined that the threshold SAR for memory effects was 9.3 W/kg. Rats exposed at 10, 9.3, 8.5 or 5 W/kg showed a reliable brain hyperthermia. Only the 9.3-W/kg treatment produced a significant memory disruption. The authors noted that at 10 W/kg this behavioral effect might have been modulated by a radiation-induced decrease in exploration. In another study, Mickley et al. [1998] repeated and extended their earlier findings. They determined that microwave induced hyperthermia produced by 600 MHz irradiation at 9.3 W/kg can cause disruption of working memory as evidenced by failure to discriminate between new and familiar objects. They also determined that a single exposure to microwave induced hyperthermia could produce thermal tolerance as evidenced by reduced hyperthermia on the second exposure and by attenuated behavioral disruptions of working memory. Finally they determined that opiod antagonism with naltrexone could partially reverse some of the behavioral effects of the microwave-induced hyperthermia. Luttges [1980] examined the effects of microwave irradiation on learning and memory in mice and found an enhancement of performance. He used a resonant microwave irradiation chamber in which mice were exposed, following daily training, to 3- GHz pulsed microwaves at approximately 18 mW/cm^2 average power levels (estimated whole-body SAR 13 W/kg) and he documented small but reliable increases in performance. The microwave induced facilitation of memory was found in both automated active-avoidance testing and in single-trial, passive-avoidance testing. D'Andrea et al. (2000) trained monkeys on a temporal response differentiation (TRD) task for food pellet reward and then exposed them to a head resonant frequency of 500 MHz. Microwave exposures were either continuous wave (CW) or pulsed (PW, 1000 pps, 5ms pd). Monkeys were exposed to average power densities ranging from 5.2 to 41.6 mW/cm^2 which produced whole-body SARs of 0.8 to 6.2 W/kg. Threshold for disruption of behavior in this study seemed to occur between whole-body SARs of 3.1 to 3.9 W/kg. At SARs higher than 3.1 W/kg the overall change was a decrease in total work. Effects on behavior seemed to occur only during

exposure and do not show any carryover to the next session. However, TRD was not significantly changed. A review of these and other studies will be given during the symposium.

SESSION 3: IN VITRO STUDIES II
Chairs: Asher Sheppard and Junji Miyakoshi

3-1

ROLE OF MODULATION IN THE EFFECT OF 2.45 GHZ MICROWAVES ON MUTAGENESIS.

A. Perrin[*1], C. Bachelet[1], P. Levêque[2], R. Malabiau[3] and J.C. Debouzy[1]. [1]Molecular and Cellular Biophysics Unit of the Health Service Research Center for Defense (CRSSA), BP 87, 38702 La Tronche cedex, France. [2]Research Institute in Optic Communication and Microwave (IRCOM), CNRS UMR 6615, 87060 Limoges, France. [3]DGA/DCN/STSN/CTSN, BP28, 83800 TOULON - Naval, France.

The objective of this work was two-fold. Firstly, to determine whether microwaves irradiation in combination with carcinogens might promote mutagenesis. Secondly, to investigate if continuous (CW) and modulated (PW) radio frequency fields effect mutation levels to different extents. Experiments were carried out on procaryotic cells of *Salmonella typhimurium* using the Ames II^{TM} assay. The latter is a short-term genetic toxicity test used to detect mutagenicity of biological mixtures or complex environment. It allows large samples to be studied rapidly. The method is based on the use of specially selected strains of *Salmonella typhymurium* bacteria containing different types of point mutations in the histidine operon. Samples were irradiated with both continuous and modulated (217 MHz) microwaves of the same frequency (2.45 GHz) and average power density .

The bacteria were exposed to the electromagnetic field both during their growing phase (16 h) and/or during the incubation time (90 min) in the presence of a mutagenic mixture of 4-nitroquinoline-N-oxide (125 ng/ml) and 2-nitrofluorene (195 ng/ml). During the incubation phase the power density was 8 mW/cm^2 corresponding to an average SAR of 3.3 W/kg (FTDT). For each experiment, a non-exposed (sham) and an exposed Ames assay were carried out simultaneously in two identical Plexiglas incubators, at 37°C. The waveguide antenna (rectangular horn) was positioned at a distance that permitted far field exposure of the samples and so as to avoid cage effects. Each assay corresponded to the repetition of the same culture conditions in 22 wells and was reproducibly repeated 10 times. Growth of the bacteria and temperature of the culture medium were determined both under and after microwave exposure.

The number of revertants remained unchanged when the bacteria were exposed to CW 2.45 GHz radiation during the growing and the mutagen treatment phases (16 h + 90 min). It decreased significantly when the cultures were exposed to PW 2.45 GHz radiation during the same incubation time ($p = 0.003$ for Students *t* test and $p< 0.001$ for Mann-Withney U-test). No significant effect was detected when irradiation took place during the period of incubation with the mutagen agents (90 min.). No difference were detected between sham and exposed bacteria density after irradiation, neither in their growing capacity after exposure. The temperatures were the same in CW and PW experiments.

In conclusion, a significant decrease in the number of mutations was observed when the bacteria were exposed to the PW electromagnetic field both during the growth phase and the mutagen treatment phase. It remained unchanged when the bacteria were exposed to CW microwave. Bacteria growth was not modified by the CW or by the PW microwave. Under these experimental conditions and contrary to expectations, the data suggests a small but “protective” effect due to exposure to the modulated electromagnetic field.

Research supported by the DGA (Direction Générale de l’Armement).

3-2

STATIC MAGNETIC FIELD HAS LITTLE EFFECT FOR GENE EXPRESSION AND LIGATION EFFICIENCY IN INVITRO. T. Nakahara[1*], M. Yoshida[1*], J. Miyakoshi[1]. [1]Department of Radiation Genetics, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan.

INTRODUCTION: Due to the recent developments in electronic technologies, daily exposure to strong static magnetic fields (SMF) is increasing. Safety issue regarding exposure to strong SMF has not been established, and this is causing public concern. We reported previously that the strong SMF exposure (10T) has no effect for cell growth and cell cycle distribution, but that exposure to 10T SMF enhanced the X-ray-induced micronucleus formation with 4Gy. Therefore, more biological data are needed to evaluate the biological effects of strong SMF.
OBJECTIVE: Firstly, we attempt to clear whether strong SMF can affect a specific gene expression. We have done DNA microarray analysis. Then, one of gene expressions changing by exposure to strong SMF is analyzed by RT-PCR and western blotting. To clear mechanisms enhancing the X-ray-induced micronucleus formation by exposure to strong SMF, we have examined effects of SMF on ligation reaction in *in vitro*.
METHODS: To assay the change of gene expression, we used UniGEM Human V Ver. 2.0 (IncyteGenomics) as a DNA microarray. Human glioma cell line MO54 after SMF-exposure or sham-exposure (6 hours) were used as a mRNA source. To assay the effect of SMF on ligation reaction, plasmid were digested with restriction enzymes (Eco RV, Bam HI and Kpn I), then mixed with "Ligation high" kit (TOYOBO) as a ligase and reaction buffer. After SMF-exposure or sham-exposure (6 hours), these plasmids were used for transformation of *Escherichia coli*. After overnight incubation, the number of colony was counted and ligation efficiency was evaluated .
CONCLUSIONS: We found a little difference in gene expression by using DNA microarray analysis. As compared with sham-exposure, expression of human gene NER (steroid hormone-nuclear receptor) was increased about two times after treatment with SMF-exposure. This difference about 2-fold increase was very small to detect it by another ways, such as RT-PCR and western blotting analysis. We also could not find the significant difference in ligation assay. These data suggest that strong SMF, up to 10T, had a little biological effect in cultured cells.
This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture, Japan and by a Grant-in-Aid from the Research for the Future Program, Japan Society for the promotion of Science.

3-3

SENSITIVITY OF OSTEOBLAST CELLS TO INHIBITION OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION BY ELF-EMF AT 14Hz. H.C. Teng, S. Cherng*, J.E. Trosko*, C.C. Chang* and B.L. Upham. *National Food Safety & Toxicology Center, and Department of Pediatrics & Human Development, Michigan State University, East Lansing, MI 48824, USA.

OBJECT: To determine if 14Hz ELF-EMF within the earth's geomagnetic field can modulate gap junctional intercellular communication (GJIC) in mouse osteoblast cells. The ELF-EMF-EMF power that the mouse osteoblast cells may absorb should be proportional to the maximum amplitude of the geomagnetic field as well as the bandwidth of the 14Hz ELF-EMF-EMF peak in a Power Density Spectrum (PDS).
BACKGROUND: Interruption of GJIC has been affiliated with many pathological endpoints including cancer, neurotoxicity, and reproductive dysfunction. The current density induced by ELF-EMF within the cells is highly correlated with GJIC, and can be calculated by cell model systems. The electromagnetic coupling hypothesis is based on the assumption that the electron transport along the respiratory chain is the

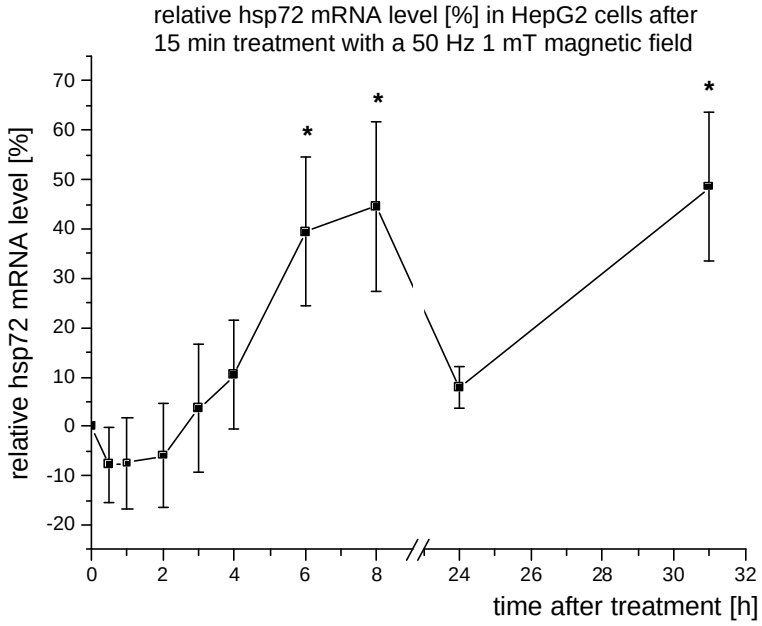
source of electromagnetic field to exert forces on the protons placed in it. Therefore, the changing current density within the cells caused by 14 Hz ELF-EMF-EMF and the geomagnetic field might also cause GJIC modulation as well.
METHOD: Part 1: We used a simple 5 cm radius helical coil, which is coiled with 200 turns of 0.45 mm diameter cooper string and 1.5 cm in height on a plastic cylinder shell for input of ELF-EMF. The ELF-EMF incubator was provided with 5% CO_2 at 98% relative humidity. Another sham cell culture incubator was at the same conditions as the ELF-EMF incubator. The Mouse Osteoblast Cells were cultured in 2 ml culture medium, supplement with 5% fetal bovine serum. The scrape loading/dye transfer (SL/DT) technique was used to measure GJIC. The microscope images of the fluorescent dye spread were digitized with a Nucleotech image analysis system and then GJIC was quantified as a fraction of the control using the Nucleotech software.
Part 2: A sensitive probe was inserted into the culture dish. The 14Hz ELF-EMF was considered as an input signal into the mouse osteoblast cells, and the output was transformed to electrical voltages by an oscilloscope. By using HP Benchlink, we collected the output data from the oscilloscope and transferred the data to Microsoft Excel as text files. Matlab and Fortran computer languages were used to do the PDS analysis.
RESULTS: Our EMF-detection system was able to detect an ELF-EMF signal at 14 Hz within the high background of ELF-EMF-noise in the cells. ELF-EMF at 14 Hz was found to inhibit GJIC. A two tail T-test indicated significance at the $p = 0.05$ level.
CONCLUSION: The inhibition of GJIC provided evidence that external ELF-EMF may couple with geomagnetic fields that created a net magnetic field, which can affect a biological system. The power density measurement of the absorption of ELF-EMF, together with the corresponding decreases in GJIC, demonstrate that biological systems can detect and are affected by weak ELF-EMF magnetic fields within high noise backgrounds. When we examine the sensitivity of the cell to magnetic fields, we may have to account for the field strengths of both the geomagnetic and applied ELF-EMF to get the most recognizable biological effect, and then we can estimate the best signal-to-noise ratio of the net magnetic fields for PDS analysis. In other words, geomagnetic field and ELF-EMF interact in maximizing the amplitude of the net magnetic field to cause a biological effect in the mouse osteoblast cells *in vitro*. The remaining question is how can we find the best combination of those factors experimentally so that we can always see the biological effect from a specific net magnetic field and ELF-EMF treatment.

3-4

50 Hz 1mT MAGNETIC FIELDS INDUCE HEAT SHOCK PROTEIN 72 mRNA IN VARIOUS CELL LINES. E. Gottwald, W. Sontag, K.-F. Weibezahn, H. Dertinger. Forschungszentrum Karlsruhe, Institute for Biomedical Engineering and Biophysics, POB 3640, 76021 Karlsruhe, Germany.

OBJECTIVE: Heat shock proteins are known to exert protective effects on cells under various stress conditions. Because it was reported that magnetic fields with field strengths in the µT-range can induce the expression of HSP72 Protein in rat cells we investigated whether 50Hz magnetic fields can induce hsp72 mRNA in various cell lines, especially those of human origin like e.g. HepG2 and Girardi Heart cells. Furthermore, we used the rat myoblast cell line H9c2.
METHODS: The cells were treated with 50 Hz magnetic fields (1 mT, 15 min) in Helmholtz coils. Controls were exposed to coils with antiparallel winding resulting in a zero magnetic field. Afterwards, cells were cultivated further for different time periods, ranging from 5 min to 31h. Total RNA was isolated and semiquantitative RT-PCR according to the "Primer Dropping"-method (Wong et al., 1994, Gottwald et al., 2001) for determination of relative hsp72 mRNA expression levels was performed.
RESULTS AND DISCUSSION: It could be shown that 50Hz, 1 mT magnetic fields applied for 15 min lead to an increase in the hsp72 mRNA level in HepG2 cells earliest after 15 min. Elevated levels decreased within 30 min after the end of exposure. A second rise in hsp72 mRNA level was observed 6 hours after the

end of the treatment and was detectable even 31 hours later. This corresponds well to the so-called first and second window of protection. H9c2 rat myoblasts reacted in a similar fashion. An increase in hsp72 mRNA was detectable 6 hours after the end of the treatment but in contrast to HepG2 cells, hsp72 mRNA levels declined immediately afterwards. The Girardi Heart cells showed no consistent behaviour after 50Hz treatment. We conclude that the induction of heat shock proteins by magnetic fields can be a useful medical approach in conditions where people are at elevated risk of ischemia or infarction.



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3-5

EVALUATION OF DNA DAMAGE IN HUMAN LEUKOCYTES AFTER *IN VITRO* EXPOSURE TO A 1.9 GHz CONTINUOUS WAVE OR AN AMPLITUDE-MODULATED RADIOFREQUENCY FIELD. J.P. McNamee, P.V. Bellier, G.B. Gajda, E.P. Lemay, B.F. Lavallée, L. Marro, S.M. Miller and A. Thansandote. Consumer and Clinical Radiation Protection Bureau, Product Safety Programme, Health Canada, 775 Brookfield Rd., Ottawa, Ontario, K1A 1C1.

The current study was undertaken to investigate whether acute exposure of human blood to 1.9 GHz radiofrequency (RF) fields, as emitted from digital PCS mobile phones, could elicit DNA damage and/or induce the formation of micronuclei in phytohemagglutinin-stimulated leukocytes. Human blood cultures were exposed for 2 or 24 hr to a 1.9 GHz continuous wave (CW) RF field (~1.18 W net forward power) or a 1.9 GHz amplitude-modulated (AM) (50Hz, 1/3 duty cycle) RF field (~3.54 W net forward power) using a series of six circularly polarized, cylindrical waveguides. Mean specific absorption rates (SAR) of 0.0, 0.1, 0.26, 0.92, 2.4, and 10 W/kg were achieved and the temperature within the cultures during exposure was maintained at 37.0 ± 0.5 ^{o}C. Concurrent negative- and positive- (1.5 Gy ^{137}Cs ?-irradiation) control cultures were run for each experiment. DNA damage was quantitated immediately after RF exposure using the alkaline comet assay and four parameters (Tail Ratio, Tail Moment, Comet Length and Tail Length) were used to assess DNA damage for each comet. No evidence of increased DNA damage was detected by any

parameter for blood cultures exposed for 2 hr to either CW or AM RF fields at any SAR tested. The formation of micronuclei in the RF-exposed blood cell cultures was assessed using the cytokinesis-block micronucleus assay. There was also no significant difference in either the binucleated cell frequency, incidence of micronucleated binucleated cells or total incidence of micronuclei between any of the RF-exposed cultures and either the sham- or incubator negative controls at any SAR tested following a 2 hr exposure period. These results do not support the hypothesis that an acute (2 hr), non-thermalizing 1.9 GHz CW or AM RF field exposure causes increased DNA damage in cultured human leukocytes. The results from the 24 hr exposure experiments will be presented at the platform session.

3-6

EXPOSURE OF HUMAN LYMPHOCYTES TO CW 830 MHz INDUCES EPIGENETIC ALTERATION ASSOCIATED WITH CANCER. M. Mashevich[1,3], D. Folkman[2], A. Kesar[2], A. Barbul[3], A. Korenstein-Ilan[1,3], L. Avivi[1], E. Jerby[2] and R. Korenstein[3]. [1]Department of Human Genetics and Molecular Medicine, [2]Department of Physical Electronics and [3]Department of Physiology and Pharmacology, Tel-Aviv University, 69978 Tel-Aviv, Israel.

BACKGROUND: The question whether exposure to radiation associated with cellular phones poses a risk factor for cancer is currently debated. In an attempt to answer this entangling problem we have examined whether *in-vitro* exposure of human peripheral blood lymphocytes (PBL) to CW 830 MHz (RF) electromagnetic fields causes abnormal mode of replication of the chromosome 17 region engaged with the segregation process of chromosomes. This manifestation of epigenetic modification is associated with hematological malignancies as well as solid tumors.

METHODS: PBL were exposed to RF in a set-up based on a parallel-plate resonator for 72 hours in a temperature range of 34.5-37.5 °C at different average absorption rates (SAR) in the range of 1.6-9 W/kg. The averaged SAR and its distribution in the exposed tissue culture flask were determined by combined measurements and numerical analysis based on a finite-element simulation code. The mode of replication was determined for the DNA sequences associated with the centromere of chromosome 17, which harbors genes important in tumorigenesis (p53, HER2-neu) by applying the fluorescence in situ hybridization (FISH) replication assay.

RESULTS: We observed a 1.5 fold increase in an abnormal (asynchronous) mode of replication of the chromosome 17 region engaged with segregation (repetitive DNA arrays associated with the centromere) when elevating the SAR level up to 8.2±0.6 W/kg. This suggests that epigenetic alterations are involved in the SAR dependent genetic toxicity. Examination of PBL of patients suffering from hematological malignancies revealed a 1.9 fold increase in frequency of asynchronous replication of centromere 17. Control experiments performed on PBL of healthy volunteers in the range of 34.5-38.5 °C (in the absence of RF radiation), showed no alterations in the mode of replication. Thus, RF exposure induced increase in the levels of the asynchronous replication of the centromeric DNA arrays via a non-thermal pathway.

CONCLUSIONS: Our findings indicate that the exposure to CW 830 MHz in the range of 1.6-9 W/kg increases genomic instability associated with cancer, as reflected from analysis of hematological malignancies. These findings should be taken into consideration in the future evaluation of guidelines for exposure.

SESSION 4: SPECIAL SYMPOSIUM II: RADIOFREQUENCY FIELDS AND COGNITION
Chair: Alan Preece

4-1

OPTIONS AMONG BIOPHYSICAL SUBSTRATES FOR OBSERVED NON-THERMAL EMF SENSITIVITIES IN BRAIN TISSUE. R. Adey. Loma Linda University School of Medicine, Loma Linda, CA 92354 USA.

Human and animal studies with ELF electric and magnetic fields, and with certain RF/microwave fields at non-thermal levels, have yielded a spectrum of behavioral and cognitive responses. Their interpretation and possible significance has required caution in both biological and biophysical perspectives. Many of these biological sensitivities run counter to accepted models of physiological thresholds based in equilibrium thermodynamics of *kT* thermal collision energies. In a physical perspective, the search also continues for biological systems compatible with a first transductive step in a range of functionally effective vibrational and electromagnetic stimuli that are orders of magnitude weaker than *kT*. Their occurrence invites hypotheses on directions for future research.

Seminal observations in the auditory system point to sensitivities based on intercellular communication as a domain function. The human auditory threshold involves a receptor vibrational displacement of 10-11m, about the diameter of a single hydrogen atom. The inner ear sensory system also suppresses noise of far larger intrinsic *kT* collision energies. It functions as though close to 0 degrees K1, consistent with domain properties of a receptor population. Similarly, behavioral electrosensitivity in sharks and rays (as low as 0.5 $nVmm^{-1)}$ is 100 times below measurable thresholds of individual electroreceptor neurons 2. Electrically inexcitable colon cancer cells also exhibit domain properties, with differential responses towards either apoptosis or cell proliferation, depending on either monolayer or multi-dimensional ("3-D") culture conditions, or when exposed to microgravity of space flight 3. Precision irradiation of human-hamster hybrid cells has shown that alpha particles damaging nuclei of a small fraction of cells indirectly induce mutation in many nearby cells through cell-cell communication involving gap-junction proteins 4.

The intercellular gutter is occupied by a glycocalyx of protruding transmembrane cell surface receptor proteins and by junctional proteins between cells. As a low-impedance pathway for tissue components of external fields, highly anionic glycoproteins have high dielectric dispersions (Dk > 106 below 1 kHz) 5. Their spatial nonlinearities suggest a role in demodulation of amplitude- and pulse-modulated RF fields. Oscillating electric and magnetic fields may act via chemical changes in extracellular fluid 6. Magnetic fields at 1 and 60 Hz destabilize rhythmic oscillations in hippocampal slices at 56 uT (0.35 to 3.5 nV mm-1), via as yet unidentified nitric oxide mechanisms involving free radicals 7

SUMMARY AND CONCLUSIONS: Findings in both excitable and non-excitable tissues emphasize the role of intercellular communication in determining stimulus thresholds. Imposed oscillating EM fields and static magnetic fields offer sensitive and unique tools for future research.

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4-2

THE IN VITRO HIPPOCAMPAL SLICE AS A MODEL FOR THE INVESTIGATION OF THE EFFECTS OF RF FIELDS ON BRAIN PHYSIOLOGY. J.E.H. Tattersall, A.C. Green*, J.R. Scott*, J.K. Deans[1] and L.F. Sundstrom*[1], *Biomedical Sciences Department, CBD Porton Down, Salisbury, Wiltshire, SP4 0JQ, UK and [1]Clinical Neurosciences Department, Southampton University, Southampton SO16 7PX, United Kingdom.

The hippocampus is involved in behaviours such as short term memory, learning, spatial awareness and epilepsy. It has a well defined structure and the neural pathways have been mapped out in great detail. Thin slices (100-600μm thick) of hippocampal tissue from mice, rats or guinea-pigs can be maintained *in vitro* in good condition for several hours and they are widely used as models of neuronal physiology, pharmacology and toxicology. The hippocampal slice preparation is a useful model of cerebral organisation for *in vitro* investigations of the cellular mechanisms underlying learning, memory and epilepsy: it is therefore a potentially powerful tool for the study of interactions of radiofrequency (RF) electromagnetic fields with these processes.

We have investigated the effects of exposure to weak RF fields on electrical activity in rat hippocampal slices. The slices were maintained in an air-liquid interface chamber and perfused with artificial cerebral spinal fluid at a tightly controlled temperature (34.0 ± 0.1°C). Exposures to RF fields were performed using a calibrated parallel plate transmission line. Extracellular field potential responses were recorded before, during and after exposure, using glass microelectrodes filled with 2M NaCl, and afferent pathways were stimulated with a concentric bipolar stainless steel electrode placed in stratum radiatum. Exposure for 5 minute periods to unmodulated 700MHz RF fields (up to 71V.m^{-1}) resulted in statistically significant increases or decreases in the amplitude of the evoked field response; sham exposed control slices showed no such change (Figure 1). The threshold field intensity for this effect was between 50 and 63V.m^{-1}. In a further series of experiments, fields of the same intensity were found to abolish epileptiform activity induced in the slice by perfusion with 4-aminopyridine (4-AP, 50-100μM) after the stimulating electrode had been removed. During the RF exposures, there was no measurable temperature rise in the fluid surrounding the slices; furthermore, FDTD modelling indicated that the SAR at the maximum field intensity used was approximately 4.4mW.kg^{-1} (Tattersall et al., 2001).

The mechanisms underlying these effects are at present unknown, but the hippocampal slice preparation is very amenable to the investigation of cellular and subcellular mechanisms. We are currently studying the effect of RF exposure on excitatory and inhibitory processes in the tissue (Deans et al. 2000) and the pharmacology of the effect.

Since the behavioural roles of the hippocampus are known, and the hippocampus can also be considered as a model for cerebral function, the implications of the RF effects for the whole animal can be investigated. For example, other experiments have indicated that exposure to unmodulated 700 and 900MHz fields, and to 900 and 1800MHz GSM fields, can affect the expression of long term potentiation (LTP) in rat hippocampal slices (Scott & Tattersall, 1999). LTP is widely regarded as being a cellular substrate for short term memory (Stevens, 1998). The changes in neuronal excitability we have observed in brain slices may also be consistent with effects on human cognitive performance reported by Preece et al. (1999) and Koivisto et al. (2000).

Finally, the hippocampal slice may be used as an *in vitro* screen to compare the effects of different frequencies and modulations of RF fields. We are currently using this approach to investigate the pulse modulation used in Terrestrial Trunked Radio (TETRA) and to compare it with the unmodulated carrier frequency (Green et al., 2002).

Supported by the Ministry of Defence, UK

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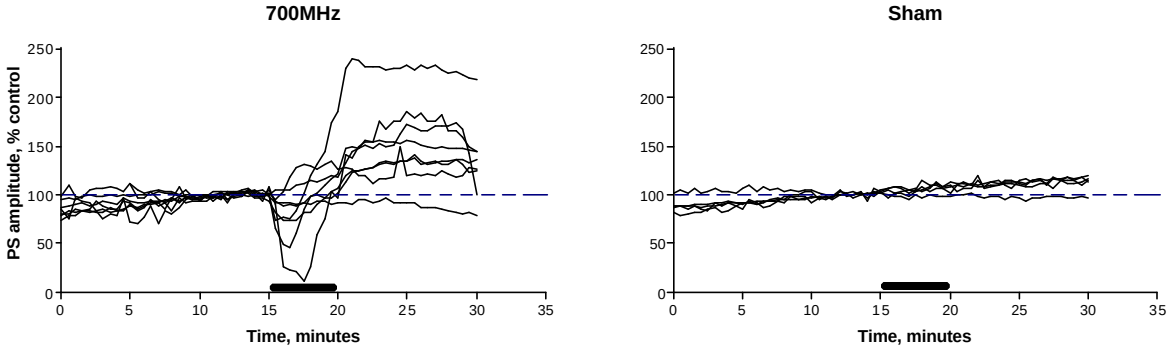

Figure 1. Changes in individual population spike (PS) amplitudes in slices exposed to 700 MHz RF (71V.m^{-1}) or sham. The solid bars indicate the time of exposure.

4-3

EFFECTS OF 380.8875MHz TETRA FIELDS ON CELLULAR CALCIUM AND ELECTROPHYSIOLOGY. A.C. Green*, I.R. Scott*, S.J. Holden and J.E.H. Tattersall. Biomedical Sciences Department, CBD Porton Down, Salisbury, Wiltshire, SP4 0JQ, United Kingdom.

OBJECTIVE: Terrestrial Trunked Radio (TETRA) uses RF fields which are pulse modulated at 17.6Hz when only one of the four time slots is occupied. A number of reports (e.g. Bawin et al., 1975) have suggested that efflux of radiolabelled calcium ions from brain tissue can be enhanced by RF fields modulated at frequencies around 16Hz. "Windows" of effective field intensity have also been reported (e.g. Blackman et al., 1989). The functional significance of this effect is unclear, however, since the source of the calcium ions remains unknown and the tissue was in poor physiological condition: for example, the effect was not blocked by the metabolic inhibitor sodium cyanide. The work reported here uses calcium sensitive dyes to measure and image calcium ion concentrations in neurones and cardiac muscle cells in real time during exposure to TETRA fields. Other experiments compare the effects of TETRA and unmodulated carrier wave on electrophysiological responses in hippocampal slices, which have previously been reported to be sensitive to 700MHz RF fields (Tattersall et al., 2001).
METHODS: For calcium imaging experiments dissociated cerebellar granule cells 6-14 (days *in vitro*, DIV) or dissociated neonatal rat myocytes 2-7 DIV are used. Cells are loaded as previously described (Green *et al.,* 1996) with either 2.5μm fura-PE3 AM for ratiometric determination of intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in granule cells, or 2μM fluo-3 AM for measurement of rapid changes of intracellular Ca^{2+} in myocytes. For experiments, cultures are maintained at 30°C in a Perspex perfusion bath within a calibrated parallel plate transmission line system (Tattersall et al., 2001) mounted on an inverted microscope. Cultures are continually perfused with a modified Locke's solution (Green *et al.,* 1996). In some experiments, granule cells are depolarised by 3 min exposures to modified Locke's solution containing 40mM K^+. Capture and analysis of fluorescence images (340nm and 380nm or 490 nm excitation >520 nm emission) and extraction of intracellular calcium concentration data are carried out with commercial hardware and either Axon Imaging Workbench or LSR's Merlin software. Intracellular calcium concentration changes are monitored before during and after a 20 min exposure to TETRA modulated RF.

Parasagittal hippocampal slices (300 μm thick) from adult Porton-Wistar rats are maintained at 32.0 ± 0.1°C in an interface chamber held within a calibrated parallel plate transmission line and perfused with artificial cerebrospinal fluid. Extracellular field potentials are recorded in CA1 stratum pyramidale using glass microelectrodes filled with 2M NaCl. Responses are evoked by a concentric bipolar stainless steel stimulating electrode placed in stratum radiatum. Exposures to RF fields are carried out in a calibrated parallel plate transmission line system, using an Agilent ESG-D signal generator connected through an amplifier and a directional coupler. Both forward and reverse power are measured during the exposures. The exposures are to unmodulated 380.8875MHz carrier frequency, 380.8875MHz modulated by a standard ETSI TETRA waveform or 380.8875MHz modulated by a 17.6Hz sine wave. The depth of modulation is 100% for both signals. In control experiments, the output of the RF source is connected to a dummy load instead of the waveguide to produce a sham exposure.
Measurements carried out by Microwave Consultants Ltd., using a phantom head exposed to a Motorola MTP300 handset in the standard CENELEC assessment positions, have indicated a maximum SAR in the brain of 0.396 $W.kg^{-1}$. FDTD modelling has been used to determine the input power to the exposure systems required to produce this SAR in the *in vitro* models. Subsequent experiments will test lower SARs to search for power "windows".
DISCUSSION: The results of this study will show whether there are effects of TETRA fields on intracellular calcium concentrations and on electrophysiological responses in brain tissue, and will compare these with the effects of the unmodulated carrier frequency. This will indicate whether there are biological effects specifically associated with the pulse frequency characteristic of TETRA. Furthermore, the results will be based on physiological responses in living cells, which will enable interpretation of the functional significance of any effects of RF exposure.
Supported by the Home Office, UK
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4-4

A STUDY OF THE EFFECT OF TETRA (TERRESTRIAL TRUNKED RADIO) SIGNALS ON COGNITIVE FUNCTION IN HUMANS. A.W. Preece, Y. Johnson* and E.J. Dunn*. Division of Medical Physics and Oncology, University of Bristol, Bristol Haematology & Oncology Centre, Bristol BS2 8ED, United Kingdom.

INTRODUCTION: A number of reports (Preece et al 1999, Koivisto et al 2000, Lee et al, 2001) have indicated cognitive changes in reaction times induced by radio frequency GSM or analogue 900 MHz exposures within ICNIRP, NRPB and ANSI-IEEE guidelines. The limitations of GSM type modulation for range and versatility have led to the introduction of TETRA for commercial and emergency service use. The modulation frequency of 17.6Hz is sufficiently close to the 16Hz indicated by Bawin et al (1996) to be associated with Ca^{++} efflux as to give concern that TETRA may have different cognitive effects on humans. Additionally, the Stewart Committee strongly recommended that low frequency modulation close to 16Hz should be avoided until the possible effects had been adequately studied.
OBJECTIVE: To devise a study of TETRA signals with a similar modulation [pattern to existing commercial systems, which is comparable with previous studies, and which will detect whether there are similar or different cognitive effects to those reported for GSM signals.

SUBJECT & METHODS: The first subject group consists of 18 members of hospital and university staff (9 males and 9 females) of mixed ages. This will be followed by a second group of medical and postgraduate science students aged 18-25.
The cognitive tests used are similar to those utilised and reported by Preece (1999) and supplied by CDR (Reading, UK) but now include a dual attention and visual tracking tasks in order to study increased cognitive loading.
The exposure system is a dummy handset consisting of a 120mm x 40mm ground plane and a resonant antenna 150mm long. This is mounted in a plastic ear defender and coaxially supplied with the signal. This consists of a synthesizer at 1760Hz gated by a ÷100 derivation and a 1:4 gate to give a square wave audio pulse with a 25% duty cycle. This is sent to the data input of a 70cm single sideband transmitter at 428MHz, and to the handset via a Bird thru-line power meter and 3dB in-line attenuator. A separate attenuated tapping from the synthesizer supplies a 1760Hz CW audio signal to the side-band generator. These two signals, together with a grounded audio input are coded via a switchbox such that three conditions are established:

A: No signal
B: CW 428MHz at 1Watt mean
C: 17.6Hz pulsed, 25% duty cycle, 1Watt mean (4Watt peak)

A randomization programme that ensures that an even number of event orders is set predetermines the administration order of the tests.
RESULTS: Average values for the particular tasks with no exposures or interventions are established for adults. Analysis in the instance will focus on intra-subject performance comparison in order to establish if TETRA signals have any effect on human cognitive function, and the precise nature of such effect.
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4-5

RADIAL-ARM MAZE PERFORMANCE IN RATS FOLLOWING REPEATED LOW LEVEL MICROWAVE (MW) RADIATION. B.L. Cobb, E.R. Adair. U.S. Air Force Research Laboratory, Human Effectiveness Directorate, Directed Energy Division, Brooks AFB, San Antonio, Texas 78235, USA.

The aim of our research was to confirm results of a 1994 publication by Henry Lai, et al, (*Bioelectromagnetics* 15:95-104) who reported a working memory deficit in rats exposed to low-level repetitive 45 min, 2450 MHz irradiation and then tested in a 12 arm, radial-arm maze. The '94 publication reported an attenuation of the MW induced learning deficit with pretreatment of both physostigmine and naltrexone hyrdochloride, but not naloxone methiodide. The authors concluded from their results that both cholinergic and opioid systems within the brain are affected by MW radiation. Memory deficits were evaluated using "error rates" (i.e. reentry's into arms already visited). The present study utilized the same exposure system (circular polarized waveguides), whole body SAR (0.6W/kg), pulse regimen, pretreatment drugs, exposure time and configuration of maze. In addition, our study included a double blind procedure and an additional dependent measure of "time to criterion". Error rates and time to criterion data for 10 consecutive test days were analyzed separately using a 3-way analysis of variance (ANOVA). Each ANOVA blocked for main effects of "Exposure" and "Drug" and a repeated factor of "test day". The results of our analyses of error rates included no significant "exposure" effect, no significant "drug" effect and no significant interactions of those two factors. There was a significant difference in "test days" ($F=8.72$, $0 < 0.001$) as expected with repeated test trial days and indicates learning. There was no significant interaction

of “test day” and the other 2 factors. The results of our analyses of “time to criterion” data included no “exposure” effect, a significant “drug” effect ($F=6.18$, $p < 0.01$), a “test day” effect ($F=14.03$, $p <0.001$) and a significant interaction between drug and test day factors ($F=2.21$, $p <0.001$). Post hoc analyses of the “Drug” factor revealed that rats pretreated with either of the two centrally acting drugs physostigmine or naltrexone hydrochloride took significantly longer to complete the maze task than those pretreated with either saline or the peripherally acting drug naltrexone methodide. We conclude from our results that we cannot confirm the earlier findings of Lai et al and in addition, two of the pretreatment compounds reduced the speed in which the rats foraged for food rewards within the maze. There is no evidence that exposure to levels of MW radiation examined here causes decrements in the ability of rats to learn this spatial memory task.

4-6

WHAT IS THE COLLECTIVE MESSAGE FROM STUDIES LOOKING AT HUMAN BRAIN FUNCTION AND CELL PHONE EXPOSURE. J.J. Morrissey, M.L. Swicord. Motorola Florida Research Labs, 8000 West Sunrise Blvd., Ft. Lauderdale, Florida 33322, USA.

OBJECTIVE: There are currently over 50 ongoing and completed studies in the WHO database that investigate human brain function & subjective disorders and exposure to cell phone radiofrequency (RF) emissions. A collective analysis of these studies will be presented.
BACKGROUND: With the continuing popularity of cell phones around the world has come an element of concern over potential human health effects due to long-term low-level RF exposure. Much of the concern initially focused on causation and/or promotion of brain tumors, although such effects do not appear to be supported by a majority of scientific evidence to date. Recently, a number of studies looking at possible influence of cell phone emissions on human brain function and subjective symptoms have been reported. There are currently 21 studies in the WHO database (http://www-nt.who.int/peh-emf/database.htm) that have reported measurement of EEG, sleep disturbances, and brain potentials in humans in response to RF exposure with signals similar to those emitted from cell phones. In addition, there are 7 studies reporting measurement of human cognitive function, 2 studies reporting measurement of hypersensitivity, and 7 studies reporting measurement of headaches and fatigue following cell phone RF exposure. While many of these studies describe certain effects, when the entire set of studies is analyzed collectively a number of inconsistencies become apparent that make interpretation with regard to a valid human health effect difficult. First, reported effects are often contrasted by studies reporting no effect under similar exposure conditions. Further, the magnitude of many reported effects is so small that the actual physiological significance must be questioned. Of critical importance, reported effects have so far not been successfully replicated by independent laboratories under exact study conditions to demonstrate the validity of the original findings, a hallmark of confirmation for any scientific study. Finally, reported effects do not seem to support one another to indicate an obvious common mechanism for RF bio-interaction, and some laboratory findings actually contradict findings from other groups.
DISCUSSION: The results of investigations regarding a possible association between cell phone RF exposure and human brain function and subjective disorders have been mixed. Although reports of effects exist, a collective analysis of the database makes it difficult to identify a consistent effect and provides even less evidence to suggest that such an effect might be adverse to human health. This has also been the conclusion of expert scientific groups from the UK, Canada, Scandinavia, and elsewhere. Many of the studies necessary to replicate the reported effects in this area are currently being performed under well-controlled exposure conditions and their completion and peer reviewed publication should greatly augment the existing database to help clarify whether any effect of cell phone RF exposure on human brain function truly exists.

SESSION 5: IN VIVO STUDIES ANIMAL I
Chairs: Larry Anderson and John Male

5-1

EFFECT OF MILLIMETER WAVES ON CYCLOPHOSPHAMIDE-INDUCED SUPPRESSION OF T-CELL-MEDIATED IMMUNITY. M. Logani, A. Anga*, A. Agelan*, and M.C. Ziskin. Richard J. Fox Center for Biomedical Physics, Temple University Medical School, Philadelphia, Pennsylvania 19140, USA.

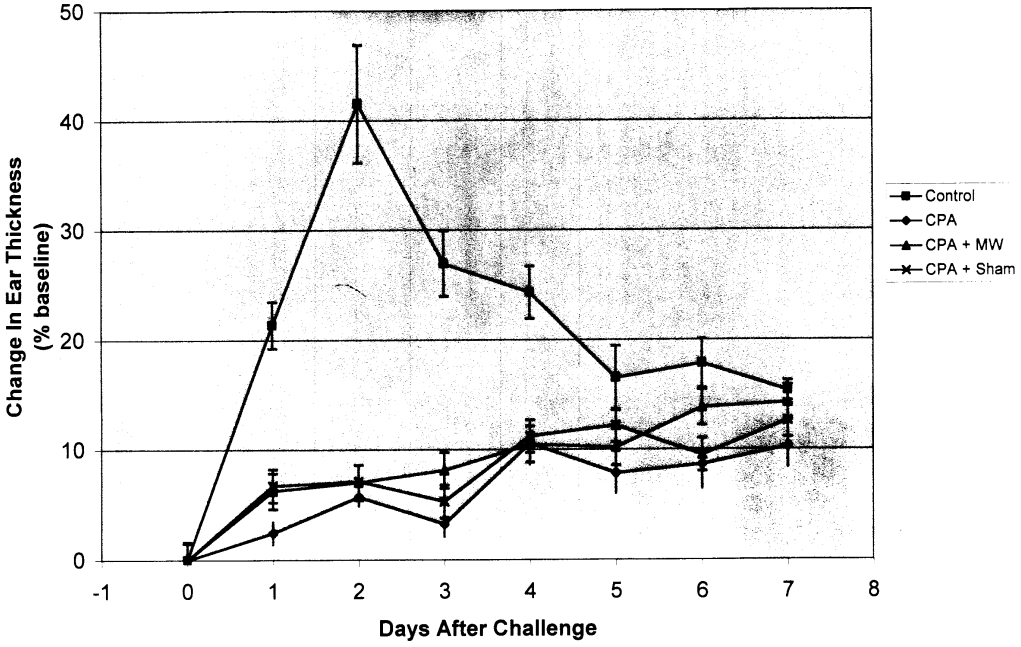
BACKGROUND: Millimeter wave therapy (MWT) is being widely used for the treatment of many diseases in Russia and other East European countries. MWT has been reported to reduce the toxic side effects of chemotherapy on the immune system. The present study was undertaken to investigate whether millimeter waves (MWs) can provide protection to T-cell-mediated immunity from the toxicity of cyclophosphamide (CPA), a commonly used anticancer drug.
METHODS: For studying the effect of MWs on CPA induced suppression of T-cell mediated immunity, a delayed type hypersensitivity (DTH) assay in mouse skin was used. The assay is comprised of two phases, the sensitization phase and challenge phase. The animals (BALB/C mice, male, 7-8 week) were sensitized with 10 µl of 2.5% dinitrochlorobenzene (DNCB) solution and challenged with 10 µl of 0.6% DNCB solution. Both solutions were prepared in acetone: 1,2-propanediol (4:1). On days 1 and 2 of the experiment, the animals were sensitized on the right ear and left foot pad, respectively. The animals were treated with CPA (60-150 mg/kg, ip) at different time intervals after sensitization. Three separate studies using 5 different doses of CPA were conducted. In the first two studies the animals were irradiated with MWs prior to or after administration of CPA. In the third study, the animals were treated with MWs and CPA on the same day (Table 1). The animals were challenged on the left ear on day 7. Four groups of animals, 10 animals per group, were used for each study. Development of the DTH reaction was determined by measuring increase in ear thickness and histological changes at the site of challenge. MWs were produced with a Russian-made YAV-1 generator. The device produced 42.2 ±0.2 GHz modulated wave radiation through a 10x20 mm rectangular output horn. The animals were restrained in plastic tubes and irradiated on the nose area. Peak SAR and incident power density were measured as 622 ±100 W/kg and 31.1 ±5 mW/cm^2, respectively.
RESULTS: Treatment of presensitized animals with different doses of CPA resulted in a significant suppression (60-85%) of the DTH reaction as compared to the untreated animals. Irradiation of animals with MWs did not provide any protection against CPA induced suppression of DTH reaction.(Fig. 1).
CONCLUSIONS: Irradiation of BALB/C mice with MWs did not inhibit CPA induced suppression of T cell mediated immunity under our experimental conditions.

Table 1 Protocol for DTH studies

	Study	Days*	CPA	MW irradiation
1	MW irradiation before CPA administration	2-4	No	Yes
		5-7	50 mg/kg/day	No
2	MW irradiation after CPA administration	4	60, 75 or 100mg/kg	
		5-7	No	Yes
3	Simultaneous application of MW irradiation and CPA	5-7	30 mg/kg/da	Yes

The animals were sensitized on day 1 and day 2 and challenged on day 7 in each study

THE WORK WAS SUPPORTED BY THE NATIONAL CENTER FOR COMPLEMENTARY & ALTERNATIVE MEDICINE; CONTRACT GRANT NUMBER: AT00492-02.



5-2

ANTI-PRESSOR EFFECTS OF STATIC MAGNETIC FIELDS IN GENETICALLY HYPERTENSIVE RATS. H. Okano[1,2], C. Ohkubo[1]. [1]Department of Physiological Hygiene, National Institute of Public Health, Tokyo 108-8638, Japan. [2]Department of Science, Pip Tokyo Co., Tokyo 101-8528, Japan.

OBJECTIVE: Our experimental studies have demonstrated that static magnetic fields (SMFs) in a few mT ranges could modulate hemodynamics and/or blood pressure (BP) under pharmacologically modified state using mammalian models [Okano et al., 1999; Xu et al., 2000; Okano and Ohkubo 2001]. Nevertheless, the possible anti-pressor mechanisms by SMFs on genetically hypertension remain unknown. To evaluate the hypothesized anti-pressor effects of SMFs in a genetically hypertensive animal, spontaneously hypertensive rat (SHR), we measured the BP and heart rate, and determined the values of their hormones and enzymes in the blood, possibly related with hypertension, with or without SMF exposure. The present study is designed to evaluate relatively weak intensities with spatial gradients of SMFs for a genetically hypertensive animal, and to show how weak SMFs can alter BP itself and BP-related biochemical substances.
METHODS: Effects of SMFs on development of hypertension were investigated using young male stroke-resistant SHRs beginning at 7 weeks of age. SHRs were randomly assigned to two different exposure groups or an unexposed group. The SHRs in the exposure groups were externally and constantly exposed to two different types of magnetic plates ranging 3.0–10.0 mT or 8.0–25.0 mT for 12 weeks. The SMFs were generated from permanent magnetic plates attached to the rat cage. The blood pressure of each rat was determined at weekly intervals using indirect tail-cuff method. Hormones and enzymes in blood, possibly related with hypertension, were monitored at the experiment period of 5 weeks in 12-week-old rats.
RESULTS AND DISCUSSION: The SMFs suppressed the development of hypertension in both exposed groups to a statistically significant greater extent for several weeks as compared with an unexposed group (Fig. 1). The anti-pressor effects were related with the extent of reduction in plasma levels of angiotensin II and aldosterone in the SHRs (Fig. 2). These results suggest that the SMFs of weak intensities with spatial gradients could be attributable to modulation of blood pressure via humoral regulation systems.
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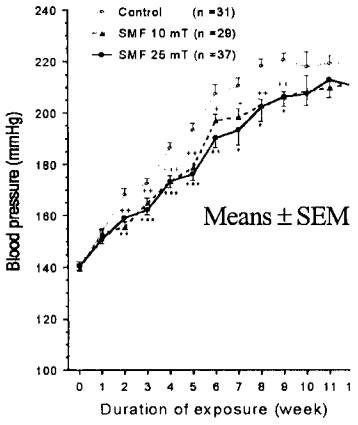


Fig. 1. Changes of systolic blood pressure with or without exposure to SMF for 12 weeks in SHR (7–19-week-old)

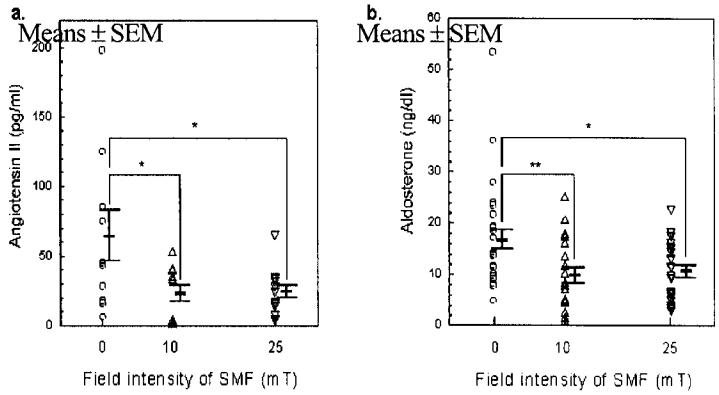


Fig. 2. Plasma values of angiotensin II (a) and aldosterone (b), with or without exposure to SMF, at the period of 5 weeks in SHR (12-week-old). *, P<0.05; **, P<0.01

25 mT vs. control, *, *P*<0.05; **, *P*<0.01; ***, *P*<0.01
10 mT vs. control, +, *P*<0.05; ++, *P*<0.01; +++, *P*<0.001

5-3

DELTA 1 AND KAPPA-OPIOID RECEPTOR SUBTYPES INVOLVED IN THE HYPOALGESIC EFFECT OF MILLIMETER WAVE TREATMENT. O. Gordiienko*, A. Radzievsky, A. Cowan*, A. Radzievsky Jr.*, M.C. Ziskin. Richard J. Fox Center for Biomedical Physics, Temple University Medical School, Philadelphia, Pennsylvania 19140, USA.

Pain relief following Millimeter Wave Treatment (MWT) has been reported by many clinicians, and quantitatively evaluated in our previous experimental studies. We have also discovered that MWT-induced hypoalgesia can be abolished by the pretreatment with nonspecific opioid blocker naloxone, which indicated the system of endogenous opioids as the possible target of MWT. However, there are three types and several subtypes of opioid receptors that have distinguishable functional and structural characteristics, and it is unclear exactly what types of these receptors are involved in the process of hypoalgesia development after exposure to MW. Thus, the main aim of the present study was to evaluate whether pretreatment with highly selective delta 1 or kappa opioid receptor blockers may influence the development of the MWT-induced hypoalgesia.

90 Swiss Webster mice were used. Millimeter waves were applied to the nose area of unanesthetized restrained mice. MW exposure parameters were: frequency = 61.22 GHz; incident power density = 15 mW/cm^2; duration = 15 min. The hypoalgesia was evaluated using the cold water Tail Flick Test (cTFT). The cTFT was performed on two consecutive days. The results of the first day were discarded ("training"). On the second day, the first test was recorded as a baseline. Following 15 min of MW or sham exposure, each mouse was tested four more times: immediately after exposure, and 15, 30 and 45 min after the exposure.

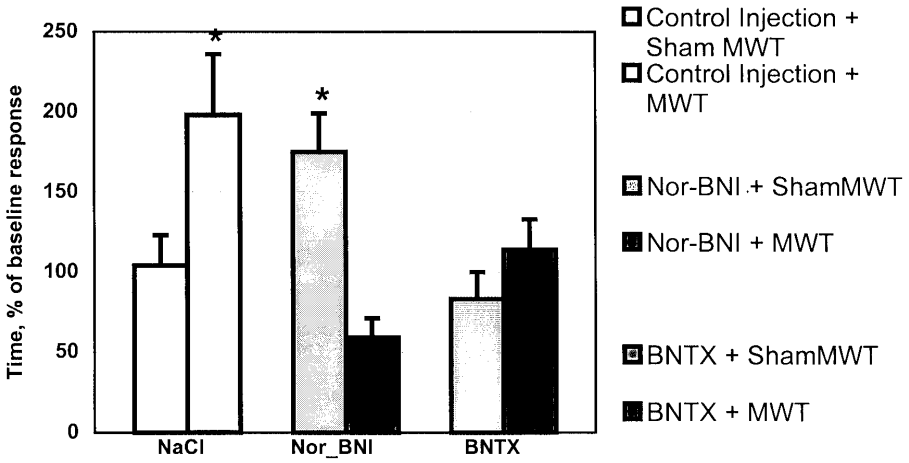


Fig.1. Hypoalgesic effect of a single MWT in mice pretreated with 0.9% NaCl (control injection), delta 1 opioid receptor blocker Nor-BNI, and kappa opioid receptor blocker BNTX. Each column represents the group mean + SEM. N ≥ 15 for each group. Asterisks indicate a statistically significant difference ($p<0.05$) of the groups in comparison with the sham-injected sham-exposed group.

Raw data were converted to percent of each mouse baseline response, and the average percent change was calculated for each animal. Post-treatment group means were compared to those of the sham group means using the Wilcoxon rank-sum test.

Norbinaltorphimine (Nor-BNI) was used as a selective antagonist for kappa opioid receptors. This agent was given subcutaneously at a dose of 20 mg/kg, 15-20 h before the mice were exposed to MW and run in the cTFT. 7-Benzylidenenaltrexone (BNTX) displays pharmacological selectivity for delta-1 opioid receptors and was also injected sc 15-20 h prior to the testing at a dose of 0.5 mg/kg. 0.9% NaCl solution was used for control injections.

The obtained data suggest (Fig. 1) that a single exposure of animals to MW results in a statistically significant hypoalgesia in the model of cold-induced pain in mice. An almost 100% increase of pain tolerance was observed after MWT in the cTFT in the group of control-injected animals. The hypoalgesic effect of MWT was completely abolished by the pretreatment with Nor-BNI or BNTX. At the same time, Nor-BNI injection itself has resulted in the appearance of the stress-induced hypoalgesia due to restraining, which was not present in the sham-injected and BNTX-injected groups.

The results of our experiments provide evidence that both types of opioid receptors, delta-1 and kappa receptors, are involved in the MWT-induced hypoalgesia. Whether the other types of opioid receptors are involved in the MWT-induced hypoalgesia, and what exact anatomical structures of the central neural system are implicated in the realization of the hypoalgesic effects of MWT, need to be determined further.

This work was sponsored by Richard J. Fox Foundation and NIH grant DHHS 1 R01 AT00493-01

5-4

INVESTIGATION OF A 1.6 GHZ WIRELESS COMMUNICATION SIGNAL FOR ITS TOXIC AND CARCINOGENIC POTENTIAL. L.B. Sasser, J.E. Morris, J.A. Creim*, B.W. Wilson, L.E. Anderson. Battelle, Richland, Washington 99352, USA.

The purpose of this study is to determine if long-term exposure to a 1.6 GHz radio-frequency (RF) field would affect the incidence of cancer in Fischer-344 rats. Timed-pregnant rats were procured and palpated for pregnancy status. Thirty-six rats were randomly assigned to each of three treatments. Two treatment groups were exposed to a far-field signal generated by a 400-watt amplifier controlled by a Motorola iridium source. A third group was sham exposed and an additional 42 animals were held as shelf-controls.

A horn irradiator was used to deliver the far field exposure (nominal SAR at 0.16 W/kg) and a similar non-energized horn was use for sham exposures. Whole body far-field exposures were initiated at 19 dg and continued for dams and pups after parturition until weaning (~ 23 days of age). Far-field exposures were for 2 hrs/day, 7 days/week. The offspring (720) of these dams became the subjects for the near-field treatments. Ninety males and 90 females were selected for each near-field treatment group as follows: (1) 1.6 W/kg, (2) 0.16 W/kg, and (3) near-field sham controls. Another 80 males and 80 females were held as shelf-controls. Near-field exposures (1.6 W/kg and 0.16 Watts/kg) were initiated when the offspring were 35±1 days of age for 2 hrs/day, 5 days/week until the rats were approximately 2 years old. The rats were confined, head first, in cylindrical tubes arranged in a carousel configuration with the RF antenna centered in the carousel. Statistically significant differences were not observed among treatment groups for number of live pups/liter, survival index at 4 days of age and at weaning, and the weaning weights (male and female) because of the far-field exposure. There were no obvious differences in clinical signs among the treatment groups after near-field exposure. Body weights of the near-field exposed male and female groups were not different from their respective sham exposed controls. However, beginning at 4 weeks and extending throughout most of the study, body weights of the cage control groups (male and female) were statistically greater than that of the near-field and sham exposed groups. The percent of animals surviving at the end of the near-field exposure were not different among the male groups. However, for the females a significant decrease in survival time for the cage control group compared to the near-field and sham control groups was observed. In addition survival time of one treated group (either near-field exposed or sham group, code not yet broken) was greater than that of the other two treatment groups.
Work supported by Motorola Corporation, Ft. Lauderdale, FL.

5-5

SUB-CHRONIC EFFECTS OF LOCAL EXPOSURE TO RADIO-FREQUENCY ELECTRO-MAGNETIC FIELDS ON THE CEREBRAL MICROCIRCULATION IN RATS. H. Masuda[*1], A. Ushiyama[*1], K. Wake[*2], S. Watanabe[*2], M. Taki[*3] and C. Ohkubo[1]. [1]Department of Physiological Hygiene, National Institute of Public Health, Tokyo 108-8638, Japan. [2]Electromagnetic Compatibility Research Section, Communications Research Laboratory, Tokyo 184-8975, Japan. [3]Department of Electrical Engineering, Graduate School of Engineering, Tokyo Metropolitan University, Tokyo 192-0397, Japan.

OBJECTIVE: A closed cranial window (CW) method enabled us to observe the cerebral microcirculation including blood-brain barrier (BBB) function, plasma velocities and leukocyte behavior in the rat for several months. Our previous results of acute exposure effects of radio-frequency electromagnetic fields(EMF) on the cerebral microcirculation showed that no noticeable changes have occurred due to EMF exposure[1)]. The present study aims to evaluate the sub-chronic exposure effects of EMF on the cerebral microcirculation in rats.
METHOD: Thirty-six male Sprague-Dawley rats (B.W. 516±10 g) having CW were divided into three groups, i.e., EMF group as rats exposed to EMF, Sham group as rats sham-exposed to EMF, and Control group as cage control rats. Intravital-microscopic study was performed under anesthetic conditions. EMF exposure system consisted of a small anechoic chamber with a monopole antenna in the center. The each head of 8 rats was positioned toward the central antenna and was locally exposed to 1,439MHz electromagnetic near-field of a simulated TDMA signal for PDC system. The intensity of EMF exposure was at 4W/kg in brain average SAR (brain peak SAR= 6.2W/kg, whole body average SAR= 0.91W/kg). The EMF exposure was daily operated for 60 minutes. The exposure was performed 5 times per week, for a period of 4 weeks. Microcirculation were measured before EMF exposure(0 week) and at the 2^{nd} and 4^{th} week of the experimental period, respectively.
RESULTS: The infection of pia mater, the regeneration of dura mater and the serpiginous change of blood vessel did not notice in any group throughout the experimental period(Figure 1). The maxmal plasma velocity of the pial venule of each group remained unchanged during 4 weeks. No significant differences

were recognized between the values from EMF group and Sham group in the number of rolling leukocytes as well as of sticking leukocytes. No extravasation of two kinds of fluorescence dyes, FITC-Dx (MW: 250,000) and sodium-fluorescein (MW: 376), from the pial venule was noticed in any groups (Figure 2).
CONCLUSION: These results suggested that no noticeable changes in the cerebral microcirculation including BBB function occurred due to our sub-chronic exposure conditions (The study was supported by funding from Ministry of Public Management, Home Affairs, Posts and Telecommunications , Japan.)
Reference.
H. Masuda, K. Wake, S. Watanabe, M. Taki, C. Ohkubo: Acute effects of local exposure to radio-frequency electromagnetic fields on the cerebral microcirculation in rats. BEMS 2001, P-51, p139-140, 2001

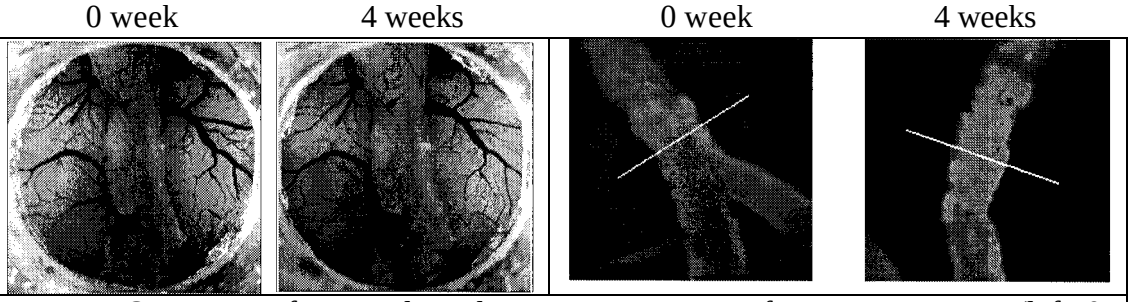


Figure 1. Overviews of a cranial window in EMF group. Before EMF exposure (left; 0 week), 4 weeks after FMF exposure(right; 4 weeks)

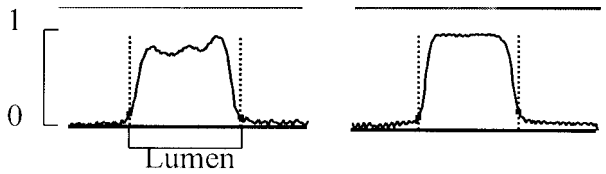


Figure 2. Intravasucular images of sodium-fluorescein in pial venules in EMF group

5-6

EFFECTS OF MAGNETIC FIELD EXPOSURE IN THE DMBA MODEL OF BREAST CANCER IN DIFFERENT SUBSTRAINS OF SPRAGUE-DAWLEY RATS. M. Fedrowitz*, W. Löscher. Department of Pharmacology, Toxicology, and Pharmacy, School of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany.

INTRODUCTION: In line with the possible relationship between electric power and breast cancer risk as well as the underlying "melatonin hypothesis", we have shown previously that 50-Hz magnetic fields (MFs) at µT-flux densities enhance mammary gland tumor development and growth in the 7,12-dimethylbenz[a]anthracene (DMBA) model of breast cancer in female Sprague-Dawley (SD) rats (cf., Thun-Battersby et al, *Cancer Res.* 59, 3627-3633, 1999). However, in contrast to our data, in a similar study conducted by Battelle in the United States, no evidence for a cocarcinogenic or tumor-promoting effect of MF exposure was found in the DMBA model in SD rats (Anderson et al., *Carcinogenesis* 20, 1615-1620, 1999). The investigators from the two studies recently discussed differences between their studies that might explain the apparent discrepancies between the results of MF exposure (Anderson et al., *Environ. Health Perspect.* 108, 797-802, 2000). The probably most important difference was the use of different substrains of SD rats; the U.S. rats were much more susceptible to DMBA but possibly less sensitive to MF than the European rats used in our studies. It has been demonstrated previously that there are inherent differences between substrains of SD outbred rats obtained in the U.S. and Europe in regard to their mammary neoplastic response to DMBA, as well as in their response to radiation (van Zwieten et al., *Eur. J. Cancer Clin. Oncol.* 20, 1199-1204, 1984).

OBJECTIVES: The goal of this work was to compare MF effects in the DMBA model in different substrains of female SD rats in order to evaluate whether the different results from our studies and the Battelle studies can be explained by the different SD substrains used.
METHODS: Two different SD substrains were obtained from Charles River. One (SD 1) was the substrain used in our previous MF/DMBA studies, the other (SD2) had never been used by us and was considered by the breeder to be genetically different from the SD 1 substrain. Preliminary experiments by our group indicated that SD2 rats are insensitive to the cell proliferation-enhancing effect of MF exposure recently reported by us for the mammary gland of SD 1 rats, which is a likely explanation for the cocarcinogenic or tumor-promoting activity of MF exposure in SD1 rats (Fedrowitz et al., *Cancer Res.* 62, 2002, in press). In the present study, two experiments were performed. In a first experiment, the two substrains (20 rats per substrain) were compared in their carcinogenic response to DMBA. DMBA (5 mg) was administered by gavage at weekly intervals up to a total of 4 applications per rat. Rats were about 50-54 d of age at onset of the experiment. Rats were palpated once weekly to assess the development of mammary tumors. After 18 weeks, all of the rats were killed for necropsy. In a second experiment, the effect of MF exposure on breast cancer development and growth was compared in the two substrains. Per substrain, two groups of 45 rats received DMBA at the dosing protocol described above and were either MF exposed or sham exposed for 18 weeks. Palpation of mammary tumors and necropsy for histological verification of grossly recorded tumors was done as in the first experiment.
RESULTS: In the first experiment, SD2 rats showed a significantly higher tumor incidence in the DMBA model than SD1 rats, substantiating the genetic difference between these substrains. In the second experiment, MF exposure significantly increased mammary tumor development and growth in SD1 but not SD2 rats.
CONCLUSIONS: The difference in the mammary neoplastic response of the SD 1 and SD2 substrains to DMBA substantiates previous studies that SD substrains may markedly differ in their sensitivity to this carcinogen (van Zwieten et al., 1984). Furthermore, as previously reported for ionizing radiation (van Zwieten et al., 1984), the present data demonstrate that SD substrains may differ in their response to MF exposure. SD2 rats resemble the SD substrain used in the Battelle studies in that these rats exhibit a high susceptibility to DMBA but not to MF, whereas the reverse is true for the MF-sensitive SDI substrain. These substrains can thus serve to evaluate which genetic factors underlie enhanced sensitivity to cocarcinogenic or tumor promoting effects of MF exposure.
This work is funded by the Deutsche Forschungsgemeinschaft (Bonn, Germany).

SESSION 6: IN VIVO STUDIES HUMAN
Chairs: Alex Thomas and Rene De Seze

6-1

HEATING OF HUMAN SKIN BY MILLIMETER WAVES: MODELING AND EXPERIMENTAL MEASURE-MENTS. S.I. Alekseev, A.A. Radzievsky, M.C. Ziskin. Richard J. Fox Center for Biomedical Physics, Temple University Medical School, Philadelphia, Pennsylvania 19140, USA.

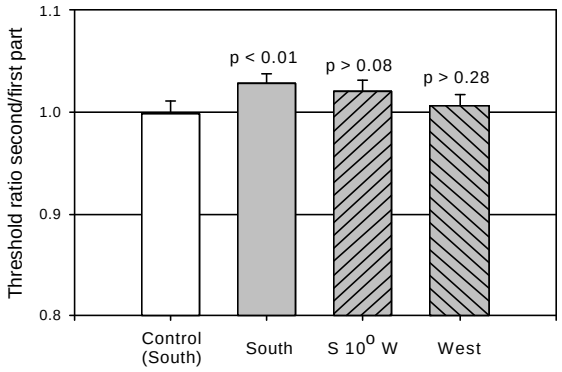
OBJECTIVES: Low intensity mm-wave exposure of the skin during mm-wave therapy and laboratory experiments is always accompanied by a small amount of heating. The latter may produce thermal effects on skin structures sensitive to temperature changes. The rate of temperature rise during irradiation is also used for determination of mm-wave absorption in the skin, i.e. dosimetry. The specific aims of this study were to measure temperature rise rates and steady-state temperature distributions on the skin surface during mm-wave irradiation and to calculate temperature profiles within the skin using models satisfying experimental data.

METHODS: Heating patterns and temperature rise rates on the surface of the human forearm skin during 75 GHz mm-wave exposures were measured with a thin (0.1 mm) thermocouple or with an infrared camera. In our calculations of temperature distributions we applied the bio-heat transfer equation to two models: 1) homogeneous skin model and 2) multilayer model consisting of three layers: epidermis and dermis, subcutaneous fat, and muscle.
RESULTS: Analysis of the transient solution of the equations showed that within the first 60 s of mm-wave exposure, the temperature rise was mostly dependent on thermal conductivity rather than on blood flow rate in the skin. At known intensities of mm-wave exposure we were able to find the thermal conductivity value satisfying experimental temperature kinetics and to use it for further calculation of the steady-state temperature rise. It was found that at low intensities (0-30 mW/cm^2) the steady state temperature on the surface of the skin was proportional to the incident power density. The calculated steady-state temperature values were strongly dependent on the blood flow rate and agreed fairly well with predictions using literature values of blood flow rates for skin, subcutaneous fat and muscle. The maximum temperature rise produced by exposure was located not at the skin surface but inside the skin. By conduction, mm-wave exposure could heat much deeper layers of the skin than the penetration depth. The temperature rise in the skin depended on the fat thickness. At a fat thickness between 2-4 mm, both models gave similar results. For dosimetry, i.e. for SAR calculation, it is very important to measure accurately the initial temperature rise rates during exposure. Our calculations indicated that the initial temperature rise rates were very non-linear and measurements of $dT/dt|_{t=0}$, using thermocouple or infrared camera were inaccurate. As was shown in real measurements, the problem became even more complicated due to the noise interference with small temperature rises. To solve this problem, we suggest finding initial temperature rise rates by fitting theoretical kinetics to experimental ones or using correction factors for thermocouple and infrared camera measurements.
CONCLUSIONS: Millimeter waves may produce thermal effects on cells, nerve endings, and other structures located in the epidermis and in the deep dermis beyond the penetration depth. The obtained data may be useful for studies of mechanisms of mm-wave effects and dosimetry.
This work was sponsored by Richard J. Fox Foundation.

6-2

THE HUMAN VISUAL DIFFERENTIAL THRESHOLD IS INFLUENCED BY THE GEOMAGNETIC FIELD. F. Thoss[1], B. Bartsch[1*], D. Tellschaft[1*], M. Thoss[2*]. [1]University of Leipzig, Carl Ludwig Institute of Physiology, 04103 Leipzig, Germany. [2]Technical University of Munich, Theoretical Chemistry, 85747 Garching, Germany.

OBJECTIVE: The geomagnetic field is used by many animals for their navigation. One of the underlying mechanisms is possibly an influence of the field on the yield of radical pairs in the triplet state, generated by the interaction of light with photopigment molecules. This radical-pair mechanism could explain the so-called inclination compass of some birds. It should influence the visual sensitivity as well. Therefore we were looking for an influence of the direction of view related to the direction of the geomagnetic field on the visual sensitivity in humans.



METHODS: The subjects were positioned in front of an illuminated screen. In the center of the screen, exactly in South direction, a small test stimulus was presented for always 0.5 s in intervals of 1 s. It started from a subthreshold value and increased in brightness step by step. At the threshold the subject pressed a key, the actual value was stored, and the procedure started again. This way the course of the threshold was recorded for twice 15 min. The experiment was

repeated on a second day. At one of both days during the second 15 min the vertical component of the geomagnetic field was compensated by the field of a pair of Helmholtz coils (field experiment). Then the remaining horizontal component was in the plain of view. The field experiment was done again with the view line to South 10 degrees to the West and to the West. From the records the mean differential thresholds were calculated and compared with each other. For this we calculated the ratio 'mean threshold of the second part of the experiment (in the field experiments under the influence of the field) divided by mean threshold of the first part of the experiment' (threshold ratio).

RESULTS AND DISCUSSION: The threshold ratio for the control experiment yields 0.999 and is not different from 1. This shows that the experimental conditions are nearly constant during the whole experiment. A significant difference from 1 must be due to additional influences as the change of the magnetic field. With the view line to the South the mean threshold for the second part of the experiment (under the field's influence) is increased by 2.8% (threshold ratio 1.028). This increase is significant with $p<0.01$. With the view line to South 10 degrees to the West the increase is 2% with $p>0.08$. For the view to the West the increase is only 0.6% with $p>0.28$.

Our results correspond with the predictions of the theorists, if we compare the visual threshold with the calculated yield of triplet molecules. During the second part of our field experiments we changed direction and strength of the geomagnetic field. For the view to the South both effects lower the yield. With the change of the view line to the West the influence of both effects decreases.

6-3

A REVIEW OF RADIOFREQUENCY BIOEFFECTS STUDIES RELEVANT TO THE CHILDREN'S ISSUE. J.A. Elder. Motorola Florida Research Labs, 8000 W. Sunrise Blvd., Fort Lauderdale, Florida 33322, USA.

INTRODUCTION: The report on "Mobile Phones and Health" (2000) concluded that children may be more vulnerable to "currently unrecognized health effects from the use of mobile phones." The child's developing nervous system and a longer lifetime of exposure were two reasons for this conclusion. The radiofrequency (RF) literature contains a number of reports addressing the "children's issue," that is, studies of laboratory animals, including nonhuman primates, exposed during periods of nervous system development and studies of lifetime exposure. The results of these studies provide information to assess potential health effects of mobile telephony emissions on children.

OBJECTIVE: The purpose of this presentation is to review the RF studies that included exposure of animals during early life to young adulthood. This review is an attempt to identify those studies pertinent to an assessment of the effects of RF radiation on the developing nervous system of children. An important aspect of this review is a comparison of brain development in animals and humans. Rodents, for example, have considerable brain development postnatally whereas humans have more brain development prenatally.

Discussion: Examples of studies included in this review are those of rodents exposed to RF radiation in which developmental endpoints such as body and organ weight, including brain weight, were measured. Other studies investigated morphological changes in brain cells, e.g., Purkinje cells in the cerebellum, in exposed rodents and nonhuman primates. Kaplan et al. (1982) exposed squirrel monkeys to three dose rates of RF radiation beginning the second trimester of pregnancy. Mothers and offspring were exposed for an additional 6 months after parturition and the offspring were exposed for another 6 months. There were no significant changes in growth rate, EEG and behavioral development in exposed offspring. Adey et al. (2000) simulated a life-long exposure to the head at a mobile phone frequency by exposing rats for about two years, beginning with exposure of fetal and preweanling rats. No effect was found on survival or number, incidence, or histological type of brain tumors. In another chronic exposure experiment, the head of rats was irradiated at a mobile phone frequency for 22 months beginning at 2 months of age; there was no effect on neoplasia in any tissue including no evidence of promotion of cranial, spinal nerve or spinal cord tumors (Zook and Simmens, 2001).

CONCLUSIONS: A review of the RF bioeffects literature revealed a substantial amount of information that can be used to make an informed decision on the "children's issue" by extrapolating the results of laboratory animal studies to exposure scenarios involving children. The results of the literature cited above provide examples that do not support the conclusion in "Mobile Phones and Health" regarding use of mobile phones by children. These results should be evaluated before considering further research needs.

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6-4

HUMAN THERMOPHSIOLOGICAL RESPONSES TO WHOLE-BODY RF EXPOSURE (100 MHz CW) REGULATE THE BODY TEMPER-ATURE EFFICIENTLY. E.R. Adair[1], K.S. Mylacraine[2], S.J. Allen[2]. [1]Air Force Research Laboratory, Human Effectiveness Directorate, Directed Energy Bioeffects Division, Brooks AFB, Tx; [2]Veridan Engineering, Inc., San Antonio, TX, USA.

INTRODUCTION: We have reported on a series of laboratory studies [Adair, et al., 1998, 1999, 2000, 2001a, 2001b] in which human volunteers were exposed for 45 min to CW RF energy (at 2 to 4 peak SARs) in three controlled thermal environments (T_a = 24, 28 and 31°C). The frequencies explored (450 and 2450 MHz) deposited RF energy close to the skin surface, resulting in SAR-dependent increases in skin temperatures but no significant change in core temperature because of efficient mobilization of autonomic heat loss responses (skin blood flow and sweating).

OBJECTIVE: To determine thermoregulatory efficiency during whole-body RF exposure of seated human volunteers at the resonant frequency, 100 MHz CW, with maximal energy penetration.

METHODS: Experimental tests were conducted inside a 22' x 22' x 32' electrically-shielded anechoic chamber lined with 6' pyramidal Eccosorb™. The maximal output power of the custom built VHF high power RF source was 1.2 kW at 100 MHz in the CW mode and the whole-body SAR was found to be 0.068 (W/kg)/(mW/cm²) [cf. Adair, et al., 2001c for details]. Subjects were seated in the far field of a dipole antenna and were irradiated dorsally. Our standardized protocol (30-min baseline, 45-min RF or sham exposure, 10-min baseline) was again used and the thermophysiological responses measured (esophageal and 6 skin temperatures, metabolic heat production, local sweat rate, and local skin blood flow) were the same as in all previous studies. Because FD-TD modeling of a seated human indicated high energy deposition in the ankle at 100 MHz, ankle skin temperature was also measured. Each of 7 volunteers (6 males, 1 female; age range 31 – 74 yr) underwent 12 test sessions in which 3 power densities (PD = 4, 6, and 8 mW/cm²) were presented in each of 3 ambient temperatures (T_a = 24, 28, and 31 °C) plus T_a controls (no RF). All physiological data were collected either continuously or sampled once per minute during each 85-min test session.

RESULTS: As was the case with earlier studies at 450 and 2450 MHz, no change in metabolic heat production occurred under any exposure condition at 100 MHz. Unlike the results of human exposure at those supra-resonant frequencies, local skin temperatures, even those on the back that were irradiated directly, changed little or not at all during 100 MHz exposure. The sole exception was the temperature of ankle skin, which increased by as much as 4 °C in some subjects (PD = 8 mW/cm²). Analysis of the change in esophageal temperature, from the end of the baseline to the end of the 45-min RF exposure period, showed modest changes (range = -0.15 – 0.13 °C). The largest increase in T_{esoph} occurred in subjects whose baseline core temperature was low (~36.0 °C) and whose sweating response was minimal. In no case did T_{esoph} of any subject exceed 37.2 °C at the end of the RF exposure period; indeed, T_{esoph} often decreased below the baseline level, especially at T_a = 31 °C, as a result of profuse sweating.

CONCLUSIONS: The thermophysiological responses of human volunteers during whole-body 45-min exposure to 100 MHz CW are different from those responses during exposure at higher RF frequencies. Little change was measured in core temperature (T_{esoph}), metabolic heat production, or most skin temperatures, even at power densities 8 times the IEEE C95.1 standard at this frequency. Thermoregulation was principally controlled by appropriate changes in evaporative heat loss (sweating) and, to a lesser extent, by changes in skin blood flow. These changes must have been stimulated by thermal receptors deep in the body rather than those located in the skin.

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5. Adair, et al., *Bioelectromagnetics*, 22:429, 2001b;
6. Adair, et al., BEMS Abstract, 23rd Annual Meeting, 112, 2001c.

6-5

OCCUPATIONAL MAGNETIC FIELD EXPOSURE AND MELATONIN: INTERACTION WITH NIGHT-AT-LIGHT. J. Juutilainen. Department of Environmental Sciences, University of Kuopio, 70211 Kuopio, Finland.

BACKGROUND: We have recently reported interesting effects of 50-Hz magnetic fields (MF) on the urinary excretion of 6-hydroxy melatonin sulfate (6-OHMS) in CD_2F_1 mice (Kumlin et al., 2000). No natural light-regulated diurnal melatonin rhythm is seen in this mouse strain, but a consistent and statistically significant day-night difference (higher levels at night) appeared in animals continuously exposed to a 100-µT MF. A possible interpretation of the finding is that MF exposure increases sensitivity of the pineal gland to light (in this mouse strain normally insensitive to diurnal light variations, the increased sensitivity would strengthen the natural melatonin rhythm).

OBJECTIVE: To test the hypothesis that MF exposure increases sensitivity to light also in humans, we reanalyzed data from a study on 6-OHMS excretion in women occupationally exposed (in a garment factory) to MFs (Juutilainen et al., 2000)

METHODS: Data from the questionnaire used by Juutilainen et al. (2000) were used for dichotomous classification of the subjects with respect to exposure to light-at-night. The questionnaire had direct questions on use of light at night in the bedroom, lamps outside the bedroom window, and dark or light curtains in the bedroom. The four-category MF exposure data from Juutilainen et al. (2000) was dichotomized (exposure or no exposure). One-way analysis of variance (ANOVA) of logarithmically transformed data and Tukey's multiple comparison test were used for statistical analysis.

RESULTS AND DISCUSSION: The lowest excretion of 6-OHMS was observed in the group of women who were exposed to both MF and light-at-night (Table 1). According to ANOVA, the differences between the four groups were significant ($p<0.0001$). According to Tukey's test, the group exposed to both MF and night-at-light was significantly different from all other groups. The effect of MF exposure was also significant among the women who had no light exposure at night. The low number of subjects who were exposed to both MF and light-at-night reduces the reliability of the data. The results support the hypothesis that daytime occupational exposure to MF enhances the effects of nighttime light exposure on melatonin production.

References.

Kumlin T, Heikkinen P, Laitinen J, Juutilainen J (2000) BEMS Twenty-Second Annual meeting, Abstract Book, 282.

Juutilainen J, Stevens RG, Anderson LE, Hansen NH, Kilpeläinen M, Kumlin T, Laitinen

JT, Sobel E, Wilson BW (2000) J Pineal Res 28:97-104.

Table 1: Nocturnal excretion of 6-OHMS into urine in women exposed to light-at-night (LAN) at home, and to low frequency magnetic fields (MF) at work.

	No LAN, no MF	No LAN, MF	LAN, no MF	LAN and MF
Number of subjects	16	32	4	8
Mean 6-OHMS, µg	9.7	5.9	9.7	2.6
SEM	1.0	0.72	2.9	0.96

6-6

GROSS PHYSIOLOGICAL MEASUREMENTS DO NOT CORRELATE SIGNIFICANTLY TO PERIPHERAL NERVE STIMULATION THRESHOLDS EXHIBITED BY HUMANS DURING MAGNETIC RESONANCE IMAGING. B.A. Chronik. Dept. of Electrical Engineering, Stanford University, Stanford, California 94305, USA.

OBJECTIVE: To determine if a simple physiological measurement, such as subject weight or local fat layer thickness, correlates significantly with peripheral nerve stimulation (PNS) thresholds exhibited by humans exposed to the switched gradient magnetic fields used during high-speed magnetic resonance imaging (MR). Presently, the onset of PNS is the dominant limitation to the application of stronger, faster gradient fields during MRI and therefore a better understanding of this phenomenon is required in order to achieve further increases in MR imaging speed and resolution. A significant correlation to a gross physiological measurement would indicate the possibility of implementing subject-specific operating limits for MRI gradient coils and would thereby allow significant increases in imaging speed for most subjects.
METHODS: Twenty-one normal subjects (16M, 5F) volunteered for this study. All experiments were conducted using an actively shielded, whole-body gradient coil system in XY oblique mode (CRM gradient subsystem, Signa CV/I, GE Medical Systems) capable of XY gradient strengths of 56mT/m. Subjects were placed in the MRI scanner supine and feet first and the subject position within the scanner resulting in maximum stimulation was found on a per-subject basis. An oscillating 64 lobe trapezoidal magnetic field waveform was applied. Threshold curves (threshold gradient strength, ΔG, required to cause stimulation as a function of the gradient magnetic field switching time,) were measured using methods described previously (1). Tissue chronaxie times (τc) were extracted from the threshold curves by taking the ratio of the curve intercept ($\Delta Gmin$) to the slope *(SRmin)*. For each subject, the following measurements were made: weight (W), body fat percentage (F%), fat layer thickness *(Fthk)*, effective radius of coronal profile *(Rcor)*, and effective radius of the axial profile (Rax). F% was measured using fat calipers and a three-point skin fold method. *Fthk* was measured directly from axial anatomie MR images of the site of stimulation (as in Fig. 1), with the thickness of the fat layer immediately beneath the skin at the site of stimulation used. Both *Rcor* and Rax were measured from subject physical dimensions. Correlation between parameters was tested by calculating the correlation coefficient and associated p-value.
RESULTS AND DISCUSSION: The mean threshold curve is shown in Fig. 2 along with the system maximum performance curve. The results of the measurements are as follows (standard error in brackets): *SRmin* = 50.1(3.1)mT/m/ms, ΔG*min* = 48.5(2.7)mT/m, τc = 1054(109) µs. These values are consistent with those from a similar study on an identical system(1). We found no convincing correlation between any physiological parameter and threshold parameter in the study. The most significant correlation (-0.33, p=0.05) was found between Rax and Gmin; however, any two parameter model fit to the dependency of these parameters resulted in an R^2 value of no better than 0.1. These results are consistent with a previous study that also noted a lack of dependence of thresholds on body weight(2). Significant correlation between thresholds and body size has been reported in electrostimulation studies(3) and this fact was partial motivation for this study. It was reported that larger subjects tended to have higher thresholds and that when parameterized by weight, the threshold level varied approximately as weight to the 2/3 power. The results of

the present study might be explained by the fact that for a given cylindrical gradient coil, the electric field increases with increasing body size(4). If the results of (3) are applicable to the sensitivity of nerves during magnetostimulation, then these two effects could be canceling each other out, resulting in thresholds independent of body size as reported here. Because of the relatively low number of subjects use in this study, it is possible that a weak correlation has gone undetected; however, the use of a physiological measure to guide operating levels for gradient systems would require the presence of a strong and direct relation that would be detectable with 20 subjects. The underlying reason for the differences in subject thresholds for gradient coil magnetostimulation remains an important and open question.

BAC was supported by NSERC of Canada through a post-doctoral fellowship.

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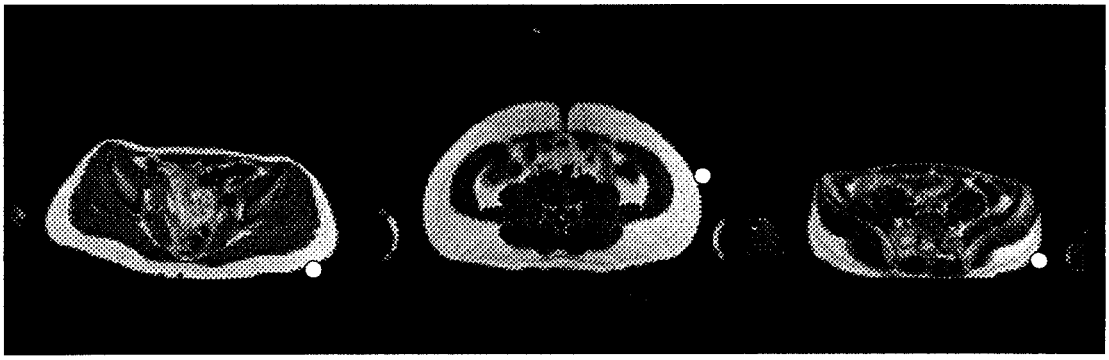

Figure 1: Axial images of stimulation sites for three of the twenty-one subjects. All images above are through the pelvic region (the middle image is at the level of the umbilicus). The reported site of stimulation in each case is noted by the circle on the image. Note the significant anatomic differences, specifically in the thickness and distribution of the fat layer.

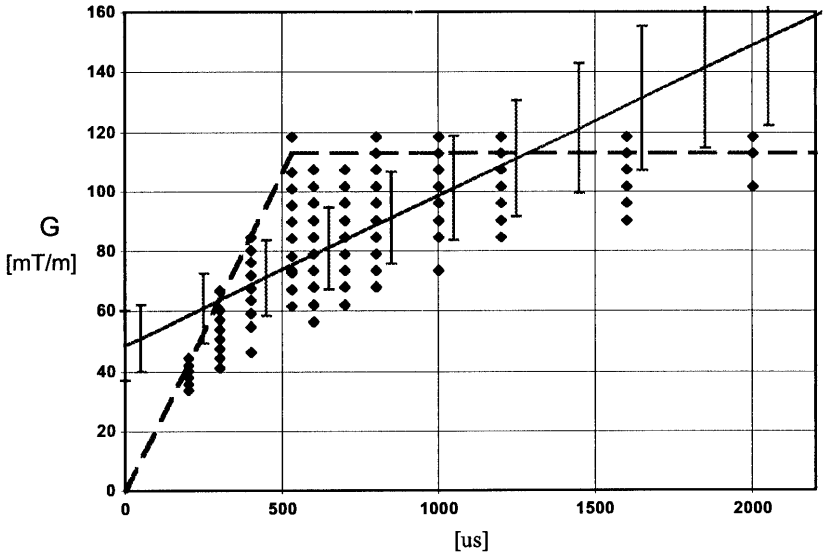

Figure 2: Mean threshold curve and error bars (± standard deviation). Dots indicate individual subject threshold measurements. Dashed line indicates performance limit of system under investigation. Dots above limit denote that a subject did not stimulate at that value.

PLENARY SESSION II:
PAIN - ETIOLOGY AND TREATMENT
Chair: Michael McLean

NEURAL SUBSTRATES OF PAIN PERCEPTION IN HUMANS STUDIED USING FMRI. R.S. Menon[1], J.S. Gati[1], A. Ploghaus[2], I. Tracey[2], S. Clare[2], J.N. Rawlins[2], P.M. Matthews[2]. [1]Laboratory for Functional MR Research, The John P. Robarts Research Institute, London, Ontario, N6A 5K8, Canada. [2]Centre for Functional MRI of the Brain, University of Oxford, Oxford OX3 9DU, UK.

OBJECTIVE: To identify the neural substrates for pain processing in humans, to correlate their activity with behavioural measures and to study potential targets for pain modulation using functional magnetic resonance imaging (fMRI).
METHODS: Functional MRI was used to study the response of normal volunteers to a graded heat stimulus, provided by a special Peltier cell in the magnet. fMRI was performed using whole brain echo-planar imaging on a 4 Tesla Varian Unity Inova whole body scanner with Siemens Sonata gradients operating at SR200 and 40 mT/m. Twelve subjects who gave informed consent were studied. In one series of experiments, pain thresholds for each subject were determined and the neural substrates identifying the areas involved in pain processing were determined. In a second set of experiments, a mismatch between the actual and expected pain stimulus was created using contrasting-color LED cues. Lights cued the delivery of painful heat, nonpainful warmth or no stimulation, but the actual stimulus delivered could be congruent or noncongruent. In this manner the anticipation of pain and actual pain processing could be separated. The imaging data were analyzed using purpose designed software methods (1,2).
RESULTS AND DISCUSSION: Expectation of pain activated sites within the medial frontal lobe, insular cortex, and cerebellum distinct from, but close to, locations mediating pain experience itself. Our first set of experiments allowed this dissociation. Anticipation of pain can in its own right cause mood changes and behavioral adaptations that exacerbate the suffering experienced by chronic pain patients. Our findings from the first set of experiments also suggest that selective manipulations of activity at these sites may offer therapeutic possibilities for treating chronic pain (1). Associative learning is thought to depend on detecting mismatches between actual and expected experiences. When painful heat stimulation was unexpected as in our second set of experiments, there was increased fMRI signal intensity in areas of the hippocampus, superior frontal gyrus, cerebellum, and superior parietal gyrus that was not found with mismatch between expectation and delivery of nonpainful warmth stimulation. When painful heat stimulation was unexpectedly omitted, the FMRI signal intensity decreased in the left superior parietal gyrus and increased in the other regions. These contrasting activation patterns correspond to two different mismatch concepts in theories of associative learning (Rescorla-Wagner, temporal difference vs. Pearce-Hall, Mackintosh).
CONCLUSIONS: Our studies have identified areas in the brain that can exacerbate psychophysical pain perception in acute and chronic pain. Searching for interventions to specifically modulate activation of these brain regions therefore offers an approach to identifying new treatments for chronic pain, which often has a substantial anticipatory or associative learning components.
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The McDonnell-Pew Program in Cognitive Neurosciences, The Canadian Institutes of Health Research, The National Sciences and Engineering Research Council of Canada.

THE ROLE OF PAIN PERCEPTION IN THE ETIOLOGY OF CHRONIC PAIN AND THE CAUSATION OF SEX DIFFERENCES. S. Lautenbacher. Physiological Psychology, University of Bamberg, Markusplatz 3, 96045 Bamberg, Germany

Pain perception can nowadays be assessed precisely (a) by inducing pain experimentally through application of heat, pressure, current, chemical agents, etc. and (b) by using psychophysical methods of assessment. The brain networks activated under such circumstances have been investigated by use of PET and fMRI techniques. Converging evidence has suggested that the thalamus, the anterior cingulate cortex (ACC), the insula and the S1 as well as the S2 cortices contribute most to the occurrence of a perception of pain (Casey and Bushnell 2000). Changes in pain perception have been observed often in cases of chronic pain. In neuropathic pain there are characteristic regional changes of pain perception known as allodynia, primary and secondary hyperalgesia. In conditions with musculoskeletal pain there are frequently more generalized patterns of pain not confined to a single region. Generalized hyperalgesia has appeared to be a regular concomitant of fibromyalgia, tension-type headache, myofascial pain, temporomandibular pain disorder and of similar conditions (Rollman and Lautenbacher 2001). However, it is still not entirely known whether the generalized hyperalgesia is cause or effect of the chronic pain. But even in the latter case it may deteriorate the chronic pain. Interestingly, the generalized hyperalgesia is more readily detected by experimental use of pressure than by use of other physical stressors. Applying pressure on soft tissue activates muscle nociceptors better than other pain inductions techniques. Just this group of nociceptors has been found to be strongly influenced by the descending pain inhibitory control system. Consequently, pressure hyperalgesia and chronic musculoskeletal pain both may be consequences of a lack of pain inhibitory control (Rollman and Lautenbacher 2001). In favour of this hypothesis characteristic sex differences in pain perception have been observed. Women are a little more sensitive to experimental pain in general but to a substantial and reliable degree when pressure is used for pain induction. Are women consequently also more vulnerable to the development of chronic musculoskeletal pain? Yes, they are. A weaker descending pain inhibitory control in women might be the reason for sex differences both in pain perception and in the risk of developing chronic pain.

References.

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MAGNETIC FIELD BASED PAIN THERAPEUTICS AND DIAGNOSTICS. A.W. Thomas and F.S. Prato. Lawson Health Research Institute and Department of Nuclear Medicine &MR, St. Joseph's Health Care (London), London Ontario N6A 4V2; and Department of Medical Biophysics, University of Western Ontario.

The issue of pain treatment is an extremely urgent health and socio-economic problem. Pain, in acute, recurrent and chronic forms, is prevalent across age, cultural background, and sex, and costs North American adults an estimated $10,000 to $15,000 per person annually. Estimates of the cost of pain do not include the nearly 30,000 people that die in North America each year due to aspirin-induced gastric lesions. In Canada, 17% (3.9 million) of people over 15 years of age suffer from chronic pain that interferes with their normal daily activities [Canadian Consortium on Pain Mechanisms, Diagnosis, and Management, CIHR Opportunities Funding, 1999]. Studies suggest that at least 1 in 4 adults in North America is suffering from some form of pain at any given moment [Chronic Pain in America, Survey conducted for American Pain Society, American Academy of Pain Medicine, and Janssen Pharmaceutica, 1999; Speaking of Pain. Survey conducted for The Arthritis Foundation and Merck & Co., 2000]. This large population relies heavily upon the medical community for the provision of pharmacological treatment. Interestingly, many physicians are now referring chronic pain sufferers to non-pharmacologically based therapies, what are now

termed 'Complementary and Alternative Medicine', in order to reduce drug dependencies and/or side effects.
One of the most reproducible results of weak, extremely low frequency (ELF) magnetic field (MF) exposure is an effect upon nociceptive processing. This observation generated the design and implementation of a specific pulsed magnetic field to be used as a therapeutic agent for the treatment of chronic pain in humans. Recent evidence suggests that pulsed magnetic fields would also be an effective complement for treating patients suffering from acute pain. Recent studies also suggest that magnetic field treatments involving the manipulation of standing balance would be effective in the determination of the aetiology of chronic pain and hence be effective in the diagnosis of the underlying disease state.
Static magnetic field devices with strong gradients have also been shown to have therapeutic potential. Specifically placed static magnetic field devices, such as the Magnabloc™ device, have been shown to reduce neural action potentials *in vitro* and alleviate spinal mediated pain in human subjects.
Human studies involving the induction of analgesia, whether utilizing pharmacology or magnetic field treatments, also need to account for the placebo response, which may explain as much as 40% of the analgesia response. However, the placebo response, or at least the central nervous system mechanisms responsible for the placebo response, may be an appropriate target for magnetic field induced therapies. Magnetic field manipulation of cognitive and behavioral processes has been well-documented in animal behavior studies and subjective-measure studies involving human subjects.

SESSION 7: RADIOFREQUENCY DOSIMETRY I
Chairs: Niels Kuster and Mays Swicord

7-1

ABSORPTION RATES INSIDE HUMAN BODY DUE TO RADIATED ELECTROMAGNETIC FIELDS OF MULTI-BAND BASE STATION ANTENNAS. A. Bitz*, M. Alaydrus*, J. Streckert*, V.W. Hansen. Chair of Electromagnetic Theory, University of Wuppertal, D-42097 Wuppertal, Germany.

INTRODUCTION: For network planning of the various mobile communication systems (GSM, UMTS) only a limited number of sites for the installation of base station antennas is available. Therefore, multi-band base station antennas with different operating frequency ranges must be used. For safety assessment the radiated electromagnetic fields in every frequency range have to be considered additively. The small number of data published so far concerns calculations for base station antennas operated in a single frequency range. Procedures applied for these studies used the Finite-Difference Time-Domain (FDTD) method for the calculation of the antennas field distribution, which can lead to unreliable results, especially if there is a large interaction between human body model and current distribution of the antenna. Further, the published data are based on a human body model which is derived from the data set of the *Visible Human Project*. The body mass of this model is about 110 kg, whereas the CENELEC standard [1] recommends a minimum body mass of 42 kg for safety assessment of workers in front of base station antennas.
METHOD: In order to treat the related electromagnetic problem we developed a technique combining the well-established FDTD method for the evaluation of the specific absorption rates inside the human body and the Hybrid^2 method for the calculation of the antenna's near field including influences of the environment [2]. The advantage of the Hybrid^2 method is a more reliable antenna analysis as compared to the FDTD method. Further, the Hybrid^2 method is able to consider the reflection and transmission properties of real wall structures, field deformations by windows and corners and other objects, thus, effects of complex antenna environments can be taken into account. The field distribution obtained by the Hybrid^2 method is used as incident field for the human body. In order to apply the FDTD method, the human body model is

enclosed by a surface with equivalent electric and magnetic currents, which have to be determined in accordance to the incident field. These currents excite the fields inside the FDTD region.
Multi-band antennas can be simultaneously operated in different frequency ranges, e.g. GSM and UMTS frequency band. Since the exposure of multiple frequency electromagnetic fields can be additive in their effects, the exposure is determined by the superposition of all specific absorption rates (SAR) for the single frequency bands. In accordance to the ICNIRP Guidelines [3] following basic restrictions should be met in order to avoid unallowable exposure conditions: $\sum_i \frac{SAR_i}{SAR_{L,i}} \leq 1$, with SAR_i being the specific absorption rate at frequency i and $SAR_{L,i}$ the limit of the specific absorption rate at frequency i.

RESULTS: This presentation will show results for the determination of safety distances of multi-band base station antennas based on a new 42 kg model in comparison to results based on the commonly used 110 kg body model. The local and whole body SAR will be presented for both body models and multi-band antennas mounted in the neighbourhood of walls, windows, house edges etc. Safety distances will be evaluated in order to keep the SAR limits recommended by the ICNIRP guidelines.

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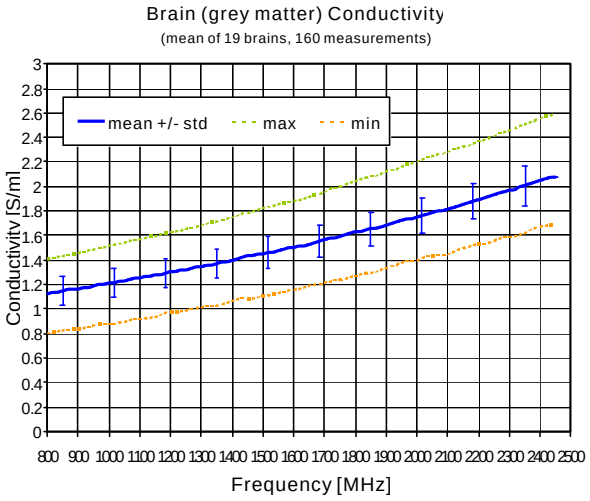
[3] ICNIRP (International Commission on Non-Ionizing Radiation Protection): "Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz)", Health Physics, vol. 74, pp. 494-522, 1998.

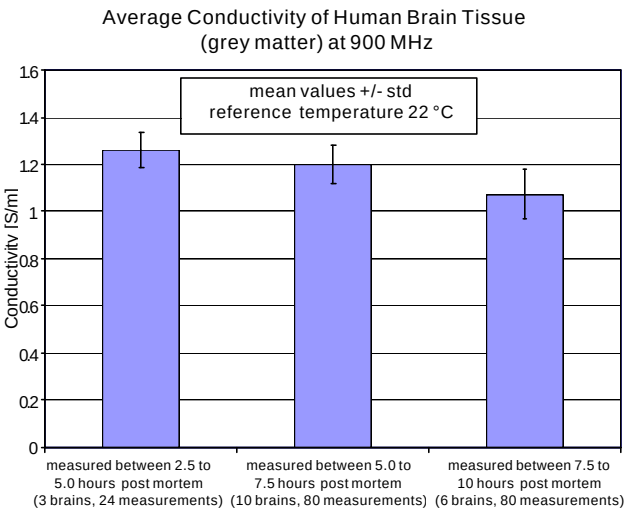
7-2

DIELECTRIC PROPERTIES (800 - 2,400 MHz) OF HUMAN BRAIN MEASURED LESS THAN 10 HOURS POST MORTEM. G. Schmid[1], G. Neubauer[1], P.R. Mazal*[2]. [1]ARC Seibersdorf Research, Austria, [2] Institute of Clinical Pathology, University of Vienna, Austria.

INTRODUCTION: A series of animal experiments indicated that the dielectric properties of brain tissue decrease within a few hours after death (1, 2). Measurements of the dielectric properties of human brain tissue reported so far were mainly performed on tissue samples more than 20 hours after death. We have measured dielectric properties at 800 – 2,400 MHz in 19 brains from humans within 3 – 10 hours after death.

METHODS: All measurements took place on the excised whole brain before the routine autopsy procedure. Relative permittivity as well as conductivity were measured at 4 well defined positions on the cortex of the temporal lobe at the right as well as at the left side. Based on autopsy results, brains of deceased patients with neurological diseases were excluded from the study. An open-ended coaxial probe (HP85070B) in combination with a vector network analyser (HP8722C) after standard air/short/water calibration was used as measurement system. The 19 samples consisted of 8 male and 11 female brains. The average age of the patients at the time of death was 70.3 years (min. 47.5 years, max. 81.8 years) and the measurements were performed at a mean post mortem time of 6.8 hours (min. 3 hours, max. 10 hours). The tissue temperatures during the measurements varied between different brains and were between 18 °C and 25 °C.





RESULTS: Figure 1 shows the mean and standard deviation of the measured electric conductivity in the frequency range 800 – 2,400 MHz, when taking into account all measured positions on all brains (160 different measurements). Figure 2 illustrates the dependence of conductivity on the time after death at 900 MHz. For this figure the 19 brains were divided into 3 groups corresponding to post mortem time when measurements were made. The mean value of conductivity at 900 MHz for each group was calculated from all measurement positions, and these mean values were normalised to a tissue temperature of 22 °C using a temperature coefficient of 1 %/°C.

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Neubauer et al., *23rd Annual BEMS Meeting*, St. Paul, Minnesota, USA, June 2001, Abstract Book p.41
The project was submitted to and accepted by the Ethic Commission of the General Hospital of Vienna and Medical Faculty of Vienna University, Austria.
This work is sponsored by MOTOROLA Inc., Fort Lauderdale, Florida, USA

7-3

FDTD DERIVED SAR DISTRIBUTIONS IN VARIOUS SIZE HUMAN HEAD MODELS EXPOSED TO SIMULATED CELLULAR TELEPHONE HANDSET TRANSMITTING 600 mW AT 835 MHz.

A.W. Guy[1], C.K. Chou[2], and G. Bit-Babik[2*]. [1]University of Washington (Emeritus), Seattle, WA 98195, USA. [2]Motorola Florida Research Laboratories, Fort Lauderdale, Florida 33322, USA.

INTRODUCTION: Gandhi et al. (1996) have shown higher peak SARs and deeper penetration in smaller head models exposed to cell phones. Schönborn et al. (1998) found no significant differences between adults and children. Gandhi and Kang (2001) reconfirmed their 1996 observations. Gandhi's results have been widely cited by the media and used by many as one of the reasons that children should minimize using cell phones. Gandhi et al. reduced the voxel sizes of an adult head by 27.8 and 57.5%, respectively to simulate 10 and five year old heads. Thus the SAR plots for the heads were based on voxel scale rather than a metric unit scale. This will cause two problems, (a) SAR averaged over smaller voxels will appear higher in high gradient regions due to improved resolution, (b) the penetration depth in terms of voxels will be greater for smaller heads even though the actual depth in metric units may be the same for all heads. This intensifies with decreasing voxel size, as the electric field is concentrated very close to the voxel corner-air interface so the averaged SAR in a corner pixel becomes increasingly larger with diminished voxel size. Our study was initiated to determine if the use of same size voxel for all heads would affect the results.
OBJECTIVES: To determine if peak SAR is higher and penetration depth is deeper in smaller heads.

METHODS: XFDTD® software (REMCOM version 5.3) was used to calculate the SAR patterns in three head models exposed to a 600 mW handset operating at 835 MHz. The GUI feature of the XFDTD® program was used to reproduce the FDTD handset and hand model used by Gandhi in a one-millimeter cubical cell mesh. The FDTD head model is based on the visible man model available from ftp.starview.brooks.af.mil. The 835 MHz permittivities and conductivities for the head tissues are from Gabriel. The handset, hand and head models were integrated into a 275x272x294-mm FDTD mesh (mesh size smaller for child heads) consisting of millimeter cubical voxels to best match the model used by Gandhi within the closest millimeter voxel. For this study we chose the worst-case exposure reported by Gandhi, a vertical handset with an attached $\lambda/4$ monopole antenna. The depth of penetration is based on the standard definition, the distance where the SAR decreases by e^2, and derived from linear regression.
RESULTS: Main results are summarized in the table (SAR in W/kg).

	Adult	10-year old	5-year old
Peak SAR in a voxel	7.66	9.78	7.97
1-g peak SAR in head	3.81	3.93	3.43
Penetration depth (cm)	4.58	5.14	4.77

CONCLUSIONS: There are small differences in the peak 1-g SARs (used for compliance limit) or depths of penetration for the three exposed heads. From these results, we conclude that the 1-g peak SAR and penetration depth in head models exposed to 835 MHz cell phones do not significantly differ in adult and child heads.
References.
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Schönborn F, Burkhardt M, and Kuster N, "Differences in Energy Absorption Between Heads of Adults and Children in the Near Field of Sources," *Health Physics*, 74(2): 160-168, 1998.
Gandhi OP, Kang G, "Effect of the head size on SAR for mobile telephones at 835 and 1900 MHz" *23rd BEMS Abstract Book*, p.52, 2001.

7-4

INDUCED ELECTRIC FIELDS IN RODENTS EXPOSED TO ELF MAGNETIC FIELDS. M.A. Stuchly, T.W. Dawson, K. Caputa. Dept. of Electrical & Computer Engineering, University of Victoria, Box #3055, Stn. CSC, Victoria, British Columbia, Canada, V8W 3PG.

OBJECTIVE: Extensive data are available on electric fields and currents induced in organs and tissues of the human body exposed to ELF fields. Numerous biological experiments have been performed on rodents. One of the most contentious issues is the association between the magnetic field exposure and childhood leukemia in view of the lack of supporting evidence from animal studies. A comparison of the induced electric fields in certain tissues (e.g., bone marrow) in experimental rodents and humans may prove to be of some assistance in addressing this perplexing question. The objective of research presented is to evaluate with high spatial resolution induced electric fields in mice and rats exposed to 60 Hz magnetic fields.
METHODS: Anatomically realistic models of rats and mice (both species, male and female) were obtained from MRI scans. The resolution (voxel size) of the models used in computations ranged from 0.85 mm to 0.35 mm for a big male rat to a small female mouse. Over 30 tissues were identified and corresponding conductivity values were allocated. The resolution of the human model is 2 mm. Numerical computations were performed for three orientations of the magnetic field with respect to the body. The previously developed and verified method, namely scalar potential finite difference, was used.
RESULTS: Table 1 gives selected measures for a few tissues for models of rodents and a human male. In all cases data are for 1 mT, 60 Hz magnetic field in the orientation that gave the greatest values of the

selected measures. For rodents, except for blood, this orientation corresponds to the field oriented from the back to front. The whole body averages for rodents are in good agreement with the previously computed data for homogeneous models (Xi et al, IEEE Trans Biomed. Engn., 41:1018-1022, 1994. However, the data for organs are obtained for the first time, and indicate that predictions based on size are not appropriate. Consistency in results for same parameter (e.g. E_{max}) in the same species can be observed, but not necessary across the species (mouse compared to rat). The differences in E_{max} are due to smaller voxels, while data for E_{avg} scales well in most tissues. Bone marrow data are quite different for female and male, which is attributable to the differences in the models.

Table 1. Induced electric field (mV/m) in body models exposed to 1 mT 60 Hz magnetic field.

Model	Rat – Male580 g			Rat – Female280 g			Mouse – Male43 g			Mouse – Female20 g			Human – Male76 kg		
Tissue	Eavg	E99	Emax	Eavg	E99	Emax	Eavg	E99	Emax	Eavg	E99	Emax	Eavg	E99	Emax
Whole body	4.8	14.7	55.3	3.6	10.0	22.9	2.05	6.2	23.5	1.6	4.5	10.0	16.2	71.4	1160
Muscle	4.7	11.8	26.4	3.8	9.1	13.7	1.8	4.5	7.5	1.7	4.0	6.0	12.8	42.9	176
Skin	5.6	16.0	55.3	4.2	12.1	22.9	2.4	6.8	23.5	1.9	5.2	10.0	16.5	73.0	1160
Blood	1.1	2.8	3.5	0.64	1.9	2.4	0.45	1.2	1.5	0.28	0.82	1.0	7.0	23.1	46.7
Bone marrow	6.0	13.5	18.3	5.0	10.9	13.9	2.6	5.8	7.8	2.2	4.7	6.1	12.9	78.0	168

CONCLUSIONS: Detailed data on induced electric fields in various organs and tissues of rodents that have been obtained provide means of scaling from animals to humans in interpretation of observed biological effects. Such scaling is valid if electric fields in tissue are a considered measure.

7-5

2-D FDTD INVERSE-SCATTERING SCHEME FOR DETERMINING MICROWAVE BREAST SKIN PROPERTIES: THE RESULTS AND THE IMPLIED OPTIMIZATION OPTIONS. M. Popovic[1], A. Taflove[2*]. [1]Department of Electrical and Computer Engineering, McGill University, Montréal, Quebec, Canada H3A 2A7. [2]Electrical and Computer Engineering Department, Northwestern University, Evanston, Illinois 60208, USA.

INTRODUCTION: The work reported in this paper is motivated by the development of a new confocal microwave technology to detect and image early-stage breast cancers. This methodology depends upon knowledge of the average dielectric properties of the local breast tissues. Patient-specific calibration of the microwave imager requires knowledge of these properties. Present results focus on dielectric properties and thickness of skin in the area of human breast. Well-documented findings have shown that skin thickness varies from patient to patient and also with location on the body of an individual patient.
Objective: A three-dimensional (3-D) inverse-scattering finite-difference time-domain (FDTD) algorithm would permit non-invasive measurement of near-surface breast tissue dielectric properties, based on the information contained in the backscattered signal recorded with a trans-receiving antenna located on the skin surface. Initial investigations are done in two dimensions. Development of a two-dimensional (2-D) FDTD algorithm allows for study of the inverse-scattering scheme and the possibilities for its optimization with a computational cost lower than that of the 3-D numerical investigations.
METHODS: A 2-D inverse-scattering algorithm based upon the FDTD method is presented for determining skin thickness and the relative permittivity $\varepsilon_{r\text{-}skin}$ and electric conductivity σ_{skin} in the microwave range. This scheme applies an iterative technique to the case of a 2-D half-space excited at its surface by an infinitely long monopole. The algorithm traces a search trajectory in the ($\varepsilon_{r\text{-}skin}$, σ_{skin}) parameter space in the following manner. A trial value of σ_{skin} is assumed and a series of FDTD forward-scattering runs is conducted to find a corresponding value of $\varepsilon_{r\text{-}skin}$ which minimizes the energy-normed error relative to the measured backscattered signal. This procedure is then repeated for a sequence of trial values of σ_{skin} which vary uniformly in equal increments within a ±50% range about the nominal value

reported in the literature. The three electric-field excitation waveforms used for the dielectric parameter reconstruction are as follows: (a) 120-ps differentiated Gaussian pulse; (b) 10-ps differentiated Gaussian pulse with a 5-ps rise-time; and (c) 5-ps rise-time ramp which has the same maximum as the peak value of the 10-ps differentiated Gaussian pulse. The robustness of the inverse-scattering scheme was tested by adding zero-mean Gaussian noise to the simulated measured backscattered signals for signal-to-noise ratios of 20dB and 40dB.

RESULTS AND CONCLUSIONS: There are two significant findings of this work. (1) The use of a time-linear (ramp) excitation yields a linear search trajectory in the ($\varepsilon_{r\text{-}skin}$, σ_{skin}) parameter space. (2) The use of a short bipolar excitation signal provides a sharp null of the energy-normed error along the search trajectory even when the backscattered signal is contaminated with a significant level of additive zero-mean Gaussian noise. Proper combination of time-linear and short bipolar pulse excitation data can yield an efficient and robust search strategy.

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7-6

60-SAMPLE RF EXPOSURE SYSTEM FOR BLOOD BRAIN BARRIER MODEL INVOLVING IMPEDANCE MEASURE-MENTS OF CELL LAYERS. J. Streckert[1*], A. Bitz[1*], H. Franke[2*], J. Goeke[3*], V. Hansen[1], F. Stögbauer[2*]. [1]University of Wuppertal, Chair of Electromagnetic Theory, D-42097 Wuppertal, Germany. [2]University of Münster, Department of Neurology, D-48149 Münster, Germany. [3]University of Applied Sciences, Department of Building Services, D-50679 Cologne, Germany.

INTRODUCTION: It is still an open question whether the permeability of the blood brain barrier (BBB) is affected by weak electromagnetic fields, e.g. by rf fields of digital mobile communication systems. For 'in vitro' investigations a cell layer model of the BBB has been developed consisting of endothelial cells and astrocytes grown on the two sides of a thin polycarbonate membrane [1]. In order to detect a possible deterioration of the physiological function of the BBB the permeability of the cell layer is investigated during exposure to rf electromagnetic fields. So far, experiments on the permeability to sucrose have relied on transport measurements. In order to allow a discussion of the mechanisms of the interaction in more detail, further experiments were designed with emphasis to the permeability of the cell monolayer to ions and charged molecules. Therefore, a novel exposure device has to be developed with an integrated system for the low frequency measurement of the electrical conductivity of the layer. The main topic in this context is the detection of interference of radio frequency electromagnetic fields with low frequency measurement signals due to possible non-linearity of the BBB-model.

OBJECTIVES: A test setup is described enabling the simultaneous exposure of up to 60 BBB samples to GSM- and UMTS-typical electromagnetic fields. Each sample holder must be equipped with a two electrode system in order to allow for individual impedance measurements. The electrodes and their leads must be shaped in a way that the lf field intersects the BBB effectively whereas the rf field remains unperturbed. Support of the cells with nutrient solution, CO_2 and constant temperature is obligatory.

METHODS: The implemented exposure system consists of two radial waveguides of 40 cm diameter and 11 mm plate distance. Both waveguides are placed together into an incubator and can in turn be used as exposure or sham exposure device. The excitation of the rf field is achieved via a shape-optimized antenna in the center of the waveguide, a 5 mm flat absorber along the perimeter reduces reflexions substantially. The 30 samples in each waveguide are arranged symmetrically near the rim. Each sample is inserted into a cylindrical plastic cartridge of 24 mm diameter and 24 mm height which is partly filled with electrolyte and also contains the disk electrodes for impedance measurements whose distance is matched to the plate distance of the waveguides. The leads of the electrodes are conducted upwards through metallic caps closing

the cartridges. Thereby, the simultaneous rf exposure and lf impedance measurement is possible at any time of the experimental stage.
RESULTS: Detailed information about input power, lf and rf field distributions, SAR values and power efficiency will be given in the talk. Further, results of the detailed analysis of the potential non-linearity of the measuring system will be discussed.
Reference.
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The support of the project by Forschungsgemeinschaft Funk, Bonn, Germany, is gratefully acknowledged.

7-7

AN OPTIMIZED METHOD TO DETERMINE EXPOSURE DUE TO GSM BASE STATIONS APPLIED IN THE CITY OF SALZBURG. G. Neubauer[1], W. Giczi[*1], G. Schmid[*1], M. Rierderer[*2], R. Coray[*2], P. Horisberger[*2], P. Krähenbühl[*2], H. Jell[*3], M. Artmüller[*3]. [1]ARC Seibersdorf Research, Austria [2]BAKOM, Switzerland [3]Amt f. Umweltschutz Salzburg, Austria.

In the last years exposure levels next to GSM base stations were becoming an increasing concern to the population. In several projects recently performed, e.g. Bergqvist et al 2001, exposure levels next to such installations were already determined, however the assessment by so - called spot measurements was restricted to rather small areas. These measurements are usually done in areas as single houses, flats or even rooms. Therefore, such evaluations can give information on exposure in the investigated area, but are not adequate to draw conclusions on the electromagnetic field levels in the complete environment of a base station. Due to several reasons like juridical requirements in some countries it becomes very often necessary to evaluate exposure at all the places where exposure may potentially exceed exposure limits set by the national or local administrations. It is extremely time consuming to evaluate exposure conditions around a typical base station by measurements only. On the other hand exposure assessment by computersimulation or calculations alone leads to considerable uncertainty budgets, therefore both, exposure simulation and exposure measurement has been applied in the case of the "Salzburg study". One of the major goals of this study was to get a picture of the NIR (Non Ionizing Radiation) exposure in the vicinity of GSM base stations, some being rather complex. For that purpose a specialised and highly sophisticated 3D computer simulation has been applied as well as specially developed measuring devices and measuring method. This method was applied in the vicinity of 13 base stations in the city of Salzburg. The first step to be done is to collect all necessary technical data of the respective base station like antenna pattern and gains, input power, frequencies and number of carriers with BCCHs (Broadcast Channel) and carriers with TCHs (Traffic Chancel) only. The base station needs to be examined visually and the topography of the environment needs to be documented. The relevant information of all buildings in an area of about 200 m around the base station also needs to be known in order to perform a reliable simulation of the field distribution around the respective base station. In the frame of this project the software "Quick_Plan" developed by the Italian company Teleinformatica e Sistemi s.r.l. was used to perform the simulations. Based on the results of these simulations that give adequate information of the outdoor field distribution around a base station, the areas inside the buildings where the highest field levels are to be expected, can easily be identified. These areas usually consist of large parts of houses, therefore, in most cases it is not possible to exactly identify the rooms with the highest field levels. Therefore, it is imperative to perform some preliminary, evaluative measurements ("prescan") at the respective places inside the buildings using broadband field meters during the second step of the procedure. It has to be taken into account that immissions from other sources, e.g. broadcast stations or radar installations may influence the results of the measurements.

Having identified the room with the highest field levels, the positions of the maximum level of the carriers with BCCHs of the considered base station are to be identified by a second "prescan". The second "prescan" is performed using a spectrum analyser and a log periodic antenna. Because the measuring engineer is forced to hold the antenna in his hands, the field distribution is influenced by this procedure. Consequently, it is not possible to rely on the absolute field levels obtained during this second "prescan". Therefore the levels of the carriers with BCCHs are determined at the obtained positions by using the method Add3D (Haider et al 2002) to finally measure the exposure levels of the considered base station. The contribution of the carriers with TCHs is also considered by calculation assuming that all slots of the respective carriers are emitting with the maximum possible power **.**

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7-8

OPTIMAL ENERGY-COUPLING TO HEAT DEEP-SEATED LESIONS IN A BODY. N. Szasz[1], O. Szasz[2*], G. Vincze[2*] and A. Szasz[23]. [1]Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. [2]Szent Istvan University, Godollo, H2103 Hungary. [3]University of Strathclyde, Glasgow, Scotland G1 IXQ, United Kingdom.

INTRODUCTION: Hyperthermia using electromagnetic fields is a rapidly developing treatment method in cancer therapy. The classical effect is based on well-focused energy absorption within the malignant tissue. There are different methods for appropriate focusing and delivery of electromagnetic energy to the desired area. The most sensitive point in designing an effective hyperthermia device is the antenna coupling. Objective: Compare the efficacy of three main antenna coupling methods**,** radiative dipole, capacitive, and inductive coupling, in order to choose the most effective applicator system for biological heating.
Theory: Calculations were made using classical Maxwellian electrodynamics, taking into account the realistic electrical parameters of the human body: conductivity (σ = 0.6-1 Ωm), relative dielectric permittivity (ε = 50-120), and relative magnetic permeability (μ = 1). The applied frequency has been chosen to optimize body-penetration, energy absorption, and technical ease.
METHODS: Maxwell's equations were solved by taking advantage of the symmetries inherent in each applicator system. For antenna arrays and inductive coupling a cylindrical arrangement is used, while a plane-parallel geometry is exploited for capacitive coupling calculations.
Results and Discussion: In general, high penetration depth and high specific energy-delivery are conflicting requirements. The lower the frequency used, the greater the penetration depth, and also the lower the energy deposition. In the case of inductive heating, the effect is very much limited by the weak magnetic interaction in bio-systems and the frequency limit of the applied coils. These limits constrain useful frequencies to be less than 24 MHz. When an antenna array is used, relatively high frequency is necessary for focused and effective heating. Capacitive coupling works in a wide range of frequencies with acceptable efficiency. It also has a self-regulated energy loss based on the inhomogeneties of the dielectric constant within the body. For example, tumors have an approximately 3 to 10-times greater complex permittivity than healthy tissue, therefore, capacitive coupling promotes approximately 2 to 3-times greater absorption of energy in the tumor than in the healthy tissue. A comparison of the methods is summarized in the table below. As the data indicates, the best efficacy is reached by capacitive coupling. Capacitively coupled electro-magnetic treatment also provides further advantages by targeting the extracellular matrix (ECM) selectively. This selectivity in heating has also proved to provide further benefits for cancer treatments[1].
References.

[1]Szasz, A., Szasz, O., Szasz N. (2001) Electro-hyperthermia: a new paradigm in cancer therapy, Deutsche Zeitschrift fur Onkologie, 33, 91-99.

Coupling Method	Typical Frequency	Efficacy	Focusing Method	Electric Field	Magnetic field	Main area of heat production
Condenser	13.56 MHz	0,225	self-focused	high	low	ECM only
Coil	< 25 MHz	0,0024	parametrical	low	high	entire tissue
Antenna-array	100 MHz	0,0345	parametrical	medium	medium	entire tissue

SESSION 8: Special Symposium III: Emerging Therapies I
Chairs: Marko Markov and James Ryaby

8-1

CLINICAL BIOPHYSICS: THE USE OF PHYSICAL AGENTS AT LOW ENERGY CONTENT IN MEDICINE. R. Cadossi. Laboratory of Clinical Biophysics. Igea. Carpi, Italy.

The therapeutic possibilities at the physician's disposal foresee the use of both chemical and physical agents. Whereas for chemicals (drugs) and high energy physical agents their use for disease treatment has been well defined and structured in the different branches of medicine and surgery, for physical agents with low energy content it has not been the same. This presentation aims at giving, for the first time, a unitary view on the areas of medicine dealing with physical agents at low energy content, that we define as "clinical biophysics".
The therapeutic use of physical means in medicine is based on the observation that biological systems are able to absorb physical energy. The specificity of the effects is inversely proportional to energy content; at energy levels increase, the effects can be described as: *i* interaction with the cell electric field, *ii* thermal effects, *iii* toxic and degenerative effects. For physical agents with low energy content, dose response curves have been demonstrated for frequency, energy, length of exposure. Low energy effects are mediated by the interaction with the micro-environment and/or the cellular membrane; no direct intracellular effects have been observed.
The cellular membrane definitely represents the most investigated interaction site; different transduction pathways for mechanical, electrical or electro-magnetic energy have been described. Whenever a physical agent is able to modulate a cellular activity, for example a membrane process, likewise drugs, the effect will be function specific rather than cell or tissue specific. This will allow to treat with the same physical agent all the conditions which are positively influenced by the activation or modulation of that cellular function. Nevertheless, we cannot foresee a specificity of physical agents similar to the one of drugs, even though it is clear that different physical agents produce different effects.
During last century, physical energy has been introduced, in particular in the form of electric currents and ultrasound, to favour bone healing. Today, several diseases, in different fields of medicine, are treated with physical agents, even though the mechanisms of action are not fully understood; thus clinical applications are more advanced than the basic research.
Unlike drugs, the effect of physical agents is local and limited to the site of application. No systemic effects have been observed following exposure to low energy physical agents.
Finally, biophysical treatments can be an important integration to pharmacology to potentiate the effects of drugs locally.
Certainly, the community of the orthopaedic surgeons has played a central role in the development and understanding of the importance of the physical stimuli to control biological activities. We should

acknowledge them the merit of understanding the clinical importance of this field of medicine. Today several clinicians are interested to utilise non-chemical means to treat different pathologies. The development of new clinical treatments with physical agents at low energy content represents an important opportunity. The identification and definition of the clinical biophysics, represents a fundamental moment of synthesis necessary to create a reference common ground for researchers of different fields.

8-2

EMERGING CLINICAL APPLICATIONS OF BIOPHYSICAL STIMULATION TECHNOLOGIES IN ORTHOPAEDICS. J.T. Ryaby. Research and Development, OrthoLogic Corp., Tempe, AZ 85281, USA.

The clinical use of electrical and electromagnetic fields for orthopaedic healing applications started in the early 1970s, with ultrasonic technologies following in the late 1980s. Since that time, clinical research on the applications of these technologies have led to FDA approved applications for treatment of fractures (non-unions and fresh fractures) and spine fusion. The acceptance of these devices in the clinical community has been aided in recent years by both basic research and several well-controlled clinical trials. Additional non-FDA approved clinical indications for these technologies have been shown for treatment of avascular necrosis, tendonitis, and osteoarthritis. The spectrum of these clinical applications clearly demonstrates the effectiveness of these biophysical stimulation devices to enhance musculoskeletal tissue healing. This paper will focus on the future directions in basic and clinical research currently underway to fully exploit the usefulness of these noninvasive biophysical stimulation technologies.

8-3

NON-INVASIVE STATIC MAGNETIC FIELD THERAPY REDUCES CHRONIC PELVIC PAIN: A DOUBLE-BLIND PILOT CLINICAL STUDY. C Brown[1], F. Ling[1], J. Wan[1], A. Pilla[2]. [1]University of Tennessee Health Science Center, Memphis, Tennessee, [2]Department of Orthopaedics, Mount Sinai School of Medicine, NY.

INTRODUCTION: Chronic pelvic pain (CPP) is one of the most common disorders in women's health. It affects one out of seven women and accounts for 10-15% of new referrals to gynecologists and family physicians. Like other pain syndromes, chronic pelvic pain is costly to the consumer and to society, accounting for 25-35% of laparoscopies and 10-15% of hysterectomies performed in the United States. This study evaluated the analgesic effect of static magnets worn continuously in women with CPP. We hypothesized that pain severity and disability ratings would significantly improve in CPP patients receiving active compared to placebo magnets. Five double blind clinical studies using static magnetic fields SMF to treat chronic pain have been reported. A single 45 min treatment with 300-500 G bipolar magnets reduced chronic pain in 50 post-polio patients by 76%. In a 4-month study of 25 fibromyalgia patients, significant improvement in pain and physical function was observed in those who slept on a mattress pad containing arrays of 1100 G (200-600G at skin surface) unidirectional ceramic magnets compared to those sleeping on a sham mattress. A second 6-month trial of 94 fibromyalgia patients confirmed significant chronic pain reduction in those sleeping on a unidirectional magnetic pad (5-6 G at skin level) compared to those sleeping on a magnetic pad with alternating polarity (0.3 – 0.9 G), sleeping on sham magnetic pads, or those following usual care. Chronic lower back pain in 20 patients was not affected by application of a flexible plastic ferrite 300 G bipolar magnet applied directly over the pain site for 6 hrs per day, 3 times per week for one week. A necklace containing 1300G unidirectional magnets was no more effective than a nonmagnetic necklace in reducing pain in 52 chronic neck and shoulder pain patients when worn continuously for 3 weeks.

METHODS: This was a double blind, randomized, parallel groups study. Subjects meeting screening eligibility criteria had sham magnets applied to two sites on the abdomen for 1 week. Those who continued to meet eligibility criteria after one week of single blind treatment were randomized to either active (500 G) or placebo magnetic devices for 2 weeks. At the end of this double-blind treatment phase, subjects were given the option of completing another 2 weeks of double-blind treatment. Ten areas located in the upper, middle, and lower abdomen were palpated for localized tender areas, or "trigger points," by the same investigator. Devices were placed on the two areas most sensitive to palpation. Active and sham magnets (BIOflex Medical Magnets) were concentric bipolar, 500 G at the surface, and 50 mm in diameter x 1.5 mm thick. Magnets were worn continuously. Outcomes were measured by the McGill Pain Questionnaire, the Pain Disability Index, and the Clinical Global Impressions scale. Investigator and patient ratings were measured at baseline, after single-blind treatment, and after 2 and 4 weeks of double-blind treatment. Change scores from baseline after single-blind treatment, and after 2 and 4 weeks of double-blind treatment were compared between the two groups using the Wilcoxon rank sum test.
CLINICAL RESULTS: Fifteen subjects receiving active magnets and 17 subjects receiving placebo magnets completed 2 weeks of double-blind treatment. Nineteen subjects elected to complete another 2 weeks of double-blind treatment, 8 active and 11 placebo. Scores on the McGill Pain Questionnaire showed consistently greater improvement among those patients completing 4 weeks of double-blind treatment who received active compared to placebo magnets. Pain intensity scores decreased by 22% in the active group and by 6% in the placebo group, but the difference was not statistically significant. Quality of pain scores improved by 40% for actives, while the placebos worsened by 3% ($P<.10$). Pain Disability Index ratings improved significantly in the active group (51%) vs. the placebo group (7%) ($P<.05$) at 4 weeks. Clinical Global Impressions-Severity scores decreased significantly in the active group (28%) vs the placebo group (10%) ($P < .05$). Similarly, CGI-Improvement scores decreased by 43% in those receiving active magnets compared to 1% in the placebo group ($P<.01$).
CONCLUSIONS: The results of this randomized double-blind, placebo-controlled trial show that SMF Therapy with 500G bipolar magnets placed on pain trigger points significantly improves disability and may reduce pain when worn continuously for 4 weeks in women with CPP. A controlled study with a larger sample of CPP patients receiving magnetic therapy over a longer period is needed to strengthen our findings. Evaluation of other chronic pain populations, with and without trigger points, receiving magnetic therapy of varying intensities, configurations, and treatment duration is warranted. These studies will determine whether magnetic therapy is useful in the management of CPP as well as in other pain syndromes commonly treated in primary care settings.

8-4

INTERIM ANALYSIS OF A PHASE 2 STUDY OF A THERAPEUTIC EMF (TEMF) DEVICE FOR TREATMENT OF CHRONIC PAIN ASSOCIATED WITH LOW BACK DISORDERS. R.N. Harden[1], T.A. Houle[1], T.A. Remble[1], J.F. Long[1], M.S. Markov[2]. [1]Rehabilitation Institute of Chicago, Chicago IL60610, USA; [2]EMF Therapeutics, Chattanooga TN 37405, USA.

OBJECTIVE: To complete an interim power analysis of results obtained in evaluating the effectiveness and safety of a therapeutic EMF (TEMF) device in chronic pain patients with lower-back disorders.
BACKGROUND: Surgical and pharmacological interventions are the primary treatment modalities employed in the management of chronic lower back disorders; however, many individuals suffering from these conditions continue to experience pain after medical interventions resulting in ever-increasing medical costs and lost work capacity. Pulsating electromagnetic field stimulation, as delivered by a proprietary technology (TEMF) designed and developed by EMF Therapeutics, Inc. (Chattanooga, TN), is a new treatment option being evaluated for chronic lower back pain.
METHODS: The original design of the study proposed 20 subjects be randomly assigned to one of four treatment groups (placebo, 5 mT, 10 mT or 15 mT). Employing a double-blind, sham-treatment controlled

design, participants were evaluated over six weeks. Subjects completed a two-week baseline period to assess the severity of pain and the use of ancillary medications. They were then randomized to one of four treatment groups (placebo, 5 mT, 10 mT or 15 mT) for six 30-minute treatments over two weeks. This was followed by a two-week follow-up period. Primary outcome measures included a self-report of pain severity using a 100 mm visual analog scale (VAS) pre- and post-treatment, and completing a twice-daily pain diary using the McGill Pain Questionnaire – Short Form (MPQ-SF).
RESULTS: An interim analysis of the data collected from the first twenty patients (5 subjects per group) was performed to assess the statistical power of the study and determine if a sufficient numbers of patients were included in the original design to achieve significant results. The interim analyses conducted on both the individual (time-series) and group (ANCOVA) levels suggested that the TEMF device operates in a therapeutic window. The interim trends observed indicated that the response to 15 mT was superior to 5 and 10 mT. For example, the reported pain levels for the placebo group remains unchanged during the two week follow up period as compared with the two weeks of baseline data. The morning pain level for the patients in the 5 mT group was slightly reduced (5% change), the patients in the 10 mT group achieved a larger reduction (14%) and the patients in the 15 mT group achieved the largest reduction (24%). The low observed effect size (difference from placebo) in the 5 mT & 10 mT groups, when coupled with the added degree of within-group variability in the 10 mT group, supported the decision to discontinue the lower EMF dose levels. Therefore, the four study groups were changed to two, the placebo and 15 mT groups.
CONCLUSIONS: Results from this interim power analysis, including estimated effect sizes, suggested utilizing 20 subjects in each group would produce significant results only with the hypothesized difference between the 15 mT and placebo groups. These results support continued investigation of the TEMF device as an effective modality for the treatment of chronic pain associated with low back disorders. Further research needs to be done to assess the efficacy of various magnetic flux densities.
This study was sponsored by EMF Therapeutics, Inc., Chattanooga, TN.

8-5

TREATMENT OF PAIN WITH A STATIC MAGNETIC FIELD DEVICE. M. McLean, R. Holcomb, S. Engstrom, N. Segal. Vanderbilt University Medical Center, 2100 Pierce Avenue, 351 MCS, Nashville, TN, 37212 USA.

OBJECTIVE: To test whether a portable static magnetic field device can reduce pain.
METHODS: The active device consisted of four cylindrical NeFeB magnets in a square array and encased in opaque hypoallergenic plastic. These and other magnetic or non-magnetic devices identical in appearance were taped to the skin overlying painful regions in patterns described in the respective protocols (seven devices over the low back; four over the knee). Efficacy of the active device was compared to a non-magnetic placebo in subjects with mechanical low back and knee pain in studies of crossover design with double masking. Patients were randomized to receive placebo or the test device first. Sets of devices were worn in a standard pattern for 24 hours on a clinical research unit and multiple pain assessments were made using the visual analog scale (VAS) and/or a verbal response score (VRS). Efficacy of the active device was compared to a second magnetic device in patients with rheumatoid arthritis was tested in a parallel group study with a one week masked period. Pain diaries were kept in all studies. Adverse effects were reported and recorded in all studies. Intactness of the blind was assessed with a questionnaire and no breaks were identified. Treatment of other conditions has been assessed in open trials.
RESULTS AND DISCUSSION: In a first pilot study, active device was superior to placebo in the treatment of mechanical low back ($p < 0.04$) and knee pain ($p < 0.02$) (Holcomb et al., Environmental Med, 8:24, 1991). In the per protocol analysis (N=77) of a follow-on study of mechanical low back pain, the active device was superior to placebo (VAS, $p < 0.05$; verbal response scale, $p < 0.02$). In patients with rheumatoid arthritis, active device was superior, but not statistically so, to a second magnetic device after one week ($p = 0.23$, $N = 64$) (Segal et al., Arch Phys Med Rehabil 82:1453, 2001). Reduction of pain at the

end of the one week masked period was statistically significant for both devices. Active device was statistically superior to the second device with respect to the Subjects' Global Assessment of Change ($p < 0.01$) and the percentage of patients feeling better or much better after one week ($p < 0.01$). These studies support the potential for using static magnetic field devices to treat pain and provide insights into the design of definitive studies. Limitations include instruments of measure, variability of pain with time and non-uniform application of inclusion/exclusion criteria. Multiple measures give a better picture of outcomes than a single measure, e.g., the VAS. Further support comes from clinical treatment of pain that demonstrates a need for accurate localization of pain from history and physical exam (see Holcomb et al., Ped Neurol 23:261, 2000). A magnetic placebo has been devised for the conduct of trials for FDA approval.

8-6

DIFFERENT EFFECTS OF TWO SIMILAR EMF SIGNALS ON NEURITE OUT-GROWTH IN CHICK DORSAL ROOT GANGLIA. B. Sisken, A. Tweheus,* A. Chan* and M. Markov. Center for Biomedical Engineering and Dept. of Anatomy and Neurobiology, Univ. of Kentucky, Lexington, Kentucky 40506-0070, USA. EMF Therapeutics, Inc., Chattanooga, Tennessee 37405, USA.

INTRODUCTION AND OBJECTIVES: For a number of years we have been testing various electric and electromagnetic field devices to determine the growth effects on sensory neurons in culture. These former studies used a chick embryo dorsal root ganglia (DRG) model to determine the extent of neurite outgrowth after exposure to DC electric fields, static magnetic fields and time-varying electromagnetic fields. While many of these previous studies were performed with low amplitude EMF, the study we are reporting today examines the effects of two similar EMF of much higher amplitudes (~2-50 times higher) on specific aspects of DRG growth (Sisken et al, 1975-2001).

METHODS: A chick embryo explant model was used to assess bioeffects by measuring various areas of growth. EMF signals were generated by TEMF-005 Animal Model (EMF Therapeutics, Inc. Chattanooga, TN). The signal consists of 120 pps fully rectified semi-sine wave. The rectified semi-sine wave maximums with magnetic flux density of 15 mT were separated by 4 ms (Signal 2) or 8 ms (Signal 1) DC components which are the result of changes in the device impedance due to the presence of different components in the electrical circuit of the generating system. In all experiments, 3-30 ng/ml nerve growth factor (NGF) was included in the culture medium since NGF is essential for normal growth and differentiation of sensory neurons.

EXPERIMENTAL DESIGN: On each surgical day DRG were dissected from 9-10 day old chick embryos and placed in 60 mm collagen-coated culture dishes containing 3 ml Neurobasal medium plus a serum-free mix of growth substances (Sigma Corp). The dishes were divided into 2 groups: a sham and an experimental using either Signal 1 or Signal 2. In each experiment five to seven DRG in each of 4 - 6 culture dishes were exposed to one of the two different signals. Sham exposures were performed by placing replicate culture dishes in the unpowered coil**.** This plan was repeated at least 4 times for each signal. The explants in the dishes were exposed to electromagnetic fields for 5 or 10 min/day for each of two days and then fixed. The fixed explants were photographed and growth areas assessed quantitatively using an image analysis technique (Shah et al, 1998). **Statistics:** Statistical analyses were performed using an Analysis of Variance followed by the Tukey-Kramer Multiple Comparison Test.

RESULTS AND CONCLUSIONS: Three growth parameters were analyzed for each DRG: total area of the explant, neurite outgrowth area and central neuronal area. Neurite outgrowth area was enhanced significantly when Signal 2 was used for either 5 or 10 min/day exposures. Results showed that growth was increased by 38% ($p<0.05$) at 5 min exposure, and 50% ($p<0.02$) at 10 min. relative to sham controls at the same NGF concentrations. Total area paralleled results found with neurite area. No differences were found in the central neuronal areas between any groups. In contrast, consistently lower values of neurite outgrowth were found when the DRG were exposed to Signal 1 even though these differences were not statistically

significant. Although the distinction between the two EMF signals is small (4 vs 8 msec delays between sine waves), the resulting differences in bioeffects is interesting and may be important.
Supported in part by EMF Therapeutics, Inc., Chattanooga, TN.

8-7

THE EFFECTS OF STRONG MAGNETIC FIELDS ON BONE FORMATION. H. Kotani[1], M. Iwasaka[1], S. Ueno[1], K. Hoshi[*2], K. Nakamura[*2] and H. Kawaguchi[*2]. [1]Department of Biomedical Engineering and [2]Department of Orthopaedic Surgery, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan.

OBJECTIVE: The induction of bone formation to an intentional orientation is a clinically viable treatment for bone disorders. Since it was reported that static magnetic fields (SMF) of tesla (T) order could regulate the shapes of blood cells and matrix fibers, the present study investigated the effects of a strong static magnetic field (8 T) on bone formation in both *in vivo* and *in vitro* systems.
METHODS: A horizontal type superconducting magnet (length = 700 mm, bore diameter = 100 mm), (Oxford, UK), which produced 8 T at its center, was used. The field distribution of the exposure system adjusted the magnetic field strength from 8.0 to 7.96 T at z = 0 to z =±10 mm at the center of the bore. The ambient temperature in the magnet was maintained by circulating temperature-regulated water in a coiled tube inside the bore.
To investigate the effect of the strong SMF on bone formation *in vivo*, we used an ectopic bone formation model. BMP (bone morphogenetic protein) / collagen pellets were implanted subcutaneously into mice. The mice were placed in the center of the magnetic bore, where constant 8.0 T SMF exposure was maintained for 60 hours. The body axes of the mice were maintained in the direction of the magnetic field. Control mice were placed and fixed under the same conditions for 60 hours without SMF exposure. After 21 days following the SMF exposure, the BMP/collagen pellets were harvested, and radiological and histological analyses were performed. X-rays revealed that the strong SMF stimulated bone formation in and around the BMP/collagen pellets.
The effects of exposure to the strong SMF (8 T) on the proliferation, orientation, and differentiation of cultured mouse osteoblastic MC3T3-E1 cells were also investigated. Cells were cultured in alpha-modified minimum essential medium (aMEM) containing 10 % fetal bovine serum (FBS) and 1 % antibiotic-antimycotic solution. The cells were positioned at the center of the magnet and exposed to 8 T magnetic fields for 60 hours. The proliferation rate of the MC3T3-E1 cells was determined by BrdU uptake. After 14 and 21 days in culture following the SMF exposure for 60 hours, the alkaline phosphatase (ALP) activity and the Alizarin-red staining of the cells were analyzed to determine the effect of SMF exposure on the differentiation of cultured MC3T3-E1 cells.
RESULTS AND DISCUSSION: The 8 T static magnetic field stimulated ectopic bone formation in and around the subcutaneously implanted BMP containing pellet in mice. The newly formed bones extended parallel to the direction of the magnetic field in the exposed group, while only small spherical shaped ossicles were observed in the non-exposed group. The bone mineral content of the exposed group was approximately 4 times greater than the non-exposed group. Histological examinations revealed that the pellets were replaced by newly-formed bone tissues including bone marrow in both the exposed and non-exposed groups. Mature bone formation was observed mainly at the periphery of the induced tissue.
After 60 hours of exposure to the static magnetic field, cultured mouse osteoblastic MC3T3-E1 cells transformed to rod-like shapes and orientated in the direction parallel to the magnetic field. Although cell proliferation was unaffected by the strong static magnetic field, cell differentiation and matrix synthesis increased, as determined by alkaline phosphatase activity and Alizarin red staining respectively.
The strong SMF stimulated bone formation in both *in vivo* ectopic bone formation models and *in vitro* osteoblast cell cultures in the direction of the magnetic field. A combination of strong static magnetic fields

and potent osteogenic agents such as BMP is a potentially viable clinical treatment for skeletal conditions such as osteoporosis and bone fractures.

8-8

TREATMENT OF OSTEOARTHRITIS WITH A NEW BROADBAND PEMF SIGNAL. W. Pawluk[1], Z. Turk[2], G. Fischer[3], W. Kobinger[3]. [1]Advanced Magnetic Research Institute of the Delaware Valley, Rancocas, New Jersey, USA, and School of Medicine, Johns Hopkins University;. [2]Maribor General Hospital, Slovenia; [3]University Graz.

INTRODUCTION: Osteoarthritis is a debilitating joint disease which affects about 40 million people in the United States of America. Osteoarthritis of the knee is a leading cause of disability in the elderly. Pharmacologic management is often ineffective. Several clinical studies using electromagnetic fields to treat osteoarthritis have been reported. One, by Trock DH et al, reported a randomized, double blind clinical trial to treat OA of the knee and cervical spine with a PEMF system. The magnetic field was a maximum 25 G; frequency varying from 5 to 24 Hz. The waveform was quasi-rectangular with abruptly rising and deteriorating wave form with a pulse burst duty cycle of up to 0.8 and up to 20 pulses per burst, depending on the frequency. There were 18 half-hour treatments in patients with OA 86 knee and 81 patients cervical spine. Evaluations were made at baseline, midway, end of treatment, and one month after completion. Tests showed extremely significant changes from baseline for the treated patients in both knee and cervical spine studies at the end of treatment and the one month followup observations. It is the purpose of this work with the QRS Salut 1 system to describe the results of a double blind knee osteoarthritis clinical study using a novel pulsing electromagnetic field (QRS, Body Fields, Des Plaines, IL). The QRS system has been primarily used in Europe where several pilot studies suggest benefits or actions in ion migration, vasodilatation, osteoporosis and osteoarthritis.

METHODS: This was a randomized double blind knee osteoarthritis (KOA) study involving 36 placebo/35 active patients. 28% were male. Mean age was 60 ± 10 years. 35% were clearly overweight. 83% had both knees affected. Subjects were entered if they had KOA with or without other comorbidities and excluded only if they could not complete the course of treatment. The QRS device induces a complex EMF waveform via large body size coils within a thin mattress or pillow pad. The coils are driven by an external generator with 10 strength settings. Peak magnetic field at the coil is approximately 400 μT within a frequency range of 1-100 kHz. The primary waveform is sawtooth-like, having a 10μsec rise time and an exponential-like fall of about 250 μsec. A burst of 3 such pulses repeating every 5 msec is applied every 30 msec in packets of three and the whole packet train is repeated every 300 msec. The polarity of the pulses is reversed every 10 seconds. The coils in the QRS device are large enough to allow maximum induced electric fields in the mV/cm range within a large tissue volume, comparable to typical bone growth stimulators. The signal is very broadband because both pulses and pulse bursts are repetitive. The primary stimulus of the QRS signal is most likely to be an induced electric field since it is well within range to be detectable by cell and/or tissue targets above background thermal and other voltage noise. Patients in both groups were treated identically, with an inactive QRS device being employed in the sham group. The QRS signal was administered by a health professional in a clinic setting daily for 8 mins twice per day for 6 weeks. Evaluations were at 0,4,6,10 weeks. Outcome measures included Knee Society Score (KSS), pain measures, CRP, P-fibrinogen and an inflammation parameter.

RESULTS: KSS improved only in the active group vs baseline, 7.3% vs 3% improvement ($P<0.05$). Knee function ($P<0.01$) and walking ability ($P<0.05$) improved significantly. Pain (28% vs 16%), general condition (6% vs 18%) and well being improved more in the active group ($P<0.01$). Medication use showed a non-significant trend to decrease in the active group. At 6 weeks P-fibrinogen showed a 14% decrease, C-reactive protein 35% decrease and blood sedimentation rate 19% decrease. Systolic blood pressure improved significantly (4% decrease vs 6% increase) after 6 wks and remained decreased for a further 4 weeks post QRS exposure.

CONCLUSIONS: The results from this study suggest that broadband ELF very low strength pulsing magnetic fields can have a physiologically significant impact on osteoarthritis of the knee. Compared to the devices discussed above, the complex QRS signal (like some bone healing devices) may couple more efficiently to the cell/tissue target than simpler sinusoidal waves. Clearly these results need to be extended, but they are promising enough to suggest that non-invasive weak pulsing electromagnetic fields may well be of importance in the treatment of osteoarthritis.

SESSION 9: RADIOFREQUENCY DOSIMETRY II
Chairs: Ken Joyner and Om Gandhi

9-1

SOME PRESENT PROBLEMS AND A PROPOSED EXPERIMENTAL PHANTOM FOR SAR COMPLIANCE TESTING OF CELLULAR TELEPHONES AT 835 AND 1900 MHZ. O.P. Gandhi, G. Kang*. Department of Electrical and Computer Engineering, University of Utah, Salt Lake City, Utah 84112, USA.

We compare the maximum allowable powers of some typical cellular telephones at 835 and 1900 MHz permitted by the SAR limits of ANSI/IEEE [1], ICNIRP [2], and the proposed modification of ANSI/IEEE which will treat pinna as an extremity tissue with considerably higher SAR limits and use the present SAR limit of 1.6 W/kg for any 1-g of tissue only for non-pinna "body tissues". It is shown that the present ANSI/IEEE guideline is the most conservative with the ICNIRP guidelines allowing a maximum radiated power that is 2.5-3 times higher and the proposed IEEE modification of treating pinna as an extremity tissue the least conservative allowing even higher radiated power by up to 50%.

Using the well-established finite-difference time-domain (FDTD) technique, we calculate the peak 1-g SAR of all tissues or of body tissues; and peak 10-g SAR for all tissues (as called for in the ICNIRP guidelines), for two anatomically-based models, i.e. the Utah model or the so-called "Visible Man" model and compare the same with the 6 mm thick plastic ear ($\varepsilon_r = 2.56$) homogenous model that has been proposed to obtain a "conservative" determination of the peak SAR limits suggested in the various safety guides. For these calculations given in Tables 1 and 2, we use both quarter wave monopole or helix type antennas and a variety of handset dimensions typical of today's telephones. Used for the calculations are the telephones held at an angle of 30° relative to the three head models. As seen in Tables 1 and 2, the SARs obtained with the plastic ear models are up to two or more times smaller than the anatomic models. This is due to a physical separation of 6 mm and absence in the plastic ear model of the high SAR region e.g. the pinna. For an anatomic model, on the other hand, the lossy ear acts as a coupler conducting the electromagnetic fields into the head resulting in higher SARs.

To alleviate the problem of lower SARs with the 6 mm thick plastic spacer (in the shape of pinna) phantoms, we propose a smoothened ear model of the human head such as that shown in Fig. 1. Since the SAR in the pinna region is substantial, the proposed phantom will use homogeneous lossy dielectric properties ($\varepsilon_r = 41.5$, $\sigma = 0.9$ S/m at 835 MHz and $\varepsilon_r = 40.0$, $\sigma = 1.4$ S/m at 1900 MHz) everywhere including the volume occupied by the smoothened pinna. For a 2 mm plastic shell of $\varepsilon_r = 2.56$ or $\varepsilon_r = 4.0$ assumed to contain the fluid for the desired dielectric properties, the calculated peak 10-g all-tissue SARs are within ±15% of the SARs obtained with realistic anatomic model of the head. A similar agreement for peak 1-g SARs within ±10% is also obtained for this thin shell lossy pinna phantom with the SARs for anatomic models at 1900 MHz, but the SARs are still considerably smaller at 835 MHz. At this lower frequency, it may be possible to use a higher conductivity fluid to get SARs to agree better with those of anatomically-realistic models.

Fig. 1. A proposed 6 mm thick smooth **lossy ear** phantom to obtain peak 1- and 10-g SARs within ±10-15% of those obtained for anatomically-realistic models. This model with the lossy ear replaced by a 6 mm thick plastic ear ($\varepsilon_r = 2.56$) gives SARs that are a factor of 2 or more lower as given in Tables 1, 2.

Table 1. Comparison of the peak 1-g SARs for all tissues (ANSI/IEEE Guidelines) and body tissues for two anatomically-based models with the corresponding 6 mm thick smooth plastic ear models.

Handset Dimensions mm	Antenna	Model	Peak 1-g SAR (W/kg)		
			1-g all tissues, anatomically-based model	1-g body tissues, anatomically-based model	6 mm thick plastic ear, homogeneous model
			1900 MHz, 125 mW Radiated Power		
22 x 42 x 122	Monopole, 40 mm length	Utah	2.38	1.02	1.00
22 x 42 x 82	Monopole, 40 mm length	Utah	2.24	1.00	0.98
22 x 42 x 82	Monopole, 40 mm length	"Visible man"	2.55	0.95	0.80
22 x 42 x 122	Helix, 20 mm length	Utah	3.05	1.32	1.26
22 x 42 x 82	Helix, 20 mm length	Utah	2.96	1.23	1.30
22 x 42 x 82	Helix, 20 mm length	"Visible man"	3.37	1.17	0.97
			835 MHz, 600 mW Radiated Power		
22 x 42 x 122	Monopole, 80 mm length	Utah	9.09	3.20	2.73
22 x 42 x 122	Monopole, 80 mm length	"Visible man"	3.43	3.29	2.80
22 x 42 x 122	Helix, 20 mm length	Utah	13.20	3.91	3.66
22 x 42 x 122	Helix, 20 mm length	"Visible man"	4.46	4.29	3.65

Table 2. Comparison of the peak 10-g SARs for all tissues (ICNIRP Guidelines) for two anatomically-based models with the corresponding 6 mm thick smooth plastic ear models.

Handset Dimensions mm	Antenna	Model	Peak 10-g SAR (W/kg)	
			Anatomically-based model	6 mm thick plastic ear, homogeneous model
			1900 MHz, 125 mW Radiated Power	
22 x 42 x 122	Monopole, 40 mm length	Utah	1.14	0.62
22 x 42 x 82	Monopole, 40 mm length	Utah	1.09	0.61
22 x 42 x 82	Monopole, 40 mm length	"Visible man"	1.03	0.56
22 x 42 x 122	Helix, 20 mm length	Utah	1.44	0.77
22 x 42 x 82	Helix, 20 mm length	Utah	1.37	0.76
22 x 42 x 82	Helix, 20 mm length	"Visible man"	1.28	0.67
			835 MHz, 600 mW Radiated Power	
22 x 42 x 122	Monopole, 80 mm length	Utah	4.03	1.96
22 x 42 x 102	Monopole, 80 mm length	Utah	3.58	2.28
22 x 42 x 122	Helix, 20 mm length	Utah	5.53	2.64
22 x 42 x 102	Helix, 20 mm length	Utah	5.02	3.27

References.
[1] American National Standards Institute (ANSI)/IEEE Std. C95.1 1992 Safety levels with respect to human exposure to radiofrequency electromagnetic fields. 3 kHz to 300 GHz. Copyright 1992 by the Institute of Electrical and Electronics Engineers (IEEE), Inc., New York, NY 10017.
[2] International Commission on Non-Ionizing Radiation Protection (ICNIRP) Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz) *Health Physics*, Vol. 74, pp. 494-5222, 1998.

9-2

CONTRIBUTION OF GSM-SIGNALS OF DIFFERENT FREQUENCIES TO THE INTERFERENCE THRESHOLD OF CARDIAC PACEMAKERS. J. Silny[1], K. Martin, V. Hombach[2]. [1]*femu*-Aachen University of Technology (RWTH), 52074 Aachen, Germany; [2]DeTeMobil Deutsche Telekom MobilNet, Darmstadt, Germany.

OBJECTIVES: It is well known that digitally modulated electromagnetic fields of a cellular GSM-phone can disturb the functions of implanted devices with electronic circuits if their distance is below 20 cm. Unknown, however, is the contribution of several independent GSM-signals to the interference threshold of an individual implanted device. In benchmark tests with cardiac pacemakers (CPMs) we investigate the contribution of one to four CW or low frequency-modulated microwave signals of different frequencies to the interference threshold of CPMs.
METHODS: More than 30 older and present-day CPMs are used in these benchmark tests. In order to reach an interference threshold in each pacemaker either one microwave signal or a sum of up to four microwave signals of different frequencies are amplified together and applied to the input of one device at a time. The individual microwave carriers have frequencies of 949.6, 950, 950.4 and 950.8 MHz, respectively, and the signals are CW or modulated with pulses of 2 Hz, 8 Hz or 217 Hz, alternatively. It is recorded which sum amplitude of the individual microwave composition is required for different specific interferences and individual pacemakers.
RESULTS: CW and especially microwaves with a low frequency-modulation can initiate different kinds of over- and undersensing or even a full inhibition of the CPMs. The lowest interference thresholds were measured at the modulation frequency of 8 Hz. The interference threshold is higher at 2 Hz modulation and much higher for the CW and 217 Hz modulation. On the whole, the older CPM models are more sensitive to microwave interference than the modern pacemakers. Nevertheless, some modern pacemakers have also

shown surprisingly low resistance to electromagnetic interference with microwaves. The amplitude of the individual microwave sources required for a specific disturbance of CPMs declines with the number of added microwave signals. In case of several microwave signals with different frequencies the interference thresholds of the CPMs result from the addition of the power densities but not by the sum of the amplitudes of the individual sources.

9-3

SAR ASSESSMENT IN THE NEAR FIELD OF BASE STATION ANTENNAS USING A HYBRID FEM / MOM TECHNIQUE. [1]F.J.C. Meyer, [1]D.B. Davidson*, [1]U. Jakobus*. [1]EM Software and Systems, Quantum House, Technopark, Stellenbosch, 7599, South Africa.

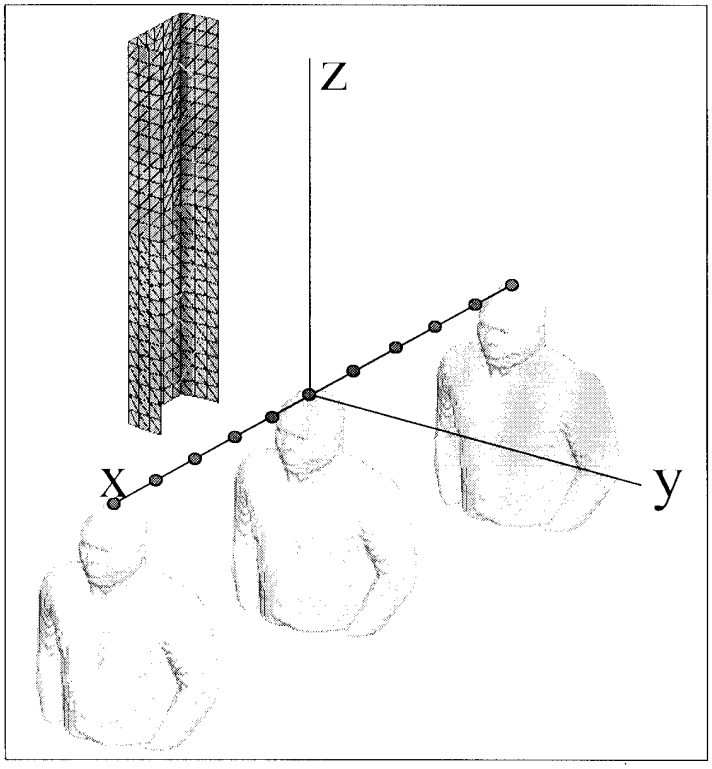
OBJECTIVES: Accurate SAR assessment in the human body situated in the near field of a base station antenna cannot efficiently be addressed using the popular Finite Difference Time Domain (FDTD) technique. The main difficulty is the large volume of free space around the base station antenna and human body that has to be included in a FDTD model (see Figure 1). The aim of this work was to couple the Method of Moments (MoM) technique with a Finite Element Method (FEM). A successful FEM/MoM implementation promised to be a highly efficient method for addressing the problem at hand. The MoM can be used to solve antenna problems involving metallic plates and wires. The FEM can be used for modeling the heterogeneous human body. The hybrid FEM/MoM would avoid modeling of the large free space region that exists in the base station / human exposure scenario due to the inherently open region formulation of the MoM.

METHOD: The standard frequency domain MoM, as implemented in the commercial code FEKO (www.feko.co.za), has been extended and hybridized with a FEM. The FEM is applied in a closed volume of finite extent. In this application the FEM region includes a volumetric human body model. The MoM is applied on the metallic surface patches and wires of the base station antenna model, and also on the surface enclosure of the FEM volume, serving as an exact radiation boundary condition for the FEM and ensuring full coupling between the antenna (MoM region) and human body (FEM region). The numerical solution of the hybrid technique is computationally intensive due to the large electrical size of the problem. A 3D tetrahedral discretization is required for the FEM human body model. This leads to a large, sparse matrix equation, consisting of millions of unknown field coefficients, that needs to be solved. A 2D triangular and 1D line segment discretization is required on the metallic surface and wire elements of the MoM base station antenna model. This leads to a relatively small, but dense matrix equation, consisting of thousands of unknown current coefficients. The sparse, FEM, matrix equation has an O(N) memory requirement and $O(N^{1..2})$ solution time dependency. The dense, MoM, matrix equation has an $O(N^2)$ memory requirement and $O(N^{2..3})$ solution time dependency. The combined solution of the FEM and MoM matrix equations is required. This was implemented using up to date linear algebra technology. The solution yields the electric field values inside the FEM region (body) from which the SAR can be calculated.

RESULTS: Validation of the hybrid FEM/MoM technique and its implementation was performed by comparison to FDTD results of a human body exposed to an incident plane wave (Figure 2). This is manageable with an FDTD method because no base station is included in the model and the free space region is thus kept to a minimum around the human body model. Further validation was performed by comparison to a MoM surface equivalence principle (SEP) implementation, for a human head exposed to a base station antenna (Figure 3). In this case the base station can be included but the body model size is limited by the rapid growth in computational requirements associated with the MoM-SEP method. The agreement is excellent for all validation tests performed. The hybrid technique was further employed to calculate averaged full body SAR as well as the SAR in critical organs in the human body, when exposed to different positions in the near field of a typical base station antenna operating at 900MHz (Figure 1 and Figure 4). A total of 60Watt radiated power was assumed with the calculation of the results in Figure 4.

CONCLUSIONS: The hybrid FEM/MoM technique is a highly efficient and accurate numerical technique for SAR assessment in humans in the near field of base station antennas.

Figure 1



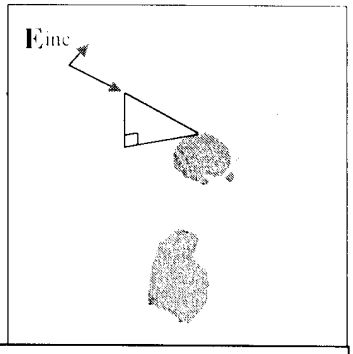


Figure 2

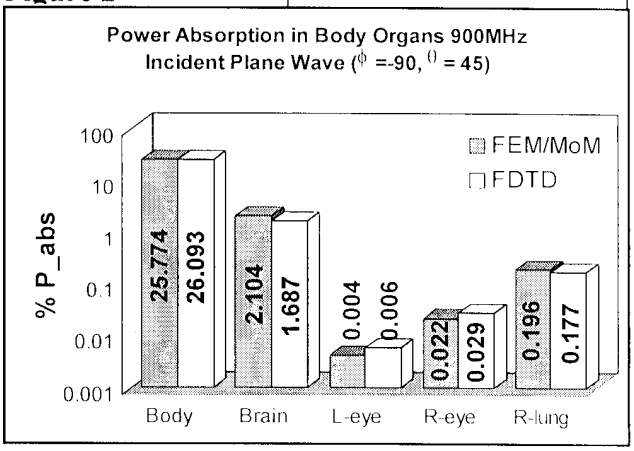
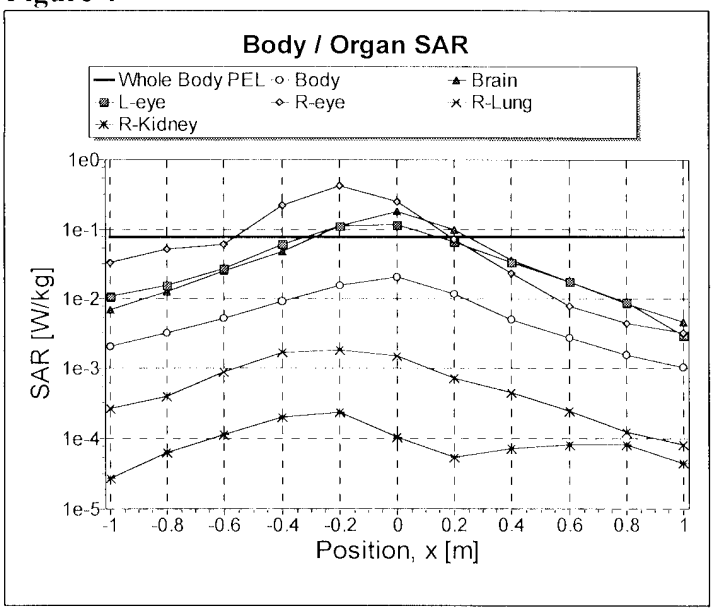


Figure 4



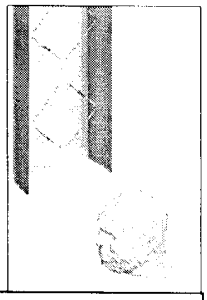
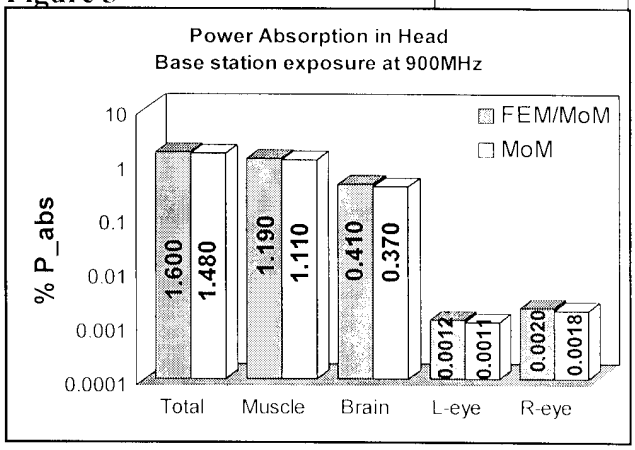


Figure 3



9-4

THERMAL MODELING OF MILLIMETER WAVE ABSORPTION IN THE NONHUMAN PRIMATE EYE AT 35 GHz AND 94 GHz. K. Foster, J.A. D'Andrea, S. Chalfin, D.J. Hatcher. University of Pennsylvania Department of Bioengineering, Philadelphia, Pennsylvania, 19140, USA.

OBJECTIVE: Chalfin et al (Health Physics, in press) have recently reported thresholds for producing corneal lesions in the primate eye from exposure to millimeter wave energy at 35 and 94 GHz. In those studies, which were conducted on five juvenile rhesus monkeys (*Macaca mulatta*), the mean fluence required to produce a threshold corneal lesion (faint epithelial edema and fluorescein staining) was 7.5 J cm^{-2} at 35 GHz and 5 J cm^{-2} at 94 GHz. This present study develops a thermal model for the exposures, to provide estimates of the thermal dose corresponding to the measured thresholds for injury expressed in terms of fluence. Its objective is to allow one to extrapolate from present data to predict thresholds for corneal injury under different exposure conditions (different frequencies or exposure duration) and at other environmental conditions.

METHODS: The model is based on the one-dimensional heat conduction equation for the cornea, which is solved both analytically and numerically (using the finite element method). Experimental data consist of thermal measurements (using an infrared camera) of the corneal temperature in five juvenile rhesus monkeys (Macaca mulatta) during exposures to 94 GHz microwave energy at several incident power levels and exposure times.

RESULTS AND DISCUSSION: The calculated temperature increases from the thermal model, based on parameter values taken from the literature, agree well with measured temperature increases, although considerable scatter is present in the data. The experimentally observed thresholds for thermal injury at both 35 and 94 GHz, expressed in terms of fluence, correspond to maximum surface temperatures of 47-48 C. This is considerably below thresholds for thermal injury reported in other tissues in previous studies. This may be attributed in part to varying definitions of thermal injury in the various studies (minimal fluorescein uptake in the cornea, vs. blistering or damage evident under histological evaluation). The thermal model, coupled with a kinetic model for thermal damage to the cornea, predicts the dependence of thresholds for corneal damage on both the irradiation time and microwave frequency.

Reference.

Chalfin C, D'Andrea JA, Comeau PD, Belt ME, Hatcher DJ (Health Physics, in press), Millimeter Wave Absorption In The Nonhuman Primate Eye at 35 GHz and 94 GHz.

9-5

EFFECT OF 2 HOUR GSM-900 MICROWAVE EXPOSURES AT 2.0, 0.5, AND 0.12 W/kg ON PLASMA PROTEIN EXTRAVASATION IN RAT BRAIN AND DURA MATER. F. Töre, P.E. Dulou, E. Haro, B. Veyret, P. Aubineau. CNRS UMR 5017, Universite Bordeaux 2, Bordeaux, France.

AIMS: Previous investigations on a possible influence of GSM microwaves on blood-brain barrier permeability have given contradictory results. The present study is a re-apraisal of this problem using well-defined experimental conditions regarding SAR levels of head exposure as well as the possible influence of stress during exposure (monitoring of arterial pressure, anaesthetized controls). The influence of 2-hour exposures to GSM-900 microwaves on plasma protein extravasation was tested at brain-averaged SARs in the range 0.12-2 W/kg. This influence was assessed both in rat brain and dura mater (the outermost meninge which is richly supplied with autonomic and sensitive nerve fibers, and which is believed to be the site of migraine mechanisms). Two age-matched animal models were used: i) normal rats, ii) rats subjected to chronic dura-mater neurogenic inflammation induced by bilateral sympathetic superior cervical ganglionectomy (considered as a model of migraine headache).

MATERIALS AND METHODS: 70 Sprague-Dawley rats weighing 250 g were used. They were divided in 6 groups: 1. Control; 2. Sham-exposed; 3. Exposed; 4. Sympathectomized, and sham-exposed, 5. Sympathectomized and exposed; 6. Positive control for protein extravasation. All rats were kept for two months in the animal house and subjected to experimental procedures when weighing 400-450 g. This delay was necessary for sympathectomized animals to develop dura-mater inflammation after sympathectomy. It was performed by removing surgically the two superior cervical ganglia innervating both brain and meningeal blood vessels. Following these two months and a week of daily progressive habituation to contention in the "rockets", all rats were catheterized in the femoral artery and vein with chronic catheters implanted 24 h before the experiment. They were then exposed or sham-exposed for 2 hours in the "rocket" using a loop-antenna placed over their head and shifted by 5° relatively to the longitudinal midline in order to expose preferentially one cerebral hemisphere. Arterial blood pressure was continuously monitored during microwave exposure. Before exposure and 15 min before its ending, a solution of bovine serum albumin bound to fluorescein-isothiocyanate (BSA-FITC) was infused via the venous catheter. At the end of the experiment, rats were killed with an overdose of anesthetic and the head was perfused through the ascending aorta with saline followed by a fixative solution. Dura mater and brain were removed for histological procedures. Dura mater was immediately mounted *in toto* for fluorescence microscope examination. Before examination, the brain was cut using a cryomicrotome and sections were treated for indirect immunohistochemistry against BSA in order to increase the intrinsic fluorescence of BSA-FITC.
RESULTS: In all animals placed in rockets, arterial pressure varied within limits (100-130 mm Hg) that are not compatible with an opening of the BBB, which is known to occur in rat for values exceeding 170 mm Hg. Furthermore, no correlation was observed between the mean level of arterial pressure and the level of extravasation in individual animals. Microscopic examination showed no noticeable BSA-FITC extravasation in control and sham-exposed groups, both in meninge and brain parenchyma. In normal exposed rats, extravasation was noticeable in the dura mater at 2 W/kg and 0.5 W/kg and in the deeper cortical layers of the brain parenchyma, directly located under the loop-antenna (mainly parietal and frontal cortex of the more exposed hemisphere). In sympathectomized sham-exposed rats, an extravasation, more prominent than that observed in normal-exposed rats, was visible not only in the dura mater, confirming our previous results, but also in various areas of the brain. This extravasation was dramatically increased in sympathectomized exposed rats, mainly on the exposed side of the brain. In these animals, the brain areas located around the antenna and the dura mater showed levels of fluorescence resembling those observed in positive controls after osmotic shock. At 0.12 W/kg there was no extravasation in the dura mater nor in the brain parenchyma.
CONCLUSION: At high and moderate SAR values (2 W/kg and 0.5 W/kg averaged over the brain), GSM microwaves induced respectively marked and discrete permeabilization of intracranial blood vessels, both in the meninge and in the brain parenchyma. This permeabilization is much more prominent in animals made "inflammation-prone" by the degeneration of their cranial sympathetic supply leading to complex trophic phenomena, which favor both the hyper-development of pro-inflammatory structures such as the parasympathetic and sensory inputs as well as mast cells, and changes in the structure of blood vessels themselves. Permeabilization was not observed at the lower averaged SAR value presently tested, i.e., 0.12 W/kg. In the outer meninge, corresponding local SAR levels can be estimated respectively at around 20, 5 and 1.2 W/kg, the last two being unlikely to induce noticeable local increase in temperature. Experiments designed to measure possible temperature increases as a function of depth are in progress.
Acknowledgments: this work was supported by the COMOBIO project of the French ministries of Research and of Industry, by France Télécom, Bouygues Télécom and the CNRS.

9-6

AUTOMATING THE ANALYSIS OF THERMOMETRIC MICROWAVE-DOSIMETRY DATA FOR THE CALCULATION OF SAR. D.D. Cox[1], S.P. Mathur[2], J.A. D'Andrea[1], S-T. Lu[2]. [1]Naval Health Research Center Detachment, Brooks Air Force Base, Texas 78235 USA, McKesson HBOC BioServices, Brooks AFB, Texas, 78235 USA.

OBJECTIVE: Automate the calculations of local specific absorption rates (SARs) using the Error Analysis of a Thermometric Microwave-Dosimetry Procedure.
METHOD: Using LabVIEW version 6.1, a virtual instrument was designed to perform the Error Analysis of a Thermometric Microwave-Dosimetry Procedure developed by Gambrill, et al.(1993). This procedure is used to perfect the calculations of SAR by correcting the errors caused by the changing thermal diffusion. The process to obtain the corrected values required manual manipulation of data inside a statistical program to match the intersect points between the pre-exposure end-point and the exposure start-point and between the exposure stop-point and post-exposure start-point. This is a timely and tedious process where large amounts of data can consume several hours to complete. The automated process is able to accept existing thermometric data files with little or no modifications and to allow the user the ability to configure exposure variables. Once the data is transferred into the program it is graphed and displayed on the computer monitor. This is where the operator can manipulate the data by the point-and-click method to complete the slope-correcting phase. The automated process also enables the user to perform calculations needed to process the necessary correction factors, tissue types, and field densities to derive at normalized SAR.
SUMMARY: Normalized SAR calculations can be performed on location with the same data acquisition computer with-in moments of exposure completion. The automation of this procedure has enabled technicians to complete SAR calculations at a fraction of the time required to perform the task manually.
CONCLUSIONS: This version of the slope program is programmed as a stand-alone program that enables the user to use the program on a non-LabVIEW system. Future updates of the slope program are planned to implement this VI as an add-on to existing data collection programs. This modification would allow for real time normalized SAR calculations to be performed as data is being collected.
Supported by the Office of Naval Research Work Unit No. 601153N.M4023. This document is approved for public release; distribution is unlimited.
Reference.
Gambrill CS, DeAngelis ML, Lu S-T. 1993. Error analysis of a thermometric microwave-dosimetry procedure. In: Blank M, editor. Electricity and magnetism in biology and medicine. San Francisco: San Francisco Press. p 593-595.

9-7

SOFTWARE TOOLS TO CALCULATE THE FAR-FIELD FOR OPEN-ENDED WAVEGUIDE, PROLATE SPHERIOD SAR, THERMOMETRIC DOSIMETRY DATA, AND A 2D-FDTD MODEL COLOR-CODER. D. Hatcher[1], D. Cox[1], J. Ziriax[1], J. D'Andrea[1], S. Mathur[2]. [1]Naval Health Research Center Detachment, Brooks Air Force Base, Texas 78235 USA, [2]McKesson BioServices, Brooks Air Force Base, Texas, 78235 USA.

OBJECTIVE: A toolkit consisting of four computer programs provide the researcher with critical experimental calculations. Packaging dosimetry related programs together allows the experimenter to rapidly and accurately solve formulas that are typically required as part of a RF bioeffects experiment.
METHOD: (1) A Microsoft Excel macro (Calculations for Open-Ended Waveguide.xls) has been written to calculate the distance to the far-field when using an open-ended waveguide. Using a formula previously developed (Gandhi 1981), the macro determines the far-field distance, in meters, from the transmitted frequency, output power, distance increments and the waveguide type. The macro calculates, displays the

distance to far-field and draws a graph that shows the power densities (mw/cm^2) at each distance interval from the beginning of the far-field. (2) SAR.EXE calculates the SAR value for any given size prolate spheroid implementing Equation 5.10 in the Radiofrequency Radiation Dosimetry Handbook (Fourth Edition) by William D. Hurt and Luis Lozano. The program (SAR.EXE) written in Labview 6.1, simply asks the user for the height (a) and width (b) of the of the prolate spheroid (cm), the starting and ending frequency and the desired frequency intervals. The user may enter up to four different sized targets for each frequency with the peak SAR and resonant frequency displayed graphically as well as numerically. (3) Another Labview 6.1 program included in this toolkit is a procedure to automate the calculations of local SAR using the Error of Analysis of a Thermometric Microwave-Dosimetry procedure by C.S Gambrill, M.L. DeAngelis, and S. -T. Lu. This program ultimately produces normalized SAR results using the slope-correction process. The program includes features such as multiple file format acceptance, graphical representations of data, point-and-click operation and data collection for further study. (4) Also included is a procedure is to produce a color-coded display of SAR values or tissue types. A second Microsoft Excel macro (ModelCoderCoder.xls) in this toolkit will color each cell in an Excel spreadsheet according to a defined color scale for either the SAR values or tissue type data. The macro will perform several operations depending on which pull-down menu option is selected. SAR Color Coder creates a color table that will display a spectrum of colors ranging from dark purple (low SAR values) to red (high SAR values). The Tissue Type Color Coder macro creates the color table as used by (Mason et al., 1995) to create anatomical models. Each tissue type is represented by an integer and a unique color from the color table.
CONCLUSIONS: These four tools greatly reduce the time to perform frequently required calculations and will minimize human error. In the future, additional dosimetry related programs will be added as they are developed. All of these programs are available from the Naval Health Research Center Detachment upon request and are published with source code as government works.
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9-8

UMTS SIGNAL SOURCE FOR RF BIOELECTROMAGNETIC STUDIES. A. Glasmachers*, J. Streckert*, S. Gencol*, D. Rozic*, H. Ndoumbé Mbonjo Mbonjo*, A. Bitz*, V. Hansen. University of Wuppertal, Department of Electrical and Information Engineering, D-42097 Wuppertal, Germany.

INTRODUCTION: The launch of the 3rd generation universal mobile telecommunication system (UMTS) is expected for the nearest future in most European countries. With regard to the large number of additional base station antennas the public acceptance may be affected by misgivings concerning suspected adverse health effects. Being aware of this apprehensiveness and aiming at the clarification of the actual risk potential of the new UMTS features on a scientific basis a number of bioelectromagnetic experiments ('in vitro' as well as 'in vivo') have been initiated by official sponsors and by industrial associations in Germany. In order to get a set of comparable results it was decided to apply a standardized signal modulation scheme for all these investigations. Recently, the specifications of this generic UMTS test signal were defined (see References). The poster explains the implementation of the signal and the features of the developed signal generator.
OBJECTIVES: The UMTS signal to be generated differs markedly from the test signals that have been applied in experiments concerning the biological relevance of the GSM system where usually low-

frequency pulsed rf signals with a repetition frequency of 217 Hz (in some cases additionally 2 Hz, 8 Hz and 1733 Hz) and pulse duty cycles of 1:8 up to 7:8 have been generated by simply pulse-modulating a conventional 900 MHz or 1800 MHz rf signal generator. On the other hand, the generation of an UMTS signal that features the properties of real-world scenarios as a representative is much more difficult and requires an 'all-in-one' solution for the signal source, i.e. rf generation, modulation, filtering and so on. This is mainly due to the UMTS typical combination of wideband coding (CDMA) and digital phase modulation technique (QPSK) together with the fast signal variations as a consequence of the power control. An electronic concept has to consider this novelty of UMTS. Moreover, the practical built-up of the signal source should imply an utmost stability of the output signal even over long periods which are typical for long-term experiments, e.g. multiple-generation reproduction studies.

METHODS: The details of the generic UMTS test signal shown in the poster were obtained by considering signal contributions with low frequency time variations of the rf envelope in a worst-case sense, being often regarded as hypothetical candidates for biological relevant agents. The electronic implementation of the generic UMTS test signal is also demonstrated in the poster. The data streams for the I- and Q-input of the phase modulator were completely calculated from the defined signal properties inclusive the root-cosine filtering and are stored in four ROM-modules with a total storage capacity of 128 MByte which are read out periodically with a repetition rate of 4.3 s at a clock time of 65 ns. The rf with a carrier frequency of 1.966 GHz is generated by a phase-lock-loop frequency multiplier (multiplication factor 512) which is stimulated by a 3.84 MHz Reference frequency crystal oscillator. By the optional use of an external Reference source carrier frequencies between 1.6 GHz and 2.4 GHz can be generated. The rf output signal of the QPSK-modulator is amplitude-modulated by driving a controllable attenuator with the predefined modulation signal simulating the fast power control. The latter is stored in a separate ROM and is read out with a peridiocity of 1min (time resolution 0.67ms, dynamic range 30 dB). A second attenuator allows for the adjustment of the average power of the output signal (resolution 1dB, dynamic range 30 dB). Thus, a UMTS-typical rf signal with a spectral width of c. 5 MHz, a power variation according to a realistic power control scheme and an average power between 10 µW and 10 mW is available at the output.
Additionally, for calibration purposes and for intermodulation tests of a following power amplifier suitable test modes were implemented.
The absolute reproducibility and stability of the generator output is guaranteed by avoiding any kind of software solution in the electronic concept.

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The project has been supported by Forschungsgemeinschaft Funk, Bonn, Germany.

SESSION 10: SPECIAL SYMPOSIUM III (continued): EMERGING THERAPIES II
Chairs: Arthur Pilla and Joseph Salvatore

10-1

ELECTROMAGNETIC AND MECHANICAL MODALITIES IN THERAPEUTIC APPLICATIONS: FROM MECHANISMS TO THE CLINIC. A. Pilla. Department of Orthopaedics, Mount Sinai School of Medicine, New York, NY, USA.

INTRODUCTION: Low intensity, non-thermal time-varying electromagnetic fields, PMF; static magnetic fields, SMF; ultrasound, US; and mechanical loading, SGP signals have proven clinical benefit when used as adjunctive therapy for a variety of musculoskeletal injuries. Clearly, each of these signals appears to have the capacity to achieve a physiologically meaningful bioeffect. The first question is why should such seemingly different modalities be effective? The second question is how to know which parameters, or even which modality, to use. This study illustrates the common dosimetry parameters for molecular, cellular and tissue targets and how both EMF and mechanical signals can be predictively configured to couple to the same target, with potentially similar or synergistic therapeutic effects.
DOSIMETRY: Bioeffects from weak EMF signals are due to the time-varying electric field, E(t) induced from either an applied time-varying magnetic field, B(t), or from streaming (electrokinetic) potentials from the mechanical signals. Most EMF clinical devices induce a peak E of 1-10 mV/cm at the treatment site. Clinical US signals also induce E in the mV/cm range due to the non-linear effects of the propagation of the pressure wave in tissue (microstreaming), with major frequency components below 1 kHz. Induced E in this case is a single repetitive pseudo-rectangular pulse with slow (millisecond) rise and fall times. Induced E from SGP is most often a slowly decaying exponential-type function having peak amplitudes in the mV/cm range and major frequency components centered below 100 Hz. The question then is whether E(t) from US, SGP and EMF signals can be detected by a biological target given kinetics and thermal noise. The cell array tissue model, which describes the dynamic electrical response of cells in real tissue configurations, allows thermal thresholds (SNR) to be evaluated for induced electric fields from any signal, and for any kinetics. In contrast, a static magnetic field may be detected at input levels far below thermal noise in an ion binding pathway via Larmor precession of the already bound and thermally shielded ion-water complex. The biophysical link between time-varying and static magnetic fields is the Larmor frequency since it couples to the same time constant(s) in the ion binding pathway as the induced E from PMF signals. Each Larmor precession frequency determines the time to reach favored orientation(s), resulting in a modulation of ion binding kinetics with a subsequent effect on the relevant biochemical cascade.
PMF SIGNALS: The validity of the cell array/SNR approach was tested at the enzyme, cellular and animal level using a clinical PRF signal retuned to couple more efficiently to the kinetics of Ca^{2+}/CaM binding. The clinical PRF signal consists of a 65μsec burst of 27.12MHz sinusoidal waves at 600 bursts/sec inducing 2G peak magnetic field and V/cm range electric fields. The retuned PRF signal was 0.2G, 500μs burst at 1 burst/sec, inducing mV/cm range electric fields with a much broader frequency spectrum. The results showed the standard clinical and retuned PRF signals caused 195% vs 217% ($P<.01$) increase in myosin phosphorylation; 46% vs 43% ($P<.01$) increase in neurite outgrowth length from dorsal root ganglia explants; and 43% vs 39% ($P<.03$) increase in torsional breaking strength in a rabbit fibula bone repair model, respectively. The predictions of this model for the effect of PRF burst width were satisfied when the burst duration was reduced to 65μs, 0.2G at 1 burst/sec, resulting in no significant effect vs controls in the myosin and nerve regeneration models. It is of interest to note that Ca^{2+}/CaM is involved in the modulation of neurite outgrowth and bone repair, suggesting that the Ca^{2+} binding model may indeed have physiological relevance in all tested models.
SMF vs PMF SIGNALS: Dosimetry for static fields compares Larmor precession frequency to the binding rate constant for the static magnetic field. Assuming Ca^{2+} bound to one water molecule in the CaM binding

site, the periods of Larmor precession corresponding to the frequency window within which the 500μsec PRF signal is effective range from ≈ 0.1 to 3 msec. This suggests magnetic fields as low as 1G, should be capable of modulating systems dependent upon Ca^{2+} binding kinetics. Both PRF (retuned, 0.2G) and static fields (2-100G) produce 200% and 50% increases in myosin phosphorylation and neurite length vs controls, respectively (P<.01).

PMF vs US SIGNALS: Both PMF and US signals were tested on bilateral fibula osteotomies in 76 mature female New Zealand White rabbits. The active limb was treated either four hours (PMF) or 20 min (US) daily and sacrificed at POD 9. The PMF was a standard clinical signal (AME). The US waveform was a 200 μs burst of 1.5 MHz sine waves repeating at 1 kHz (Duarte). The incident US intensity was 30 mW/cm2, well within the non-thermal range. All fibula pairs were tested in torsion to failure at room temperature. Results at POD 9 for both US and EMF treatment modalities show both modalities were effective in accelerating the biomechanical evolution of fracture repair vs their respective contralateral controls.

CONCLUSIONS: The analyses presented in this study strongly suggest, if ion/ligand binding with known kinetics is the EMF-sensitive target, PMF, SMF, US or SGP signals may be predictively configured to achieve adequate dosimetry for a physiologically meaningful bioeffect. This may explain why many of the PMF and US signal variants, as well as relatively weak static magnetic fields appear capable of producing meaningful clinical effects. The approach utilized here may also lead to a rationale for the use of programmed (vs target state) and/or combined modalities to achieve optimum therapeutic effect.

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10-2

STATIC MAGNETIC FIELDS IN THE TREATMENT OF HUMAN MALIGNANCY. J.R. Salvatore. VA Medical Center, Phoenix, AZ.

Static magnet field therapy is used and promoted for the treatment of a variety of medical conditions, although the data to support or refute efficacy in these clinical settings is inconclusive. Electromagnetic fields have wide application in medical diagnosis and treatment, particularly in Neurology, Orthopedics/Rheumatology, Psychiatry, and pain control. Laboratory and clinical studies suggest that magnetic fields can either promote or retard growth of neoplastic cells, and that static magnetic fields may alter blood flow and enhance the efficacy of cancer chemotherapy. In cancer medicine, a Phase I clinical trial is done to generate a toxicity and safety profile of an anti-neoplastic therapy. Since there is insufficient data in the clinical literature on the effects of static magnet therapy in patients with malignant disease, a Phase I clinical trial to assess the safety of a static magnetic field applied during treatment of advanced human cancer would help to develop such a safety/toxicity profile. These results would be required before more extensive clinical trials of the efficacy of static magnetic fields in this clinical setting can be conducted. The objective of such work is to observe if Grade 3 or 4 toxicities occur with a static magnetic field (using the NCI CTEP V. 2.0 Common Toxicity Criteria), and to determine if there are any previously unknown toxicities.

10-3

ANTICANCER ACTIVITY BY MAGNETIC FIELDS: A POTENTIAL EMERGING THERAPEUTIC APPLI-CATION. S. Tofani. Department of Medical Physics, Ivrea Hospital, ASL 9, 10015 Ivrea - Italy.

The possibility that magnetic fields (MF) may contribute to cancer research and therapy has recently been investigated. MF of more than 1 mT have been hypothesized to exert anticancer activity. A mechanism for this activity is the possible MF influence on free radical recombination processes that activate p 53 gene dependent survival mechanisms [Tofani, 1999]. Extremely low frequency (ELF) MF of 100 mT were used to inhibit tumor growth in mice [de Seze, et al, 2000] Static MF of 110 mT were used to enhance chemotherapy in mice [Gray et al, 2000].

A multi-center Italian Research effort has investigated whether magnetic fields may have possible applications in oncology. As a result it has been shown [Tofani et al 1999; 2001a; in press] that appropriately modulated magnetic fields (MF) generated as shown in [Tofani, 2002] (static MF with a superimposition of ELF at 50Hz), with an average intensity higher than 3.59 mT cause from 40 to 50% tumor growth inhibition in nude mice, bearing a subcutaneous human colon adenocarcinoma induced by WiDr cell injection, exposed 70 min daily for 5 week for 4 consecutive weeks. Inhibition of tumor growth was reported together with a decrease in tumor cell mitotic index and proliferative activity. An increase in apoptosis and corresponding reduction of immunoreactive p 53 expression was also reported. Gross pathology, hematoclinical/hematological and histological examinations did not show any toxic or abnormal effects.

Similarly, it was also shown [Tofani et al, in press] that in the same *in vivo* model system the MF treatment significantly increased survival time (31%) of the nude mice exposed daily to 5.5 mT MF, starting 24 hours after WiDr cell inoculation.

Lastly, significant inhibition on spread and growth of lung metastases in exposed nude mice bearing a subcutaneous human breast tumor MDA-MB485 [Tofani et al, 2001b; submitted] was reported.

Data from the above cited *in vivo* experiments on the efficacy of MF as antitumor agent (tumor growth inhibition and survival) show that MF exposure affects early tumor growth (exposure starting on day 1 of tumor transplant) as well as advanced tumors (exposure starting when tumor was palpable, i.e. on day 15 or later). As well as it affects the metastatic processes too [Tofani et al 2001b; submitted].

New experiments aimed at a better understanding of the biophysical mechanisms and at the consolidation of anticancer activity data for possible future therapeutic application are in progress. The possible interference between MF and chemotherapeutic agents is also under investigation.

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10-4

CHEMOTHERAPY AND STATIC FIELDS: FROM THERAPY TO OVERDOSE. J.R. Gray*, C.H. Frith*, J.D. Parker*. Conundrum Project, 8816 Westwood Avenue, Little Rock, Arkansas 72204, USA.

INTRODUCTION: Mounting evidence suggests that static fields may provide therapeutic benefit. For example, static fields have previously been reported to enhance the in vivo affect of a single adriamycin (ADM) treatment against cancer (Gray, Frith, Parker, 2000). The study reported here illustrates the high-level of pharmacological synergism these fields may provide.
OBJECTIVE: Examine the ability of applied external static fields to potentate the in vivo affect of a two-dose regimen of ADM against murine mammary adenocarcinoma.
METHODS: Forty-four female B6C3F1 mice with implanted murine mammary adenocarcinoma were divided into four groups of 11 animals each on day 1 of the study. The animals in all four groups were then treated with 8-mg/kg ADM on day 3, and again on day 5, of the study. The Group A animals were not exposed to static fields at any time during the study. The animals in Groups B-D were exposed to static fields for four-hours following each ADM treatment. The
Group B animals were placed in a cage with a negative 15 kV charged screen placed above to expose them to a constant static electric field of approximately 80,000 V/m. The screen was encapsulated and did not create measurable ionic current flow to the animals. The Group C animals were exposed to the same 80,000 V/m field, but the field polarity was reversed each 15 minutes. For the Group D animals, the north-seeking pole of a 12.7-mm diameter by 5.1-mm long neodymium magnet was glued directly over each tumor, and the animals were placed in individual restraining tubes for 4-hours after each ADM dose to prevent removal of the magnets. The magnets exposed the Group D animals to a static magnetic field ranging from approximately 0.338 T at the skin surface to 0.044 T around the heart area.
RESULTS: Some of the animals in the three field exposed groups began dying on day 10 of the study (see Table 1). Following this, more than half of the animals in Groups C and D died by day 12, with additional deaths for Groups B-D through day 28. This was completely unexpected. ADM treatment at this level is known to be safe for these animals. Also, the tumors in these Groups were still small and not a factor in the deaths. The first animal death in Group A occurred on day 30, and this was from the affect of the tumor (all tumors in this Group were very large by this time). Necropsy revealed heart enlargement consistent with congestive heart failure in all of the animals dying during study days 10-25 (all in Groups B-D), but not in the single Group A animal dying on day 30.
CONCLUSIONS: Both the static electric and static magnetic fields increased the animals' cardio-cellular reaction with the ADM to a lethal level. Delayed congestive heart failure, presenting a few days after treatment, is the classic symptom of ADM overdose. That appears to be the effect created by both field types although, as shown by the Group A animals, the ADM dose level was safe. Since the ADM molecule does not carry a dominant electrical charge it is likely that the fields were not directly affecting the ADM, but were instead increasing cellular reaction with the ADM. To our knowledge, this is the strongest in vivo influence ever observed from a non-ionizing field of any type. Based on the typical ADM LD 50 for this

animal, we believe both static fields almost doubled the ADM/cellular reaction. We also have reason to believe that these fields can potentate the in vivo reaction of at least some other chemotherapy agents. This technology seems to present a highly desirable modality. It may be possible to treat cancer patients with significantly smaller than normal systemic doses of chemotherapy, then use controlled application of static fields to significantly increase cell kill only in tumor areas.

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This work was supported by the family of Gary Lynn Gray.

Table 1: Animal Fatalities

Day		GA	GB	GC	GD
0	Implant all tumors				
3	1st ADM injection, all groups				
5	2nd ADM injection, all groups				
10					XXX
11				X	X
12			XXX	XXXXXX	XX
13					X
18			X		
19			X		
22			X		XX
25					X
27					X
28			X		
30		X			

X = animal death

10-5

BRIEF DAILY EXPOSURES TO A PULSATING MAGNETIC FIELD SAFELY SUPPRESSES TUMOR GROWTH AND ANGIOGENESIS. I.L. Cameron[1], W.E. Hardman[2], M.S. Markov[3], C.D. Williams[3]. [1]University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7762, USA; [2]Pennington Biomedical Research Center, Louisiana State University, 6400 Perkins Road, Baton Rouge, Louisiana 70808, USA; [3]EMF Therapeutics, Inc., 1200 Mountain Creek Road, Suite 160, Chattanooga, Tennessee 37405, USA.

BACKGROUND: A number of beneficial effects of extremely low frequency electromagnetic fields (EMF) have been reported in the last two decades, especially in the treatment of musculoskeletal disorders and pain. There are however few reports of treatment of tumors by EMF. EMF Therapeutics, Inc., Chattanooga TN, USA, has developed a new approach to cancer therapy based on the application of a therapeutic electromagnetic field (TEMF).

MATERIALS AND METHODS: The study was designed to explore the effects of daily treatment with a fully rectified 60 Hz sinewave transferred to 120 pulses per second TEMF, at a range of TEMF intensities and times, on growth and vascularization of established and measurable murine 16/C mammary adenocarcinomas growing in C3H/HeJ mice. Tumors were allowed to grow for seven days until the tumor mass reached 100 mg before the treatment started. Applied magnetic field intensities were 0, 10, 15 or 20 mT. Animals (10 per treatment group, 20 in the control group) received treatment for 0, 3, 10 or 40 min daily or twice daily. The fast growth of tumors in the control group limited the length of the experiment to 12 days. The extent of vascularization was evaluated by the expression of CD31 in the tumors. Tumors from all mice were cryosectioned, immunohistochemically stained using an antibody against the vascular

endothelial cell determinant CD31 and the extent of tumor vascularization, viable area and necrotic area was measured by morphometrics.
RESULTS AND DISCUSSION: Assessment of tumor volume after nine days of treatment revealed significant suppression of tumor growth under all TEMF exposure conditions. Twice daily exposure was no more effective in tumor suppression than a single daily exposure. The largest reduction in vascularization was observed in the tumors exposed to 15 mT while the largest inhibition of tumor growth was observed in the mice exposed to 20 mT. The magnetic fields significantly reduced the percentage of CD31 staining in the tumors of all three groups: by 39% (at 10 mT), 68% (at 15 mT) and 62% (at 20 mT) thus indicating a reduction in vascularization. At initiation of TEMF treatment, the tumor volumes of all groups were approximately equal, however, the final tumor volume of the control group was 3567 mm^3 while the final tumor volume of groups exposed to TEMF was 2945 mm^3 (for 10 mT), 2807 mm^3 (for 15 mT) and 2563 mm^3 (for 20 mT). After 12 days of treatment mice exposed to 15 and 20 mT had fewer tumor associated deaths than did the control mice. The necrotic area in the tumors of the control group was 25% and increased to 32% for 10 mT, 42% for 15 mT and 36% for 20 mT treatment. Thus, a 10 min daily exposure to 15 or 20 mT fields significantly reduced tumor growth and tumor vascularization and was associated with a concomitant increase in tumor necrosis. The observed maximum of effects at 15-20 mT suggests the possibility of an amplitude window, consistent with results already reported in several papers.
CONCLUSIONS: The results suggest that the blood vessel network in the growing tumor is a target for TEMF and that TEMF therapy may be a simple and safe adjuvant to increase the efficacy of conventional therapy for vascularized and growing tumors.
This study was supported by EMF Therapeutics Inc.

10-6

EFFECTS OF PULSED RF ENERGY ON POSTMASTECTOMY ARM LYMPHEDEMA. H.N. Mayrovitz*, N. Sims*, J. Macdonald*. Department of Physiology, College of Medical Sciences, Nova Southeastern University, Ft. Lauderdale, Florida, 33328, USA.

OBJECTIVES: Arm lymphedema (L-E) that occurs following mastectomy and related cancer treatment often develops gradually, and if untreated tends to worsen. Therapeutic measures, such as complete decongestive therapy (CDT) which includes compression and massage, are effective in reducing L-E in some patients. A physiological aspect of properly applied massage is its promotion of lymphatic drainage by expanding collateral lymphatic channels that connect to normally functioning lymphatic collectors. This provides useful alternative lymphatic pathways to accommodate drainage of excess lymph that is blocked from its normal routes. It was reasoned that if a simple method were available to facilitate collateral lymphatic enlargement then it might initially augment CDT outcomes and possibly provide patients with a longer-term continuous therapy option. Because previous work[1-2] showed that low-energy pulsed radio-frequency therapy at 27.120 MHz increased skin blood flow, likely via enlargement of vascular channels, it was hypothesized that this approach might also serve to similarly affect lymphatic channels. We therefore sought to determine if such an approach might have a positive impact on L-E reduction. As this therapy has not been previously used for L-E, the research was exploratory, with the goal to determine if treatments alone would provide evidence of potential efficacy.
METHODS: Seven post-mastectomy patients with unilateral arm L-E were studied. They were treated 4-6 times for 60 minutes over a 2-week interval using pulsed RF (27.120 MHz at 700 pps, Magnatherm, IME). The dual heads of the device were placed over the affected arm about one cm. from the skin. Arm volumes were measured before starting treatment and prior to the start of each follow-up treatment. Edema volume was calculated as the difference between the volume of the affected (treated) arm and the control arm. Percent edema was calculated as edema volume divided by control arm volume. Skin blood perfusion (SBF) was measured by laser-Doppler using a thin non-metallic laser-Doppler probe placed on the affected arm at

a standardized site midway between the wrist and elbow. Transcutaneous oxygen tension ($TcPO_2$) was monitored with $TcPO_2$ probes placed on the affected and control arm.

RESULTS: Arm volume data (mean ± sem) showed that the initial percent edema of 24.5 ± 7.3%, was significantly reduced to 18.5 ± 6.3% ($p<0.01$) after one treatment, with further reductions occurring through the fourth treatment. Four treatments were associated with a reduction in percent edema to 56.2 ± 8.4% of its initial value. The largest effect appeared to occur early in the treatment sequence. SBF significantly increased after about 30 minutes of treatment, continued to rise and was maintained for at least 20 minutes after treatment was terminated. Thus, SBF showed a progressive increase from its baseline value of 266 ± 10 a.u. (Friedman test, $p<0.001$) and was significantly greater than baseline after 30 minutes of treatment (371 ± 38 a.u., $p=0.018$ Wilcoxon test). By 60 minutes, SBF reached 705 ± 122 a.u., which was on average 4.10 ± 0.87 times greater than baseline. Contrastingly, $TcPO_2$ did not significantly change in either the treated or control arm. Prior to initiation of treatment, $TcPO_2$ values were not significantly different between affected and treated arms (72.7 ±6.9 vs. 64.1 ± 6.4 mmHg).

CONCLUSIONS: The initial results are encouraging, especially in view of the fact that the women in this pilot study had already received CDT therapy and had long standing residual lymphedema. Volume reduction was rapid and significant and occurred using only ~10% of the device maximum power. It is unknown if different power levels would change the observed short-term outcome. The role of the observed SBF increase during individual treatments, in mediating the lymphedema reduction, is not known. However, an intriguing possibility is that mechanisms similar to those that cause SBF to increase, also act to increase lymphatic flow by expanding collateral channels or by enhancing functional activity of lymph vessels. Although these initial findings are encouraging and the method tested may prove to be a useful compliment to current therapeutic practice, final conclusions must await further and expanded placebo controlled tests that are currently underway.

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10-7

A NON-INVASIVE PULSED ELECTROSTATIC FIELD (ETG) REDUCES HAIR LOSS IN BREAST CANCER PATIENTS AND ITS BEHAVIORAL IMPLICATIONS: A PILOT CLINICAL STUDY.

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INTRODUCTION: Alopecia (baldness) is a common and distressing consequence of cyclophosphamide/ methotrexate/ fluorouracil (CMF) chemotherapy for breast cancer, the standard treatment protocol used in New Zealand. Hair cells multiply rapidly and chemotherapeutic agents produce an anagen effluvium by killing actively mitotic cells in the hair matrix. Hair loss occurs immediately or in 7 to 14 days. Studies indicate a concern about hair loss occurs in as many as 91% of patients undergoing CMF for breast cancer. Hair loss during the time of treatment can result in significant psychological trauma. Breast cancer diagnosis and subsequent therapy may elicit responses such as depression, anxiety, hostility, decreased self-esteem, hopelessness, denial and loss of control. The end result could be a reduction in the effectiveness of the prescribed medical treatment. Options to alleviate hair loss include cold caps, no longer considered appropriate as they may provide a sanctuary for malignant cells, and pharmacological agents, such as Minoxidil, which do not prevent hair loss. Previous reports by Maddin et al. showed efficacy and safety of Electrotrichogenesis (ETG) in which a pulsed electrostatic field is non-invasively applied to the scalp in

men with androgenetic alopecia. The objective of this study was to determine whether or not a similar pulsed electrostatic field can prevent or reduce hair loss for patients undergoing CMF chemotherapy.
METHODS: Patients were included if they were age ≥19 to 65 years with a clinical diagnosis of breast cancer and awaiting CMF chemotherapy; an ability to understand the protocol and provide informed consent; a willingness to undergo a medical examination as required. The study was restricted to women. Exclusion criteria were: a history of hair thinning or alopecia during the previous 12 months; other conditions such as scarring alopecia, dermatitis or psoriasis. Also excluded were subjects with a history of rheumatic, connective tissue, cardiovascular, liver, or kidney disease, endocrine dysfunction and/or psychiatric or emotional disorders, as well as those who had used topical Minoxidil within the past 3 months. Other exclusion criteria included any metal implants in the skull or the presence of a heart pacemaker or participation in any other investigational drug or device evaluation. The ETG device (CTC, Inc., Vancouver, BC) consists of an ergonomically designed reclining chair with an adjustable half-spherical hood containing insulated metal plates attached at the head end which emits a pulsed electrostatic field. The subject sits under the hood for a twelve-minute treatment session and no active element is in contact with the scalp during this time (air-coupled). The applied signal consisted of a millisecond time-scale pulse repeating at 8 Hz, inducing a peak electric field just outside the scalp of 5 V/cm. Two twelve-minute ETG treatments were administered twice weekly, two days apart, commencing two weeks prior to chemotherapy for six weeks. This was followed by one ETG treatment each week for 12 weeks, then 2 weeks of twice weekly treatments followed by once weekly until 6 weeks after the end of the chemotherapy regime. Efficacy assessments included photographs; quantitative assessment of hair loss using a standard brush technique; and subject, investigator and nurse's assessment of hair loss, all collected weekly.
CLINICAL RESULTS: Of the fourteen women enrolled, the mean age was 43 (25-59). In addition to chemotherapy, all had undergone previous surgery and some had undergone radiotherapy. Thirteen subjects were evaluated. One withdrew due to non-compliance. Quantitative assessment of hair loss by counting of the number of hairs collected in a brush shows there was no significant difference between the mean number of hairs collected before and after the completed course of chemotherapy treatments. The subjective assessments by the subject, nurse, and investigator, as well as the photographs, were rated on a scale of 0-4, with 0 total hair loss, 4 slight hair increase and 3 no change. Hair loss was negligible (<15%) in 12 of 13 subjects (92%), and approximately 50% in one subject. This is to be compared with the reported 50 ± 20% mean hair loss for patients undergoing standard CMF therapy. There were no side effects reported.
CONCLUSIONS: This pilot study suggests adjunctive treatment with a pulsed electrostatic signal (ETG) substantially reduces or prevents hair loss occurring during chemotherapy for the treatment of breast cancer in women. Since hair loss generally accompanies chemotherapy, the implications for widespread use and adoption of ETG therapy are substantial and may well impact on the overall clinical effectiveness of many chemotherapy regimens.
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10-8

MODULATOR EFFECTS OF STATIC MAGNETIC FIELD EXPOSURE ON MICROCIRCULATION AND SYSTEMIC CIRCULATION IN ANIMALS. C. Ohkubo[1], H. Okano[1,2], S. Xu[1,2], J. Gmitrov[1,3]. [1]Department of Physiological Hygiene, National Institute of Public Health, Tokyo 108-8638, Japan. [2]Department of Science, Pip Tokyo 101-8528, Japan, [3]IV. Internal Medicine Clinic, L. Pasteur University Hospital, Kosice, Slovak Republic.

OBJECTIVE: Static magnetic fields have been used in pain clinic for many countries. However, the physiological mechanism is still unclear. The studies have been carried out to elucidate the exposure effects of relatively weak static magnetic fields on microcirculatory system in animals.

METHODS AND RESULTS: A series of *in vivo* studies[1-12)] on microcirculation and systemic circulation in animals have been made since 1996. Effects of local or whole body exposures of static magentic fields (SMF) on cutaneous microcirculation within a rabbit ear chamber under conscious conditions[1-4, 9)] and skeletal muscle microcirculation under anesthetized conditions in mice[7)] were studied intravital-microscopically. Concurrent with intravital-microscopic study, blood pressure and heart rate were also monitored[2, 5, 6, 8, 9-11)]. Rabbits having a transparent ear chamber were subjected to microphotoelectric plethysmography. Following the acute exposures (less than 1 min) of SMF, the spontaneous and continuous rhythmic fluctuation of microvascular blood flow due to vasomotion was modified. There was no dose-response relationship between the extent of vasomotion changes and power levels of SMF (1, 5, 10, 100 mT). One mT of SMF for 10 min modulated the vascular tone biphasically [1,4)]. Sub-chronic exposure of SMF (180 mT) for several weeks induced enhancement of vasomotion and dilatation of arterioles[3)]. Under pharmacologically induced low vascular tone, acute exposure (10 - 30 min) of SMF (1-5.5 mT) evoked vasoconstriction, in contrast, under pharmacologically induced high vascular tone it did vasodilatation[4, 8, 11)]. For 10 min of whole body exposure to SMF (1- 10 mT), the capillary blood flow velocity of tibialis anterior muscle was accelerated and carotid blood pressure was elevated from its low level due to anesthesia[7)]. These changes in blood pressure and/or heart rate including microcirculatory parameter were also recognized in rabbits under conscious conditions at higher doses of SMF(more than 200 mT) which was applied to the skin surface ares of the carotid sinus[5,6,9,10)] as well as in genetically hypertensive rats under conscious conditions at lower doses of whole body exposure of SMF(3-25 mT) [12)].

CONCLUSION: The results mentioned above indicate that relatively weak SMF can modulate beneficially both micro- and macrocirculation under untoward conditions. These results suggest that the SMF in a certain intensity can modulate vascular tone beneficially due to modification of vasomotion, biphasically.

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SESSION 11: INVIVO STUDIES ANIMAL II
Chairs: Bernard Veyret and Ronald Seaman

11-1

IN VIVO EXPOSURE TO GSM-MODULATED 900 MHZ FOR 1,2 OR 4 WEEKS DOES NOT AFFECT MOUSE PERIPHERAL LYMPHOCYTES. L. Gatta*, R. Pinto*, V. Ubaldi*, L. Pace*, P. Galloni*, G. Lovisolo*, C. Marino*, C. Pioli*. ENEA, Biotechnology Unit, Rome, I-00060, Italy.

INTRODUCTION: In the last few years, the use of mobile phone telecommunications increased exponentially and general public concerns generated a large discussion on potential hazards associated with the exposition to electromagnetic fields generated by wireless communications. Studies on lymphocytes exposed to RF describe effects ranging from inhibition of cell proliferation to complete lack of effects. In

the present study, we investigated the effects of 1, 2 or 4 weeks *in vivo* exposure to 900 MHz GSM-modulated fields on spleen cells. Cell counts, proliferation rate, cytokine production, cell phenotype and the expression of early activation markers were evaluated.
METHODS: C57BL/6 mice have been exposed in a TEM cell to GSM signals, 2h/day, for 7, 14 or 28 days. Mice from exposed groups received 1 or 2 W/kg (whole body average). Dosimetric analyses have been performed through experimental evaluation of the SAR in phantoms and *in vivo*. The power loss has been monitored during all the experiments. Sham-exposed mice were treated as SAR-exposed mice with the exception that the signal was off. Normal un-manipulated mice were used as further control to discriminate the possible effects of the stress.
Spleen cells were counted and stimulated with anti-CD3ε and anti-CD28 mAbs or LPS. Culture supernatants were analyzed by ELISA to evaluate cytokine production (IL-2, IFN-γ). Cell proliferation has been evaluated by ^{3}H-TdR uptake. Spleen cells were also analyzed by flow cytometry to identify different subpopulations and activation markers.
RESULTS AND DISCUSSION: Results show that the number of nucleated cells and the frequency of T ($TCR\alpha\beta^{+}$) and B ($B220^{+}$) lymphocytes, and macrophages were not affected by 1, 2 or 4 weeks exposure to 1 or 2 W/kg. Within the T cell population also the ratio between CD4 and CD8 cells was similar in sham-, 1 W/kg- and 2 W/kg-exposed mice. T cell proliferation was analyzed in response to anti-CD3 alone or anti-CD3 and increasing concentrations of anti-CD28 mAbs whereas B cells were stimulated with LPS. Results show that 1, 2 or 4 weeks exposure does not affect T and B cell proliferation as well as the expression of activation markers (CD69, CD25). Cytokine production (IL-2 and IFN-γ) was also not affected by the exposure.
CONCLUSIONS: Our data show that under the experimental conditions adopted the exposure to 900 MHz GSM-modulated does not induce considerable changes in the frequency of spleen cell populations, in cell proliferative responses, in cytokine production and activation markers. Altogether, our findings indicate that GSM-exposure does not affect peripheral lymphocytes.

11-2

EFFECTS OF PULSED HIGH FREQUENCY EMF EXPOSURE DURING PREGNANCY ON OFFSPRING OF WISTAR RATS. J. Buschmann[*1], J. Streckert[*2], A. Bitz[*2], V. Hansen[2]. [1]Fraunhofer Institute of Toxicology and Aerosol Research, Hannover, Germany, [2]Bergische Universität, Wuppertal, Germany.

OBJECTIVE: to investigate the effects of a higher power density of pulsed high frequency EMF exposure during pregnancy on offspring of rats. The exposure of the rats was performed in special waveguides. These waveguides allowed the exposure of the cage area to a homogeneous electromagnetic field in order to keep variations of the exposure due to movements of the animals low. The modulation of the radio frequency 890 MHz carrier signal was selected in a way that the spectrum contained GSM typical frequency components occuring at 1,733 kHz, 217 Hz, 8 Hz and 2 Hz (and integer multiples thereof) beside the carrier signal. A power density of 60 W/m^2 in the cage area as the highest athermal value as determined in pilot studies was investigated.
METHODS: Pregnant Wistar rats, Crl:(WI)BR, were exposed from day 0 p.c. (11.00 a.m.) through day 20 p.c. (7.00 a.m.), 20 h per day (approx. 11.00 a.m. - 7.00 a.m.). On the days of exposure, dams were housed individually in special metal free cages with plastic lid and glass drinking tubes in waveguide systems. Cage positions were changed daily in order to achieve similar exposure conditions for all animals. A sham exposed control group was run concurrently. The investigations were performed as a double blind study. The exposure units were completely electromagnetically shielded. Consequently, all external electromagnetic influences could be excluded as well as the influence from the exposed unit on the adjacent control unit. The presence/absence of the rf field was controlled by a computer and values were recorded

every 5 minutes. The study was conducted following OECD-Guideline 414 "Teratogenicity". At day 20 p.c., dams were sacrificed to determine potential prenatal toxic effects of the exposure. The following reproductive parameters were determined: uterine weight, number of Corpora lutea, implantation sites (after ammonium sulfide staining in uteri without visible implantations), number and position of early and late resorptions, number and position of live and dead fetuses, sex, position and individual weight of live fetuses as well as individual placental weight. Fetuses were examined for external anomalies. One half of the fetuses were examined for skeletal anomalies. The remaining half of the fetuses were examined for visceral anomalies using WILSON's sectioning technique.

RESULTS AND DISCUSSION: The animals showed no abnormal clinical symptoms during the study. No mortality occurred and there were no signs of abortion or premature delivery in exposed and sham-exposed dams. Macroscopic adspection of maternal organs did not indicate any pathological changes. No statistically significant influence was found on total body weight gain and maternal weight gain except uterus, although the values in the exposed group are lower than in the sham exposed group.

There was also no statistically significant influence of exposure on pre- and postimplantation loss, number of implantation sites and live fetuses and fetal and placental weights (except a significant decrease in the placental weight of male fetuses). However, all values in the exposed group were decreased compared to the sham exposed control group.

Since even the corresponding data for the above-mentioned parameters of the control group are just at the lower limit of the historical control data, an influence of the housing conditions, which due to the position of the cages within the exposure units in this study were different from those applied in routine studies, cannot be excluded. In this case, the slight differences between the two groups may be due to an interaction of these housing conditions and an onset of first unspecific effects of the exposure on prenatal development of the exposed animals.

No influence of the exposure could be detected on the incidence of single external, visceral and/or skeletal anomalies, the number of fetuses with these anomalies, and the number of litters with affected fetuses, with the only exception of an increase in the number of fetuses and litters showing slightly dilated ureter in the exposed group. However, this finding is considered a sporadically occurring variation in the used strain of rats, and the observed incidence in the control group is still within historical control data.

Except one complex malformation in the control group (ectopic omphalocele, ologodactylia, missing diaphragma), the remaining observed anomalies are not considered to be malformations, but sporadically occurring variations. All anomalies were found in both groups, including the control group, and/or in an incidence which is characteristic for the used strain of Wistar rats.

There was no effect on the incidence of incomplete ossification, which sporadically occurred in both groups in an incidence which is characteristic for 20 day old fetuses of the used strain of Wistar rats. The number of calcified ossification centres was also not influenced.

CONCLUSIONS: The exposure of gravid Wistar rats [Crl:(WI)BR] from day 0 - 20 p.c. to a far field of an antenna transmitting a radio frequency 890 MHz carrier signal modulated in a way that the spectrum contained GSM typical frequency components occuring at 1,733 kHz, 217 Hz, 8 Hz and 2 Hz (and integer multiples thereof) beside the carrier signal at a field strength of 60 W/m² did not significantly affect most of the investigated maternal and fetal parameters. The observed (statistically non-significant) effects mainly on maternal weight gain, pre- and postimplantation loss, and fetal and placental weight may, however, represent the onset of an unspecific adverse effect of exposure on prenatal development.

11-3

THE EFFECT OF CHRONIC EXPOSURE TO 835.62MHZ EMCW OR 847.7MHZ CDMA ON THE INCIDENCE OF SPONTANEOUS TUMORS IN RATS. M. La Regina[1*], E. Moros[2], W. Pickard[3], W. Straube[2], J. Roti Roti[2]. [1]Division Of Comparative Medicine, and [2]Radiation Oncology Center Washington University School of Medicine, St. Louis, Missouri, USA, 63110. [3]Department of Electrical Engineering, Washington University, St. Louis, Missouri 63156, USA.

OBJECTIVE: The study used a whole animal model to determine if chronic exposure to 835.62 MHz Frequency Modulation Continuous Wave (FMCW) and 847.74 MHz Code Division Multiple Access (CDMA) radio frequency radiation from cellular phones contribute to the development of spontaneous tumors.

METHODS: Eighty male and eighty female F344 rats, were randomly placed in each of three irradiation groups (480 rats total): the Sham Group received no irradiation; the FMCW Group exposed to 835.62 MHz FMCW; or the CDMA Group exposed to 847.74 MHz CDMA. The study began when animals were 6 weeks old and continued for 2 years. The irradiation chamberettes held 40 rats for exposure. Each chamberette was equipped with an amplifier and four antennas to provide the electromagnetic signal, and 4 carousels onto which rats in tubular restrainers were positioned. The sham chamberette was identical but without the amplifier. The electromagnetic signal was generated by proprietary equipment provided by Motorola Corp. At necropsy all major organs were evaluated grossly and taken for histopathology including brain, spinal cord, adrenal glands, esophagus, heart, stomach, intestines, kidney, liver, lung, cervical lymph nodes, nasal cavity, reproductive organs, pancreas, salivary gland, spleen, thymus, thyroid, trachea, and urinary bladder. Numbers and types of tumors and incidence of hyperplasia for each organ were compared to the sham group. If incidence in treatment groups exceeded the sham group, a chi-square analysis was performed with significant p value of 0.05 or less. Length of survival and final body weight was analyzed using analysis of variance. A p value of 0.05 or less was considered significant.

RESULTS: There were no significant differences among final body weights for either males or females in any group.

Average Final Body Weights (in grams) with Standard Deviation Females:

Sham	FMCW	CDMA	P- Value* analysis of variance
271.25± 37.39	272.38± 36.92	270.19±34.21	0.93

Average Body Weights (in grams) with Standard Deviation Males:

Sham	FMCW	CDMA	P- Value* analysis of variance
386.63± 36.83	374.88± 38.02	379.19± 41.37	0.16

Likewise, there were no significant differences in survival days among any of the groups for either males or females.

Average Number of Days Survived with Standard Deviation Females

Sham	FMCW	CDMA	P- Value* analysis of variance
692± 69	689± 103	697± 74	0.83

Average Number of Days Survived with Standard Deviation Males

Sham	FMCW	CDMA	P- Value* analysis of variance
708± 44	680± 92	692± 80	0.06

Comparison of the sham group tumor incidence with historical controls showed that incidence was within expected ranges, suggesting that were no adverse influences during the course of the study. No significant differences were found for any tumor in any organ (including brain and spinal cord) between treated and sham animals when analyzed by chi square analysis.

CONCLUSION: Chronic exposure to 835.62 MHz Frequency Modulation Continuous Wave (FMCW) or 847.74 MHz Code Division Multiple Access (CDMA) radio frequency radiation had no effect on survival, body weight or incidence of spontaneous tumors in F344 rats.

11-4

EFFECTS OF A 50HZ MAGNETIC FIELD ON THE IMMUNE STATUS OF THE MOUSE, MUS MUSCULUS: LONG AND SHORT TERM EXPOSURE. L. De Jager, G.J. Van Zyl, H.J. Geyer, L. De Bruyn. School of Health Technology, Technikon Free State, Bloemfontein, South Africa.

INTRODUCTION AND OBJECTIVES: Recent studies on the influence of low frequency electro-magnetic fields on biological systems suggest that there may be an interaction between magnetic fields (MF) and biological systems. The immune system, with natural defense mechanisms, protects the body against infections and, in addition, the immune system plays an important role in the immune surveillance mechanisms of the body against tumor cells. It has been suggested that MF exposure affect the immune system; Mevissen observed *in vivo* immunosuppressive effects of magnetic fields in rats. In a previous study we demonstrated a shortened life-expectancy in mice exposed to a time-varying power line magnetic field. The mice died at an earlier age because of non-specific illnesses. These findings prompted us to evaluate the *in vivo* effect of long- term and short term exposure to a time varying 50 Hz magnetic field on the immune status of a mammal, namely the mouse.

MATERIALS AND METHODS: An exposed and sham- exposed group of mice, *Mus Musculus* of the BALB/c strain were used in the study. The mice were housed in two laboratories under controlled conditions. The mice in the exposed group were exposed for 24 hours per day, to a rms 50 Hz magnetic field randomly varying between 0.5 and 77µT with an average of 2.75µT. Groups of mice were exposed or sham-exposed for one week, 14 weeks and 12 months. After the exposure period the quantitative status, as well as the qualitative status, of the immune system of the mice were determined. The B- lymphocytes ($CD19^{+}$), T-helper cells ($CD8^{+}$), T- suppressor ($CD4^{+)}$ and natural killer ($CD56^{+)}$ were determined quantitatively by means of immuno- phenotyping in conjunction with whole blood cell counts. The capacity of T- helper and B- lymphocytes cells to be activated was determined by means of the blast cell transformation test. Results were statistically analyzed by means of the Student's *t* or Mann- Whitney tests.

RESULTS AND DISCUSSION: Statistically significantly differences between the number of cells in the subpopulations of lymphocyte in the exposed and sham-exposed groups were found. After 14 weeks of exposure the MF exposed group of mice showed a significantly decrease in the total number of lymphocytes, as well as in the T- helper cells and T suppressor cells, as well as a statistically significantly difference in the relation of $CD4^{+}$ to $CD3^{+}$. A suppression of T, as well as B- cell proliferative capacity was found in the exposed group after 14 weeks of exposure. The same tendency, thus a statistically significantly decrease in the total number of lymphocyte cells and T suppressor cells, was found in the MF exposed group after one year of exposure. The relation of $CD4^{+}$ to $CD3^{+}$ also showed a statistically significantly change after one year of exposure. After one week of exposure (short term exposure) a tendency towards an increase in the total number of T lymphocytes was noted, as well as in some of the other sub populations; T suppressor cells, and T helper cells. The differences were, however not statistically significant.

The immune surveillance system has repeatedly been proposed as a potential link in MF effects on carcinogenesis. In evaluating the potential role of MF effects on the immune system, long term *in vivo* studies are essential to accommodate a possible humoral effect that would be missed *in vivo* and/or short term studies. In this study we found that a decrease in the number of cells in sub populations of lymphocytes as well as in the proliferative capacity of the T and B lymphocyte cells after long term exposure. Our results thus indicate that long- term exposure to a time varying magnetic field has an effect on the immune system of mice that could lead to a decrease in the capacity of the immune surveillance system, these observations are in line with those of Mevissen who reported on immunosuppressive effects of

MF exposure in rats. After short- term exposure a simulation effect on the immune system was observed and we could thus conclude that the response of the immune system to magnetic fields depend on the duration of exposure. More work is needed to understand the biological significance of the observed effects.

11-5

SINGLE EXPOSURE TO PULSED MICROWAVES AT 0.6 W/KG DOES NOT MODIFY LOCOMOTOR ACTIVITY OR ACOUSTIC STARTLE IMMEDIATELY AFTER EXPOSURE.

R.L. Seaman, S.P. Mathur*, and A.M. Phinney*. McKesson Clinical Services and USAMRD Microwave Bioeffects Branch, Brooks AFB, Texas 78235 USA.

OBJECTIVE: Spontaneous motor activity and acoustic startle were used to test for immediately manifested effects in rats exposed for 30-min to microwave pulses.
METHODS: Twelve male Sprague-Dawley rats weighing 508±54 g (mean±st.dev.) aged 4-5 months were used. Six animals were exposed to 1.25-GHz 6-μs microwave pulses at 10 Hz for 30 min at specific absorption rate (SAR) 0.6 W/kg. Six animals were sham exposed under otherwise identical conditions. Spontaneous motor activity was measured for 5 min in a 40.8x40.8 cm AccuScan activity monitor, starting within 6 min after exposure. Interruptions of infrared beams in the monitor were detected under computer control to provide measures of horizontal movement, vertical movement (rearing), and stereotypy (rapid, repeated movements). Immediately after activity testing, whole-body startle response to 20-ms bursts of 120-dBA broadband noise was measured. Prepulses 0, 3, 6, or 12 dBA above a 70-dBA background level were delivered to produce prepulse inhibition (PPI) of the startle response. Wilcoxon rank sum (Mann-Whitney U-test), Friedman two-way ANOVA by ranks, and nonparametric Newman-Keuls multiple comparisons were used to test for differences.
RESULTS: Colonic temperature increased on average by 0.20 and 0.52°C for sham- and microwave-exposed groups, respectively; however, the increase was not different between the groups (2-tailed t-test, p=.65). No significant difference was found between the groups for overall activity, horizontal activity (number of movements, distance), or vertical activity (number of counts, number of movements). No significant difference was found between the exposure groups for overall stereotypy activity or the number of stereotypy episodes. The total duration of stereotypical movements was different between groups (Wilcoxon p=.037) with the duration of the sham-exposed group greater than that of the microwave-exposed group (medians: 61.2 and 48.6 s, means 60.8 and 47.3 s). No significant difference between the exposure groups was found in amplitude of the startle response or its within-session habituation. Significant differences were indicated in PPI of startle (Friedman $F(6,5)=19.43$, p=.0016). The differences were all found to be in prepulse intensity and did not depend on the type of exposure.
CONCLUSIONS: Only one behavioral variable that was measured in this study was affected by the 30-min, 0.6-W/kg-microwave exposure with the pulse parameters used. This indicated that the exposure used did not affect the neural circuits underlying the majority of behaviors, or did not affect them enough to be reflected in the measures used. The reduction in duration of stereotypical movements after microwave exposure might indicate a change in the basal ganglia circuits that are involved in motor stereotypy. However, the reduction may be of minor biological significance because it was not accompanied by a significant change in two other measures of stereotypy.
Supported by the U.S. Army Medical Research and Materiel Command under contract DAMD17-94-C-4069 awarded to McKessonHBOC BioServices. The views, opinions and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy or decision. In conducting this research, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the NRC Institute of Laboratory Animal Resources.

SESSION 12: MEDICAL APPLICATIONS
Chairs: Art Thansandote and James Lin

12-1

MULTIPLE MILLIMETER WAVE TREATMENTS REDUCE THE SYMPTOMS OF NEUROPATHIC PAIN IN MICE. A. Radzievsky, O. Gordiienko*, A. Cowan*, A. Radzievsky, Jr.*, S.I. Alekseev, M.C. Ziskin. Richard J. Fox Center for Biomedical Physics, Temple University Medical School, Philadelphia, Pennsylvania 19140, USA.

The hypoalgesic effect of Millimeter Wave Treatment (MWT) is a well-known phenomenon. It was reported by many Eastern European clinicians, and was quantitatively evaluated in our laboratory using various experimental models of acute and chronic pain. At the same time, neuropathic pain, in addition to being resistant to traditional pharmacological treatments, was found to be resistant to a single MWT as well. Taking in consideration that some success was previously reported in patients with neuropathic pain after multiple MWT, the **primary aim** of the present study was to quantitatively evaluate the possible hypoalgesic effect of multiple MWT in mice with neuropathic pain following experimentally induced unilateral Chronic Constriction Injury (CCI) to the common sciatic nerve.
The behavioral testing of the surgically induced mechanical allodynia in mice following the CCI was conducted using the Wire Surface Test (WST). Three main indexes were evaluated: the number of Paw Protective Movements (PPM) during 5 min of the observation period; the Total Time (TT) the injured paw was held above the surface; and the Total Vertical Activity (TVA) of the experimental animals during the testing. The results of WST on day 9 and day 10 after the surgery (before the first MWT) were averaged and were taken as a baseline of the test. MWT was started on day 10 after the surgery, when the neuropathy was fully developed and the allodynia symptoms were relatively stable.

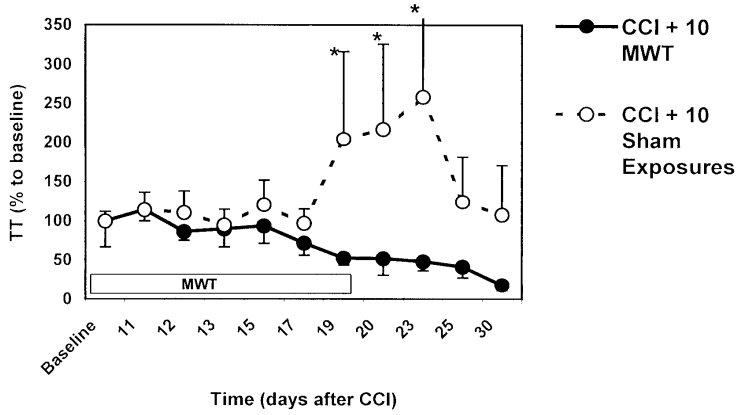

Fig.1. Effect of multiple MWT on the level of allodynia in the Wire Surface Test. CCI – Chronic Constriction Injury; TT – Total Time the injured paw was held above the surface. Data presented as the groups means ± SEM. N = 8 for each point. Asterisks indicate a statistically significant difference ($p<0.05$) of the exposed group in comparison with the sham control group.

Millimeter waves were applied to the nose area of unanesthetized restrained mice. MW exposure parameters were: frequency = 61.22 GHz; incident power density = 15 mW/cm^2; duration = 15 min. A single MWT was provided on each of ten consecutive days. The WST was conducted twice a day (15 min before and 30 min following the MWT), and also once a day for 10 days following the course of the treatment.
Our experiments have demonstrated that a statistically significant hypoalgesic effect of multiple MWT is detectable only after the 8th exposure to millimeter waves (Fig. 1.). The TT index in the group of treated mice was reduced by more then 50%, while in the sham-exposed animals this index increased more than 2

times. The same tendencies were discovered for the number of PPM (Fig.2.). However, the MWT induced hypoalgesia is not long lasting. On 6th day following the last MWT the differences between the indexes became statistically insignificant. The TVA in the treated and in the untreated mice had not changed significantly in both groups during the period of treatment.

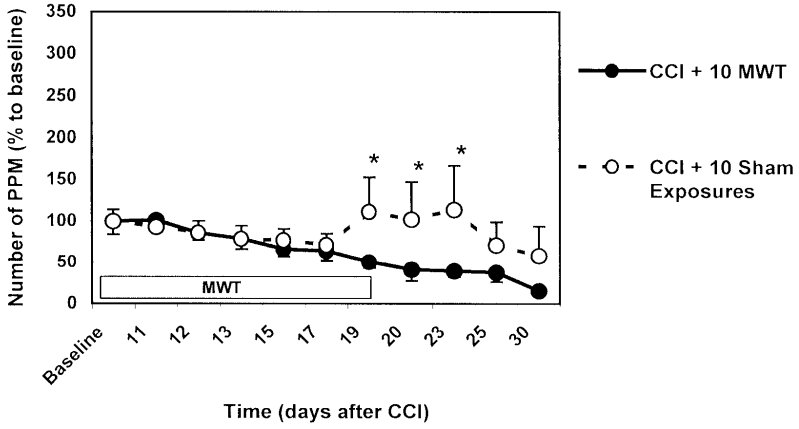

Fig.2. Effect of multiple MWT on the level of allodynia in the Wire Surface Test. CCI – Chronic Constriction Injury; PPM – Paw Protective Movements. Data presented as the groups means ± SEM. N = 8 for each point. Asterisks indicate a statistically significant difference ($p<0.05$) of the exposed group in comparison with the sham control group.

Our results demonstrated that multiple MWT could reduce the symptoms of neuropathic pain. The MWT-induced hypoalgesia is apparent only after the multiple exposures, which suggests the possibility of accumulation of the treatment effects of MWT. The hypoalgesia was short lived and, most probably, associated with opioid mechanisms, as we have previously shown for other types of pain. Since neuropathic pain mechanisms involve disturbances in various pathways and different types of receptors, it may be worthwhile to consider the combination of MWT with pharmacological agents targeting these non-opioid receptors as an option for clinical practice.

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12-2

MAGNETOFECTION: ENHANCING AND TARGETING GENE DELIVERY WITH SUPERPARAMAGNETIC NANOPARTICLES AND MAGNETIC FIELDS.

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BACKGROUND: The true benefits of gene therapy can only be realized if the limitations posed by insufficient gene transfer efficacy and specificity are overcome. Moreover, assigning function to the recently decoded primary sequences of vertebrate genomes affords rapid and highly efficient gene transfer techniques *in vitro* amenable to high throughput automatization.

OBJECTIVES: To exploit the attractive forces of magnetic gradient fields on superparamagnetic particles to potentiate the efficacies, improve the kinetics and dose response profiles of gene transfer processes and target gene delivery by application of magnetic fields. Similar approaches have been described previously in magnetic targeting of classic drugs and have been used with some success in the treatment of cancer patients (Lübbe et al., 1998, *Cancer J.* 11:104-105; Lübbe et al., 1996, *Cancer Res* 56:4686-4693).

METHODS: Superparamagnetic iron oxide nanoparticles were manufactured with polyelectrolyte surface coatings to allow their association with gene vectors by salt-induced colloid aggregation. State-of-the-art

gene vectors were associated with these particles by simple mixing in salt-containing buffers. Association was evaluated by dynamic light scattering, zeta potential measurements and electron microscopy and was quantified with radioactive-labeled components. Magnetic devices with neodymium-iron-boron (Nd-Fe-B) permanent magnets (remanence Br = 1080 - 1150 mT) matching the dimensions of cell culture dishes, upon which these were positioned during transfection, were constructed for *in vitro* gene delivery. For *in vivo* gene delivery, rectangular-shaped Nd-Fe-B magnets were positioned in direct contact with the target tissue of gene transfer, such as blood vessels, the intestine or the stomach followed by luminal injection of superparamagnetic gene vectors.

RESULTS AND DISCUSSION: Synthetic and viral gene vectors associated quantitatively with polyelectrolyte-coated superparamagnetic nanoparticles by salt-induced aggregation. The magnetic gradient fields of the devices constructed for cell culture experiments were sufficient to sediment superparamagnetic gene vectors on target cells quantitatively within a few minutes. As a consequence, the required process times of gene transfer were decreased from hours to minutes (Fig. 1, left), the dose-response profiles were greatly improved (Fig. 1, right), and gene transfer efficiencies were raised up to 5 orders of magnitude. Noteably, gene transfer was confined („targeted“) to an area defined by the shape of the magnetic fields of the applied magnetic devices (Fig. 2). This held true for any gene transfer technique examined (viral and nonviral). Gene delivery to otherwise non-permissive cells and tissues was achieved. Most importantly, magnetic field-guided local transfection *in vivo* in the gastrointestinal tract and in blood vessels was feasible. The principle of magnetic drug targeting is universally applicable to gene vectors.

CONCLUSIONS: With the the drastically lowered vector dose and the short incubation times required to achieve high transfection/transduction efficiency and the possibility of gene delivery to otherwise non-permissive cells, magnetofection is an ideal tool for *ex vivo* gene therapy approaches and for screening purposes where the available vector dose, the required process time and the sustainable costs of the procedure are limiting factors. Also for *in vivo* gene- and nucleic acid-based therapies, magnetofection may become a strong choice where local treatment is required. . Specific and efficient magnetic targeting of interior body regions upon systemic vector administration will require the generation of focussed strong magnetic fields, a challenge for physical and medical sciences.

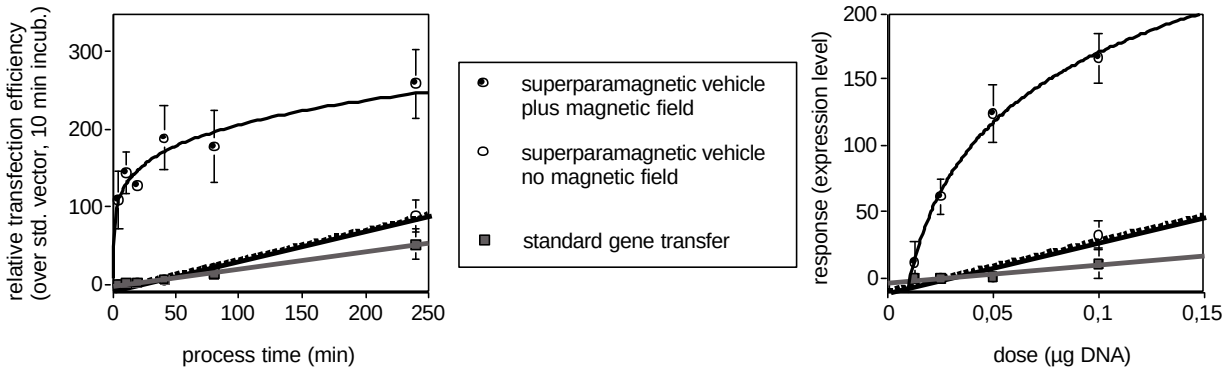

Figure 1: The transfection reagents GenePorter (left) and Lipofectamine (right) were incubated with plasmid DNA (squares) containing the luciferase gene as reporter according to the instructions of the manufacturers or with plasmid DNA previously mixed with polyethylenimine-coated superparamagnetic iron oxide nanoparticles (circles). NIH3T3 mouse fibroblasts were incubated with these preparations in the presence (filled circles) or the absence of a magnetic field for the specified durations (left) or with serial dilutions of these preparations for 15 min (right). Reporter gene expression was determined after 24 hrs by measuring the luminescence arising upon substrate (luciferin) addition to cell extracts.

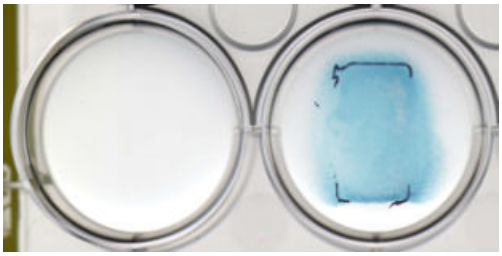

Figure 2: NIH3T3 cells were incubated for 5 min with a recombinant adenovirus carrying the lacZ reporter gene at a multiplicity of infection (MOI) of 200 with a Nd-Fe-B magnet attached under the culture dish (left) or without magnet (right). The virus preparation was previously associated with polyethylenimine-coated superparamagnetic iron oxide nanoparticles. NIH3T3 cells are non-permissive for adenovirus infection as they do not express the virus receptor (CAR). By magnetofection, this limiation can be overcome, and gene transfer is confined to an area defined by the shape of the magnet attached under the dish (rectangular area in the right dish).

12-3

COMPUTATIONAL AND EXPERIMENTAL STUDIES OF A 2.45 GHZ ANTENNA FOR MICROWAVE THERMAL ABLATION TREATMENT. P. Bernardi,[1], J.C. Lin,[2] M. Cavagnaro,[1*] S. Pisa,[1*] and E. Piuzzi.[1*]. [1]Department of Electronic Engineering, University "La Sapienza" of Rome, Via Eudossiana 18, 00184 Rome, Italy; [2]Department of Electrical and Computer Engineering, University of Illinois at Chicago, (M/C 154) 851 South Morgan Street, Chicago, IL 60607-7053, USA.

INTRODUCTION: Transcatheter thermal ablation is increasingly used as a minimally invasive therapeutic technology. Microwave energy has been investigated for its potential in producing larger and deeper lesions. In contrast to lower frequency technologies, microwave energy is delivered through a radiating antenna mounted to the tip of a catheter. Tissue heating is produced exclusively by absorption of radiated microwave energy in the biological dielectric. Therefore, microwave energy is more effective and has greater versatility for producing ablative lesions. Accordingly, minimally invasive transcatheter microwave technology has been suggested as a method to improve the depth of thermally induced ablative lesions [1].
OBJECTIVES: This paper presents a numerical and experimental study of a miniature microwave antenna designed to operate at 2450 MHz for thermal ablation therapy. In order to analyse the antenna performance (impedance matching, transmission efficiency, etc.), power deposition pattern, and temperature increase in a muscle phantom, the catheter antenna has been evaluated and compared, numerically and experimentally.
METHODS: The antenna investigated in this study is a miniature sleeved slot (or choke-slot) antenna, which consists of two slots and a matching sleeve or choke, mounted to a miniature coaxial cable. In the experiment, the antenna was inserted into the center of a cylindrical phantom, filled with muscle-equivalent tissue [2]. The phantom was placed inside a constant temperature water bath. The pattern of SAR distribution, in three-dimensions, was obtained from transient (~5 s) temperature data measured using a fiber optic temperature sensing system (Luxtron 3000). Positioning of the temperature probes was controlled by a micromanipulator. In addition, holding the water bath at a constant physiological temperature, steady-state temperature measurements were taken. To simplify the analysis, the numerical SAR study, based on a FDTD code, exploited the cylindrical symmetry both of the antenna and of the experimental phantom. The time course of temperature increase was numerically evaluated by solving the bio-heat equation in the cylindrical geometry, through an explicit finite-difference approach.
RESULTS AND CONCLUSIONS: Numerically computed and experimentally measured SAR data, along the antenna axis at different radial distances from the antenna surface, are in excellent agreement. Similar results were obtained for temperature evolutions in the muscle phantom, as a function of power application. These results indicate that the SAR distribution has a pattern that accents heating of tissues toward the distal

end of the catheter antenna. The shape of the SAR and temperature distributions are such that the miniature catheter antenna could give rise to lesions that are most suitable for ablation of a larger tissue volume.

References
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[2] S. Pisa, M. Cavagnaro, P. Bernardi, and J.C. Lin, "A 915-MHz Antenna for Microwave Thermal Ablation Treatment: Physical Design, Computer Modelling and Experimental Measurement", IEEE Trans. on Biomedical Eng., vol. 48, n. 5, pp. 599-601, 2001

12-4

THERAPEUTIC NIGHTTIME ELECTRICAL STIMULATION IN CHILDREN WITH MYELO-MENINGOCELE. S.W. Ryan, J.L. Walker, R. Davis, T.R. Coburn. Shriners Hospitals for Children, Lexington and University of Kentucky Division of Orthopaedic Surgery, Lexington, Kentucky, 40502, USA.

INTRODUCTION: One in a thousand children are born with spina bifida and myelomeningocele. For most, this results in some paralysis in the lower extremities and bowel and bladder dysfunction. Nighttime low-level electrical stimulation has been advocated to improve muscle strength and bowel/bladder function in children with myelomeningocele and other neuromuscular disorders. The purpose of this study was to evaluate the changes in motor function, balance and sensation in children with thoracolumbar (TL) or lumbosacral (LS) level neurological deficits due to myelomeningocele with transcutaneous electrical stimulation (TES) in addition to physical therapy.
METHODS AND MATERIALS: This was a non-blinded, non-randomized prospective study with the subject serving as his/her own control with pre and post treatment assessments. Fifteen children ages 4-12 years with TL or LS myelomeningocele were selected because of their lower extremity weakness, enrollment in a stable physical therapy program, and no plans for surgery. They had all documented clinical compliance at our institution and made a commitment for one year of treatment and follow-up. Subjects were treated with nighttime electrical stimulation using a BMR NT2000 stimulator 6 nights per week. Families were instructed in electrode placement over the trunk and gluteal muscles with TL level and gluteal and quadriceps muscles for LS level neurological deficits in their children. Follow up phone calls and clinic visits were performed throughout the study period to answer any questions, provide solutions to any problems and to encourage compliance. Pre and post treatment assessments included physical exam, manual muscle testing, measurement of range of motion, walking video, WeeFIM (a standardized assessment of physical function in children), monofilament sensory exam, Progressive Ambulation Scale and Tinnetti balance classification. The results were analyzed by descriptive comparisons of subjects, pre and post treatment.
RESULTS: Of the 15 children who enrolled in the study, only 7 completed 9 months of treatment and are the subjects of this analysis. None finished the full year. Of the 2 children that walked household distances in long leg braces and walkers, 1 improved from swing-through to reciprocal pattern and improved his gait velocity. Both children who walked with short leg braces and no assistive devices, improved their hip flexor and quadriceps strength by ½ to 1½ grades. They had better control of foot progression and less trunk sway in walking. 2 children walked with short leg braces and crutches. 1 improved her lateral sway when walking. The other had no improvement in gait or strength but he was also the one subject who had a component of lower extremity arthrogryposis. 2 children walked with short leg braces and reverse walkers. One progressed to crutches and the other improved hip control and gait velocity. There were no significant changes in fine touch sensation or sitting balance (all were able to sit independently against resistance). One child improved 1 grade on the Progressive Ambulation Scale. There were no significant changes in WeeFIM scores for self care or locomotion. Families consistently reported improved bowel continence.

Reasons cited for not completing the study were difficulties complying with the intensive treatment schedule, problems with TES units that went unreported for weeks between visits, unexpected surgery or fracture, and one who was lost to follow-up. No skin problems were reported with the electrodes.
CONCLUSIONS: Small gains were made in strength, gait, and reported bowel continence in children with myelomeningocele who used nighttime TES for at least 9 months. However, compliance with such an intensive regimen (6 of 7 nights) was poor and ultimately all families discontinued it within one year.
This study was funded by the Kosair Charities Foundation, Louisville KY

PLENARY SESSION III: EPIDEMIOLOGY
Chair: Joachim Schuz

EPIDEMIOLOGICAL METHODS – STRENGTHS AND LIMITATIONS AS APPLIED TO HEALTH EFFECTS OF ELECTROMAGNETIC FIELDS. M Feychting. Institute of Environmental Medicine, Karolinska Institutet, Box 210, S-171 77 Stockholm, Sweden.

Epidemiology is the science of disease occurrence and distribution of determinants of disease in human populations. It provides the most direct evidence of whether environmental exposures affect the risk of disease. However, epidemiological investigations are useful only to the extent that their results are accurate. Knowledge of basic principles and sources of error in epidemiological studies are essential for anyone evaluating results from such studies.
This presentation will review basic epidemiological study designs, and discuss potential sources of errors, with special Reference to studies of electromagnetic fields. The different sources of bias can be grouped into three categories; selection bias, misclassification (information bias), and confounding. Selection bias occurs if the probability of being included in the study is related to both the exposure and the disease. This source of bias is usually not a problem in prospective cohort studies, but may be an important limitation in case-control studies, not only related to the way controls are chosen, but also to participation rates.
Misclassification of the exposure or the disease is likely to affect the majority of studies. The potential impact on the study results depends not only on the extent of the misclassification, but also on the type of misclassification and the prevalence of the exposure (or the disease). It can range from virtually no effect on the study results at all, also when the misclassification is extensive, to creation of a spurious effect or concealment of a true effect. Misclassification can also affect the shape of dose-response patterns.
Confounding is often mentioned as the most serious source of bias in epidemiological studies. However, the potential problem with confounding is often exaggerated. A confounder must both be associated with the exposure and at the same time be a risk factor for the disease over and above what is occasioned by the association with the exposure. For a confounder to account for an observed excess relative risk the requirement is a strong covariance with the exposure and an association with the disease that is stronger than that observed for the studied exposure.
Epidemiological studies are often perceived as easy to understand and conduct. This is more or less a delusion, which will be illustrated in this presentation.

ELF AND PUBLIC HEALTH: REVIEWS AND RESEARCH PRIORITIES. L.I. Kheifets. World Health Organization, CH-1211 Geneva, Switzerland.

In the past year several key reviews of ELF Electromagnetic Fields have been completed. These include: cancer hazard identification by International Agency for Research on Cancer (IARC) and a report by an expert Advisory Group of the National Radiological Protection Board in the United Kingdom (AGNIR) on

the Neurodegenerative Disease. I will present these evaluations, discuss their conclusions and compare them to previous reviews.
In 1996, the World Health Organization (WHO) established the International Electromagnetic Fields (EMF) Project to address the health issues associated with exposure to EMF. The IARC review addresses the issue of whether it is feasible that ELF-EMF pose a cancer risks. The next step in the process is a rigorous health risk assessment that will incorporate, as appropriate, description of sources and exposures, dose-response evaluation and calculation of attributable risk and to evaluate evidence for other (non-cancer) diseases. WHO should finish this part of the risk assessment in 2003. I will present and solicit comments on plans for the WHO Risk Assessment that will be published as an Environmental Health Criteria (EHC) Monograph. WHO's EMF Project aims to help national authorities balance the benefits of electrical technology against possible health risks, and to help them decide what protective measures, if any, may be needed. EHC will consider various policy recommendations for protection of human health.
Finally, I will discuss current WHO research priorities in the ELF area.

REVIEW OF RF/MICROWAVE EPIDEMIOLOGY STUDIES, WITH EMPHASIS ON POTENTIAL HEALTH RISK FROM WIRELESS COMMUNICATIONS DEVICES. M.L. McBride, British Columbia Cancer Agency, Vancouver, B.C., V5Z 4E6 Canada.

Studies to date: There has been a recent increase in public concern over health effects with exposures to radiofrequency(RF)/microwave frequency electromagnetic fields, particularly with increasing use of wireless communications devices over the past two decades. A number of epidemiologic studies have been conducted to investigate potential health effects of exposure to these fields. Studies of RF/microwave fields and adult cancers have been conducted in occupational groups with reputed high exposures such as military personnel, electronics workers, medical workers, and other industrial groups with high exposures. Types of exposures ranged from extremely low frequency to radiofrequency, microwave, and ionizing radiation, complicating interpretation of results. Other studies have examined groups of cellular phone users, and residential exposures. These include studies of adult and childhood cancers, and other health outcomes such as adverse reproductive outcomes, congenital anomalies, and nonspecific complaints such as headache, fatigue, and RF sickness syndrome
RESULTS: Results of the occupational studies are conflicting; however, methodologic limitations make interpretation of these results problematic. None of the few investigations of the risk of childhood cancer conducted to date are methodologically sufficiently robust to provide quality information on the effect of RF fields on risk. However, results of three larger well-designed studies of cellular phone users (one cohort, two case-control) published in 2000 and 2001 do not indicate an increased risk of brain cancer or other cancers with cellular phone use, although more follow-up was recommended.
Proposed and ongoing studies: Several studies of cellular telephone users are ongoing, the largest of which is a prospective, case-control study of risk of cancers of the brain, salivary glands, and leukemia, sponsored by the International Agency for Research on Cancer, involving 13 countries in Europe, North America, and the Pacific Rim.
Methodological issues: There have been several methodologic problems in earlier studies, which limited the validity of results. These include overall design issues, such as difficulties in identification of exposed groups or outcome, lack of information on confounders, lack of statistical power, and problems in exposure assessment. Problems in exposure assessment include: identification of relevant exposures, multiple exposures at different frequencies, and difficulties in measurement of exposure, including lack of information on individual exposures.
CONCLUSIONS: Most of the earlier epidemiologic studies are of limited value, mainly because of methodologic limitations, in particular with respect to exposure assessment. More recent studies, including the large multi-country case-control study overseen by the International Agency for Research on Cancer, will provide more definitive results. No high excess risks were observed in any studies of adequate design, and in general, no statistical trends with different levels of exposure were observed. At this time, the

epidemiologic evidence for a health risk with exposures to RF/microwave electromagnetic fields is inadequate for evaluation, and will need to be reviewed in conjunction with biologic and animal evidence for causative relationships.

SESSION 13: SPECIAL SYMPOSIUM IVA: COMBINED EFFECTS OF ELECTROMAGNETIC FIELD EXPOSURE AND OTHER AGENTS.

Chairs: Vijayalaxmi and Junji Miyakoshi

13-1

GENOTOXIC EFFECTS OF ELF AND RF FIELDS IN CULTURED MAMMALIAN CELLS. J. Miyakoshi[1]; M. Yoshida[1], T. Nakahara[1], K. Wake[2], and M. Taki[3]. [1]Graduate School of Medicine, Kyoto University, Kyoto, [2]Communications Research Laboratory, Independent Administrative Institution, [3]Graduate School of Engineering, Tokyo Metropolitan University, Tokyo, Japan.

OBJECTIVES: In *in vitro* studies, there have been contradictory reports regarding the effects of electromagnetic fields at low flux densities or low specific absorption rate (SAR). We have designed and manufactured several pieces of equipment for exposure of cells to high-density (5 to 400 mT) ELF and high SAR (5 to 100 W/kg) RF electromagnetic fields. This paper reviews our studies on the genotoxic effects of ELF and RF electromagnetic fields.

RESULTS AND DISCUSSION: ELF Fields: Exposure to an ELF field at 400 mT induced mutations in the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene in human melanoma MeWo cells. The mutation frequency increased with an increase in both the exposure period and the induced current intensity. Mutations induced by X-rays were enhanced by ELF field exposure. No significant increase in mutation frequency occurred when DNA replication was inhibited during ELF field exposure. Errors in DNA'replication are suspected of causing the mutations produced by ELF field exposure. We investigated the effect on the mutation frequency of long term exposure to a 5 mT ELF field at 60 Hz. CHO-K1 cells were exposed or sham-exposed to a 5 mT ELF field for up to 6 weeks, with or without X-irradiation (3 Gy), and the mutation frequency was analyzed. Long term exposure to a 5 mT ELF field did not increase the mutation rate, suggesting that the threshold for mutation induction is greater than a magnetic density of 5 mT. However, X-irradiation followed by long term exposure to a 5 mT ELF field did produce an increase in the mutation rate. These results suggest that exposure to ELF fields greater than 5 mT may promote X-ray-induced mutations. In agene expression study, exposure of PC12-VG cells to a 400 mT field enhanced the expression of the ß-galactosidase gene, following stimulation with forskolin. The effect of the ELF field was inhibited by treatment with a specific PKC inhibitor and by Ca^{2+} channel blockers. Enhanced expression of the neuron derived orphan receptor (NOR-1) gene was also observed on exposure of CHO-K1 cells to a 400 mT ELF field, but not onexposure to a 5 mT field. Exposure of cells to a high-density 400 mT ELF field may affect signal transduction, resulting in enhanced gene expression. These effects were not observed at lower magnetic densities, such as 5 mT, suggesting that there may be a threshold of over 5 mT.
RF Fields: The effects of RF fields at 2.45 GHz on cell proliferation, mutations and DNA strand breaks were examined. The exposure device was based on a TE_{01} circular waveguide cavity. We observed inhibition of CHO-K1 cell proliferation after exposure to RF fields for 2 days at the highest average SAR (100 W/kg) and a decrease in cell survival in both MO54 and CHO-K1 cells after 24 h exposure. When MO54 cells were exposed to electromagnetic fields up to an average SAR of 50 W/kg for 2 h, no effect on HPRT mutations was observed. In the alkaline Comet assay for DNA strand breaks, no significant difference in tail moment was observed for sham-exposure and RF exposure at a SAR of 100 W/kg for 2 h. These results suggest that exposure to RF fields with a high SAR can induce cytotoxicity, but is unlikely to induce genotoxicity.

13-2

***IN VITRO* INVESTIGATIONS OF POTENTIAL INTERACTIONS OF RADIO FREQUENCY RADIATION WITH OTHER PHYSICAL AND CHEMICAL AGENTS.** M. Meltz. Department of Radiation Oncology and Center for Environmental Radiation Toxicology, University of Texas Health Science Center at San Antonio, Texas 78229, USA.

The question of whether or not radio frequency radiation (RF) exposures of *in vitro* cell culture systems are associated with biological effects has been extended in several laboratories to look for potential interactive effects between RF and other physical and chemical agents. The studies have included assessments of induction of chromosome aberrations, sister chromatid exchanges, phenotypic mutations, DNA repair, and cell transformation. Effects have also been examined on cell proliferation and the kinetics of mitosis. A number of these studies will be described, in the context of the biological "activities" that could also be perturbed if an interactive effect were to be observed.
The presentation of this work is supported by the Air Force Office of Scientific Research, Grant No. F49620-01-1-0349.

13-3

LACK OF TUMOR PROMOTION POTENTIAL OF THE ELECTROMAGNETIC NEAR FIELD USED FOR CELLULAR PHONES (900 MHz and 1.5 GHz) ON RAT LIVER AND MOUSE SKIN CARCINOGENEISIS. T. Shirai[1], K. Imaida[1,5], M. Taki[2], S. Watanabe[3], K. Wake[3], J. Wang[4] and O. Fujiwara[4]. [1]Department of Pathology, Nagoya City University Medical School, Nagoya, Japan [2]Tokyo Metropolitan University, [3]Nagoya Institute of Technology, [3]Communication Research Laboratory, Tokyo Japan, [5]Department of Pathology, Kagawa Medical University, Kagawa, Japan.

INTRODUCTION: The number of users of portable cellular phones has rapidly expanded in many countries and the potential health hazards, especially any tumorigenic or tumor promoting effects of the electromagnetic fields (EMF) that they use, are of great concern. Therefore the influence of 900MHz and 1.5GHz EMFs were investigated with respect to rat liver carcinogenesis and mouse skin carcinogenesis, using specific organ bioassay models.
Promoting potential on rat liver carcinogenesis: 929.2MHz or 1.439GHz EMF time division multiple access (TDMA) signals for the PDC (Personal Digital Cellular, Japanese cellular telephone standard) system were directed to rats through a quarter-wavelength mono-pole antenna. Peak SARs of 929.2MHz and 1.439GHz within the liver, the target organ, were 2.0-1.7 and 1.91-0.98 W/kg, respectively, while whole-body average SARs were 0.8-0.58 and 0.68-0.45 W/kg, respectively. Exposure was for 90 min. a day, 5 days a week, for 6 weeks, to male F344 rats, given a single dose of diethylnitrosamine (200mg/kg, i.p.) 2 weeks previously. At week 3, all rats were subjected to a two-thirds partial hepatectomy. At week 8, the experiments were terminated. Liver tumor promoting 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 and sham-exposed rats. The values were not significantly altered by EMF exposure, showing that both 900MHz and 1.5GHz EMF lack promoting effects on rat liver carcinogenesis in this model (Imaida et al., Carcinogenesis., 19:311-314, 1998; Imaida et al., Jpn J Cancer Res., 89:995-1002, 1998).
Promoting potential on mouse skin carcinogenesis: Ten-week old ICR-1 female mice were treated by painting at 100μg/100μl acetone per mouse with 7,12-dimethylbenz[a]anthracene (DMBA) on pre-shaved dorsal skin. One week later, they were divided into 4 groups as follows: EMF exposure group (DMBA-EMF), sham-exposure group (DMBA-Sham), 12-O-tetradecanoylphorbol-13-acetate (TPA, 4.0 μg/ 200 μl

acetone/mouse), as a positive control group (DMBA-TPA), and non-treatment control group (DMBA-Control). The skin local peak SAR was 2.0 W/kg and the whole body average SAR was 0.084 W/kg, 90 min. a day, 5 days a week, for 19 weeks. At Week 20, animals were sacrificed and skin tumors were analyzed histopathologically. The incidences of skin tumors in DMBA-EMF, DMBA-Sham, DMBA-TPA and DMBA-Control groups were 0/48 (0%), 0/48 (0%), 29/30 (96.6%), 1/30 (3.3%), respectively. The numbers of tumors per mouse in DMBA-TPA and DMBA-Control groups were 18.8 $\pm$ 13.4 and 0.1 $\pm$ 0.5, respectively. These data clearly demonstrated that near field exposure to 1.5GHz EMF, used for cellular phones, did not exert any enhancing effect on mouse skin tumorigenesis initiated by DMBA (Imaida et al., *Carcinogenesis*., 22:1837-1841, 2001).

13-4

JOINT ACTIONS IN RATS OF A CHEMICAL BRAIN TUMOR PROMOTER WITH EITHER DIGITAL OR ANALOG MOBILE PHONE FIELDS. R. Adey, Loma Linda University School of Medicine, Loma Linda, CA 92354 USA.

OBJECTIVE: Two life-term studies of brain tumor promotion in a rat model (Adey et al., *Rad Res.*, 152:293-302, 1999; Adey et al., *Cancer Res*., 60:1857-1863, 2000) have tested known promoting actions of n-Ethyl-N-nitroso-urea (ENU). In both studies, we also examined the separate and combined actions of ENU and a simulated mobile phone field, using a digital signal in one study and an analog signal in the other. We examine findings. They are consistent with a disturbance in the normal balanced equilibrium between DNA damage and DNA repair, leading to options for either apoptosis or unregulated cell growth.
METHOD: Fischer 344 rats received a single IV dose of ENU (4mg/kg), or a sham dose of buffer solution, on pregnancy Day 18. Pregnant dams were randomly assigned to groups (ENU-dosed/ sham RF field; ENU-dosed/RF field; ENU-sham/ RF field; and ENU-sham/sham RF field). Offspring were assigned to the same groups in approximately equal numbers. Experiment 1 exposed rats (n = 236) to the North American Digital Cellular (NADC) 836 MHz field, with 50 Hz Time Division Multiple Access (TDMA) modulation, "slot" average brain SAR 1.0-1.6 W/kg (time-averaged SAR 0.33-0.53 W/kg) 2h/day, 4days/week, from pregnancy Day 19 until death or experiment termination at 709 days. Experiment 2 similarly exposed rats (n = 540) to an 836 MHz analog (FM) simulated cell phone field, modulated with "balanced speech", deviation ±12.5kHz. An antenna power of 2.5±0.1W produced the same average brain SARs as the "slot" average in Experiment 1. The experiment was terminated at 731 days.
RESULTS: In Experiment 1, when compared with ENU alone, a significant tumor reduction occurred in rats jointly exposed to ENU and microwave fields, and dying from a primary brain tumor before experiment termination ($P < 0.015$, 1-tailed). Overall, similar but non-significant field-induced trends towards tumor reduction occurred [spontaneous tumors ($P < 0.08$, 1-tailed), ENU-induced tumors ($P < 0.08$, 1-tailed)]. In Experiment 2, no FM-mediated changes were mediated in number, incidence or types of either spontaneous or ENU-induced CNS tumors.
DISCUSSION AND SUMMARY: A 90% reduction in ENU-induced brain tumors follows a single dose of ionizing radiation 24h preceding ENU (Warkany et al., *J Natl Cancer Inst*., 56:59-64, 1976). A confirming study also noted radiation-induced activation of the DNA repair enzyme alkylguaninine-DNA-6-alkyltransferase (Stammberger et al., *Carcinogenesis.*, 11:219-222, 1976). Precision cellular irradiation has shown that alpha particles damaging nuclei of a small f: action of cells indirectly induce mutation in many nearby cells through cell-cell communication involving gap-junction proteins (Zhou et al., *PNASci.*, 98:14410-14415, 2001) suggesting cell sensitivities as a domain function. Differential responses towards apoptosis or cell proliferation depending on either monolayer or multidimensional ("3-D") culture conditions, or the microgravity of space flight (Jessup et al., *In Vitro Cell Dev Biol-Animal.*, 36:367-373, 2000), also support domain organization as a critical determinant in epigenetic carcinogenesis.
Our animal studies were supported by the Motorola Corporation.

13-5

EFFECT OF GSM-900 EXPOSURE ON NOS-II EXPRESSION IN RAT C6 GLIOMA CELLS. I. Lagroye, E. Haro, P-E Dulou, B. Billaudel, B. Veyret. PIOM/Bioelectromagnetics Laboratory, ENSCPB/EPHE, Pessac, France.

INTRODUCTION AND OBJECTIVE: Production of nitric oxide (NO) by glial cells after stimulation has been shown to be responsible for neuronal cell death. Moreover, an increase in the rate of cell death in the adult nervous system is involved in neurodegenerative diseases.
The objective of this study was to determine whether exposure to GSM-900 microwaves could elicit the expression of the inducible isoform of the Nitric Oxide Synthase (NOS2) or interfere with LPS plus cytokine-induced NOS2 expression in C6 rat glial cells.
MATERIAL AND METHODS: Two days before the experiment, C6 cells were plated in Petri dishes at a density of $5x10^4$ cells/dish. At the day of experiment, half of the samples were treated with a cocktail of lipopolysacharide (LPS 10 µg/ml) and cytokines IFNγ (50 U/ml) plus TNFα (50 ng/ml) before Petri dishes were placed in the wire-patch antenna.
In vitro exposure to GSM-900 was performed using wire-patch antennas. Temperature monitoring was used to evaluate the SAR experimentally during experiments. After a 3-hour temperature stabilisation, RF generator was turned on to generate GSM-900 signal at 0.2 W/kg. Exposure lasted 48 hours. Sham exposed samples were run in the same way in a non-activated wire-patch antenna placed in a second, identical incubator.
Following RF or sham-exposure, the cells were harvested for western blot analysis using RIPA buffer. Proteins were extracted from cell lysate and the concentration was determined by the Bradford reaction. Protein samples (10 µg) were separated by SDS-PAGE (7.5 % polyacrylamide), electroblotted and probed with mouse anti-NOS2 (Transduction Laboratories). Immunoreactive bands were visualised using ECL Western Blotting System® (Amersham-Pharmacia Biotech) and exposure to autoradiography film. Two to three independent experiments were performed in a blind manner.
RESULTS: A basal level of NOS2 was detected in C6 cells, although inter-experiment's variation could be seen. A 48-hour treatment with LPS plus CK increased the expression of the enzyme by a factor 5 (figure 1). Exposure to GSM-900 at 0.2 W/kg for 48 hours was shown to decrease the expression of NOS2 compared to sham exposure. Co-exposure to GSM and LPS plus cytokine reproducibly decreased the effect of LPS plus cytokine treatment on NOS2 expression by about 40% (figure 2).
DISCUSSION AND CONCLUSIONS: These data show that exposure to GSM-900 at low SAR is able to interfere with a cocktail of LPS plus cytokines for the induction of the NOS2 enzyme. The 40% decrease observed may indicate that a 900 MHz signal could modulate the inflammatory process induced in a rat astroglial cell line.
This work was supported by the European Community (Reflex® Project), the Aquitaine Council for Research and the CNRS.

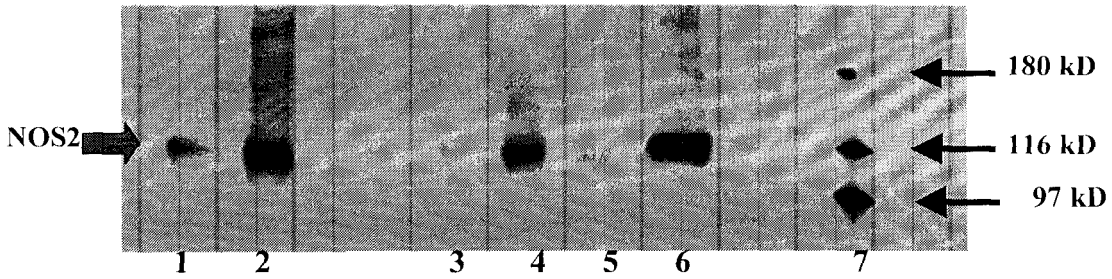


Figure 1: Representative blot for NOS2 expression after RF and/or LPS plus cytokines exposure. Lane 1: untreated C6 cells (negative control); lane 2: cells treated with LPS plus cytokines (positive control); lane 3:

cells exposed for 48 hours to GSM-900 at 0.2 W/kg; lane 4: cells exposed to RF and LPS plus cytokines for 48 hours ; lane 5 sham-exposed C6 cells; lane 6 : sham-exposed C6 cells treated with LPS plus cytokines for 2 days; lane 7 : molecular weight markers. Arrow on the left stands for p^{130} NOS2

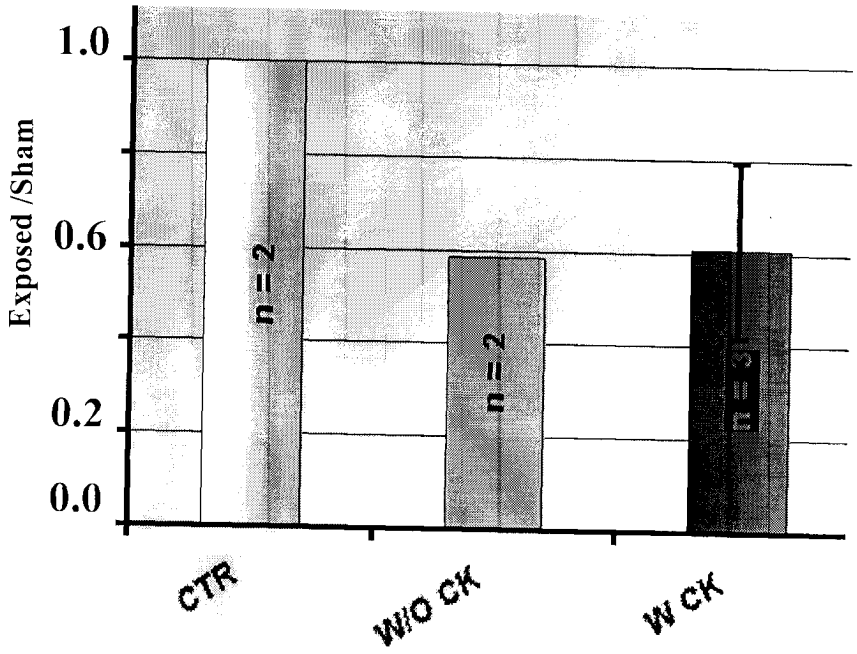


Figure 2: Effect of GSM-900 on the NOS2 expression. A 40% decrease was measured in samples exposed to GSM-900 either without (W/O CK) or with (W CK) LPS plus cytokine (CK) treatment. CTR is 100% of NOS2 expression either in sham-exposed samples or after CK treatment for the evaluation of the effect of GSM-900 alone or in combination with CK, respectively.

13-6

NEURODEGENERATION FROM MICROWAVE AND NEUROTOXIN EXPOSURE. R.L. Seaman, S.-T. Lu. McKesson Clinical Services and USAMRD Microwave Bioeffects Branch, Brooks AFB, Texas, USA.

OBJECTIVE: To investigate effects of microwave exposure on neurodegeneration in the CNS nigrostriatal system using toxin-induced degeneration in animal models. These models represent the basal ganglia and brainstem degeneration indicated in certain veterans.

METHODS: Two animal models are being studied in which mitochondrial ATP production is inhibited by specific action of neurotoxins on electron transport. In one model, 4-month old male Sprague-Dawley rats are injected intraperitoneally with 3-nitropropionic acid (3-NP). For a range of 3-NP doses, degeneration processes occur selectively in the caudate-putamen to provide a model of Huntington's disease. In the other model, male Lewis rats are implanted with osmotic pumps to deliver systemic rotenone chronically. With the proper dose of rotenone, degeneration occurs primarily in the substantia nigra and caudate-putamen to provide a model of Parkinson's disease. After animals are treated with neurotoxin in our work they are exposed to 6-μs, 1.25-GHz pulses generated by an FPS-7 radar transmitter at 10 Hz repetition frequency. Nigrostriatal function is assessed after neurotoxin and microwave treatments by testing spontaneous locomotor activity, acoustic startle, and forelimb use. Neuronal structure is assessed by means of light and electron microscopy.

RESULTS: Combined effects of microwave exposure and 3-NP have been seen in prepulse inhibition of acoustic startle and in the ultrastructure of caudate-putamen output neurons. This study is currently being

replicated. The rotenone model is being developed for use with microwave exposure by establishing appropriate dose and duration of delivery.

CONCLUSIONS: In the 3-NP model, combined effects of microwave exposure and 3-NP are not always synergistic as one might expect from previous reports of neuropathology after microwave exposure. Both the 3-NP and rotenone models provide the means by which combined actions of environmental chemicals, physical trauma, and/or electromagnetic fields can be studied *in vivo*. These environmental factors and their applications can be selected to simulate military occupational exposures. Use of neurotoxin-induced neurodegeneration models also allows level, duration, and sequence of simulated exposures to be controlled and studied.

Supported by the U.S. Army Medical Research and Materiel Command under contract DAMD17-94-C-4069 awarded to McKessonHBOC BioServices. The views, opinions and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy or decision. In conducting this research, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the NRC Institute of Laboratory Animal Resources.

SESSION 14: Special Symposium IVB: Presentation of REFLEX Results
Chair: Franz Adlkofer

14-1

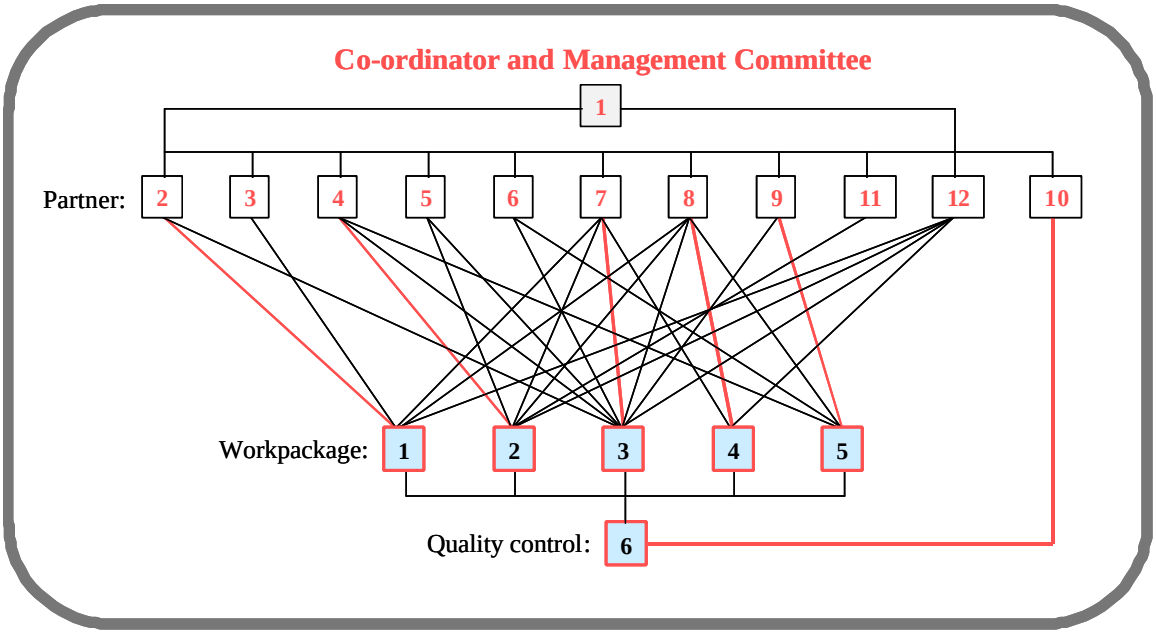
RISK EVALUATION OF POTENTIAL ENVIRONMENTAL HAZARDS FROM LOW ENERGY ELECTROMAGNETIC FIELD EXPOSURE USING SENSITIVE IN VITRO METHODS (REFLEX) - INTRODUCTION.

F. Adlkofer[1], H. Dertinger[1], R. Tauber[2], R. Fitzner[2], K. Schlatterer[2], O. Jahn[3], H.W. Ruediger[3], E. Diem[3], S. Ivancsits[3], A.M. Wobus[4], J. Czyz[4], A. Trillo[5], A. Ubeda[5], D. Leszczynski[6], S. Joenväärä[6], R. Kuokka[6], J. Reivinen[6], H.A. Kolb[7], F. Bersani[8], C. Franceschi[8], I. Lagroye[9], B. Veyret[9], N. Kuster[10], J. Schuderer[10], F. Clementi[11], D. Fornasari[11], C. Gotti[11], C. Maercker[12]. [1]VERUM Foundation, Munich, Germany; [2]Dept. Clinical Chemistry, University Hospital Benjamin Franklin, Free University of Berlin, Germany; [3]Div. of Occupational Medicine, University of Vienna, Austria; [4] In vitro Differentiation Group, IPK, Gatersleben, Germany; [5]Insalud, Ramon y Cajal Hospital, Madrid, Spain; [6]STUK - Radiation and Nuclear Safety Authority, Helsinki, Finland; [7]Institute of Biophysics, University of Hannover, Germany; [8]Dept. of Physics, University of Bologna, Italy; [9]PIOM, Ecole National Supérieure de Chimie et de Physique, Bordeaux, France; [10]IT'IS, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland, [11]Dept. of Medical Pharmacology, University of Milan, Italy, [12]Resource Center for Genome Research, Heidelberg; Germany.

INTRODUCTION: Although the biological effects of EMF exposure have been under study for the past 30-40 years, no consensus has been achieved with respect to either findings or their interpretation. The reasons for this are numerous: difficulties in measuring EMF exposure at the putative sites of action, vast differences in exposure and experimental conditions and the complete lack of agreement on biological endpoints appropriate for study. In the REFLEX project essential central elements in the function of various cell systems and in the development of disease are investigated using the most powerful molecular biological tools currently available. This will ensure a better understanding of the biological effects of EMFs, which is the prerequisite for an objective assessment of potential health hazards and perhaps also of potential therapeutic effects.

PROJECT OBJECTIVES AND STRUCTURE: Twelve research groups from all over Europe cooperate within the REFLEX project. The following five priority areas condensed in workpackages are investigated under strictly controlled conditions : 1. Direct and indirect genotoxic effects of EMFs. 2. Effects of EMFs on differentiation and function of embryonic stem cells. 3. Effects of EMFs on gene expression and protein targeting. 4. Effects of EMFs on the immune system.

5. Effects of EMFs on cell transformation and apoptosis. In a 6th workpackage the exposure conditions of various cell lines to EMFs including dosimetry and temperature are monitored in order to obtain reproducible and hence reliable data. The workpackages are interconnected on scientific grounds. It is, therefore, obligatory, that various partners using different methods, but pursuing the same research direction cooperate in one workpackage. This is documented in the diagram: Number 1 to 6 in blue: Workpackages as described above, number 1 to 12 in red: Contributing laboratories as shown in the author's list and in the table.



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7 Institute for Biophysics, University of Hannover, Germany
8 Dept. of Physics + Dept. of Experimental Pathology, University of Bologna, Italy
9 PIOM, Ecole Nationale Superieure de Chimie et de Physique, Bordeaux, France
10 IT'IS, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland
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14-2

EXPOSURE SYSTEMS AND DOSIMETRIC QUALITY CONTROL IN THE REFLEX PROJECT.

J. Schuderer, W. Oesch, R. Mertens, U. Frauenknecht, N. Kuster. Foundation for Research on Information Technologies in Society (IT'IS) & Laboratory for Integrated Systems, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland; Schmid & Partner Engineering AG, Zurich, Switzerland.

OBJECTIVE: The objective was to meet the requirements defined in [Kuster, 2000] for as many setups utilized in the REFLEX project as possible. The applied signals represented worst-case environmental exposure conditions for GSM, DCS as well as ELF magnetic field exposures in terms of SAR levels as well as amplitude modulation. The requirements to be satisfied included field homogeneity, high dynamic range, minimum temperature and vibration loads, double blind protocols, compact design at large loading volume, etc. Furthermore, all relevant exposure and environment parameters needed to be continuously monitored during exposure. Partner laboratories without any setup or with existing ones that did not meet the standard had to be equipped with novel optimized exposure setups. An additional requirement was that the quality of the exposure could be remotely monitored and maintained, since most laboratories do not have RF know-how available in-house.

METHODS: Exposure setup evaluation and optimization was performed using numerical techniques. The simulation platform SEMCAD including its thermal solver extension served for the analysis of the RF setups. Details such as precise meniscus models at the solid/liquid interface as well as all plastic parts of the dishes and dish holder have been accounted for. The results were carefully verified using the near-field scanner DASY3 equipped with the latest RF probe technology. The temperature response of the medium was assessed with a highly resistive thermistor probe. The ELF setups were also analyzed and optimized with Mathematica 3.0 evaluating the corresponding analytical equations based on the law of Biot-Savart. Field verification was also conducted with the DASY3 scanner equipped with a highly sensitive 3-axis Hall probe. Unwanted electric fields were characterized with the use of a 3-axis ELF E-field probe, and vibrations were assessed with a 1-axis accelerometer. The signal is generated by an arbitrary function generator (Agilent 33120A), an RF signal generator (Rhode & Schwarz SML02) combined with a frame generator (SPEAG DCU) or an ELF current source (custom made). The data acquisition system (Agilent 34970A) is used to multiplex the inputs from the RF, temperature and airflow sensors. The software, written in Visual C++, generates the complicated environmental exposure schemes consisting of up to four independent random events, as well as controls and monitors all devices and sensors. The GUI has been realized as a very user-friendly built-in state machine. Prior to the experiment, it performs redundant verification checks to verify that all devices within the system operate within its specifications. During the exposure all parameters are monitored every 10s. All communications between the computer and the devices are noted with time stamps. This allows evaluation of the entire experiment every day. In addition, it enables to reconstruct the entire experiment if needed.

RESULTS: Five RF and four ELF setups have been installed in the laboratories of the consortium. The novel RF system is based on a dual resonant waveguide system installed in a standard incubator (carrier frequency: 1800MHz; dynamic range: 0.1mW/kg – 100W/kg; deviation from uniformity < 30%; arbitrary modulation signals with a 16k point length and a frequency of less than 15 MHz; prediction of temperature load; blinded design). The ELF setup consists of a dual 4-coil ELF system which also fits in a standard incubator (dynamic range: 0.02mTrms - 3.6mTrms with frequency components from DC –1.5kHz; deviation from uniformity: < 1%, stability and drift: <0.01%, vibration; << 0.1G; E-fields < 2V/m; loading volume: 16cm x 16cm x 23cm; shielding: µ-metal box; monitoring of current (field) and temperature). The permanent control of the exposure is realized by the analysis of the measurement data sent to Zurich in an encoded file. Predefined signal types can be applied (RF setup: CW, 217HZ pulse, GSM basic, DTX only, GSM Talk and GSM environment; ELF setup: 50Hz sinus; power line, 50Hz consumer devices and 16 2/3Hz railway signals). The signals can be furthermore modulated by arbitrary field on/off cycles.

In addition, the wire patch cell setup [Lace, 1999] and the STUK resonator [Toivo, 2001] are utilized for the experiments conducted at 900 MHz. Furthermore, two coil systems developed by Insalud, Ramon y Cajal Hospital, Madrid as well as the University of Bologna are also used.

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14-3

GENOTOXIC EFFECTS OF EXTREMELY-LOW-FREQUENCY ELECTROMAGNETIC FIELDS ON HUMAN CELLS IN VITRO. H.W. Rüdiger[1], S. Ivancsists[1], E. Diem[1], A. Pilger[1], F. Bersani[2], O. Jahn[1]. [1]Div. of Occupational Medicine, University of Vienna, Austria; [2]Dept. of Physics, University of Bologna, Italy.

METHODS: Human diploid fibroblasts and human blood lymphocytes were exposed to continuous or intermittent vertical ELF electromagnetic fields (50 Hz, sinusoidal, 24 h, 1000 µT). Occurrence of DNA single and double strand breaks was determined using the alkaline and the neutral comet assay.
RESULTS: In contrast to continuous ELF-EMF exposure, intermittent fields reproducibly induced a significant increase of DNA strand breaks, predominantly double strand breaks, being highest at 5 min. field-on/10 min field-off. The response was dose dependent beginning with 70 µT (Figure 1) and its intensity varied between individual cell strains.
Conclusion: We conclude from our data that intermittent ELF-EMF may produce clastogenic effects in a dose-dependent manner.

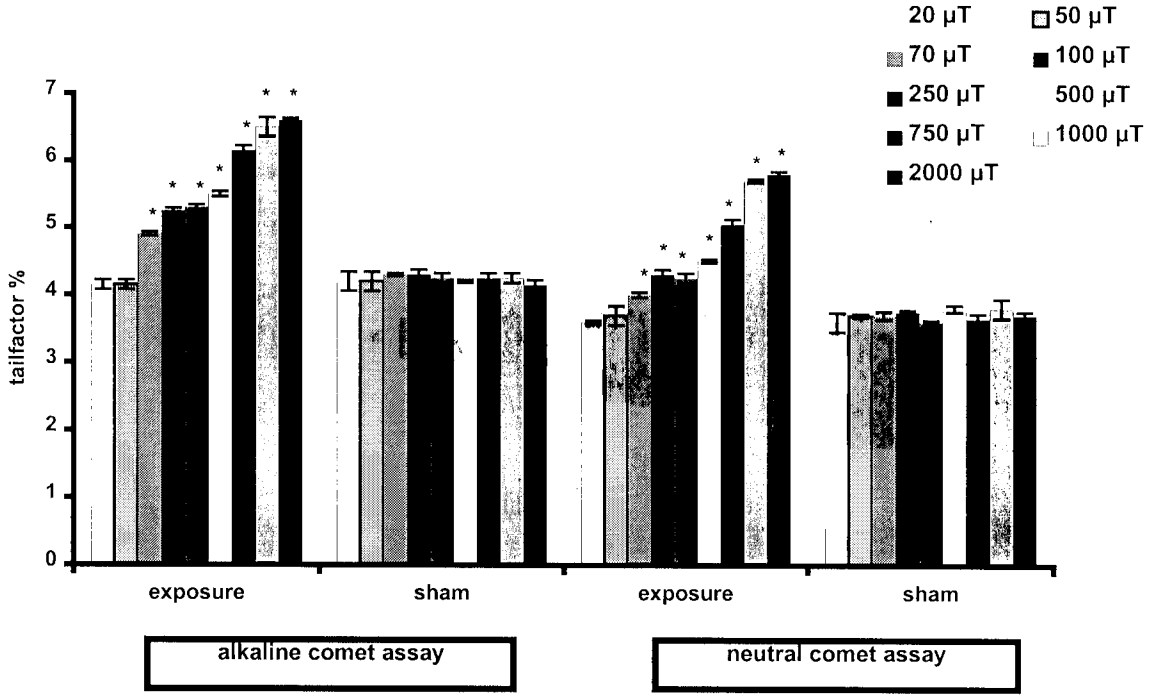

Figure 1: Exposure response: alkaline and neutral Comet Assay tailfactors of ELF exposed fibroblasts (50 Hz sinus, 24 h, intermittent 5 min on/10 min off). * $p < 0.01$ exposed versus sham-exposed

14-4

GENE EXPRESSION PROFILING STUDIES ON GLOBAL cDNA ARRAYS SHOW SENSITIVITY OF HUMAN AND MOUSE CELL LINES TO ELF-EMF EXPOSURE. C. Maercker[1], J. Czyz[2], S. Ivancsits[3], H.W. Ruediger[3], O. Jahn[3], E. Diem[3], A. Pilger[3], A. Rolletschek[3], J. Schuderer[4], N. Kuster[4], K. Guan[2], A. Trillo[5], E. Bazán[5], D. Reimers[5], D. Fornasari[6], F. Clementi[6], K. Schlatterer[7], R. Tauber[7], R. Fitzner[7], F. Adlkofer[8], A.M. Wobus[2]. [1]Resource Center for Genome Research (RZPD), Heidelberg, Germany. [2]In vitro Differentiation Group, IPK, Gatersleben, Germany. [3]Div. of Occupational Medicine, Vienna, Austria. [4]IT'IS, Swiss Federal Institute of Technology (ETH), Zurich, Switzerland. [5]Insalud, Ramon y Cajal Hospital, Madrid, Spain. [6]Dept. of Medical Pharmacology, University of Milan, Italy. [7]Dept. Clinical Chemistry, University Hospital Benjamin Franklin, Free University of Berlin, Germany, [8]VERUM Foundation, Munich, Germany.

METHODS: Human diploid fibroblasts, mouse embryonic stem (ES) cells (wildtype and p53 deficient), human promyelocytic cells (HL60), human neuroblastoma cells (NB69, with or without all-trans-retinoic acid (RA) induction), were exposed to ELF-EMF with different on/off cycles. Afterwards the cells were tested for viability, cell growth, genotoxicity, or gene expression by cell assays or molecular assays on DNA, RNA, or protein level. Cells which showed significant changes were exposed again under the same conditions. Total RNA, representing all expressed genes in a cell, was isolated from these cells to prepare labeled cDNA probes for hybridization of whole-genome cDNA arrays (Human Unigene RZPD-2, about 75.000 genes and ESTs (expressed sequence tags); Mouse Unigene RZPD-1, about 25.000 clones), followed by an extensive image analysis with the help of several software tools and databases.
RESULTS: Elf exposure (2.3 mT, 5min on/30 min off, 6 h) of mouse ES cells and subsequent RT-PCR analysis showed a significant up-regulation of egr-1, p21 and c-jun in p53 deficient cells, but not in wildtype cells. Also ELF-EMF (50 Hz sinusoidal, 1000 µT, 5 min on/10 min off, 24 h) causes DNA single and double strand breaks in human diploid fibroblasts, shown by the comet assay. Currently it is not clear how far NB 69 cells are influenced by ELF-EMF (50 Hz, 100-2000 µT, 5 min on/5 min off, 16 h; or 100 µT, no on/off cycles, 42 h). There are hints that ELF-EMF might positively influence the proliferation of NB 69 cells. Also genes of the nicotinic receptor gene family were shown to be up-regulated by Northern analysis. These experiments are currently repeated on protein level with the help of radiolabeling of different receptor molecules. At the moment, RNA from exposed and sham-exposed human fibroblasts, NB 69 cells and HL 60 cells is profiled on the human cDNA array, whereas the mouse ES cell probes are hybridized to Mouse Unigene RZPD-1. The hybridization of the fibroblast samples is already finished and directs to a significant influence on the expression of certain signaling molecules by the applicated fields.
CONCLUSIONS: Complex hybridization of cDNA arrays allows us to investigate the expression of all known genes of an organism within one single experiment. Therefore, it is a very strong method to investigate the influence of ELF-EMF on mammalian cells. First hints done by single assays showing that central signaling pathways are influenced by these fields, could be confirmed by gene expression profilings so far. This is documented by a list of up- or down-regulated genes in treated cells in comparison to sham-exposed controls. Further experiments with RNA from different cell lines will reveal important insights in cellular behaviour upon exposure, and also be an important requirement for more specialized assays performed by experts in the different REFLEX groups.

14-5

RF-EMF GENOTOXIC EFFECTS. K. Schlatterer, R. Tauber, R. Fitzner**.** Dept. Clinical Chemistry, University Hospital Benjamin Franklin, Free University of Berlin, Berlin, Germany.

OBJECTIVE: In order to standardize analytical regimes the European REFLEX consortium performs studies under highly standardized and quality controlled technical conditions. The different partners use and exchange well defined cell culture models for different experimental test designs in order to maximize the analytical output and to independently control the results.
BACKGROUND: One goal of the project is the evaluation of potential genotoxic effects with strong correlation to cell growth and cancerogenesis on different cell lines by radiofrequency electromagnetic fields. For the detection of direct genotoxic effects the single cell gel electrophoresis (Comet assay) is used. By use of the alkaline Comet assay single as well as double strand breaks can be detected. Another test system used in mutagenicity studies for the determination of direct genotoxicity is the micronucleus test. Micronuclei formation is induced by clastogens following DNA damage.
METHOD: The human promyelocytic leukaemia cell line HL60 was continuous wave-exposed to 1800 MHz at SAR 1.3 and 1.0 W/kg over 24 hours. Other exposure protocols will be additionally performed. HL60 cells were cultured in RPMI 1640 medium with 10% FCS under temperature- and pH-control conditions at 37°C. For radiofrequency exposure experiments the initial seeding density per 35 mm petri dish was 7.5×10^5 cells. Exposure and sham-exposure were performed double-blinded. DNA strand-breaks were examined by means of the single cell gel electrophoresis (SCGE), i.e. Comet assay. Applying the alkaline Comet assay, single as well as double strand-breaks can be detected. After exposure, cells were monitored immediately for DNA breakage by use of a modification the alkaline Comet assay (McNamee et al., 2000). 1000 cells per slide were scored manually by fluorescence microscopy for Comet formation according to Diem et al. (1999), generating a tail factor by a simple mathematical approach. This tail factor allows to quantitatively compare the induction capacity of different environmental noxes concerning DNA-stand-breakage. The micronucleus testing was performed by means of a flow cytometry method.
RESULTS: The tail factor determined in these experiments is significantly increased for exposed HL60 cells as compared to sham-exposed controls. As positive control for DNA strand-breakage the tumour initiator 7,12-dimethylbenz[a]anthracene (DMBA), was used at a concentration of 25 µg/ml culture medium (0.01 mM). The effect of 1800 MHz radiofrequency exposure exerts that of DMBA clearly. As DMBA was solubilized in dimethylsulfoxide (DMSO), we also performed sovent control experiments. Evaluation of the tail length showed that treatment of cells with DMBA causes a significantly higher tail factor as compared to the DMSO solvent control, which is in accordance to previous studies performed for Hep G2 cells (Yusuf et al., 2000). The micronucleus test results are preliminary.
DISCUSSION: A DNA damaging effect of radiofrequency radiation on different cell lines has been discussed controversely so far. Lai and Singh had described DNA strand breakage in rat brain cells directly after exposure to microwaves (2450 MHz) in 1995. Using different cell lines and different radiofrequency signals in other model systems, no such effect could be reproduced by other groups. Experiments are ongoing, but our results obtained so far indicate, that there might be a different susceptibility of different target tissues regarding direct genotoxic effects. This increased susceptibility may be correlated with an altered DNA damage repair capacity.
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14-6

EFFECTS OF MOBILE PHONE RADIATION ON GENE AND PROTEIN EXPRESSION IN VITRO. D. Leszczynski[1], B. Billaudel[2], J. Czyz[3], P-E. Dulou[2], K. Guan[3], E. Haro[2], S. Joenväärä[1], R. Kuokka[1], I. Lagroye[2], A. Meister[3], J. Revinen[1], B. Veyret[2], A.M. Wobus[3], Q. Zeng¤. [1]STUK-Radiation and Nuclear Safety Authority, Helsinki, Finland; [2]PIOM, Ecole Nationale Supérieure de Chimie et de Physique, Bordeaux, France; [3]In vitro Differentiation Group, IPK Gatersleben, Germany.

BACKGROUND: Energy deposited in tissue by mobile phone is very small and, although it was shown to elicit some biological responses, it is still uncertain whether it could pose any significant health risk. Several studies have demonstrated that mobile phone radiation activates cellular stress response, which is known to regulate, among others, expression of a variety of genes. Thus, if the changes in gene expression induced by mobile phone radiation will be indeed proven, they could lead to changes in protein expression, followed by changes in cell physiology and, eventually, by some health-related responses.
OBJECTIVES: To determine whether *in vitro* exposure of cells to mobile phone radiation causes changes in gene and protein expression.
MATERIAL AND METHODS: Three different experimental protocols were used:
STUK/Finland: human endothelial cell line EA.hy926 was exposed for 1h to 900MHz GSM signal, average SAR 2W/kg, mRNA and proteins were analyzed using cDNA Expression Array (gene expression) and 2D-electrophoresis with PDQuest analysis (protein expression),
PIOM/France: rat C6 glioma cells were exposed for 48h to 900MHz GSM signal, SAR 0.2W/kg, some cells were co-treated with cocktail of LPS + γIFN + TNFα, expression of nitric oxide synthase-II (NOS-II) was examined using western blot,
IPK/Germany: mouse embryonic pluripotent stem cells ($p53^{+/+}$ and $p53^{-/-}$) were exposed for 6h and 48h (5min. on/30min. off) to 1800MHz GSM signal, SAR 1.5 W/kg and 2.0W/kg, gene expression was analyzed by RT-PCR
RESULTS: cDNA Expression Array compared the expression levels of up to 3600 genes in EA.hy926 cells. In addition to many minor changes induced by mobile phone radiation, several gene products showed significant changes in the expression levels (at least 2-fold increase/
decrease). These six genes encoded regulatory proteins involved in various cellular signaling systems (stress proteins & kinases, mitochondrial energy and apoptosis regulators, PKC-dependent signaling pathway). Image analysis of silver stained 2D-electrophoresis gels revealed that among all of the detectable protein spots some 15% of them have altered their expression level. In addition there were many minor changes which were at the present arbitrarily excluded from the analysis.
Basal and induced (LPS+γIFN+TNFα) level of NOS-II expression in C6 cells declined following exposure to mobile phone radiation,
Exposure of $p53^{+/+}$ stem cells to mobile phone radiation did not affect expression of genes involved in regulation of stress response, cell cycle and apoptosis. However, the $p53^{-/-}$ stem cells responded to irradiation by a small, but significant, up-regulation of c-jun, c-myc and p21 mRNA levels.
CONCLUSIONS: Our results suggest that mobile phone radiation may alter expression of various genes and proteins encoded by them. Gene/protein expression changes were detected using varying irradiation times (1 - 48h), SAR levels (0.2 - 2.0W/kg) and cell systems (human, rat, mice) what suggests that they are not a peculiarity of a particular experimental set-up, but might have a broader biological significance. Interestingly, the observed varying responses of cells with different genotype composition (p53+/+ and p53-/- stem cells) suggest that the response and possibly its severity might be influenced by the cell's genotype. Whether the detected changes in gene/protein expression will be subsequently followed by physiological changes is still to be determined.

14-7

DO ELF OR RF FIELDS AFFECT THE APOPTOTIC PROCESS? DATA FROM THE REFLEX PROGRAM. F. Bersani, B. Billaudel[1], M. Capri, J. Czyz[2], P-E. Dulou[1], K. Guan[2], E. Haro[1], S. Joenvaara[3], R. Kuokka[3], N. Kuster[4], I. Lagroye[1], D. Leszcynski, A. Meister[5], J. Reivinent[3], J. Schuderer[4], B. Veyret[1], A.M. Wobus[2], Q. Zeng[2], [1] PIOM, Ecole Nationale Supérieure de Chimie et de Physique, Bordeaux, France; [2]In vitro Differentiation Group, IPK, Gatersleben, Germany; Dept. of Physics, University of Bologna, Italy; [3]STUK, Radiation and Nuclear Safety Authority, Helsinki, Finland; [4]IT'IS, Swiss Federal Institute of Technology, Zurich, Switzerland.

OBJECTIVES: Apoptosis is a complex and highly regulated phenomenon. Any alteration of this process (alteration in signaling pathways, key genes, or proteins) may be involved in various pathologies such as cancer or neurodegenerative diseases and apoptosis could be a major target in therapeutic treatments. To date, only sparse data on the effect of electromagnetic fields, especially radiofrequency (RF) fields, on cell apoptosis are available. The objective of the REFLEX consortium was to determine whether extremely low-frequency (ELF) or RF fields could influence the apoptotic process in vitro, per se, or after previous treatment with known apoptosis inducers.
METHODS: Four laboratories within the consortium dealt with the occurrence of apoptosis in different cell types after exposure to ELF or RF fields. Exposure lasted from 1 to 48 hours. RF exposure included GSM-900 (basic) and GSM-1800 (basic, DTX, or talk) signals at SARs levels ranging from 0.1 to 2 W/kg. ELF exposure was done at 50 Hz, 0.1, 1 and 2.3 mT. Intermittence was introduced in some experiments. Chemicals that were used to induce apoptosis were 2-deoxy-D-ribose, staurosporine or camptothecin, depending on the cell system used. According to the expertise of each partner, various markers were used to detect apoptosis. The loss of mitochondrial membrane potential, the presence of phosphatidylserine on the outer leaflet of the plasma membrane, the appearance of a sub-G1 population, or caspase-3 activity were investigated using flow cytometry. The expression of the bcl-2 gene involved in the regulation of apoptosis was detected by RT-PCR.
RESULTS: Data from the REFLEX consortium showed no significant effect of RF fields on apoptosis in cells of the immune system (human peripheral blood mononuclear cells from young and elderly people, human U937 cells), and in EA.hy926 human endothelial cells. The signaling pathways involving blc-2 was not affected in either p53+/+ or p53-/- embryonic stem cells tested after exposure to RF or ELF fields. The activity of caspase 3 was not altered in EA.hy926 cells. In all systems tested, intermittence in the signals did not elicit apoptosis. Moreover, apoptosis induced by chemicals was not affected by further exposure to RF fields. Data also suggest that for the exposure conditions tested, field effects were not substantially affected by the cell genetics (embryonic stem cells), or the age of the donors (human peripheral blood mononuclear cells).
CONCLUSIONS: Overall, the results from the REFLEX programme do not bring evidence so far that either ELF or RF fields interfere with the integrative apoptotic process in cultured cells.

14-8

SUMMARY OF THE FINDINGS OBTAINED IN THE REFLEX PROJECT AND FUTURE PERSPECTIVES. F. Adlkofer[1], H. Dertinger[1]. [1]VERUM Foundation, Munich, Germany.

Preliminary outcome of the REFLEX project: Although only half of the REFLEX research period has passed, there is already enough evidence to claim that electromagnetic fields affect living cells at the DNA level and that the observed biological effects are of non-thermal nature. Based on the data related to research on biological effects of extremely low frequency electromagnetic fields (ELF-EMF) which have been

obtained in the REFLEX project so far, it can be stated that a genotoxic effect of ELF-EMF on primary cell cultures of human fibroblasts is to be considered as proven. DNA strand breaks at a significant level are produced by ELF-EMF at a flux density as low as 70 µT and there is a strong correlation between the increase in DNA strand breaks and the increase in micronucleus frequencies. With regard to the genotoxic effect of ELF-EMF a considerable inter-individual variance exists. Furthermore, there is evidence that ELF-EMF influences the expression of genes in embryonic stem cells of mice, if the stem cells are deficient of the p53 gene, the so-called guardian of the genome. In these cells the regulatory genes egr-1, p21, c-jun and bcl-2 are up-regulated after exposure to ELF-EMF. Since the flux density must be as high as 2.3 mT before a significant difference in gene expression between exposed and sham-exposed stem cells can be observed, it is not clear yet, how to assess the biological relevance of the findings. At present the data suggest that it may be the genetic background whether or not stem cells respond to ELF-EMF. No differences in DNA synthesis, cell cycle and apoptosis between exposed and sham-exposed primary human peripheral blood mononuclear cells were observed after ELF-EMF exposure. More data dealing with cell proliferation and gene expression in various transformed cell lines need to be confirmed before firm conclusions can be drawn.

With respect to radiofrequency electromagnetic fields (RF-EMF), it is not proven at present that RF radiation produces genotoxic effects in living cells, even if some data support such an assumption. Energy deposited in living tissue by RF-EMF under real-life conditions may be insufficient to break chemical bonds. On the other hand, RF-EMF at a SAR of 1.5 W/kg is able to upregulate the expression of early genes, such as p21, c-jun and c-myc, in p53-deficient embryonic stem cells, but not in wildtype cells. After lowering the SAR value to 0.11 W/kg, no influence on the mRNA levels of these genes was observed anymore. Obviously, whether or not embryonic stem cells respond to RF-EMF is dependent on their genetic background and also on the field strength of RF-EMF. Additional evidence that RF-EMF may alter the gene and protein expression comes from another REFLEX study which demonstrates that a one hour exposure to RF-EMF at a SAR of about 2 W/kg changes the protein expression levels of numerous, yet largely unidentified proteins in a transformed human endothelial cell line. Among the proteins, the expression of which were transiently increased, were the heat shock protein-27 (hsp27) and p38 mitogen-activated protein kinase activity (p38MAPK). The process of protein expression was followed by a transient increase in phosphorylation of hsp27. Further data are available suggesting that RF-EMF exposure diminishes the expression of the receptor (FGF-R1) for the basic fibroblast growth factor (bFGF) in human neuroblastoma cells and in neuronal stem cells and the expression of NOS2 in neuronal and astroglial cell lines. No effect of RF-EMF on human peripheral blood mononuclear cells representing the immune system and on apoptosis in various cell types was observed.

While single and double DNA strand breaks never have been shown in *in vitro* studies before, more or less relevant hints on the modulation of the gene expression through EMFs in *in vitro* studies have already been observed by other authors [(Blank and Goodman 1997; Ivaschuck et al. 1997 (RF-EMF; PC12 rat pheochromocytoma cells); Goswami et al.1999 (RF-EMF; Mouse embryonic fibroblasts); Harvey and French 1999 (RF-EMF; human mast cell line); Arvidson et al. in Goteborg 2001 (ELF-EMF; neuronal stem cells)]. Even if we do at present neither understand the mechanism of the EMF induced genotoxicity and gene expression nor their biological significance, there is no doubt, that these findings deserve utmost scientific attention. Fortunately, the present picture of EMF in vitro research although far from being complete is good enough to draw conclusions where to future research efforts should be directed.

Conclusions and future perspectives: The search for molecular, subcellular and cellular mechanisms influenced by EMF should be continued with priority.

Since the significance of these mechanisms for human health are not yet known, the central question is, whether they are relevant for the whole organism and, if so, for the pathogenesis of diseases.

Genomics and proteomics may be the most powerful tools which should be applied in the search for and the understanding of EMF-induced mechanisms.

A first step may be to make use of the findings provided by the Human Genome Project in order to locate the genes which are responsive to EMFs.

Proteomics may be applied to search for biological markers of RF-EMF effects that are urgently needed for epidemiological studies in order to improve their reliability.
On the basis of *in vitro* results sound scientific hypotheses should be generated the validity of which would have to be further investigated in the whole body system of man and animal.
The new UMTS technology must be included in further research.
With such a program and the engagement and enthusiasm of scientists like those carrying out the REFLEX project it should be possible to reach the goal envisaged: A breakthrough in EMF research within a foreseeable time.
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SESSION 15: Mary Ellen O'Connor Memorial Student Session
Chairs: James Ryaby and Andrei Pakhomov

15-1

IN VIVO EXPOSURE SYSTEM OPERATING AT 1900 MHz. C. Argiolas[1,2]*, F. Apollonio[2]*, L. Ardoino[1]*, R. Pinto[1]*, G.A. Lovisolo[1]*, and G. D'Inzeo[2]. [1]Section of Toxicolgy and Biomedical Sciences, ENEA C.R. Casaccia, Rome, 00060 Italy; [2]Dept. of Electronic Engineering, "La Sapienza" University, Rome, 00184 Italy.

OBJECTIVE: This work describes the structure and the realization of an exposure system based on a resonant cavity with rectangular cross section (Burkhardt et al, 1996; Curto et al, 2001), designed to expose *in vivo* biological samples (mice) at the frequency of 1900 MHz. Power coupling to the structure has been realized through magnetic loops: a power divider supplies four loops in order to generate specific electromagnetic (EM) field distribution along the system. In this way it is possible to expose, in correspondence of peaks, up to three couples of animals with a good level of Specific Absorption Rate (SAR) uniformity.
MATERIAL and METHODS: The dimensions of the structure cross section have been chosen in order to guarantee only the propagation of the TE_{10} fundamental mode at the frequency of interest (1900 MHz), while the distance between the closing walls (length of the exposure system) has been determined in order to have a standing wave pattern with about five half-guide wavelength (L=510 mm). The transfer of EM energy into the resonator has been realized by means of the introduction of conducting loops placed in a plane normal to the magnetic field lines, at half wave length starting from the closing metallic walls, corresponding to the positions of maximum of magnetic field inside the cavity (Ramo et al, 1994). The choice of using multiple loop antennas has been proposed in order to have a uniform distribution inside the structure, in spite of the presence of the biological targets, which attenuate a propagating EM field (see Figure 1). The cavity characterization has been performed by numerical simulations, based on FIT (Finite

Integral Technique) codes, modelling the exposure system with and without targets inside. Numerical dosimetry has been carried out modelling the biological targets with six cylinders forms (radius =10 mm, length =60 mm). The targets are plastic vessel filled by about 25g of equivalent biological material (e_r=53.2, s=1.94 S/m, ?=1000 kg/m^3).

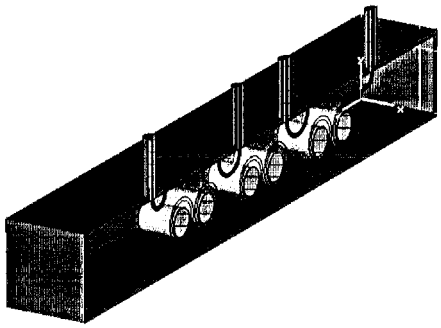

Figure 1 Schematic representation of the exposure system with six homogeneous phantoms

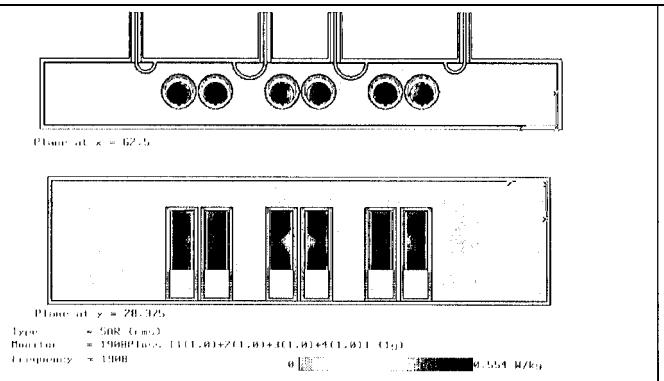

Figure 2. Sar distribution inside the targets. Sectional (upper) and longitudinal (lower) views

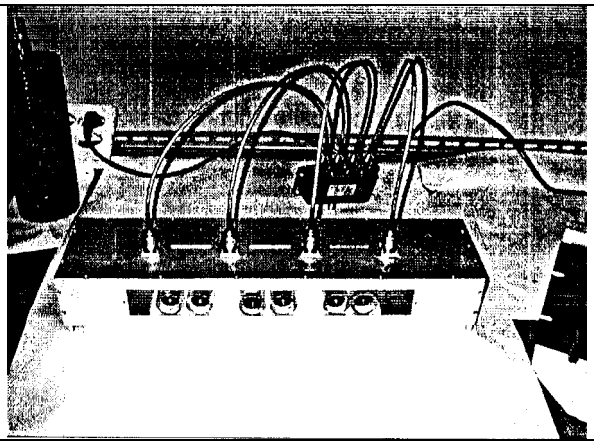

Figure 3. Frontal view of the exposure system

RESULTS and DISCUSSION: Particular attention has been paid to optimize power matching between the power divider and loop antennas. The loops are positioned in correspondence of the maximum of magnetic field. Different configurations of targets positioning have been tested to obtain the most homogeneous SAR distribution (see Figure 2). The cavity is presented in Figure 3, in frontal view with mobile panel opened to show inner configuration with mice phantom samples.

CONCLUSIONS: An exposure system based on a resonant structure has been presented. The system is suitable to expose, with a good efficiency, *in vivo* biological targets.

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15-2

INTERFERENCE ASSESSMENT OF CARDIAC PACEMAKERS IN ELF MAGNETIC FIELDS.
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OBJECTIVES: In spite of extensive research the question of a possible interference of strong ELF magnetic fields with cardiac pacemakers (CPMs) is still unanswered. For instance, the effective induction loop areas which served as the basis for previous studies vary from 200 to 570 cm^2. Furthermore, the actual interference thresholds of present day cardiac pacemakers in ELF magnetic fields are unknown. Consequently, our objectives were: A) To apprehend the magnetic induction in CPM systems on the basis of a theoretical as well as an experimental approach. B) To be able to specify practical and plausible values for the effective induction loop area of CPM systems. C) To quantify the interference thresholds of modern cardiac pacemakers in ELF magnetic fields of different frequencies.
METHODS: Based on the results of the theoretical approach on the magnetic induction in CPM systems, experiments in a realistic model of a human thorax were carried out. The tank model was filled with a physiological saline solution and placed in the center of a Helmholtz coil arrangement. Both a left- and a right-pectorally implanted dual chamber CPM system were simulated. The shape of the simulation loops was derived from X-rays of actual CPM patients. To ensure a correct 3D reproduction of the CPM system, X-rays from both the sagittal and the frontal plane were taken into account. Each of the simulation loops was placed in the thorax model which was exposed to the magnetic field of the Helmholtz coil arrangement. From each of the voltages induced into the four CPM simulation loops the effective induction loop area of the respective CPM system could be determined. From these results and the results of benchmark tests with modern cardiac pacemakers (cf. BEMS 2001) we were able to calculate thresholds for cardiac pacemaker systems exposed to ELF magnetic fields.
RESULTS: The theoretical as well as the experimental approach clearly indicate that there are two loops (the CPM lead tissue loop and the body loop) responsible for the magnitude of the disturbance voltage at the input of a CPM. Therefore, earlier assumptions of a CPM system as a closed induction loop are inadequate. The effective induction loop areas, frequently believed to reach a maximum of 570 cm^2, ranged from 100 to 221 cm^2 depending on the CPM system's implantation position and the position of the lead head. The left pectorally implanted, atrially controlled, unipolar CPM system turned out to be the worst case. Here, the interference thresholds for the magnetic induction varied between 552 and 20 µT (RMS) for magnetic field frequency ranges between 10 and 250 Hz. Comparable or even stronger fields are produced by a number of sources of ELF magnetic fields, e.g. electronic article surveillance (EAS) systems. Thus, interferences of ELF magnetic fields occuring in the everyday life with implanted CPMs cannot generally be ruled out.
Reference.
Scholten A, Silny J (2001): The interference threshold of unipolar cardiac pacemakers in extremely low frequency magnetic fields. *J Med Eng Technol* 25: 185-194

15-3

REGULATION OF CELL VIABILITY AND PROSTAGLANDIN E_2 SECRETION BY SPECIFIC 7.5 HZ ELECTROMAGNETIC FIELD STIMULATIONS ON OSTEOBLASTS.
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OBJECTIVES: The aims of the present work were (a) to show 7.5 Hz PEMF can increase cell viability, (b) to examine the response of different time constant 7.5 Hz PEMF stimulation on rat osteoblasts, (c) to determine the shortest exposure time to a 7.5 Hz PEMF, that is necessary to increase the cell viability'in *vitro*, (d) to elucidate the effect of PGE2 release on osteoblasts after 7.5 Hz PEMF stimulation.

METHODS: In the experiment, 5 ml confluent rat osteoblasts cultures were treated with trypsin-EDTA and then seeded into 35 mm tissue culture wells (Corning, NY, USA) by seeding density of 1 x 105 cells / well. The culture media used was Dulbecco's modified EAGLE's medium (HyClone, UT, USA) supplemented with 1% fetal bovine serum (Gibco, Paisley, UK), penicillin G sodium 100 units/ml and streptomycin 100 mg/ml (Sigma, St. Louis, MO, USA). The dishes were incubated at 37°C in an atmosphere supplemented with 5% CO2 for 24 hours to facilitate the attachment of osteoblasts. The experiment was divided into two groups: firstly, dishes were received 20 minutes per day at different time constant (694, 432, or 268 us) PEMF stimulation for 1 day, 2 days, or 3 days at 2 mV/cm with different exposure conditions or sham-exposure. Secondly, dishes were subjected to various exposure time stimulations (20 minutes, 1 hour, 3 hours, 9 hours, and 24 hours per day) at selected time constant (432 us) PEMF stimulation for 1 day, 2 days, or 3 days. The cell viability of each culture well was assayed by MTT test after 24 hours (including exposure time) of the PEMF or sham exposure. The supernatants were harvested before the MTT assay, and analyzed by ELISA for the secretion of PGE2.

RESULTS AND DISCUSSION: It shows that osteoblasts viability can be increased significantly by this specific 7.5 Hz PEMF with different time constant for 20 min/day after 3 days exposure. It seems that PEMF exposure time (only 20 min/day may cause significant increasing of cell viability as shown in Figure 1) on osteoblasts in vitro might differ from the therapeutic time (usually need 8 hours/day or more) in vivo or clinical fracture healing model. Longer exposure time (9 hours/day) does not get better cell viability increase than the shorter. If we stimulated the osteoblasts continuously for 24 hours/day, cell viability would be decreased significantly. It probably is caused by over dose of energy, which is absorbed by osteoblasts in vitro, and there is no circulation system, soft tissue and hard tissue to reduce the energy. Or it is because longer treatment (8 hours/day) of PEMF enhanced osteoblasts differentiation in vitro [Lohmann et al. 2000]. However, there are more evident proofs that PEMF exposure time in vitro is shorter than animal model or clinical trail, and PEMF stimulate osteoblast proliferation and growth factor synthesis [Goodman et al. 1995; Cleary SF 1993]. It just needs a short time as 20 rain/day to increase cell viability and regulate PGE2 secretion (See Figure 2). Different exposure time of 2 mV/cm PEMF can influence the cell viability conspicuously ($p<0.05$; ANOVA), and it seemed that the viability of osteoblasts decreased with the increase in the exposure time. The different kind of time constant PEMF didn't have differences between them significantly, but they did stimulate the osteoblast growth obviously. This research indicates that the growth of osteoblasts by PEMF stimulation is at least partly due to the regulation of the synthesis and secretion of PGE2.

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Cleary SF (1993) *Am Ind Hyg Assoc J* 54, 178-185.

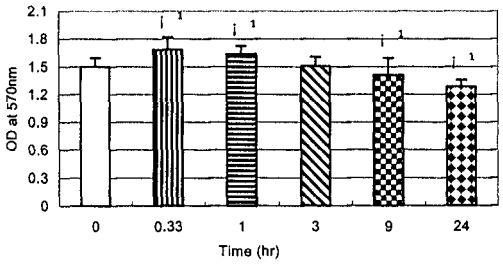

Figure. 1 Time course for the length of 432 us time constant PEMF exposure for 3 days in osteoblasts viability presented by MTT-formazan production. Zero time of exposure corresponds to non-treated cultures (sham). Asterisks indicate statistical significance ($p<0.05$).

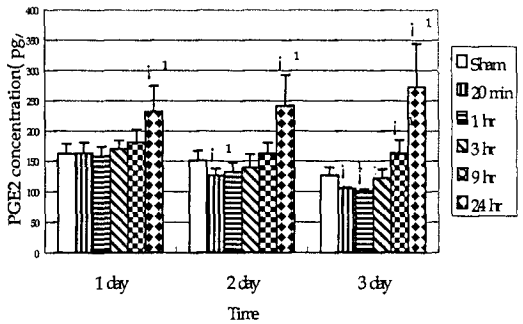

Figure. 2 PGE2 secretion of different exposure time per day of 7.5 Hz, 2mV/cm, 432 us time constant PEMF for lday, 2 days and 3 days on osteoblasts. Asterisks indicate statistical significance ($p<0.05$).

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15-4

QUANTITATIVE ANALYSIS OF RAT TENDINITIS HEALING: IMPACT OF A THERAPEUTIC PULSED ELECTROMAGNETIC FIELD. [1]B.J. Wetzel, [1]G. Nindl, [2]D.N. Vesper, [2]J.A. Swez, [3]M.A. Sandrey, [1]M.T. Johnson. [1] Terre Haute Center for Medical Education, Terre Haute, IN 47809, USA. [2] Department of Physics, Indiana State University, Terre Haute, IN 47809, USA. [3] Department of Athletic Training, West Virginia University, Morgantown, WV 26506, USA.

OBJECTIVE: The goal of the current study was to determine the natural time course of rat tendinitis healing and to evaluate the use of a pulsed electromagnetic field (PEMF, Electro Biology Inc., Parsippany, NJ) as a possible therapeutic modality to accelerate tendon healing.
BACKGROUND: Tendinitis is a result of painful inflammation of tendon fibers that accounts for almost half of all occupational injuries in the United States. No effective treatment exists to eliminate inflammation that causes pain, which can result in re-injury, chronic soreness and loss of function. Electromagnetic field therapy is well known to be a successful treatment modality for some musculoskeletal diseases and therefore this study was designed to test PEMF efficacy in tendinitis.
METHODS: Tendinitis was induced in Harlan Sprague Dawley rats by collagenase injections into the Achilles tendon, and tendons were collected at four times during the first week following injury and once per week for the following three weeks. Application of PEMFs involved exposing the animals for four hours daily to 0.12 mT pulsed fields. To determine the extent of inflammation, histomorphometric analysis of paraffin embedded tendons was used. This method involves stereological digital quantitation of the cell numbers on hematoxylin-and-eosin-stained histological sections. To determine the extent of edema, rat ankle widths were measured with calipers. In addition, tendon wet weights were compared with tendon dry weights after lyophilizing for 24 hours.
RESULTS: We first established a chemical rat tendinitis model of the hind leg Achilles tendon and determined the time course of natural healing. Histological analysis of tendon sections showed that the total number of tendon cells nearly doubled by 24 hours post-collagenase injury, reached a maximum two weeks after injury and then gradually declined. At week four, the numbers of cells were comparable to the cell numbers 24 hours after injury. Analysis of edema showed a maximum at the 24-hour time point with a continuous decline until the end of the first week, when pre-injury values were reached. PEMF exposure had no significant effect on total tendon cell numbers and on the extent of edema as determined by ankle size over the first week post-injury.
CONCLUSION: Our data indicate that edema is maximal 24 hours after injury. This time course was confirmed by histological analysis showing early massive cell infiltration into the damaged area. However, total tendon cell numbers peaked two weeks after injury. This can be explained by fibroblast proliferation, which precedes the construction of new tendon material. PEMFs did not affect the parameters selected to monitor tendon healing during the first week after injury and therefore does not seem to influence the acute phase of tendon healing. Future experiments are planned to characterize the time course of appearance of specific inflammatory cell types and to evaluate the effect of PEMFs on these cells.

15-5

SIMULATING ENVIRONMENTAL GSM FEATURES FOR USE IN BIOEXPERIMENTS. R. Mertens*, W. Kainz*, N. Kuster. Foundation for Research on Information Technologies in Society (IT'IS), Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland.

INTRODUCTION: Actual exposure conditions of mobile phone users vary significantly due to numerous factors. GSM system features like handover, power control and discontinuous transmission mode (DTX) lead to different output power levels and modulation schemes during a phone call. The usage of these features depends on connection quality, network conditions, user speed and the number and durations of speech pauses of the user. Bioexperiments with the aim of applying actual exposure conditions should implement these features in their signals.
OBJECTIVE: Artificially generated signal variances incorporating the mentioned system features should rely on representative data but be easily applicable and should be driven by the premise of maximizing the significance of negative findings with respect to the safety of the tested technology. The objective was to develop an algorithm that fulfills theses criteria using computer based random generators.
METHODS: The generated model was compared to measured mobile phone output power levels driving along routes in an urban area [1]. Using the χ^2-hypothesis test on different statistical distributions revealed the most probable distribution for the occurrence of handovers, the power variances and the pausing in speech. The corresponding parameters were drawn using maximum likelihood estimates.
RESULTS: For a fast moving user on a highway in a rural area the times between handovers follow an exponentially decreasing distribution with a linear regression coefficient of 0.0233. Thus handovers occur on an average of every 43 s. Events that require a readjustment of power output also follow an exponentially decreasing distribution and occur on an average of 12 s. The level change needed to ensure connection quality is 6 dB. The actual output power is adjusted in steps of +/- 2 dB staying at a level for about 4 s. The analysis of business phone calls revealed an average pause duration of 8 s. The system presented takes all these facts into account and consists of six independent random variables and distributions.
DISCUSSION: The presented algorithm is easily applicable using a computer controlled system for the exposure setup, and its spread of simulated exposure conditions is wide enough to reasonably cover current as well as future utilization of GSM technology. The generation of the artificial system model presented is based on the most current data and is therefore highly suitable for all bioexperiments using actual exposure conditions in GSM systems. The presented algorithm is also applicable to UMTS systems. A revaluation can be done by fitting the model with representative network data and by expanding the system with additional UMTS features.
Reference.
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[2] P. Zimmerman, CNET internal report (1997)

15-6

IN VITRO EXPOSURE SETUP FOR ELF MAGNETIC FIELDS ENABLING FLEXIBLE SIGNAL SCHEMES AND DOUBLE BLIND PROTOCOLS. J. Schuderer*, W. Oesch*, N. Kuster. Foundation for Research on Information Technologies in Society (IT'IS), Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland, Schmid & Partner Engineering AG, 8004 Zurich.

INTRODUCTION: The Project REFLEX, sponsored by DG XII under Framework V of the European Union, aims to investigate the molecular and functional responses of living cells to EMF *in vitro* in five research areas: (1) Genotoxic Effects, (2) Effects on Differentiation and Function of Embryonic Stem (ES)

Cells and Tumor Cells, (3) Effects on Gene Expression and Protein Targeting, (4) Effects on the Immune System, (5) Effects on Transformation and Apoptosis of Cells.
OBJECTIVE: The objective of this study was to design, optimize and build an exposure setup for ELF magnetic fields. The requirements were: 1) homogeneous B-field over a large volume of up to > $2mT_{rms}$, 2) flexible signal schemes enabling the generation of conditions simulating worst-case exposures to power lines, consumer equipment, railways, etc. in terms of field strengths and harmonics, 3) intermittence of exposure, 4) sham and control in the same incubator, 5) double blind protocol, 6) continuous monitoring of all environmental parameters.
METHODS: The evaluation, optimization and fine tuning of the ELF-setup was performed using numerical methods (Mathematica 3.0) by solving the analytical equations based on the law of Biot-Savart. The results were carefully verified using the near-field scanner DASY3 equipped with a highly sensitive 3-axis Hall probe. Unwanted electric fields were characterized with the use of a 3-axis ELF E-field probe, and vibrations were assessed with an accelerometer.
RESULTS: The setup consists of two four-coil systems, each of which is placed inside a μ-metal box. The shielded design of the chamber guarantees noninterference between the two units, which can therefore be kept inside the same incubator. The currents in the bifilar coils can be randomly switched parallel for field exposure or nonparallel for sham control by the computer. This procedure is used to apply double blind protocols and additionally to avoid temperature artifacts between exposed and control coils, since they are heated by the same current.
An inhomogeneity of less than 1% in the magnetic field over the exposure area of 16cm x 16cm x 23cm was achieved. In order to avoid additional exposure to nonwanted electric fields that result from the induced voltage over the coil, a metallic shielding box between coil and Petri dishes was integrated. The electric fields at the location of the dishes could thereby be reduced to less than 2V/m. Two fans per chamber have been mounted to guarantee enough atmospheric exchange of the coil boxes. The airflow temperature is monitored with highly accurate DIN 1/10 Pt100 probes fixed on top of the fans. The actual magnetic field is periodically monitored and regulated via measurement of the coil currents at low temperature sensitive resistors.
Current is provided by a stable current source that is based on four audio amplifiers and allows arbitrary field variations in the range from DC – 1.5kHz. The signal is generated by a computer controlled function generator. Noise and drift cause field deviations of less than 0.01% from the target value.
The vibration load at the location of the Petri dish is at its maximum when the field frequency comes close to a mechanical resonance and has been assessed to be less than 0.1g.
This study was supported by the 5th Framework Program of the European Union as well as by the Swiss Government.

15-7

EXPOSURE FUNCTIONS FOR UNIFORM DOSIMETRY IN RATS AT 902MHZ AND 1747MHZ.
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INTRODUCTION: Lifespan exposure at uniform dose is one of the basic requirement in the PERFORM A project. PERFORM A consists of two „classical" combined chronic toxicity and carcinogenicity two-year bioassays conducted at RCC Ltd, Switzerland at both GSM frequency bands (GSM900 and DCS1800) as well as a co-carcinogenicity study using DMBA induced Sprague Dawley rats at GSM900 at ARCS, Austria. An additional co-carcinogenicity study using DMBA induced Sprague Dawley rats will be carried out simultaneously at Hangzou University, China.

OBJECTIVES: The objective of this study was to assess the incident exposures for 902MHz and 1747MHz as function of sex, age and weight in order to minimize the variations of whole-body as well as organ based SAR levels for uniform dosimetry during the 2-year exposure period starting at the age of 5-8 weeks.
METHODS: The evaluation of the setup was performed by numerical dosimetry using the FDTD simulation platform SEMCAD. The animal phantoms generated for the purpose of this study were 1) male Sprague Dawley of age 7weeks weight 200g (slice separation of 0.575mm), 2) pregnant female Sprague Dawley of weight 275g (slice separation of 0.595mm), 3) male Sprague Dawley of weight 370g (slice separation of 3mm), 4) female Sprague Dawley with a mammary tumor in plum, weight 500g (slice separation of 0.53mm) and 5) male Sprague Dawley of weight 566g (slice separation of 0.525). Up 100 different regions were discriminated. SEMCAD was extended to automatically assess the averaged, maximum, minimum SAR values for whole-body as well as every organ and tissues respectively. In addition, the values for the spatial peak SAR averaged over 0.01g, 0.1g and 1g were compared. In the first step, detailed but numerically optimized models of the two physical exposure setups were generated. They were optimized with respect to accuracy and numerical efficiency. In the second step, these numerical setups were extensively validated by comparing the performances with detailed measurements of the two physical setups using different dummy rats in two different setup configurations: (A) synthetic as symmetrical phantoms and (B) restraining tubes as rat-like phantoms. The experimental evaluations had been conducted using the near-field scanner DASY4 equipped with the latest probe technology as well temperature measurements. In the third step, the detailed rat phantoms were placed in the setup representing averaged positions and postures in the restraining tubes.
RESULT AND CONCLUSIONS: The whole-body and organ averaged SAR as function of age and weight are not identical requesting a compromise. On the other hand, the differences between the sexes are only minor. These findings enabled to implement a power control function to adjust the incident field depending on the animal weight in order to guarantee fairly uniform exposure during the entire lifespan of the animal.
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Fröhlich J., et al., 2001, Exposure setups for the simultaneous exposure of a large number of rats for risk assessment studies at the mobile communication frequencies 902MHz and 1747MHz, in Proceedings of 5th Int. Congress of the European BioElectromagnetics Association (EBEA), Sept., Helsinki, Finland.
The study was supported by the 5th Framework Program of the European Union, the Swiss Government (BBW), the Mobile Manufacturers Forum (MMF) and the GSM Association.

SESSION 16:
MARY ELLEN O'CONNOR MEMORIAL STUDENT SESSION
Chairs: Ruggerio Cadossi and Carl Blackman

16-1

EXPOSURE SETUPS FOR SIMULTANEOUS EXPOSURE OF 17 RATS FOR RISK ASSESMENT STUDIES AT 902MHz AND 1747MHz. N. Nikoloski*, W. Kainz*, M. Frauscher‡, J. Froehlich*, N. Kuster*. *Foundation for Research on Information Technologies in Society (IT'IS), Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland; ‡ Seibersdorf Research GmbH (ARC) , A-2444 Seibersdorf, Austria.

INTRODUCTION: Two types of exposure setups for rats had to be developed for the European project PERFORM A: a large toxicological/carcinogenic setup as well as a simultaneous replication setup in the context of health risk assessment of low level exposure to the RF of mobile phones.
OBJECTIVES: The objective was to develop, optimize and build two exposure setup for rats. One should operate at 902 the other at 1747MHz. The experiments shall be conducted double blind at three different

power levels, whereby the high dose shall be marginally below the thermal stress level. The high dose will be assessed in a separate pre-study. The environmental requirements were (1) enough light for the animals and (2) the same airflow for all 17 animals. The dosimetric requirements were (1) an as uniform as possible distribution of the SAR within the animal, with the whole body SAR varying less than 2dB over its lifetime, (2) the SAR distribution of each organ shall be well specified, (3) the deviation of the whole body SAR between animals in the same wheel should be as minimal as possible. In addition all relevant technical and biological parameters during the experiment (e.g., temperature, humidity, oxygen, field strength) will be monitored every ten seconds. This will be controlled by the "*Perform A*" software written in C++
METHODS: The evaluation, optimization and fine tuning of the setup was performed using the FDTD simulation platform SEMCAD including its thermal solver extension. The results were carefully verified using the near-field scanner DASY4 equipped with the latest probe technology.
RESULT AND CONCLUSIONS: Various concepts for exposure setups have been studied. All these concepts were evaluated with respect to efficiency, homogeneity of exposure, size, GLP compliance and cost. The concept which meets all constraints best is a Ferris-wheel with H-polarization. H-polarization has been found to be the only condition which provides reasonable homogeneity of exposure for different sizes of the animals. The realized prototype-concept of the rat setup is a circular cascade of 17 sectorial wave-guides all excited by a loop antenna placed in the center. A main task was to develop a loop-antenna which handles 200W CW / 400 W peak, has a homogeneous circular field distribution, low losses and a well matched impedance. For the 1800MHz the concept had to be further enhanced by reducing the distance of the plates for a certain diameter around the exciting antenna in order to guarantee single mode excitation and by developing a more sophisticated setup shielding. The distance between the short cut and the rats as well as the diameter of the inserted circular plates for the 1800MHz band have been optimized for maximum uniformity of exposure. Electromagnetic sealing of the openings is achieved using easy to handle, custom made stainless steel covers. E-Field sensors were introduced to enable exact monitoring of the exposure as well as for drift compensation. The final prototype of the setup has been carefully evaluated by numerical and experimental means.
The study was supported by the 5th Framework Program of the European Union, the Swiss Government (BBW), the Mobile Manufacturers Forum (MMF) and the GSM Association.

16-2

CHARACTERIZATION AND DOSIMETRY OF THE PERFORM A *IN VIVO* EXPOSURE SYSTEM AT THE MOBILE COMMUNICATION FREQUENCIES 902MHz AND 1747MHz.
S. Ebert*, J. Froehlich*, W. Oesch*, R. Mertens*, N. Kuster. Foundation for Research on Information Technologies in Society (IT'IS), Swiss Federal Institute of Technology (ETH), Zurich, CH-8092 Zurich and Schmid & Partner Engineering AG, CH-8004 Zurich, Switzerland.

INTRODUCTION: The European project PERFORM A is a large toxicological/carcinogenic study in the context of health risk assessment of low-level exposure to the RF of mobile phones. Within this project two types of exposure setups for mice had been developed. With these setups two "classical" combined chronic toxicity and carcinogenicity two-year bioassays are currently being conducted at the Fraunhofer ITA in Germany at both GSM frequency bands, GSM900 and DCS1800, and a co-carcinogenicity study using Eμ-Pim 1 transgenic mice is currently being conducted at GSM900 at RBM in Italy.
OBJECTIVE: The exposure systems for mice operate at the mid band of the GSM 900MHz frequency band (i.e., 902 MHz), and at the mid band of the DCS 1800 MHz system (i.e., 1747 MHz), applying a complex GSM signal into a resonant structure of the exposure setup.
The setups were developed with respect to several requirements: the experiments are conducted double blinded at three different power levels, whereby the high dose is marginally below the thermal stress level, determined in a pre-study prior to the main study. The setups of these large scale *in vivo* studies include a total of more than 1500 animals in group sizes of 65 animals and had to satisfy GLP conditions. The

dosimetric requirements were: (1) the field distribution inside the animal should be as good as possible, and the whole body SAR should vary less than 2dB over its lifetime, (2) the SAR distribution of each organ shall be specified. All relevant technical and biological parameters during the experiment (e.g., temperature, humidity, oxygen, field strength) are monitored at all times. The main study started in October/November 2001. This presentation shows results from the pre-study and the dosimetry.
METHOD: The dosimetric evaluation of the setup was performed using the most advanced FDTD simulation platform SEMCAD and the near-field scanning system DASY3 equipped with the latest probe technology. The SAR assessment was performed with a temperature method using a highly resistive thermistor probe and a commercially available multi-channel fluoroptic temperature probe measurement system from Luxtron.
RESULTS: The basic concept of the mouse setup was adopted from the "Ferris-Wheel" developed by Motorola at 900MHz [1] but has been greatly optimized. The technical data of the setup was presented at the last BEMS conference [2]. This presentation shows the results regarding the characterization of the setup design, regarding the thermal stress threshold findings of mice exposed to 902MHz and 1747MHz cw signal and regarding the dosimetry of the setup. The PERFORM A exposure setup has been carefully evaluated by numerical and experimental means. Through the combination of highly sophisticated measurement techniques and excellent simulation tools together with high-resolution animal models it is possible to determine the applied SAR in the mice more precisely than ever before. Numerical and experimental characterization showed the occurrence of higher modes due to the principle design of the setup. With a temperature measurement technique in dummies these higher modes were characterized and a method was developed to suppress the resulting effect as well as possible. The thermal stress threshold was determined by performing online body temperature measurements in mice exposed to 902MHz and 1747MHz cw signal at different exposure levels. These temperature measurements in combination with the dosimetry showed that the thermal stress threshold is at an SAR value of about 5W/kg at 902MHz cw and about 6W/kg at 1747MHz cw.
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16-3

ARE ELF MAGNETIC FIELD EFFECTS ON CYTOSOLIC CALCIUM IN JURKAT CELLS DEPENDENT ON CELL CYCLE? C.R. McCreary, F.S. Prato. Lawson Health Research Institute and Department of Medical Biophysics, University of Western Ontario London, Ontario, CANADA, N6A 4V2.

INTRODUCTION: A number of studies have examined the effect of ELF MF on the kinetics of the cell cycle but we are unaware of any studies that have examined the dependence of an ELF MF effect on the phase of the cell cycle. It is possible that cells may be more sensitive to ELF MF effects during specific phases of the cell cycle, similar to cell killing efficacy of ionizing radiation. Previous results from our laboratory have suggested that the effects of extremely low frequency magnetic field (ELF MF) exposure on cytosolic calcium concenlration ($[Ca^{2+}]_c$) and calcium signalling are confounded by distribution w/thin the cell cycle [McCreary *et al*, 2002].
OBJECTIVE: To determine if ELF MF effects on $[Ca^{2+}]_c$ and calcium signalling are dependent on the phases of the cell cycle (G0-G1, S, and G2-M).
METHODS: The human lymphocytic cell line, Jurkat E6.1 clone, was synchronized using either serum starvation or a double thymidine block. The distribution of the cell culture within the cell cycle was determined using propidium iodide labelling and flow cytometery. ELF MF exposure experiments were

performed with enriched G0-G1, S, and G2-M phase cell cultures. We used a custom designed spectrophotometer to measure $[Ca^{2+}]_c$ in cells suspended in conditioned RPMI 1640 medium containing 10% foetal bovine serum. Two magnetic field exposures were compared: zero static MF (Null) and the combination of 60 Hz, 100 µT sinusoidal plus 78 µT static MF (AD+DC). All MF were applied in the vertical direction. Cells were cultured in a mu-metal box to reduce ambient static and ELF MF. The $[Ca^{2+}]_c$ in Jurkat cells loaded with Indo 1-AM was determined using ratiometric techniques. In all experiments, cells were exposed to a null static magnetic field condition (<0.4 µT) for the first 5 min and this was used for normalisation of subsequent data in each experiment. To stimulate a calcium signal, a-CD3 was introduced into the cell samples after 1200s of exposure and the calcium signal was monitored for another 1200s. A number of calcium parameters were compared using multivariate analysis (SPSS) and results were confirmed by a non-parametric test (Mann-Whimey U p-values reported).

RESULTS: When Jurkat cells were synchronized by serum starvation, a significant decrease in normalised $[Ca^{2+}]_c$ between 555-615s was observed when G0-G1 phase enriched cell samples were exposed to the AC+DC (n=16) field in comparison to the Null group (n=14; p = 0.028). When S phase enriched cell samples (n = 5 for both AC+DC and Null groups) were compared, a significant decrease in normalised $[Ca^{2+}]_c$ at t=-615s (p=0.047) and t=2100s (p=0.028) was observed. However, no significant differences were detected when the cell samples were G2-M phase enriched. To increase the enrichment of the cell culture, Jurkat cells were synchronized by double thymidine block. No significant differences were detected when the cell samples were S phase enriched.

DISCUSSION: We have determined the effect of ELF MF exposure on $[Ca^{2+}]_c$ and calcium signalling in Jurkat cell culture enriched with cells in S phase by two techniques. Despite greater S phase enrichment when the cells were synchronized using thyimidine, ~65% of the cells were in S phase compared to ~42% achieved with serum starvation, no differences in $[Ca^{2+}]_c$ or calcium signalling were detected when thymidine was used. It is possible that the thymidine treatment interfered with the ELF MF effects observed when serum starvation synchronization was used. Future experiments using elutriation techniques to separate cells according to phase within the cell cycle will be completed to determine if ELF magnetic field effects on cytosolic calcium in Jurkat cells dependent on cell cycle.

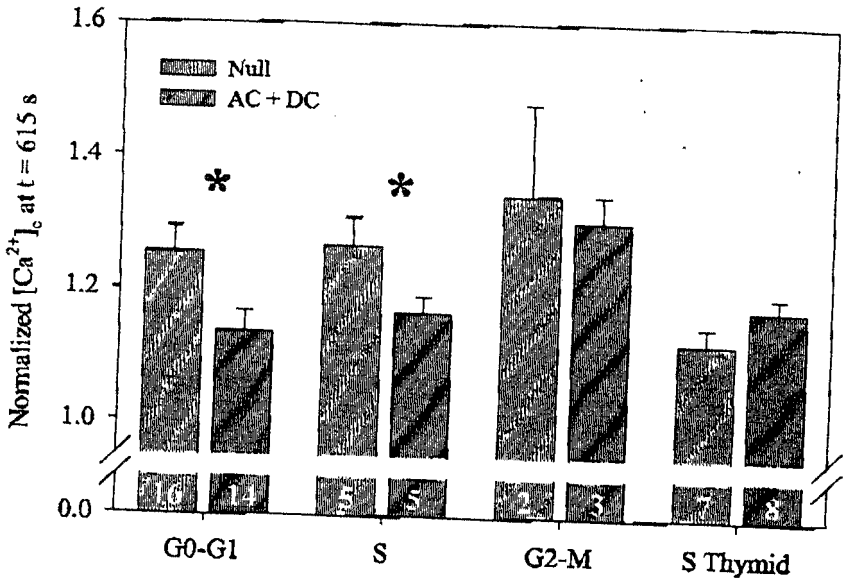

Figure 1. Preliminary results suggested ELF MF effects on $[Ca^{2+}]_c$ were apparent only when Jurkat cells were enriched with cells in G0-G1 or S phase through serum Starvation. The number at the bottom of the bars indicates the sample size and error bars represent the standard error of the mean. The asterix indicates p<0 05 when Null and AC+DC groups were compared.

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This work was funded by the Canadian Institutes of Health Research.

16-4

REPEATED MAGNETIC FIELD SHIELDING INDUCES ANALGESIA IN MICE. D.C. Desjardins[*1,2], A.W. Thomas[2], C.M. Cook[2], F.S. Prato[2]. [1]Neuroscience Program; [2]Department of Medical Biophysics, University of Western Ontario, London, Ontario, Canada; [2]Lawson Health Research Institute and Department of Nuclear Medicine & MR, St. Joseph's Health Care (London), London, Ontario, Canada N6A 4V2.

INTRODUCTION: A single exposure of mice to ELF magnetic fields has been shown to attenuate opioid induced analgesia [1] whereas repeated daily exposures have been shown to induce analgesia [2]. We have recently observed that a single exposure of mice to a magnetically shielded environment produced by placement in a μ-metal box can also attenuate opioid induced analgesia [3].
OBJECTIVE: It was the objective of this study to determine if daily repeated exposures of mice to the environment produced by a μ-metal box would induce analgesia.
METHODS: Adult male Swiss CD1 mice [Charles River, Canada] were placed in an opaque Plexiglas™ lined μ-metal box or an opaque Plexiglas™ box (sham condition) for 1 hour a day for 10 consecutive days. The μ-metal box attenuates ambient magnetic fields from 0 to 60 Hz by a factor of 125 or more [3]. Nociception was measured as the latency of a foot lifting/lick to an aversive thermal stimulus (50 ±0.5° C) before (pre) and immediately after (post) placement in the boxes. Two experiments were conducted, one in which thermal latency was tested on each of the ten days and one in which testing was only performed on days 1, 5 and 10 but exposure was for 10 consecutive days as in the first experiment. Post "exposure" latency values were normalized (post/pre) to pre-exposure values and significance was tested using ANOVA with $p<0.05$.
RESULTS: An analysis of variance showed a significant between subjects effect by exposure condition [$F_{(1,21)}=10.81$, $p<.001$, $Eta^2=.34$] where those subjects exposed to the μ-metal box showed a significant increase in latency as compared to the control subjects. In experiment one a multivariate analysis showed a significant within subjects interaction between days of exposure and exposure condition [$F_{(1,9)}=1.942$, $p<.05$, $Eta^2=.64$]. The latency times of the μ-metal exposed group dropped to 80% of pre-values after the first exposure which corroborates former findings [3] and then increased to 160% as compared to pre-values after exposures of days 3, 4 and 5. By day 10 post values were not different from pre-values. No such results were seen in the control group (see Figure 1 for results of Experiment 1). Results of experiment 2 confirmed those of experiment 1 showing again a decrease in post latency values on day 1 (70% of pre-values), increase on day 5 (180% of pre-values) with a return to pre-latency values on day 10.
DISCUSSION: These experiments further suggest that shielding from ambient magnetic fields affects opioid related behaviors in a manner similar but not identical to effects from exposure to increased ELF magnetic fields (MF). The initial reduction on day 1 was consistent with previous data [3] as was the induction of analgesia on day 5 [2]. Further experiments are needed to compare and contrast the effects of ELFMF exposure and ELFMF shielding on opioid function.

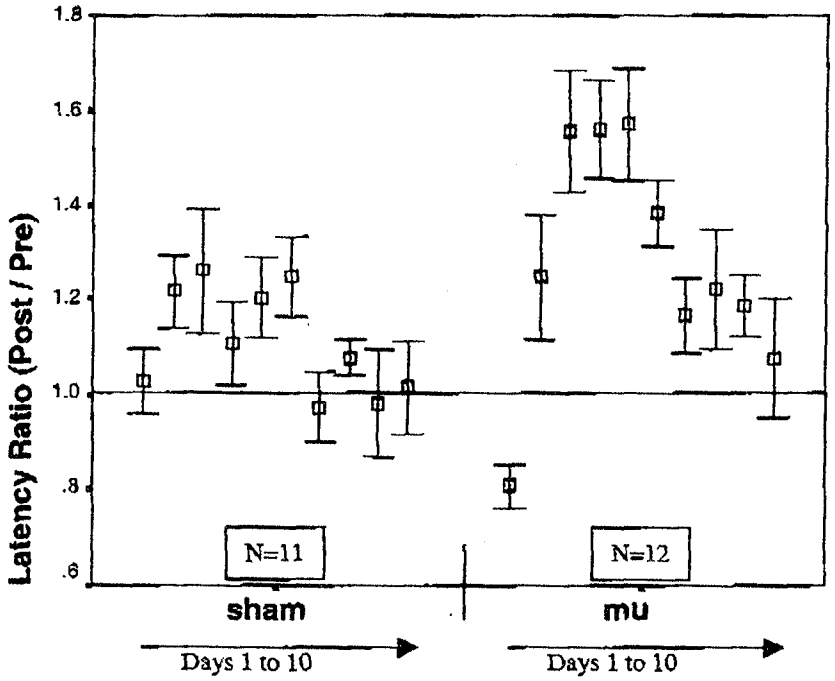

Figure 1: Results of Experiment 1. The latency ratio values immediately after replacement in control box or g-metal box were normalized for each mouse. This figure shows a significant between-subjects effect by exposure condition [$F_{1,21}$)=10.81, p<.001, Eta^2=.34] and a significant within subjects interaction between days of exposure and exposure condition [$F_{1,9}$)=1.942, p<.05, Eta^2=.64]. Each point on this figure represent the ratio of individual post/pro values for each test day and the error bars represent the SEM.

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Funded in part by the Canadian Institute of Health Research.

16-5

ELECROCUTANEOUS SENSATION: THRESHOLDS OF PERCEPTION, ADAPTATION, AND TISSUE-INTERNAL FIELDS. G. Lindenblatt, G. Schell, and J. Silny. femu - Research Center for Bioelectromagnetic Interaction, Aachen University of Technology, Germany.

INTRODUCTION: Perceptions of prickling and itching are well-known everyday phenomena triggered by electrical charges passing the skin. Depending on the strength of the stimulus, these perceptions can intensify to a feeling of pain. In order to avoid this, contact voltages and leakage currents must be limited. The assessment of a threshold current, representing the upper limit of an acceptable leakage current, is a common method. However, this parameter depends on the utilized experimental setup, certain environmental factors, and the decision behaviour of the test person.

OBJECTIVES: The tissue-internal current density value at the perception threshold as a measure of sensitivity against the non-adequate provocation of the cutaneous receptors was determined.
METHODS: Two kinds of experiments on the perceptibility of currents were conducted with consenting test persons: (1) The adaptation to electric currents was determined. (2) The absolute current threshold for a specific experimental setup was measured.
Using a numerical model of the fingertip (the place of the current injection) we calculated the current density inside the tissue at the depth of the irritated receptors. The computer model follows the used experimental setup.
Varying the thickness of the different skin tissue layers we aimed to obtain a worst case-value of the current density threshold at the place of the receptors which are provoked by the current.
RESULTS AND CONCLUSIONS: The found current threshold (at 50Hz, the European power supply net frequency) is 170 µA at the 50%-border. This value is corrected for an adaptation which occurs during a test sequence. With this input value the numerical calculations result in a current density of 30 $\mu A/cm^2$ inside the tissue at the place of the irritated mechanoreceptors as the result of the worst case-estimation.
We conclude that on the one hand the limiting values of ~ 500 µA for a leakage current, which are specified in a number of precautionary regulations for the low frequency range, are much too high. In fact, depending on the situation, a sensation of pain can be triggered already below this value. Instead the specification of a current density value seems more reasonable as it does not depend on the specific experimental setup and it reflects the real situation inside the organ when triggering a perception.

16-6

AN INVERSE ALGORITHM TO DETECT NEURAL ACTIVITY AT UP TO FOUR LOCATIONS USING MEG. Q.X. Li*, O.P. Gandhi. Department of Electrical and Computer Engineering, University of Utah, Salt Lake City, UT 84112, USA.

OBJECTIVE: Neuromagnetic imaging techniques using the measured MEG data have been used to visualize electrical activity in the human brain, for studies of the human brain function in response to various stimuli or for memory and cognition. The neural activity manifests itself in terms of two to four localized equivalent current dipoles (ECDs). During the last twenty years, several inverse algorithms have been discussed to determine the location, orientation, and magnitude of the ECDs. However, a homogeneous sphere model of the head is mostly used for such inverse algorithms [1]. An important aspect of inversion problem is to have good anatomically-realistic forward models such as those that have previously been developed for dosimetry of EM fields. For the present paper, we have used a shaped model of the head represented by 6 mm voxels in the first instance, and the quasi-static impedance method [2] to calculate the forward magnetic fields at 74 locations of the MEG sensors (e.g. Bio-magnetic Technologies Inc., San Diego, CA). We show that the external magnetic fields at the sensor locations are erroneous if secondary currents due to the conductivity of the surrounding tissues are ignored, but are affected very little by using a shaped but homogeneous model rather than the detailed heterogeneous model. This obviates the need for modeling the patient-specific anatomic details as long as the shape of the head is properly modeled. Whereas the use of such a model for locating two ECDs over limited subvolumes of the brain (1.8×2.4×2.4 cm) has previously been described [3], we have extended the work for up to four ECDs that may be located over much larger subvolumes in the two halves of the brain. Our goal is to extend the search for the ECDs to the entire volume of the brain.
METHODS: In this study, we postulate two to four dipole sources that are located arbitrarily in the two halves of the brain over two subvolumes of dimensions 1.8×3.0×6.0 cm represented by 100 voxels each. We further assume that the depth of the individual sources may vary from 3.0 to 4.2 cm from the scalp. It is recognized that the measured MEG signals may be corrupted by white Gaussian noise. Noise levels with signal to noise ratio (SNR) of 2 are added to the signals for each of the 74 magnetic field sensors which are distributed equally between the two sides of the head. A total of 300 repeated measurements are averaged to

improve the SNR. Based on FOCUSS algorithm [4], the iterative minimum norm algorithm and Tikhonov regularization are used in first step to resolve the location of the sources from the $\mathbf{A}\vec{J}_{est.} = \vec{B}_{meas.}$ matrix equation, where $\vec{B}_{meas.}$ is the MEG measurement data, **A** is the forward problem matrix which is calculated by impedance method for the shaped homogeneous head model, and $\vec{J}_{est.}$ is the estimated source vector which is projected in x, y, and z directions. The iterative minimum norm algorithm updates the **A** matrix at each iteration step, and obtains several possible locations after seven to ten iterations. We then use weighting functions to calculate their geometrical center and thus obtain the source locations. In second step, we use pseudo-inverse of matrix **A** to calculate the source orientation and magnitude based on the locations of the sources obtained as a result of step one.

RESULTS AND DISCUSSION: In Table 1 we give the assumed and estimated source locations, angles, and the errors in calculations by this inverse algorithm. The algorithm is able to locate two to four sources with error in estimated positions of less than one voxel (for 80% of the cases there is no position error), when one to two sources for each side of the brain are assumed. Typically, error in magnitude of the ECD is less than ±3 percent, and the orientation error is less than $\pm 5^{o}$ both for angles Φ and Θ. For ECDs with error in estimated positions, the error in magnitude of the ECD is less than ±15% , and the error in orientation is less than $\pm 12^{o}$ of angle both for Φ and Θ. In order to improve the accuracy even further we intend to interpolate the measured MEG data from the 74 measurement sensors to many additional intermediate sites (perhaps a total of 100 - 200 sites for each side of the brain).

We are presently extending the algorithm so that the search will cover the whole brain or a substantial part thereof.

Reference.

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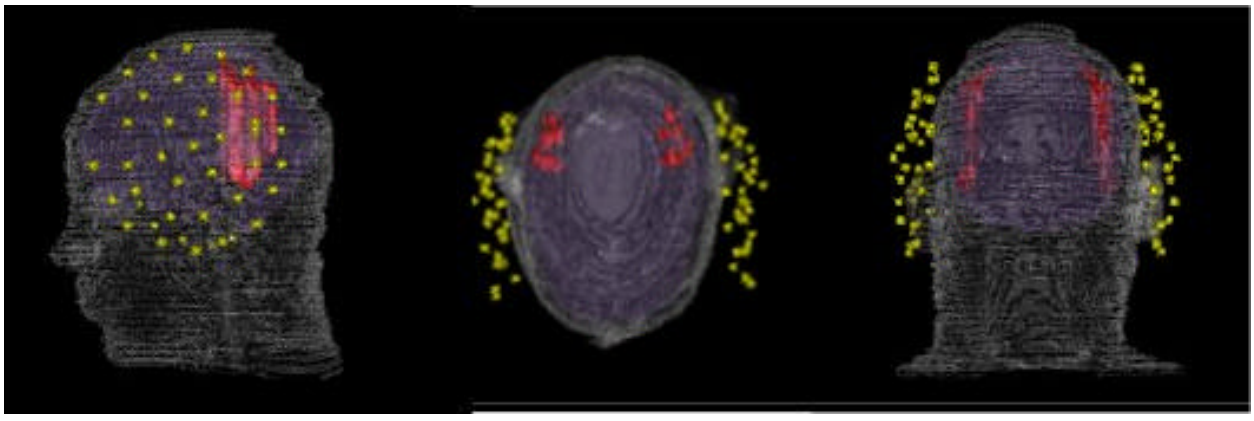

Figure 1. Three visualizations of the human head model, and positions of sources and sensors. From left to right: The *y*-*z* plane, the *x*-*y* plane, and the *x*-*z* plane.

TABLE 1. COMPARISON OF ASSUMED AND ESTIMATED FOUR ECDS

Assumed Values				Estimated Values				Error in			
Position (Coordinates)	Ampli-tude	Φ (deg.)	Θ (deg.)	Position (Coordinates)	Ampli-tude	Φ (deg.)	Θ (deg.)	Position (Coordinates)	Ampli-tude (%)	Φ (deg.)	Θ (deg.)
(14,20,17)	1.3	50	35	(14,20,17)	1.30	50.3	34.9	(0,0,0)	0.18	0.25	-0.07
(15,21,21)	0.5	290	70	(15,21,21)	0.50	291.5	70.1	(0,0,0)	0.28	1.46	0.06
(29,20,17)	1.1	340	35	(29,20,17)	1.09	340.4	35.4	(0,0,0)	-0.99	0.43	0.37
(27,21,21)	0.8	90	130	(27,21,21)	0.80	92.1	130.2	(0,0,0)	-0.51	2.06	0.16
(12,20,18)	0.6	40	125	(12,20,18)	0.61	40.7	125.9	(0,0,0)	2.05	0.66	0.87
(15,24,17)	1.1	120	50	(15,24,17)	1.10	119.9	50.4	(0,0,0)	-0.12	-0.11	0.42
(30,20,18)	1.0	40	40	(30,20,18)	0.94	36.0	43.1	(0,0,0)	-5.53	-3.97	3.07
(28,24,17)	1.2	52	50	(28,24,18)	1.36	50.1	48.9	(0,0,1)	13.35	-1.9	-1.11
(13,20,21)	1.7	40	45	(13,20,21)	1.70	40.2	44.5	(0,0,0)	0.19	0.18	-0.5
(14,21,17)	0.3	120	130	(14,21,17)	0.30	115.5	131.2	(0,0,0)	-0.87	-4.49	1.24
(30,21,17)	1.1	240	55	(30,21,17)	1.10	239.4	54.9	(0,0,0)	-0.32	-0.6	-0.14
(27,21,21)	1.3	20	160	(27,21,21)	1.30	19.7	161.0	(0,0,0)	-0.3	-0.28	0.98
(15,20,17)	0.5	240	25	(15,20,17)	0.50	244.3	23.0	(0,0,0)	-0.09	4.29	-1.99
(12,24,21)	1.8	120	30	(12,24,21)	1.80	119.3	29.9	(0,0,0)	0.15	-0.67	-0.15
(29,22,17)	0.7	40	125	(29,22,17)	0.71	40.7	125.8	(0,0,0)	0.99	0.72	0.78
(27,24,21)	1.2	20	30	(27,24,21)	1.19	19.2	30.5	(0,0,0)	-1.12	-0.79	0.52
(15,20,21)	1.3	150	105	(15,20,21)	1.30	150.0	105.0	(0,0,0)	0.13	-0.02	-0.05
(13,24,18)	0.4	40	30	(13,24,18)	0.40	40.0	30.1	(0,0,0)	0.11	-0.02	0.12
(30,20,19)	1.3	150	25	(30,20,19)	1.39	153.3	25.0	(0,0,0)	7.08	3.28	0.03
(27,23,20)	0.6	70	150	(28,22,21)	0.57	81.5	138.7	(1,-1,1)	-5.79	11.45	-11.31
(13,20,21)	1.3	40	35	(13,20,21)	1.29	40.9	35.3	(0,0,0)	-0.76	0.86	0.27
(14,24,21)	1.2	30	50	(14,24,21)	1.20	28.9	49.9	(0,0,0)	-0.06	-1.08	-0.07
(27,22,21)	0.7	200	45	(27,22,21)	0.69	199.9	45.3	(0,0,0)	-1.24	-0.13	0.27
(29,24,18)	0.9	70	70	(29,24,18)	0.90	68.8	70.2	(0,0,0)	-0.47	-1.18	0.24

16-7

EFFECTS OF HIGH POWER 900 MHz MICROWAVES ON NEUROTRANSMISSION IN THE RAT BRAIN. A.L. Mausset*[1], H. Hirbec*[1], J. Vignon*[1], A. Privat*[1] and R. de Seze[2]. [1]Laboratoire de Développement, Plasticité et Vieillissement du Systéme Nerveux - INSERM U336 - Université Montpellier II - Place Eugéne Bataillon - 34095 Montpellier CEDEX 5 - France. [2]INERIS - DRC - Toxicologie - Parc ALATA BP 2 - 60550 - Verneuil en Halatte France.

OBJECTIVES: The use of cellular phones in close vicinity of the head raises the problem of interaction between radiofrequency electromagnetic fields (RF) and the brain. In this study, we investigated the potential effects of RF on neurotransmission systems and their consequences on gliosis and behavior in the rat.

METHODS: Rats were maintained in specific Plexiglas holders and exposed to RF thanks to individual loop antennas placed above their heads. These antennas were connected to a power amplifier, which emitted 900 MHz RF, and allowed to control the energy received by tissues (quantified by Specific Absorption Rate or SAR).

In order to investigate the effects of RF on neurotransmitter (NT) level and gliosis, we used immunohistochemistry method to label GABAergic neurons and GFAP-immunoreactive astrocytes respectively. We performed semi-quantitative image analysis to compare the GABA or GFAP quantities in control and exposed animals.

To study the potential effect of RF on NT receptors, we performed autoradiographic experiments. Different NT receptors were labelled with specific radioligands: [^{3}H]TCP for NMDA glutamate receptors, [^{3}H]Muscimol for $GABA_A$ receptors and [^{3}H]BTCP for dopamine transporters. Binding levels were quantified by image analysis in different brain structures and compared between control and exposed rats. The precise biophysical parameters of the binding were determined, for each receptor, by binding experiments on different brain structure homogenates.
We also studied the effect of RF exposure on the rat locomotor behavior in an open field experiment.
RESULTS: By immunohistochemistry, we showed that an exposure to high SAR (4 and 32 W/kg) for 2 h induced a dose-dependent decrease in GABA content in the cerebellum.
Autoradiographic experiments showed that an exposure to a SAR of 4 W/kg for 15 min induced a decrease in [^{3}H]TCP and [^{3}H]Muscimol binding and an increase in [^{3}H]BTCP binding. The structures affected by the RF exposure were mainly the cortex, the striatum and the hippocampus, depending on the type of receptor investigated.
The binding studies showed that the decrease in [^{3}H]TCP and [^{3}H]Muscimol binding was correlated with a decrease in the receptor affinity for this ligand. The increase in [^{3}H]BTCP binding was rather due to an increase in the maximal number of accessible sites.
Although striatum was significantly affected by RF exposure, we did not observe any significant effect on the rat locomotor behavior.
However, we noticed a strong glial reaction in the exposed rats, since the GFAP quantity increased dramatically 72 h after the RF exposure in the cortex, the cerebellum and the hippocampus. This result indicates that RF exposure may induce a neuronal injury.
CONCLUSION: As a conclusion, our results show, for the first time, that a short duration exposure to high SAR 900 MHz RF is sufficient to induce some important modifications on both neurotransmission systems and glial reaction.
This work was funded by France Telecom R&D and the French Ministry of National Education and Research (COMOBIO project of RNRT, 99.S.0168).

PLENARY SESSION IV: MECHANISMS OF INTERACTION
Chair: Abraham R. Liboff

PHYSICAL MECHANISMS FOR ELECTROMAGNETIC FIELD TRANSDUCTION IN BIOLOGY. S. Engström, Vanderbilt University, Nashville, TN, USA.

The low field effect of the radical pair mechanism. Geminate radical pair recombination offers multiple possibilites for magnetic field detection. Many models up to this point have focused on mechanisms that become relevant only for fields of several mT and this has somewhat dampened the interest in this mechanism. Recent work on the free radical low field effect moves this limit firmly into the μT range. A complete model of this effect requires detailed assumptions about the participating chemical species, but general conclusions are available for a broad range of chemical composition.
Interpretation of hypotheses in experimental and theoretical work. Many experiments are hypothesis-driven, but only if the hypothesis can be rejected by the experiments, and if the complement to the hypothesis is interesting is some sense, is any definite ground gained. Theoretical rejection of physical mechanisms abound in the field of bioelectromagnetics – What positive conclusions (if any) can be drawn from such work? Resonant models for field detection – what constitues strong experimental evidence for a resonant interaction?
Animal navigation is now well established in the scientific literature with many species that have a documented sensitivity to geomagnetic-level fields. Do the theoretical models developed in this field have general significance for electromagnetic field bio-effects? The plausible models in the Literature tend to

build a rather complex 'sensor' around a relatively simple transduction mechanism. It can be argued that the sensitivity of these experiments in some cases is a small fraction of the field amplitudes involved: Even with clever sensory models, the physical detection mechanism has to be able to usefully resolve the components of the geomagnetic field.

Transcranial magnetic stimulation now has a substantial clinical literature as a therapeutic modality to treat behavioral diseases and epilepsy. Here, it appears that the physical mechanism is not actively sought since it is assumed that induced electric fields exite neurons. The mechanistic problem that is investigated is the subsequent biochemical problem.

Co-factors in electromagnetic field experiments. The uneven nature of many experiments in bioelectromagnetics makes one wonder if there is a major common factor that is being overlooked or not uniformly controlled for. One such possibility is light. Some experimental evidence is suggesting that light may indeed be a co-factor for field detection and possibly the transduction mechanism itself. Other factors are possible: contamination with ferromagnetic particles, cell state and passage number, the presence of relevant non-equilibrium processes, transients, naturally occurring static field components.

EM FIELDS: BIOLOGICAL TRANSDUCTION MECHANISMS. M. Blank. Department of Physiology and Cellular Biophysics, Columbia University, New York, New York 10032, USA.

INTRODUCTION: Weak low frequency electromagnetic (**EM**) fields affect a wide range of biological processes from simple enzyme reactions to the far more complex gene induction and protein synthesis, as in the stress response (1). One expects EM fields to initiate these processes by interaction with moving charges (2, 3), and that the moving charge interaction (**MCI**) mechanism is most apt to apply to electron transfer reactions because of their high charge/mass ratio.

REACTION KINETICS: EM fields accelerate the cytochrome oxidase reaction (4, 5), which clearly involves electron transfer, and the Na,K-ATPase reaction (6, 7), where estimates of the charge velocity suggest that they too are electrons. To test the applicability of the MCI mechanism in a chemical system, we have studied the Belousov-Zhabotinski (**BZ**) reaction (8), the oxidation of malonic acid in Br^-, BrO_3^- and Fe^{+2}/Fe^{+3} catalyst. The BZ reaction is accelerated with no enzyme, membrane or calcium ions present (9). EM fields accelerate all three reactions similarly, and in ways that are compatible with the MCI mechanism:

- % acceleration varies inversely with intrinsic reaction rate, showing competition between the applied magnetic field and intrinsic chemical forces at the electron.
- % acceleration rises steeply at the onset of the effect, followed by a much slower decline at higher amplitudes, suggesting a threshold event.
- the threshold appears to be well below 5mG.
- variation of % acceleration with frequency shows a broad maximum that appears to be related to the specific electron transfer reaction.

TRANSCRIPTION: Since EM fields initiate transcription, in particular the stress response (1), it is likely that they do so by interacting with electrons moving within DNA (10, 11). The velocity of charge movement in Na,K-ATPase is similar to electron transfer in DNA (3), so the forces that affect enzyme reactions may be large enough to cause changes in DNA. A 900 base pair segment has been identified in the hsp70 promoter that is needed for the response to EM fields (12). Removal of the segment eliminates the response, and transfection into a promoter construct renders it EM field responsive (13). Assuming that EM fields initiate transcription by generating repulsive forces within DNA that cause chain separation, it is possible to show that repulsive forces are greatest near the sequences needed for transcription by EM fields (14).

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POSSIBLE EFFECTS OF HIGH FREQUENCY LOW AMPLITUDE ELECTRO-MAGNETIC WAVES ON MECHANISMS FOR ERROR CORRECTION IN DNA SYNTHESIS. R.D. Astumian, Dept. of Physics, University of Maine, Orono, ME 04469

There is strong theoretical evidence that very low amplitude high frequency waves cannot directly influence most biological processes. Any effect would be swamped by intrinsic noise in the environment. One possible exception may be a process that repeats many times in its completion. An obvious example is a kinetic proofreading mechanism where a protein repeatedly interogates a DNA sequence, moving back and forth over the same set of DNA bases many times. It is conceivable that a weak oscillating field, by slightly biasing this motion could have a significant impact on the fidelity of DNA transcription, and hence on the health of the organism. We will examine this possibility using recent theoretical developments of "Brownian ratchets", where a zero average input, coupled with a system asymmetry and thermal noise, can be converted to net directed motion. Preliminary results of this analysis show that indeed fields smaller than might be expected cannot be ruled out as possibly being a causal agent for a real biological effect. Nevertheless, the threshold for such fields, while surprisingly small, is still many orders of magnitude larger than fields typical in the environment.

SESSION 17: EPIDEMIOLOGY
Chairs: Maria Feychting and Jutta Brix

17-1

RESIDENTIAL AND OCCUPATIONAL EXPOSURE TO 50 HZ MAGNETIC FIELDS AND HEMATOLOGICAL CANCERS. T. Tynes[1,2*], T. Haldorsen[2]. [1]Norwegian Radiation Protection Authority, Osterás, Norway, [2]Kreftregisteret, Oslo, Norway.

OBJECTIVE: The aim of this population based nested case-control study was to test the hypothesis that exposure to electromagnetic fields from high-voltage power lines increases the incidence of hematological cancers in adults aged 16 and above.
METHODS: The study population comprised subjects aged 16 and above who had lived in a residence situated in a broad corridor around a high-voltage power line in 1980, or one of the years from 1986 to 1996. The cases were incident cases of hematological cancers that were diagnosed 1980-96 and reported to the Cancer Registry of Norway. Two controls were matched to each case by year of birth, sex, municipality and first year entering the cohort. Twa (time-weighted average) exposure to residential magnetic fields generated by the power lines was calculated for the exposure follow-up from January 1, 1967 until diagnosis by means of a computer programs in which distance from a residency to the line, line configuration, and current load were taken into account. Exposure was analysed using cut off points at 0.05 and 0.2 microtesla (μT). In addition exposure to magnetic fields at work was classified by an expert panel who assessed

magnetic field exposure by combining branch and occupation into one of three levels: < 4 hours, 4-24 hours and > 24 hours per week above background (0.1 µT). The categories were cumulated over the occupationally active years for the exposure follow-up January 1, 1955 until diagnosis, and cut off points at 18 and 31 category-years were evaluated.
RESULTS AND DISCUSSION: The analysis of the two upper residential magnetic field categories showed non-significant elevated odds ratios (ORs) for all leukemia combined (OR 1.31, 95% confidence interval (CI) 0.70-2.45 and OR=1.51, 95%CI 0.75-3.03). Acute myelocytic leukemia, acute lymphatic leukemia and chronic lymphatic leukemia also showed non-significant elevated ORs. For chronic myeloid leukemia we had too sparse data to perform an evaluation. For lymphoma a non-significant decreased odds ratio was seen in the upper exposure category. For multiple myeloma non-significant elevated ORs were seen. Occupational exposure showed no significant association with any site. For CLL non-significant elevated odds ratios were seen in the two upper occupational exposure categories.
CONCLUSION. Our findings provide no firm support to an association between residential or occupational expsoure to magnetic fields and hematological cancer.

17-2

EXPOSURE ASSESSMENT ERRORS IN DISEASE RISKS ASSOCIATED WITH THE ELF MAGNETIC FIELD MAGNITUDE (RESULTANT): A POSSIBLE CAUSE OF IN-CONSISTENCIES AMONG EPIDEMIOLOGIC STUDIES. J.D. Bowman, P.B. Shaw. National Institute for Occupational Safety and Health, Cincinnati, Ohio, 45226 USA.

BACKGROUND: Reviews of occupational epidemiologic studies of ELF magnetic fields and cancer have frequently concluded that the results are "inconsistent". Although meta-analyses have reported the average risks for brain cancer and leukemia to be significantly elevated, inconsistencies between individual studies are often cited as evidence that these associations are not causal. However, disease risks from known causes can vary substantially between studies due to exposure assessment errors. Since EMF epidemiologic studies virtually all measure the ELF magnetic field magnitude (*i.e.* the broadband resultant), they are especially liable to exposure assessment error because this is not the biologically-effective exposure metric in any of the biophysical mechanisms proposed so far. Induced current mechanisms depend on the frequency spectrum and spatial orientation as well as the ELF magnitude. Free radical mechanisms are also sensitive to the static magnetic field magnitude B_o. Ion parametric resonance (IPR) depends on all the above, plus the relative orientation of the static and ELF vectors. For these three mechanisms, other magnetic field metrics are much better correlated with the biophysical effects than the ELF magnitude (1). This paper estimates the bias and variance in disease risks potentially due to measuring exposure with the ELF magnitude if one of these three mechanisms is actually causing the biological effects.
Theory: Consider a study population where exposures to a biologically-effective metric X can be fit to the ELF magnitude by linear regression: $B_{ELF} = a + bX + e$ where the residual $e \sim N(0,s^2)$ and $R^2 = 1/(1+s^2/b^2s_X^2)$. If X causes a disease with relative risks $RR = \exp(ßX)$, an epidemiologic study that measures a subject's exposures to the ELF magnitude will observe a risk coefficient $ß^* = ßR^2$, that is biased towards the null. The exposure assessment error also inflates the variance of risk estimates, decreasing the power of the study. This theory is a variant of classical measurement error, where exposure measurements $Z = X+e$ and the bias $ß^*/ß = 1/(1+s^2/s_X^2)$.
METHODS AND RESULTS: The analysis uses half-shift measurements taken on 25 electrical workers with the Multiwave® System III, a personal three-axis waveform monitor for 0-3000 Hz magnetic fields. The workers were line workers, electricians and welders from a federal research laboratory and a jet engine factory (2). The exposure metrics below were calculated from 1/30 sec. waveforms captured every second, for a total of 154,945 3D wave-form measurements. The measurements were grouped by occupational category for the calculation of the bias.

Mechanism	Exposure metric	Bias in disease risks associated with B_{ELF}		
		Line workers (N=58,913)	Electricians (N=41,688)	Welders (N=54,344)
Induced currents	dB/dt	0.993	0.862	0.879
Free radicals	$B_o^2 + B_{ELF}^2$	0.069	0.003	0.293
IPR with Ca^{2+}	Lednev metric (3)	0.462	0.731	0.094

DISCUSSION: If epidemiologic studies measuring the resultant were conducted on this sample of workers, any disease risks due to ELF magnetic fields would be biased towards the null. The amount of bias varies with occupation and the mechanism. If induced currents causes the disease, exposure assessment errors would be relatively small, but would be substantial for the free radical and IPR mechanisms. Since these errors also vary among the three occupations, epidemiologic studies with different occupational compositions would have increased chances of inconsistent findings. These classical measurement errors are found when exposures are measured on all cases and controls. Studies with job-exposure matrices are liable to Berkson error, which may not have bias but does increase the variance in risk estimates. Whether the apparent inconsistencies among disease risks associated with occupational magnetic fields are due to exposure assessment errors can be tested by epidemiologic studies with new instruments like the Multiwave III that measure biologically-based exposure metrics.

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17-3

COMPARISON OF VARIOUS SAFETY GUIDELINES FOR EAS DEVICES WITH PULSED NON-SINSOIDAL MAGNETIC FIELDS. G. Kang*, O.P. Gandhi. Department of Electrical and Computer Engineering, University of Utah, Salt Lake City, UT 84112, USA.

Limits of human exposure to electromagnetic fields at low frequencies are set in terms of induced current densities (ICNIRP, 1998) or induced electric fields for various tissues (under consideration by IEEE). Compliance with these limits is used to evaluate human exposure to electromagnetic fields from devices such as electronic article surveillance (EAS), radiofrequency identification (RFID) and other low-frequency security systems using time-varying magnetic fields in the frequency band 0-300 kHz. While induced current densities (or electric fields) are easy to assess for compliance with the safety guidelines for sinusoidally-varying magnetic fields of some of the commonly encountered solenoid-type deactivators or pass-by EAS or RFID systems (Gandhi and Kang, 2001), many other security systems including some for libraries use pulses or damped sinusoidal magnetic fields. Two different procedures have been suggested in both the ICNIRP and the proposed IEEE guidelines for assessing compliance with the safety limits for such

pulsed or nonsinusoidal exposures. Two procedures are (a) to treat the exposure as a multifrequency exposure with various frequency components derived from Fourier components or (b) to calculate the peak induced current densities or electric fields which should be less than $\sqrt{2}$ times the basic restriction at the equivalent frequency $f = \frac{1}{2} t_p$ where t_p is the duration of the pulse or peak excursion of the exposure field. We have used the impedance method code to assess the exposure for a couple of assumed but typical pulsed nonsinusoidal magnetic field exposure situations that may be encountered for such EAS or RFID systems. Used for the induced current calculations is the anatomically-based 31-tissue, $2 \times 2 \times 3$ mm resolution, Utah model of the human described in Gandhi and Kang (2001). The geometry used for the pass-by EAS system is similar to that of Fig. 3 in Gandhi and Kang (2001) in that the EAS system is assumed to use a pair of coplanar rectangular coils each of dimensions 50×60 cm with an overlap of 10 cm in the middle. Also the lower rung of the bottom coil is assumed to be 20 cm off the ground plane. The human model with the proximal side of the torso (armpit) at a distance of 20 cm from the coplanar coils is assumed to be placed centrally in the nonuniform magnetic fields of the EAS system.

In order to evaluate the implications of using either of the two procedures suggested in the ICNIRP guidelines, we have considered two different types of currents sketched in Fig. 1 that may be fed in phase into the two coplanar coils. The current waveform in Fig. 1a is a damped sinusoid $I(t) = e^{-100t} \sin(2\pi ft)$ where f = 300 Hz, while that in Fig. 1b is a set of three triangular pulses of width 1.6 ms each. Each of the two assumed currents may be represented either in terms of the Fourier spectra or as an individual pulse to compare the 1 cm^2 area-averaged peak induced current densities for the brain and the spinal cord against the basic restrictions given in the ICNIRP guidelines (1998). The total or the individual "normalized" current density factors T thus obtained using the two suggested procedures are given in Table 1 where

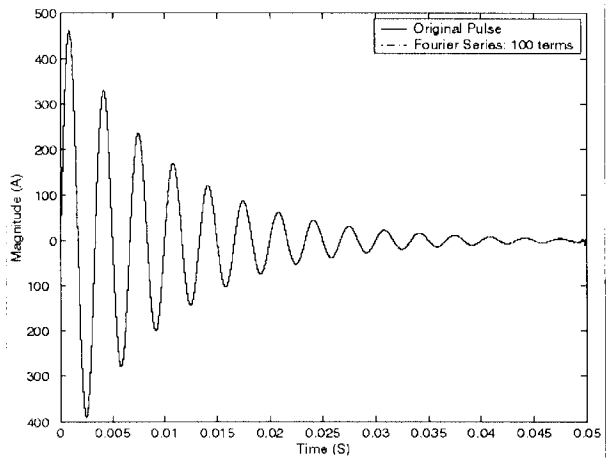
$$T = \sum_{i=1}^{N} \frac{J_i}{J_{L,i}} \quad \textbf{(1)}$$

where J_i is the current density induced for frequency f_i (corresponding to each of the Fourier components) and $J_{L,i}$ is the induced current density restriction for the corresponding frequency f_i. From Table 1, it may be seen that the two methods give considerably different values of T by a factor as large as 1.75 for the two current waveforms considered here. In fact, the three triangular pulse system would pass if method 2 is used but would not comply with the ICNIRP guideline if method 1 based on the multifrequency Fourier analysis is used.

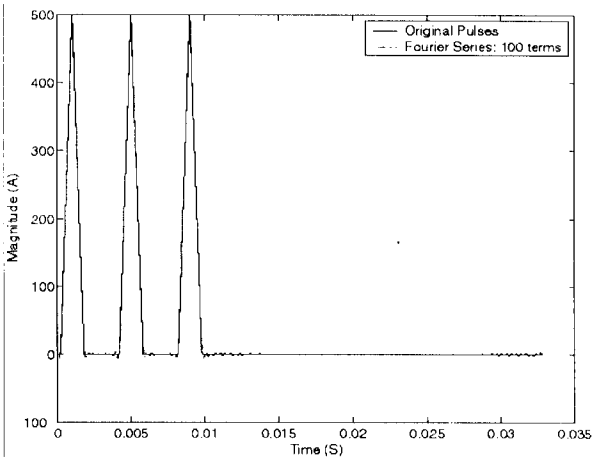
Table 1. The calculated values of the induced 1 cm^2 area-averaged "normalized" 1 cm^2 area-averaged current density factors T (from Eq. 1) for comparison with the ICNIRP limit of 1 for the CNS tissues i.e. the brain and the spinal cord.

No. of Frequency Components N	$T = \sum_{i=1}^{N} \frac{J_i}{J_{L,i}}$			
	For damped sinusoidal current of Fig. 1a		For three triangular pulses of Fig. 1b	
	For Brain	For Spinal Cord	For Brain	For Spinal Cord
50	0.403	0.747	0.462	0.855
100	0.455	0.843	0.533	0.987
150	0.472	0.874	0.559	1.035
200	0.482	0.890	0.569	1.054
200	0.286	0.530	0.325	0.602

- This method calculates the peak induced current density treating the largest pulse as a sinusoid of time period $2t_p$ and compares the same with $\sqrt{2}$ times the basic restriction at $f = \frac{1}{2} t_p$.



a. A damped sinusoid.



b. Three triangular pulses.

Fig. 1. Two assumed input currents for the pass-by EAS system made of two coplanar rectangular coils each of dimensions 50 × 60 cm with a central overlap of 10 cm.

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17-4

SLEEPING DISORDERS AND DEPRESSIVE SYMPTOMS IN WOMEN LIVING NEAR A 735 KV LINE. P. Levallois[1], M. Dumont[2*], R. Boyer[3*], D. Gauvin[1*], S. Gingras[1*]. [1]Unité de recherche en santé publique, Centre Hospitalier Universitaire de Québec, Beauport, (Québec) Canada G1E7G9. [2]Laboratoire de Chronobiologie, Hôpital du Sacré-Coeur, Université de Montréal, Montréal (Québec) Canada H4J1C5. [3]Centre de recherche Fernand-Seguin, Hôpital Louis-H Lafontaine, Montréal (Québec) Canada H1N3V2.

OBJECTIVES: 735 kV powerlines increase residential exposure to electric and magnetic fields (EMF) for people living nearby. Several studies have suspected effect of EMF on depressive symptoms and there is some plausibility that chronic EMF could lead to sleeping disorders. Recently, we found that aged and overweight women living near high power lines had lower sulfatoxymelatonin excretion (Levallois et al 2001). The objective of this study was to compare symptoms of insomnia and depression in women living near and far away from a high powerline.
METHODS: In the Québec city area, 221 living within 200 meters of a 735 kV line and 195 women living at least 400 meters from any power lines were recruited. Participants, aged between 20 and 74, were required to wear an EMDEX Lite meter for 36 hours to measure personal exposure to extremely low frequency magnetic field and residential exposure to extremely low frequency electric field was assessed by spot measurements. Sleeping disorders in the last month were evaluated with the Jenkins questionnaire and depressive symptoms with the Center for Epidemiologic Depression scale (CESD). Information on general health status and lifestyle was gathered by questionnaire.
RESULTS: Magnetic field exposure over 24 hours was three times higher for women living near a power line than women living farther away ($p<0.001$) and residential electric field intensity was nearly twice in those living near the line ($p<0.001$). Sleeping disorders and particularly, <wake up feeling tired> was more frequent in the last month for those living near the line (OR=2.25, 95%CI:1.19-4.28) but no dose-response relationship was found with magnetic or electric intensity. This result was not explained by other variables

taken into account. Proportion of participants with high CESD score (>16) was similar in the two groups (p=0.64) and no modification effect of age or BMI was found.
CONCLUSIONS: No effect on depressive symptoms was found in association with exposure to electric and magnetic fields. A slight excess of frequency of difficult awakenings was found in the group living near the line but without any relationship with the intensity of the fields. Globally this study is reassuring, especially in the perspective of our previous findings of a possible effect of magnetic fields on melatonin secretion.
Reference.
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Hydro-Québec and Electricité de France supported the melatonin study from which these results are derived.

17-5

LEUKEMIA RISK IN CHILDREN LIVING CLOSE TO RAILROADS. J. Schüz[1]*, J.P. Grigat[2]*, K. Brinkmann[2] and J. Michaelis[1]*. [1]Institute for Medical Biometrics, Epidemiology and Informatics, University of Mainz, D-55101 Mainz, Germany [2]Forschungsverbund EMV biologischer Systeme, Technical University of Braunschweg, D-38023 Braunshweig, Germany.

OBJECTIVES: So far, epidemiological studies on childhood cancer and residential exposure to extremely-low frequency electric and magnetic fields (ELF-EMF) focused on power-frequency fields (50/60 Hz). In some countries, including Germany, a different frequency level is used by electric trains (16 2/3 Hz) and, thus, magnetic fields at this frequency were neglected in these studies. High peaks of 16.7 Hz magnetic fields were expected to occur in residences close to railroads. In residences close to electrical installations of the "Bundesbahn" (the German railway system), e.g., high-voltage power lines or substations, there was also a chance of elevated average magnetic fields.
METHODS: 24 hours magnetic field measurements at 16.7 Hz were included in nationwide case-control study on childhood leukemia and residential magnetic fields. In total, we conducted measurements for 489 cases and 1240 controls. We used the FW2a field meter for measurements (Physical Systems Inc. Bradenton, Fl).
RESULTS: Average (median) 16.7 Hz magnetic fields above 0.2 µT were a very rare event and observed in only 0.5% of all residences of controls. The respective prevalence of peak (95th percentile) magnetic fields was 2.6%. While there was a weak, but insignificant association between childhood leukemia and average magnetic fields (Odds Ratio (OR) 1.9, 95%-confidence interval (CI) 0.4-8.9), no association was seen with peak fields (OR 0.7, CI 0.3-1.6). Combining magnetic fields at 16.7 Hz and 50 Hz lead to risk estimates that were similar to those derived for 50 Hz magnetic fields alone.
CONCLUSIONS: Because of the small number of exposed children, our study neither provides noticeable evidence for an association between childhood leukemia and exposure to 16.7 Hz magnetic fields, nor can a small increase in risk be excluded. It is reassuring, however, that after including exposure to 16.7 Hz magnetic fields in our risk analysis, the results for 50 Hz magnetic fields were only marginally altered. Therefore, neglecting magnetic fields produced by railroad traffic in childhood cancer studies on ELF-EMF appears to have only little effect on the overall outcome.
This study was funded by the German Federal Ministry for the Environment, Nuclear Safety and Nature Preservation.

17-6

HEALTH COUNCIL OF THE NETHERLANDS REPORT ON MOBILE PHONES. E. van Rongen. Electromagnetic Fields Committee. Health Council of the Netherlands, 2500 BB The Hague, The Netherlands.

OBJECTIVE: To provide an analysis of any health risks associated with the use of mobile phones.
METHODS: A thorough analysis of the relevant scientific literature was performed and the validity of papers was assessed on the basis of clearly formulated criteria.
RESULTS AND DISCUSSION: The reports starts off with clarifications of some controversial issues. First, the distinction between biological or physiological effects and health effects is clarified. An effect of an EM field on an isolated biological system does not necessarily imply that such a field will also lead to adverse health effects in an organism. The human body has a great capacity for adequately dealing with external influences. Second, it is not correct to assume that clearly discernible and possibly biologically effective low-frequency components are present in the electromagnetic signal of a base station or of a mobile telephone. It is therefore highly unlikely that interference with bioprocesses such as natural brain waves operating at such frequencies will occur.
Following this, a number of health-related issues is dicussed:

- Model studies on temperature changes in the head show that temperature in brain tissue will rise maximally 0.2°C. This does not give reason to revise the existing recommendations on partial body exposure limits.
- Various nonspecific symptoms, such as headache, dizziness and insomnia, have been linked to EM field exposure. However, this type of symptoms is very general in nature and may have all kinds of causes. It is believed that the EM fields generated by mobile telephones cannot be regarded as a cause of such symptoms.
- In a few human volunteer studies, subtle changes have been found in certain cognitive functions, such as memory and reaction speed. Although this suggests an influence from EM fields on brain activity under certain conditions, the effects involved are extremely small and reversible. They are, therefore, not regarded as harmful to health. The available data does not indicate an adverse effect on cognitive abilities, even in people who make frequent use of mobile telephones.
- Several studies have shown that exposure to a GSM signal during sleep exerts effects on natural brain activity. However, the data on this are equivocal and no correlation has been observed with increasing field strength. It is nevertheless striking that, in one of the studies, changes in brain activity during sleep were found following exposure prior to falling asleep. It is felt that, on the basis of these findings, there is no reason to suppose that the effects, in so far as they are real, lead to health problems. Similar effects on brain activity have also been found as a result of caffeine use and natural hormonal fluctuations.
- One of the greatest fears of mobile phone users is, that their habit will increase brain tumour risk. Fortunately, no indications have been found in epidemiological studies for a link between mobile telephone use and the incidence of brain tumours in general. Although in some studies a weak association was observed between telephone use and the occurrence of certain tumours on the side of the head where, as indicated by the subjects, the telephone was normally held, this association is not significant and the studies had a number of important methodological shortcomings. Most animal studies also do not show a relation between EM field exposure and tumour growth or development. In only one study an effect has been found, but the experimental design was such that no satisfactory conclusions can be drawn. Although at present there is no reason to assume any relation between mobile phone use and tumour development, the phones might not have been in use for a sufficiently long period of time to get really adequate data on this long-term effect.
- There is no evidence to suggest that electromagnetic fields from mobile telephones have any effect on the cardiovascular system, the endocrine system and the immune system.
- A number of studies from the 1970s and 1980s seem to indicate that exposure to EM fields affects the permeability of the blood-brain barrier. However, recent research has been unable to reproduce these

effects. Therefore it is concluded that there are no scientific grounds to assume the existence of such effects.

- It is unlikely from a developmental point of view that major changes in brain sensitivity to EM fields still occur after the second year of life. Consequently, there is no difference in sensitivity of children's and adult's brain to EM fields. This gives no grounds to recommend that mobile telephone use by children should be limited.
- The scientific information concerning the above-mentioned non-thermal effects provides no reason to apply the precautionary principle and lower the SAR limits for partial body exposure.
- The use of a handsfree set generally leads to considerable reduction in the amount of energy absorbed by the head. Although it is possible that the cable of an handsfree set acts as antenna and transports energy to the head, this will only under highly uncommon circumstances result in local SARs near the limit values. Exceeding these values will not occur. Sending and receiving SMS messages does not result in significant exposure of any body part, since the phone is held away from the body to look at its screen. The use of protective covers is deemed not useful and promotion of phone-mounted objects that are claimed to absorb EM fields is misleading.
- Since mobile phones may interfere with medical electronic equipment, the immunity of such devices should be increased. Meanwhile, care should be taken not to use a mobile telephone at short distance of vital equipment.
- A prohibition for the non-handsfree use of mobile telephones by drivers of motorized vehicles should be extended to drivers of all vehicles. However, since diversion of attention by carrying on a conversation by the phone probably has a much larger negative influence on road safety, it should be promoted that long or difficult calls only be handeld when the vehicle is put to a stop in a safe place.

Finally, as an alternative way of recommending precautionary actions, many suggestions are being made for further studies, especially for research that might be performed in the Netherlands.
The report 'Mobile telephones; an evaluation of health effects' (publication no. 2002/01E) can be obtained at www.gr.nl.

SESSION 18: MECHANISMS AND MODELING
Chairs: Frank Barnes and Ben Greenebaum

18-1

THE APPLICABILITY OF CURRENT AND PAST BIOLOGICAL RESEARCH ON RADIOFREQUENCY RADIATION TO NEW TECHNOLOGY (3G): PORTABILITY REQUIRES A MECHANISM. M.L. Swicord[1], J.J. Morrissey[1], J.A. Elder[1], A.R. Sheppard[2]. [1]Motorola Florida Research Lab, Plantation, Florida 33322, [2]Asher Sheppard Consulting, Redlands, California, USA.

INTRODUCTION: About 300 biological studies have been initiated using various mobile telephony signals. Even though these studies are comprehensive in addressing current technologies they do not and cannot cover the infinite number of possible frequencies and modulations in use. For example, the majority of these studies utilize generic type GSM signals (e.g., steady pulse amplitude and regular pulse pattern), but by no means cover all GSM modulation characteristics now in use or planned (variable pulse amplitude and irregular pulse patterns). Forthcoming third generation (3G) wireless devices will combine current technologies and the future may bring additional variations. Is it necessary to test all possible combinations of frequency and modulation to determine whether or not each signal type will or will not cause adverse health effects, or is current research portable to new technologies? The answer to this question depends on the mechanism of interaction of the radiofrequency (RF) energy with the biological system.
DISCUSSION: A thermal mechanism depends only on the amount of energy absorbed and thus its frequency dependence is predictable. The amount of energy absorbed will depend on the electrical

properties of the tissue and the geometrical interaction with the biological object, both of which will cause well-established frequency variations. There is no modulation dependence for a thermal mechanism. A non-thermal mechanism, on the other hand, would be expected to exhibit frequency-dependent responses, modulation-dependent responses or both. Several workshops have been held recently on mechanisms. Conclusion of these workshops will be reviewed. One held in May 2001 in Washington DC considered the plausibility of various proposed mechanisms (for a summary see the Research section at www.mmfai.org/). This group concluded in part, "For exposures to RF energy from sources in the general environment and from use of mobile telephone devices, the only clearly plausible mechanisms for RF interactions with biological systems involve heating." This workshop evaluated physical mechanisms of interaction from a theoretical basis only. Subjects considered included: temporal and spatial temperature gradients, alteration of membrane potential, membrane rectification, polarization of structures or molecules, RF pumping and chemical kinetics, magnetic dipole interactions, coherence and cooperative interactions. No mechanism, except for thermal gradients, was considered plausible at environmental exposure levels, but most require further theoretical evaluation to determine their limitations.
In addition to theoretical examination of proposed mechanisms, a second approach to a determination of possible mechanisms is to study a repeatable biological effect and establish the biochemical and biophysical event that causes this response. There are a number of publications that report effects at non-thermal levels, but these reported findings do not build a consistent or connected body of data. Confirmation of non-thermal results must come from either independent replication or from established biological or biochemical connections in which the occurrence of one finding would predict the second. Science builds upon the mechanistic knowledge gained in one experiment by using it to generate the hypotheses for another experiment. In the absence of any plausible biophysical mechanism to explain reported non-thermal "positive" findings and in the absence of any consistent or repeatable results at non-thermal levels one must conclude that the only established mechanism for adverse health effects is an increase in temperature due to absorbed RF energy.
CONCLUSIONS: To determine whether experimental findings are applicable to all current and proposed (3G) technologies, one must understand the basic physical mechanism that is causing any biological response. At the present stage of RF research, only thermal effects and neurostimulation by RF fields and currents have been established. At present, the absence of experimental and theoretical evidence for non-thermal mechanisms related to an adverse health effect leads one to conclude that current research findings are portable to all existing and new technologies.

18-2

A MECHANISM OF INTERACTION BETWEEN ELF ELECTROMAGNETIC FIELD AND DIPOLES PRESENT IN BIOLOGICAL MEMBRANES. R. Schiavo[*2], I. De Sena[*1], M. Liberti[*2], G. D'Inzeo[2] and A. Ramundo-Orlando[*1]. [1]Institute of Neurobiology and Molecular Medicine-CNR, Via Fosso del Cavaliere, 00133-Rome, [2]ICEmB @ University "La Sapienza", Via Eudossiana, 18 00184-Rome, Italy.

OBJECTIVES: In the recent past, authors presented an interaction model between ELF electromagnetic (EM) fields and cellular membrane, based on Larmor precession theory. This model justified, with the action induced on the dipole of stearylamine (SA), the direct involvement of charges of SA on the lipid membrane surface observed in enzyme-loaded unilamellar liposomes (Ramundo-Orlando et al.I and II, 2000).
In the present paper the role played by the different physical parameters involved in the model is analysed, carrying out a further spectral analysis in order to clarify the modalities of EM coupling with any specific biological dipole. In this paper attention has been focussed on the polar head group of phosfatydilcoline (DPPC): $(CH_3)_3$-N^+ (M = 59).
METHODS: A Fourier analysis of Lz (z component of the angular moment of the dipole) exposed to ELF EM fields was carried out; the frequency spectrum of instantaneous power and energy transferred from the

EM field to the dipole was estimated as well. Results had lead to a better understanding of the mechanism of interaction.

Figure 1 shows the L_z spectrum for a signal frequency of 7 Hz, a static magnetic field (B_{DC}) of 50 μT and a mass number (M) of 59, which correspond to a Larmor frequency of 6.5Hz. Analysing the L_z spectrum it is possible to say that B_{DC} and M impose a component corresponding to the Larmor oscillation frequency f_L. The superimposed AC magnetic field at frequency f_i produces harmonics at frequency nf_i. interestingly besides these harmonics, we can detect the cross-modulation products $f_L \pm nf_i$.

For a better representation and analysis of this excitation phenomenon, we realised a so-called "mode-map" (Figure 2). In this map (specific for each Larmor frequency characteristic of the analysed system), we have in the x-axis the impressed signal frequency, and in the y-axis the frequency values of harmonics that should appear in L_z spectrum.

Moving to power spectrum it is interesting to notice that all the cross-modulation components seem to decrease of intensity and impressed component seems to become the dominant one (see Figure 3).

DISCUSSION: Our mechanism studied the transfer of power from an EM field to a biological dipole. We chose to study the interaction of the EM field by means of a Fourier analysis of forces (angular moment) exerted on the dipole and power transferred. By this analysis it is possible to evaluate the characteristics of the EM interaction with biological dipoles evaluating the EM energy coupling and evidencing the role played by the single physical variables involved in the model.

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Ramundo-Orlando, F. Mattia, A. Palombo, and G. D'Inzeo, "Effect of low-frequency, low-amplitude magnetic fields on the permeability of cationic liposomes entrapping carbonic anhydrase. II. No evidence for surface enzyme involvement", Bioelectromagnetics, 21:2000

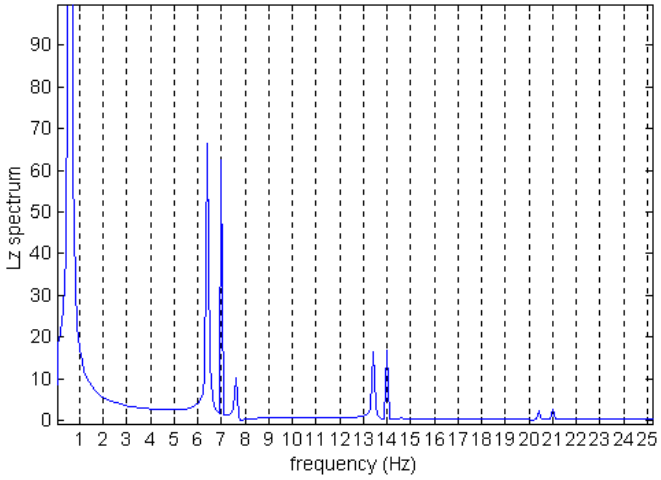

Figure 1: Lz spectrum for f_L=6.5 Hz and f_i=7 Hz; we can detect the Larmor oscillation frequency, the impressed frequency and its harmonics (14,21 Hz) and the following cross-modulation products: f_i- f_L at 0.5 Hz, $2f_i$- f_L at 7.5 Hz, $3f_i$- f_L at 14.5 Hz, f_i+f_L at 13.5 Hz, $2f_i$+ f_L at 20.5 Hz.

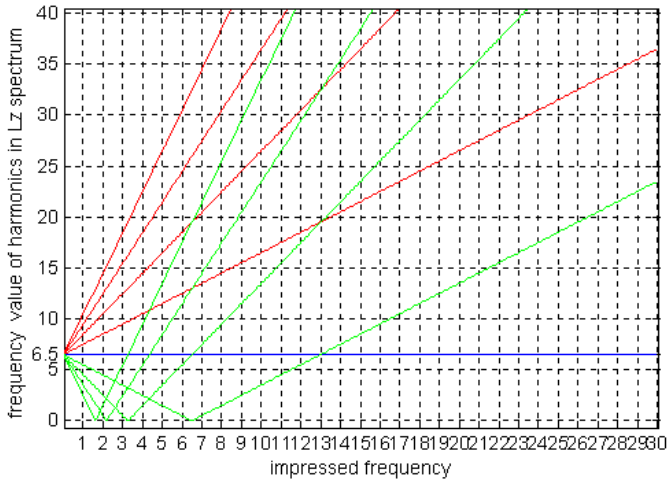

Figure 2: Mode-map for Larmor oscillation frequency f_L=6.5 Hz.

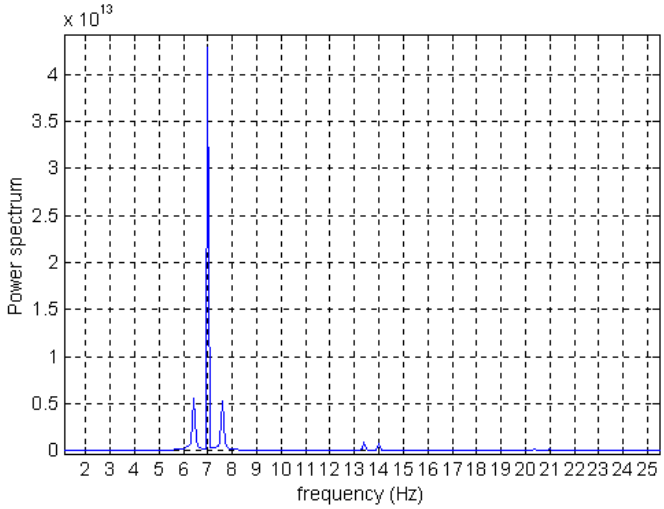

Figure 3: Power spectrum transferred from magnetic field to dipole for f_L=6.5 Hz and f_i=7 Hz; the harmonic at the impressed frequency is the dominant one.

18-3

ENZYMATIC ACTIVITY OF IMMOBILIZED CATALASE ON ELECTRODE UNDER MAGNETIC FIELDS. M. Iwasaka[1], M. Yaoita[2], S. Ueno[1]. [1]Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan. [2]Department of Bioscience, Tokyo University of Science and Technology, Yamanashi 409-0913, Japan.

INTRODUCTION AND OBJECTIVE: The effects of magnetic fields on the enzymatic activity of catalase have been discussed for over three decades. Catalase is a magnetically sensitive biological molecule, presumably due to its paramagnetic characteristics. Recently, an improved biochemical measurement technique under real-time conditions in a magnet produced absolute negative results. In our previous study, we also carried out real-time optical measurements of the catalytic decomposition of hydrogen peroxide by catalase under static magnetic fields of up to 8 T, which resulted in both positive effect and no effect of magnetic fields depending on the hydrogen peroxide concentrations. In the present study, a catalase-immobilized platinum black electrode was used to measure the catalytic decomposition of hydrogen peroxide by catalase.

METHODS: In order to observe the enzymatic reaction of catalase electrochemically, a hydrogen peroxide-sensor was developed, which immobilized catalase molecules on the porous surface of a platinum black electrode. The platinum black of electrode catalyzes decomposition of hydrogen peroxide (H_2O_2) on the surface of the electrode. The decomposition of a H_2O_2 generates O_2, $2H^+$, and two electrons (e^-). The current of the working electrode, which was caused by the electrons, is corresponding to the decomposition of H_2O_2. The catalase immobilization on platinum black of electrode decreases the amount of H_2O_2, which reaches the platinum black, because the H_2O_2 is decomposed by the catalase as well as by the platinum black. In the catalase immobilized electrode, a decrease and an increase of the catalase activity results in an increase and a decrease of the electrode's current, respectively. We used a horizontal type of superconducting magnet, which produced magnetic fields of up to 8T at its center.
RESULTS AND DISCUSSION: The electrochemical system was inserted into the magnet's bore after the electrode's current was stabilized, and a spontaneous increase in the current was observed. The increase in the current by the magnetic field exposure was reproducible when the electrodes went back and forth between 0 T and 8 T fields. Subsequently, as the superconducting currents of the 8 T superconducting magnet were swept between 8 T and 0 T, the sensor's current changed in correspondence with the varying magnetic fields. The catalase sensor's current was monitored continuously by a potentiostat. During the magnetic field exposures, the sensor's current increased nearly 200%. The consumption of H_2O_2 by catalase resulted in a decrease in the working electrode's current. The clear increase in the sensor's current indicated that the catalytic activity of catalase decreased under magnetic field exposure of up to 8 T. Furthermore, to confirm the effect of magnetic field exposure, immobilized catalase molecules were heated and inactivated with boiling water, and the electrode with inactivated catalase was exposed to magnetic fields of up to 8 T in the electrochemical system. The heat-inactivated catalase electrode showed no change during magnetic field exposure and the active-catalase immobilized electrode showed a current increase during magnetic field exposure. The probable reason for the observed changes in the sensor's current under magnetic fields is related to the immobilized catalase on the electrode. The observed changes in the sensor's current under magnetic fields was probably attributed to conformational or catalytic changes in the immobilized catalase on the electrode during catalytic decomposition of hydrogen peroxide.

18-4

THERMODYNAMIC THEORY AND EXPERIMENTAL METHODS FOR DETECTION IN VITRO OF NONLINEAR INTERACTIONS OF RF ENERGY WITH BIOLOGICAL CELLS. Q. Balzano[1], A. Sheppard[2]. [1]Consulting Scientist, Motorola Florida Research Labs, 8000 W. Sunrise Blvd., Fort Lauderdale, Florida 33322, USA. [2]Asher Sheppard Consulting, Redlands, California, 92373 USA.

INTRODUCTION: The reported significance of low frequency modulation at non-heating exposure levels cannot be understood in terms of the bulk properties of an aqueous dielectric system. The observations could be the result of nonlinear interactions between the RF field and a small fraction of biomolecules [Illinger 1982 Bioelectromagnetics 3:9-16], although direct physical observations have not found nonlinear interactions involving biological matter above about 10 MHz. Because of their highly polar nature, water molecules in a RF field undergo rotational motions that subsequently transfer energy to the thermodynamic system through collisions. RF energy absorption is represented thermodynamically by an increase in the entropy S of the bosons (photons and vibrational modes) that ends (dS=0) when the incident radiation and the molecular oscillators are in thermodynamic equilibrium. For a linear system, the spectrum scattered from a RF-exposed biosystem has no spectral features other than those of the incident signal.
THEORY: We considered a system of biological cells in thermodynamic equilibrium at temperature T that are exposed to a flux dM/dt of RF photons at angular frequency ? in a cavity. The system, which has vibrational energy at frequencies $?_{k,}$ and a chemical potential energy μ, has this number of interacting photons and vibrational states:

$$n(h?_k,T,\mu) = [(1 - a)\cdot f_k^{-1}\cdot h?\cdot dM/dt+1]\cdot[exp\ss(h?_k - \mu) -1]^{-1}, \quad (1)$$

where a is the fraction of dissipated energy (a = 0 for totally elastic photon interactions), and f_k is the transition probability for oscillators with energy $h? = h(?_k - ?_{k-1})$. Also, $\ss = (kT)^{-1}$, k, the Boltzmann constant; h, the Planck constant; $h = h\cdot(2p)^{-1}$. Eq. (1) states that at equilibrium all energy entering the cavity is either dissipated or reradiated by the oscillators of the system. The case dM/dt = 0 yields the usual Planck formula. If totally transparent to RF, $f_k = 0$ and the first term diverges as a consequence of ignoring cavity losses. For a square-law nonlinearity, the equilibrium condition
$(\partial [n(h?_k,T,\mu) + n(h?_{2k},T,\mu)] / \partial t = 0)$ leads to this expression

$$(1-?)\, dM/dt = ?_k\{n(h?_k,T,\mu)\cdot[exp\ss(h?_k - \mu)-1]-1\} + ?_{2k}\{n(h?_{2k},T,\mu)\cdot[exp\ss(h?_{2k} - \mu)-1]-1\}, \quad (2)$$

that has linear and quadratic terms with photons at frequencies $?_k$, $?_{2k}$, respectively, where ? represent the energy lost in the linear and the nonlinear interaction, and $?_k$ and $?_{2k}$ are transition probability rates. Additional frequencies at 2O, $?_k \pm O$, and $2?_k \pm O$ appear when the incident RF signal is amplitude modulated at O ($O << ?_k$). Linear interactions of the RF field with vibrational modes introduce lines at $? \pm nO$, n = 1, 3… whereas lines resulting from non-linearities would appear at $m(? \pm 2nO)$, m = 2, 3….
EXPERIMENTAL METHOD: Balzano [Bioelectromagnetics 2002 in press] previously proposed direct spectroscopic detection of lines at n? and $n(? \pm O)$, n = 2, 3, …, but a different technique is needed to detect energy at frequencies $? \pm 2nO$, that is, lines lying very close to the incident frequency ? . We propose a cavity operating in the TM_{010} and TM_{011} modes in order to provide nearly constant magnetic excitation of an annular exposure volume of as much as 90 cm^3 containing the biological sample that is excited with a 100% amplitude-modulated carrier. Reradiated energy is picked up by two loop antennas located to provide signals 180º out of phase in order to reduce noise in the side bands originating within the sample. A modulation analyzer and digital signal processing permit detection of the harmonic at $? \pm 2nO$ to a limit estimated as –120 dB below the incident amplitude modulated signal.
CONCLUSIONS: A thermodynamics-based analysis shows that if there are nonlinear interactions involving vibrational energy, previous experimental techniques designed to detect harmonics of the carrier need to be extended to permit detection of energies of $mh(? \pm 2nO)$. A cavity operating in the TM_{010} and TM_{011} modes, digital filtering and low-noise amplification provide a practical way to observe such energies in biological cells down to very low levels.

18-5

AN ANALYTICAL STUDY ON THE SENSITIVITY AND FREQUENCY RESPONSE OF VOLTAGE-GATED ION CHANNELS IN CELL MEMBRANES TO ELECTRO-MAGNETIC FIELDS. F. Apollonio[1*], M. Liberti[1*], G. D'Inzeo[1]. [1]Department of Electronic Engineering, ICEMB at "La Sapienza" University of Rome, 00184 Rome, Italy.

OBJECTIVES: In order to better understand the biological effects of low level electromagnetic fields (around and below the values fixed in the current safety guidelines), it is now a common approach to perform biophysical models of the interaction mechanisms at cellular level: in particular ionic channels, regulating the current fluxes through cell membrane, are among the basic biological units to be modelled (Apollonio, Liberti, D'Inzeo, Tarricone 2000). In this work the attention has been focussed on voltage-gated ion channels, whose random fluctuations among different conformational states depend on transmembrane voltage; the channels are described by means of stochastic models. An analysis on the sensitivity of the model to membrane variations as well as a study on the model frequency response have been performed.
METHODS: In general the conductance of a voltage-gated ion channel can be written as:

$$g_i(V_m,t) = g_i m^M h^H \qquad (1)$$

where γ_i denotes the open conductance of the channel, m and h denote the activation and inactivation variables of the channel and M and H are the number of activating and inactivating gates (subunits), respectively. In standard models of channels kinetics, derived from the classical Hodgkin-Huxley model, m and h are assumed to be continuous deterministic variables. In stochastic models, voltage-gated channels are modelled as discrete-state Markov chains with transition rates between the different conformational states dependent on transmembrane voltage (Hille 1992). In particular the channel is modelled by a set of N states and a transition rate matrix Q, whose elements are the transition rates regulating the kinetic of the process. Each state in the model represents a possible conformation for the channel (open or close), and transitions among states represent structural modifications, associated to energetical changes. The Markov model corresponding to the kinetics of potassium channel is reported in Figure 1. The channel is modelled by means of five different states, of which only one is considered open (state 5); each state is represented by four equal subunits whose behaviour is regulated by the transition rates α and β.

RESULTS: A simple exogenous electromagnetic (EM) signal (for example a sinusoidal waveform) acting on the channel, can be characterised by two main parameters: amplitude and frequency. In terms of amplitude, the EM signal being responsible for a variation of the transmembrane voltage (V), acts on the channel model as a variation of the transition rates (Apollonio, Liberti, D'Inzeo, Tarricone 2000). Therefore it seems interesting to investigate which is the minimum transmembrane variation detectable by the model. Table I has been obtained referring to ergodic properties of the model averaging for three seconds and 250.000 realisations of the automaton representing the potassium channel of Figure 1. From the table it can be seen that variation in transmembrane voltage lower than 20 μV give rise to values of open probability which fall in the uncertainty of the open probability calculated for the physiological condition. In terms of frequency response, the channel model is able to detect low frequency components (below 1 kHz) in the spectrum of the incoming EM signal. Moreover considering an external EM signal consisting in the sum of two sinusoidal signals (30 and 40 Hz frequency, for example) the channel is able to detect the two different contributions, giving rise to a frequency resolution of 10 Hz, in its frequency response (see Figure 2).

DISCUSSION: The reported results give specific indications about channel sensitivity on membrane voltage and frequency response in terms of ability of the channel to detect low frequency components and frequency resolution. Further studies will be able to identify the optimum simulation conditions (observation time, number of realisations, as well as observation domain i.e. time or frequency) in order to set the channel sensitivity and the frequency response.

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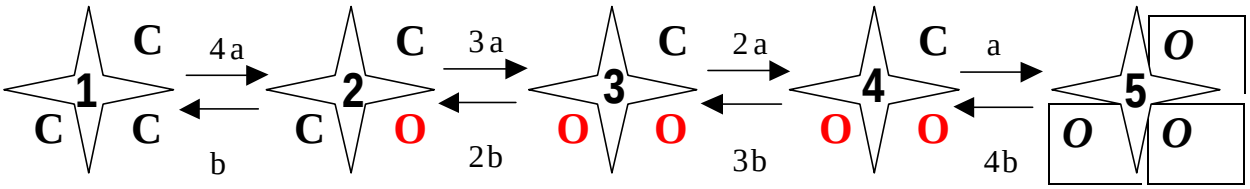

Figure 1. Markov model relative to potassium channel. The transition rates α and β represent respectively the probability for the single subunit to change its configuration from closed (C in the figure) to open (O) and viceversa. The channel is open (State 5) when all the four sub-units are in the O conformation.

	Phys.	1 μV	10 μV	20 μV	50 μV
Open prob.	min=0.4188 max=0.4196	0.4192	0.4191	0.4188	0.4186

Table I. Comparison of the mean open probability for 250000 realisations of three seconds for the potassium channel in physiological and in exposure conditions.

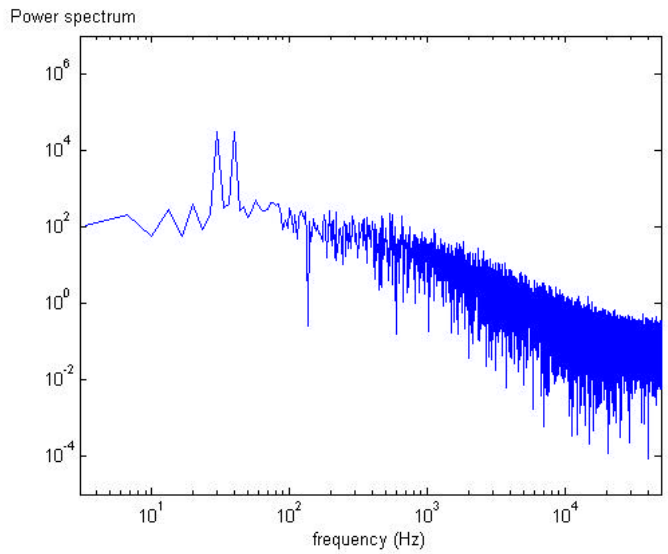

Figure 2. Power spectrum of potassium channel exposed to an EM signal with two sinusoidal components of 30 and 40 Hz.

18-6

A THEORETICAL STUDY OF THE EFFECTS OF INHOMOGENIOUS RF FIELDS IN THE VICINITY OF MEMBRANES. F. Barnes, Y. Kwon. Department of Electrical and Computer Engineering, Campus Box 425, University of Colorado Boulder, Colorado 80309-0425 USA.

OBJECTIVE: To explore possible theoretical models for the effects of highly inhomogeneous radio frequency fields on biological systems.
BACKGROUND: One of the open questions in the study of possible health effects of the electromagnetic fields from cell phones is "Are there theoretical reasons to believe that the fields from cell phones can effect biological system by mechanisms other than heating?" There are experimental results that are not as yet explained as effects of heating [DePomeraiD:radiation] in spite of the fact that some experimental result that were first thought to be non thermal have now be shown to depend on the rate of temperature rise [BarnesF:tempRate]. Additionaly experimental measurements have failed to show rectification by way of a shift in the voltage across a cell membrane upon exposure to RF fields at frequencies above about 10MHz. [PickardW:RadioEffects] The later is thought to be the result of limitations of the distance an ion can be moved in half a cycle at high frequencies. At this time mechanism other than heating have not been confirmed as a source of changes in biological systems upon the application of R.F.
METHOD: A theoretical model has been constructed of the various forces that affect the motion of both charged and uncharged molecules in the vicinity of the surface of a membrane. These forces include Van der Waals forces, Coulomb forces, solvent effects, diffusion or osmotic forces and dielectorphoric forces. Numerical methods are used to calculate the effect of these forces.
RESULTS: The calculations show that under the assumption that we have made that the fields in the low dielectric materials such as the membranes are approximately 30 times higher than the electric fields in high dielectric fluids such as the intra-cellular and inter cellular fluids. As the separation of membranes are often only a few nanometers and large protein molecules are know to project well beyond the average position of the lipid membrane surface the fields are expect to vary rapidly in space when examined on the scale of nanometers. Under these conditions we estimate the forces that are most likely to be effected by the

application of RF fields at cell phone frequencies of 900MHz and 2 GHz are the dielectrophoric forces and that these forces could lead to changes in the rate at which both charged and uncharged molecules move to the surface of the membrane. See Figures 1-2. The currents generated by these forces will be compared to those are expected from the background biological fields and diffusion.

DISCUSSION: It is expected that the currents of either ions or molecules such as neural transmitters that are generated by the applied RF fields will have to be on same order as the natural currents if they are to be biologically significant. For ion currents these currents would be expected to be on the order 0.01 ampers per meter squared and for molecules such as neural transmitters they could be much smaller. It is known that significant amplification may occur in the vicinity of cell membranes and thus relatively small signal can lead to major changes in the cell activity. Amount the biological studies that this theoretical work suggests are of interest are those looking for changes in growth rates and signaling such as might effect stress reactions and the immune system.

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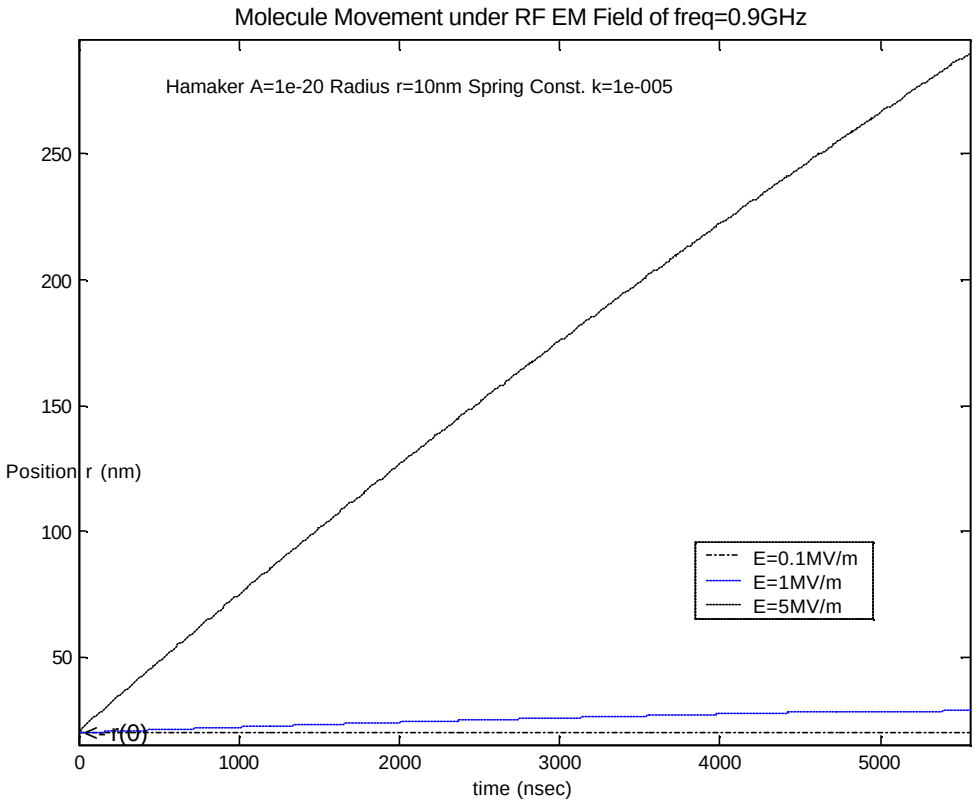
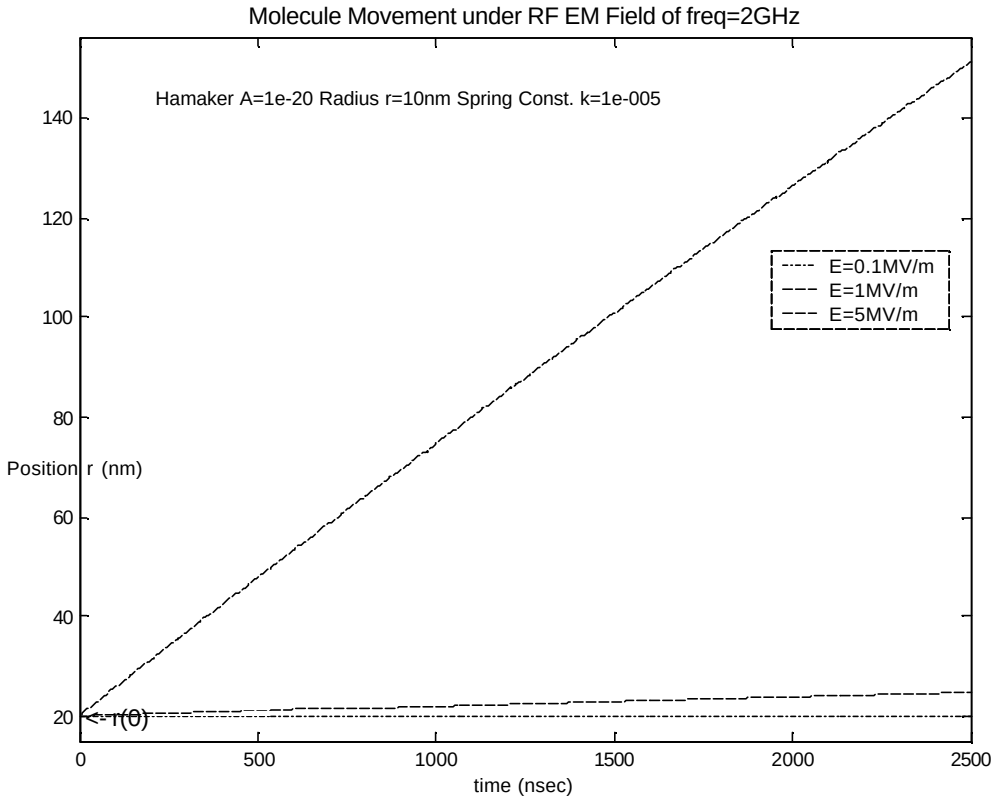




POSTERS

INFORMATICS: ANALYSIS DATABASES – ELF/RF

P-1

BIOELECTROMAGNETICS: **JOURNAL UPDATE.** B. Greenebaum. Dept. of Physics, University of Wisconsin-Parkside, Kenosha, Wisconsin 53141-2000, USA.

Comparative statistics for 2001 and prior years concerning manuscripts received and accepted, geographical and subject matter distribution, and review times will be presented at the meeting. Preliminary indications are that 114 manuscripts were received in 2001, just slightly below the 120 received in 2000. Preliminary figures show that submissions from outside North America (mostly Europe and Japan) appear to continue to be about the same proportion of the total (~25% vs. ~30% in 2000). ELF papers (~45% vs. ~42% in 2000) continue to lead RF papers slightly (~36% vs. ~32% in 2000), with the remainder of the submissions concerning relatively low frequency pulsed fields and other subjects. The Society purchased extra pages in issues published at the end of 2001, as well as at the beginning of 2002, in order to shorten the time accepted papers were waiting at the publisher. This had the desired effect, but by the meeting abstract deadline, reliable numbers for the decrease in the backlog at the publisher or for changes in the typical length of the review cycle were not available. These will be presented at the meeting.
Beginning in February, Dr. Rene de Seze replaced Dr. Jukka Juutilainen as one of the Associate Editors. The Society's Board of Directors expressed its great appreciation for Dr. Juutilainen's years of service, and

the editor regrets that the press of other duties led him to resign. Similar appreciation went to Drs. Mays Swicord, Sheila Galt and Jan Wallezcek, who ended service on the Editorial Board.
The editors again thank the many members who acted as reviewers for their work, as well as the authors for their submissions.

P-2

REVIEW OF PROMISING CLINICAL RESEARCH ON THE QUANTRON RESONANCE SYSTEM - AN ELF PEMF FIELD. W. Pawluk. Advanced Magnetic Research Institute of the Delaware Valley and School of Medicine, Johns Hopkins University, Rancocas, New Jersey 08073, USA.

The QRS has been in use for about 20 years in Europe. Research results are available to demonstrate biological and clinical human benefits. Various studies provide evidence of benefits or actions in ion migration, vasodilatation, osteoporosis and osteoarthritis. Research in the USA is also being carried out to evaluate use in the plastic surgery setting for wound healing.
The QRS device induces a complex EMF waveform via large body size coils within a thin mattress or pillow pads. The coils are driven by an external generator with 10 strength settings. Peak magnetic field at the coil is approximately 400 µT within a frequency range of 1-100 kHz. The primary sawtooth waveform is a burst of 3 pulses, each having a 10µsec rise time and an exponential-like fall of about 250 µsec duration. Pulses repeat every 5 msec within the burst. The pulse burst is repeated every 30 msec in packets of three and the whole packet train is repeated every 300 msec. The polarity of the pulses is reversed every 10 seconds.
The coils in the QRS device are large enough to allow maximum induced electric fields in the mV/cm range within a large tissue volume, comparable to typical bone growth stimulators. The signal is very broadband because both pulses and pulse bursts are repetitive. The primary stimulus of the QRS signal is most likely to be an induced electric field since it is well within range to be detectable by cell and/or tissue targets above background thermal and other voltage noise.
The University of Munich evaluated a case series of 74 subjects. 37% had degenerative arthropathy, 34% vertebral degenerative problems, 16% pain syndrome and 11% respectively each with inflammatory joint disorders and sleep disturbance. 71% classified their conditions as severe or very severe. 68% self-reported good/very good results. After a one year follow-up 85% claimed a benefit in pain reduction. Medication consumption decreased from 39% at 8 weeks to 88% after 8 weeks.
A controlled study was conducted on high performance fitness for exercise (the Deutscher Solar and Fitness Verband). 34 active and 41 control subjects were tested. Measurements were made prior to exercise, then prior to QRS, after QRS and after a second fitness exercise. Body Balance (BBM), lactate, blood gasses and vital signs were measured. BBM and lactate were significantly better in the QRS group ($P<0.001$). BBM was no different after training in both groups but was positive after QRS exposure. Lactate levels allowed a longer time to anerobic threshold in the QRS group. O2 and pO2 levels and pulse rates did not show significant differences. Results indicate enhanced endurance for exercise.
Four subjects had their retinal vascular flow studied using the Heidelberg Retina Flowmeter pre and post 8 minutes of QRS. Pearson correlation of 0.75 was found for field strength between QRS intensity setting 2 (low) and 8 (high) in flow rate. Flow rates increased by 10% and 39% respectively, pre and post exposure.
A study was carried out in Heidelberg to determine change in ion charge in 40 treated and 40 control turkeys after a daily 2 hr whole body exposure. Results showed an increase in sodium concentration of 222 mg/dl (5.8%) and calcium of 12 mg/dl (9.8%) and a negative change in potassium of 31 mg/dl (-224.6%).
A controlled orthopedic clinic study was conducted in Germany on patients with objective documentation of osteoporosis. After 18 months of treatment using the QRS twice a day, there was a demonstrated statistically significant improvement in bone density measured by DEXA scanning.
In a randomized double blind knee osteoarthritis (KOA) study involving 36 placebo/35 active patients at Maribor Hospital, Slovenia the QRS was used daily for 16 minutes for 6 weeks. 28% were male. Average

age was 60 +/- 10 years. 35% were clearly overweight. 83% had both knees affected. Evaluations were at 0,4,6,10 weeks. Measures included Knee Society Score, pain measures, CRP, P-fibrinogen and an inflammation parameter. KSS improved only in the active group ($P<0.01$) vs placebo group ($P<0.05$). Knee function ($P<0.01$) and walking ability ($P<0.05$) improved significantly. Pain, GC and well being improved more in the active group ($P<0.01$). Medication use was better. After 6 weeks P-fibrinogen, C-reactive protein and blood sedimentation rate decreased significantly. Systolic blood pressure improved significantly after 6 wks and remained decreased for a further 4 weeks without QRS exposure.

P-3

THE MMF BIOELECTROMAGNETICS RESEARCH PROGRAM. M.L. Swicord[1], T. Persson[2*], S. Lang[3], M. Milligan[4*]. [1]Motorola Corporate Research Laboratory, Ft. Lauderdale, Florida 33322, USA. [2]Ericson Research, Ericsson Radio Systems AB, SE-164 80 Stockholm, Sweden. [3]Nokia Research Center, FIN-00180 Helsinki, Finland. [4]Mobile Manufacturers Forum, B-1030 Brussels, Belgium.

The MMF (Mobile Manufacturers Forum) is an international association of radio equipment manufacturers whose members include Alcatel, Ericsson, Mitsubishi Electric, Motorola, Nokia, Panasonic, Philips, Sagem, Siemens and Sony. The MMF was formed in 1998 to jointly fund key research projects, as well as to cooperate on standards, regulatory issues and communications activities concerning health and mobile phones. The research program of the MMF is adopted from the WHO Research Agenda that recommends areas in which further studies are needed or would be useful. In supporting research, the MMF seeks to sponsor projects jointly with national and international health and scientific research bodies. The MMF also encourages all research findings to be published in peer-reviewed scientific journals to ensure openness and transparency in our research programs. The following constitutes the main parts of the current MMF research program.

PROGRAM 1 - PERFORM A: This program includes work in 6 European countries and is led by the Fraunhofer Institute in Hannover, Germany. The work, partly funded by the MMF, the GSMA (the association for GSM operators) and the European Commission under the 5th Framework program, includes:

Perform-Al Two combined toxicity/carcinogenicity studies of 900 MHz and 1800 MHz GSM signals in B6C3F1 mice at three dose levels.

Perform-A2 Two combined toxicity/carcinogenicity studies of 900 MHz and 1800 MHz GSM signals in WISTAR rats at three dose levels.

Perform-A3 Evaluation of 900 MHz GSM wireless communication signals on DMBA-induced mammary tumors in Sprague Dawley rats

Perform-A4 Evaluation of 900 MHz GSM signals on Lymphoma induction in *PIM 1* transgenic mice.

PROGRAM 2 - PERFORM B: The Perform-B program involves both *in vivo* and *in vitro* replication studies.. This work, partly funded by the MMF the GSMA and various governments, includes:

Perform-B1 Activity of the enzyme ODC in cell cultures following RF exposure. This study is a replication of work by the Litovitz group suggesting a temporary increase in ODC activity in L929 fibroblasts after exposure to NAD phone signal

Perform-B2 Genotoxicity studies following RF exposure. This study is a replication of work undertaken by the Maes group suggesting increased sister chromatid exchange in human lymphocytes.

Perform-B3 Spatial working memory in rodents. This study is a replication and extension of Lai et al research suggesting decreased maze performance ability in rodents after RF exposure.

PROGRAM 3 - INTERPHONE : International case-controlled study of cancer in relation to mobile telephone use. This study is a major international epidemiological study of head and neck tumors. One of the largest studies of its kind, the study involves 13 countries including 7 from the EU. The MMF is funding part of the study along with the GSMA, the European Commission's 5th Framework program and various other government sources.

PROGRAM 4 - Human studies

The MMF has agreed to support a research program to be conducted at the Karolinska Institute in Sweden. The study programs to be undertaken includes: Effects on sleep and EEG, Effects of RF exposure during sleep, Skin hypersensitivity response, Reaction time, Headaches and Blood pressure.

PROGRAM 5 - DMBA Replication

This program is a replication study paralleling Perform–A3 and is being conducted by the Zhejiang University Medical School, Hangshou, China.

PROGRAM 6 - UK Research Program

The UK Government has announced the establishment of an EMF research program and the MMF has committed to contributing towards the sponsorship of the program.

PROGRAM 7 - Mechanism studies

This program involves a number of individual theoretical studies examining the plausibility of different mechanisms for RF interaction with the human body.

MMF's dosimetry measurement research program

In addition to the above mentioned programs, the MMF is sponsoring a number of dosimetry and measurement related research programs details of which will be presented at the conference. Additional information is available on the MMF web site at: http://www.mmfai.org

P-4

COMMON CHARACTERISTICS OF HIGHLY SIGNIFICANT EXPERIMENTAL RESULTS IN ELECTROMAGNETIC FIELD BIOEFFECTS RESEARCH. R.B. Goldberg, W.A. Creasey, M.N. Collier, B.H. Kleinstein. Information Ventures, Inc., 42 South 15th Street, Suite 700, Philadelphia, Pennsylvania 19102-2299, USA.

INTRODUCTION: Concern about potential adverse biological and health effects of new electromagnetic technology (for example, wireless communications signals) motivates funding of ongoing exploratory studies. The purpose of such research is to find whether biological effects exist, and where no effects are found, to have reasonable confidence that none exist. In planning exploratory studies, the existing body of completed work should be evaluated critically and comprehensively to identify experimental models and conditions most likely to yield evidence of biological effects.

OBJECTIVE: To determine characteristics of experimental systems that have yielded positive findings (significant reported effects). Our approach is based on the hypothesis that biological responsiveness to electromagnetic fields is highly dependent on the state of the target organism, and therefore that studies reporting strong effects collectively describe a limited set of experimental conditions required for a biological response.

METHODS: Information Ventures' *EMF Database*, covering electromagnetic field bioeffects research literature published since 1975, was used to identify relevant studies. A unique feature of the *EMF Database* is the inclusion of an original abstract for each record that provides a detailed account of the research, including experimental details and numerical results with statistical data when provided in an author's text. The *EMF Database* was searched for p values in the abstract record field in a numerical range of 0.001 or less to identify those studies reporting numerical results of high statistical significance. In the context of this evaluation, p values were not necessarily taken as a definitive indicator of a study's value, but rather as a way of easily identifying reports claiming robust bioeffects. This subset of records was further searched and analyzed to identify common characteristics of studies with significant results as an indication of possible trends in experimental design and effective electromagnetic field conditions.

RESULTS: Approximately 1,000 records (of about 30,500 *EMF Database* records) include p values of 0.001 or less. After eliminating studies where strong correlations represent only methodological details (e.g., positive controls, correlations between surrogate measures such as wire code and magnetic field levels), the remaining studies show some interesting common characteristics. Studies using DC electric fields and low frequency pulsed magnetic fields to promote bone and soft tissue healing emerge strongly

from this type of p value analysis with approximately 130 records. Many other research studies reporting highly significant results share a characteristic with bone healing papers in that they are studies in which whole animals, plants, or complex in vitro systems (e.g., mixed cells from organ explants rather than established cell lines) are exposed to electromagnetic fields. Behavioral endpoints and their biochemical correlates represent a substantial portion of these positive results. Other strongly positive whole animal (or human subject) studies involve complex biological endpoints such as changes in immune or hematopoietic system characteristics, heart rate variability, developmental abnormalities, and disease correlates with geomagnetic field fluctuations. Reports of successful treatments or animal studies with potential clinical applications also appear in substantial numbers. Based on key-word searching, about three quarters of the studies reporting highly significant p values are based on whole animal rather than cell responses (whereas in the *EMF Database* as a whole about two thirds of the records are concerned with cell-level effects). Conclusion: As a tentative working hypothesis, we believe the literature suggests that complex whole animal or organ systems, while often more expensive and difficult to use experimentally, may be more responsive to electromagnetic fields than isolated cells. This is in accord with a number of theoretical mechanistic studies that stress the potential role of cell aggregates as "antennas" that intensify the response to weak signals. If this is true, the "reductionist" approach that dominates current biological research may be suboptimal for finding electromagnetic field bioeffects. We are currently in the process of using the *EMF Database* to analyze threads of experimental work in order to determine which research areas with highly significant results have been replicated by more than one research group, which studies involve effects produced by one predominant type of electromagnetic field condition (in regard to frequency, intensity, and waveform) as opposed to those in which effects are reported over a range of field characteristics, and common characteristics of the minority of cell-level studies reporting highly significant results.

INSTRUMENTATION AND METHODOLOGY – ELF

P-5

Abstract withdrawn by Author.

P-6

RADIAL TRANSMISSION WAVEGUIDE FOR SIMULTANEOUS EXPOSURE OF 24 RATS AT 900 MHZ. L. Puranen*, A.-P. Sigvonen*, A. Turunen*, T. Toivo*, K. Jokela. STUK Radiation and Nuclear Safety Authority, P.O. BOX 14, 00881 Helsinki, Finland.

OBJECTIVE: Female Wistar rats are exposed to GSM-phone radiation at 900 MHz in a research project where combined effects of low-level electromagnetic fields and drinking water mutagen are studied (CEMFEC). Three groups, each consisting of 72 rats, are exposed at whole-body-averaged Specific Absorption Rate (SAR) (time-averaged over the pulse period) of 0 W/kg (sham-exposure), 0.23 W/kg and 0.9 W/kg, two hours a day, five days per week for a life time or two years. Rats live in the exposure chambers during the week from Monday morning to Friday afternoon and are moved at weekends to their normal cages. Therefore, exposure chambers were designed so that rats can freely move in cages and food and drinking water is provided for the rats.
METHODS: Due to the limited space and large number of rats (216) to be exposed, a radial transmission waveguide (RTW) was chosen for an exposure chamber. RTWs have been successfully used in other biological studies [1]. The designed RTW consists (Fig. 1) of two horizontal circular aluminium plates (3 mm thick) with a diameter of 150 cm and a separation distance of 15 cm. The plates are short-circuited at

the edges and, to reduce the reflections, absorbing material was attached to the inner surface of the short-circuiting wall. The source of the electromagnetic field is a tuneable monopole antenna attached to the centre of the bottom plate. The electric field is vertical and the magnetic field is azimuthal. The upper plate is perforated near the centre and above rat cages to enable ventilation. Twenty-four truncated wedge shaped cages (length of 350 mm and width of 85 mm and 170 mm) made of durable plastic material can be placed in the RTW. Plastic pins attached to the bottom plate help in positioning the cages. Only one hatch, size of two cages, was made to the upper plate. A special mechanism consisting of an aluminium rim around the outer sidewall of the chamber was designed in order to raise the upper plate. In raised position the hatch can be turned above the cages. In the lower (exposure) position a flexible RF shielding seal attached to the lower edge of the sidewall provides a galvanic contact to the lower plate. The seal is not important for the internal fields; it is useful for prevention of RF emission.

Nine RTWs and three special aluminium racks were constructed. In each rack three chambers are placed on top of the other (Fig. 2). A GSM900 phone controlled by a computer was used as a signal source (pulse duration 0.577 ms, pulse period 4.615 ms, repetition frequency of 217 Hz). The output RF power was divided by a four-way RF power divider and fed through variable attenuators to four high power RF amplifiers. Bi-directional RF power meters monitored the input and reflected power of the exposure chambers.

Electric (E) field distributions and components were measured by using a miniature isotropic E-field probe and a small (16 mm) dipole sensor. Whole-body-averaged SAR of rats was determined with analytical calculations, numerical methods (FDTD) and calorimetrical measurements with rat phantoms. Three different phantoms simulating rats of different sizes were made of plastic LDPE bottles (130 ml, 280 ml and 550 ml). The bottles were filled with muscle simulating liquid made of 53.6% distilled water, 45,0% purified sugar and 1.4% salt (NaCl).

RESULTS AND DISCUSSION: The measured return loss of the chambers varied from 10 dB to 30 dB depending on the position, size and orientation of the rat phantoms. The variation of the E field was less than ±1% (1 S.D.) in the vertical direction and less than ±10% in the azimuthal direction, which indicated that no higher order modes were present. Other E-field components were at least 20 dB smaller than the dominant vertical component. A standing wave pattern in the E field was obtained in the radial direction due to the thin absorber. SAR determinations revealed that SAR depends strongly on the orientation of the rat. If the rat remains in the horizontal position the SAR varied from -60% to +40% with respect to the SAR in the Reference position where the rat is with its long axis in the radial direction in the centre of the cage. The maximum SAR (+430%) occured with the long axis in the vertical direction. SAR depended also on the mass of the rat: For a young rat (100g) the SAR was 75 mW/kg and for an adult rat 45 mW/kg per 1 W of input power in the Reference position.

The developed exposure system enables us to place 216 rats in separate cages in nine exposure chambers on three racks requiring only 9 m^2 of floor area. Rats can freely move in their cages where food and drinking water is provided ad libitum most of the time.

Reference.

Hansen VW, Bitz AK, Streckert JR. *RF exposure of biological systems in radial waveguides*. IEEE Tr. Electrom. Comp1999, 41: 487-493.

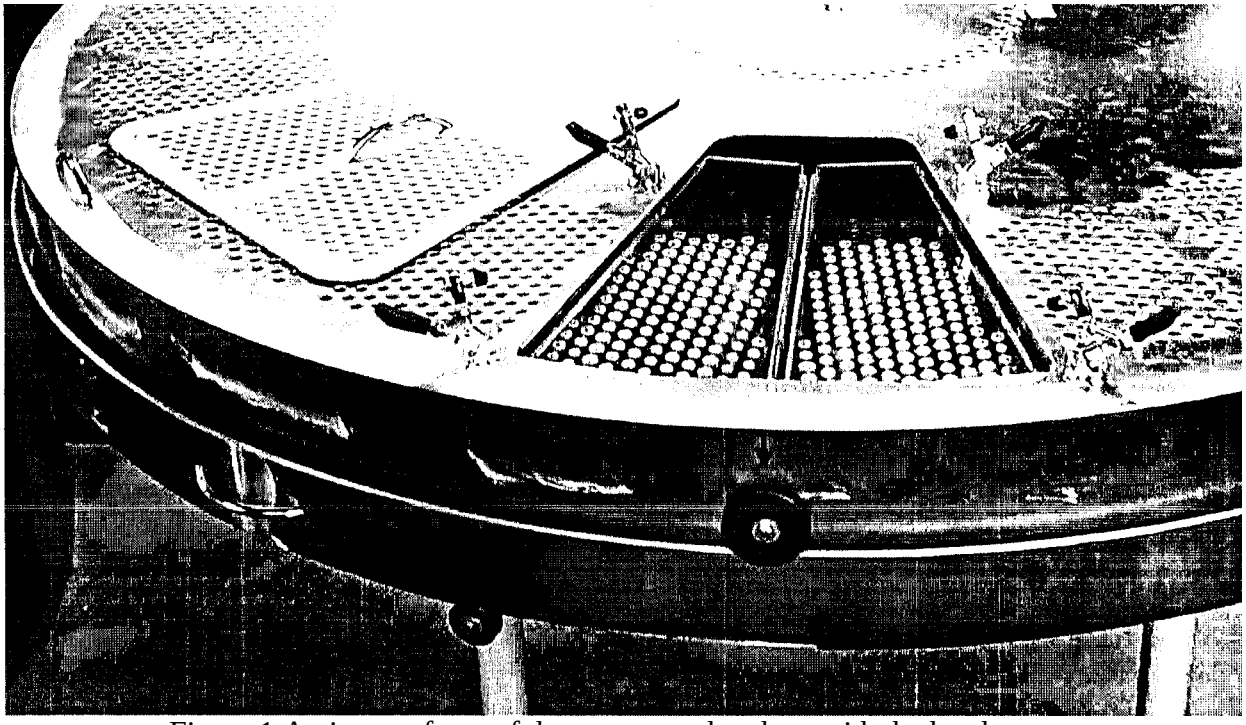


Figure 1 A picture of one of the exposure chambers with the hatch open.

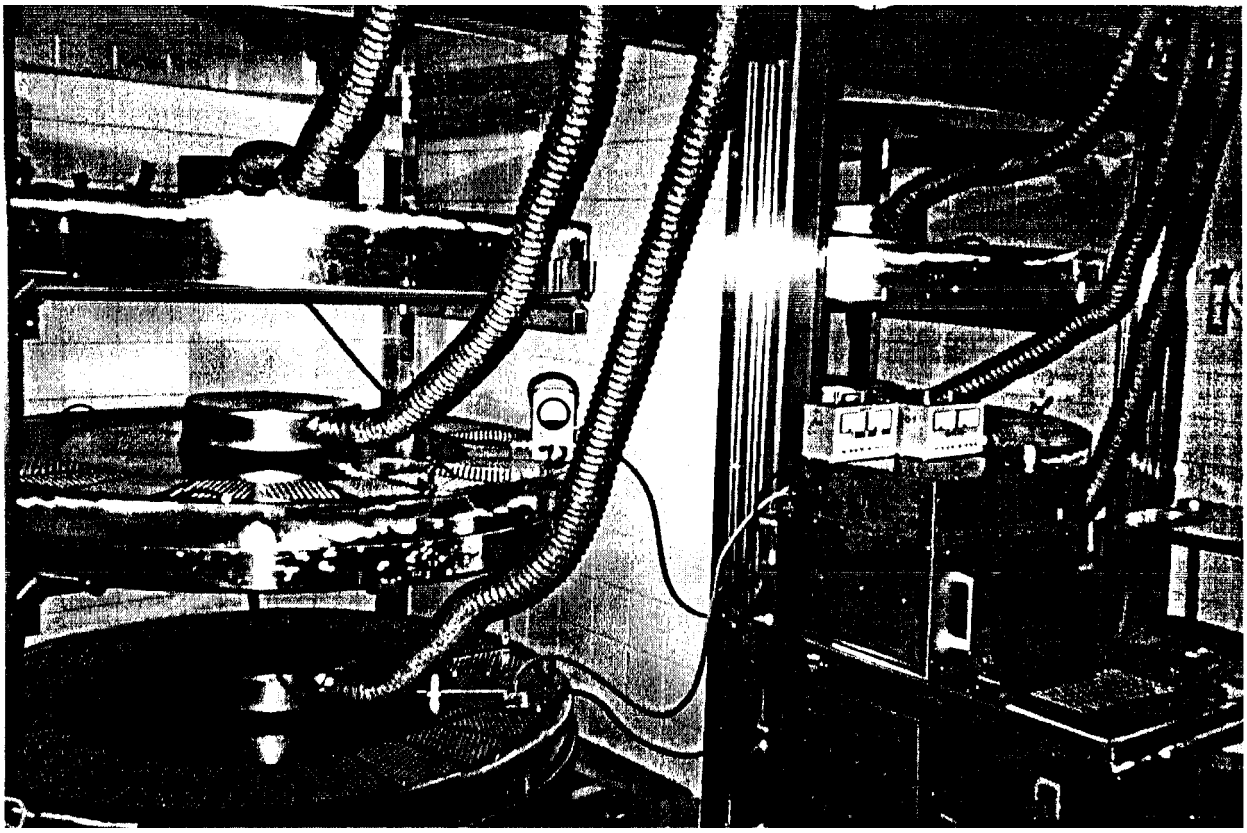


Figure 2 A picture of one of two racks with exposure chambers and the ventilation system.

P-7

AN IN VITRO EXPOSURE SYSTEM AT 2 GHZ USING HORN ANTENNA AND ANECHOIC CHAMBER. S. Uebayashi[1*], Y. Tarusawa[1*], T. Iyama[1*], M. Sekijima[2*], T. Nojima[1*]. [1]NTT DoCoMo Inc., Hikari-no-oka, Yokosuka-shi, Kanagawa, 239-8536, Japan. [2]Mitsubishi Chemical Safety Institute Ltd., Hasaki-machi, Kashima-gun, Ibaraki, 314-0255, Japan.

INTRODUCTION: We developed an *in vitro* exposure system using a horn antenna and dielectric lens in an anechoic chamber. The culture case, in which culture dishes are laid out, is sealed and connected by pipes to the main unit of an incubator set outside the anechoic chamber. This configuration enables exposure of large quantities of cells to a homogeneous electric field. The electric field and SAR (Specific Absorption Ratio) in culture fluid are measured and compared with numeric calculation (FDTD) results.
OBJECTIVE: The objective is to develop exposure equipment that establishes a wide and homogeneous electric field, and to control with high accuracy the electric field and culture atmosphere. The frequency is 2.1425 GHz, which is the mid frequency of the IMT-2000 downlink band, and the power density is 1 $\mu W/cm^2$ to 2 mW/cm^2. The density of approximately $1\mu W/cm^2$ corresponds to a field around the base station of a cellular system, and 1 mW/cm^2 is the standard value of ICNIRP, FCC, and ARIB in Japan.
METHOD: Figure 1 is an overview of the exposure equipment. The horn antenna and dielectric lens are covered by anechoic material. The culture case is set on the exposure table. The case has a two-layer structure. High temperature (40°C) air circulates in the outer layer of the culture case to maintain a constant culture temperature (37°C) and prevent condensation. Two anechoic chambers (each is 2.6 m X 4.0 m X 2.7 m) are built, one for the group exposed to the electric field and the other is sham exposed. The exposure equipment and the culture case are constructed from a plastic material with a low dielectric constant and low conductivity.
RESULTS: Figure 2 shows the measured electric field in the exposure area. Three miniature and mutually orthogonal dipoles (each element length is 4 mm) are used as a sensor. Figure 2 shows that the dielectric lens is effective in forming a wide and homogeneous electric field. The deviation of the electric field in the exposure area (30 cm X 30 cm) is ±1.5 dB. Figure 3 shows the calculated electric field in the culture fluid of 49 Petri dishes in the exposure area. The diameter of the Petri dishes is 40 mm and the height of the fluid is 3.0 mm. Although the radiated electric field is homogeneous, the field in the fluid fluctuates widely (the deviation is ±3.0 dB) because the wavelength in the fluid is short and there are reflected waves from the surface of the fluid. There was a close agreement between the measured and calculated values.
We will investigate the possible biological effects of exposure to electromagnetic radiation on the genome wide expression profile in human cell lines, using this *in vitro* exposure system.

Fig. 1. Overview of the exposure

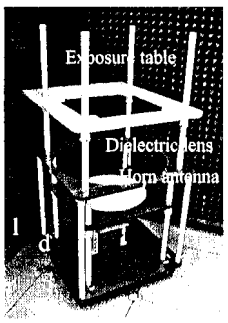
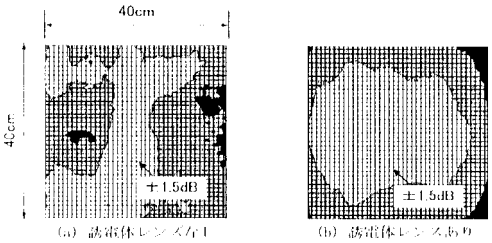


Fig. 1. Overview of the



a.Without Dielectric b.With Dielectric

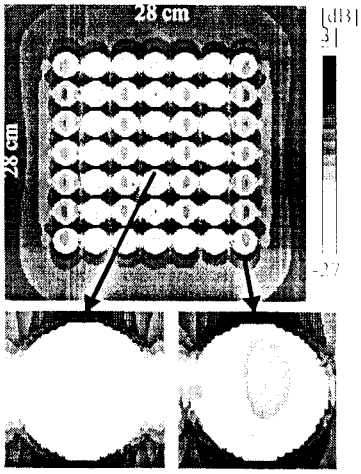


Fig. 3. Electric field in the

exposure	Fig 2. Electric field in the exposure	culture

P-8

ANATOMICALLY REALISTIC THERMOREGULATION ANALYSIS SYSTEM. D.A. Prelewicz[1*], W.C. Arcieri[1*], D.A. Barber[2*], J.M. Ziriax[3]. [1]Information Systems Laboratories, Inc. Rockville, Maryland 20852, USA. [2]Information Systems Laboratories, Inc. Idaho Falls, Idaho 83404, USA. [3]Naval Health Research Center Detachment, Brooks Air Force Base, Texas 78235, USA.

INTRODUCTION: The ANTHERM™ (ANatomical THERmal Model) prototype analysis system was developed to simulate thermal response in three-dimensional anatomically realistic models using tissue-coded data files for phantoms, animal carcasses, laboratory animals and humans. Results generated to date using ANTHERM™ show excellent agreement with analytical solutions and experimental data. The ANTHERM™ software is available in an open source environment.

OBJECTIVES: Project objectives were to develop a prototype thermal analysis system to simulate anatomically realistic models, to assess the analysis system against experimental data, and to demonstrate the performance of this system on a human model. At present, only passive mechanisms are modeled, making the system useful for RF dosimetry investigations. Active thermoregulation heat generation and transport mechanisms are being added.

METHODS: The ANTHERM™ prototype system is a Fortran-90 computer code for steady-state and transient thermal analysis of three-dimensional anatomical models, which are set up automatically using tissue-coded data files openly available from the microwave dosimetry programs at Brooks Air Force Base (P. A. Mason, et. al., 1995). The anatomical data files and the thermal models are all voxel based in Cartesian coordinates, the natural coordinate system for the medical images from which the data files were developed. The spatial distribution of the heat source can be input from the results of an FD-TD calculation (J. M. Ziriax, et. al. 1999) of the specific absorption rate (SAR), or from the output of a source generator utility code. Minimal information on the heat source magnitude, boundary conditions and run parameters is supplied by the code user. A combination of forced convection, natural convection and radiation heat transfer boundary conditions can be specified on the exterior surface of the anatomical body. Best estimate values of thermal conductivity and specific heat were determined from the literature and included in a library of tissue thermal properties. An algorithm was added to ANTHERM™ to automatically adjust surface heat transfer areas in Cartesian geometry to better model the curved surfaces typical of anatomical structures. A Krylov subspace Bi-Conjugate Gradient Stabilized linear solver was implemented as the steady-state solution technique. The MPICH message passing interface routines were used to parallelize the explicit transient solution. Comparisons to analytical solutions for a uniformly heated 101 mm phantom sphere demonstrate that the code accurately predicts the governing thermal processes. In particular, the excellent comparisons with analytical solutions show that no loss in accuracy occurs using Cartesian coordinates to model anatomical objects with curved surfaces. An assessment was also performed against microwave exposure data for Sprague-Dawley rats (T. J. Walter, et. al., 1998). In these experiments male Sprague-Dawley rats were exposed to high power microwave (HPM) heating and low power microwave (LPM) heating. Power densities were 1,700 mW/cm^2 (HPM) and 170 mW/cm^2 (LPM) at 2.06 GHz. Comparisons were made against measured hypothalamic and cortical temperatures during the microwave exposure. The observed phenomena of continued rise in local cortical temperature after the HPM power source was turned off was predicted. A demonstration calculation was also performed for 800 MHz microwave exposure of a human head.

CONCLUSIONS: Assessments and a sample application show that this prototype code is useful for thermal analysis of anatomical models. Anticipated applications for the system include design of protective garments and wearable wireless devices, hyperthermia and hypothermia treatment planning, forensic analysis, and medical training simulators.

References.
P. A. Mason, et. al., "Database created from magnetic resonance images of a Sprague-Dawley rat, rhesus monkey, and pigmy goat", *FASEB J.*, 9: 434-440 1995.
J. M. Ziriax, et. al., "Comparison of FD-TD and Experimentally Determined Local SAR Values in a Rhesus Monkey Model", *Electricity and Magnetism in Biology and Medicine*, Bersani, Kluwer Academic/Plenum Publishers, 1999.
T. J. Walters, et. al., "Regional Brain Heating During Microwave Exposure (2.06 GHz), Warm-Water Immersion, Environmental Heating and Exercise", *Bioelectromagnetics*, 19: 341-353, 1998.
This work was supported by the Office of Naval Research, U.S. Navy under a Small Business Innovation Research (SBIR) contract.

P-9

USE OF HIGH RESOLUTION DIGITAL CAMERA AND IMAGE ANALYSIS TO DETERMINE NEURITE OUTGROWTH OF DORSAL ROOT GANGLIA. P.P. Resig[1], B.F. Sisken[2], P.C. Fischer[3*], J. Smith[2*], J. Shropshire[2*], E. Herbst[1]. [1]Herbst Research, Inc., Edgewater, New Jersey 07020. [2]Center for Biomedical Engineering, University of Kentucky, Lexington, Kentucky 40506. [3]Zedec Technologies Inc., Morrisville, North Carolina 27560.

OBJECTIVE: To improve image quality for use in image analysis of neurite outgrowth in explanted chick dorsal root ganglia (DRG). Image analysis requires a minimum level of image quality to obtain measurable results. The overall reliability of the study is improved with higher resolution images. These higher quality images may produce smaller standard deviations with fewer images being required. Hence, fewer experimental trials would be needed to determine statistical significance.
METHODS: A Redlake MASD ES1.0 MegaPlus digital camera was mounted to the camera port of a Nikon Diaphot inverted microscope via a 0.6x C-mount adaptor lens (Nikon USA, Melville, NY). The camera has a resolution of 1018 pixels by 1008 pixels with 8-bit depth gray scale and the ability to capture up to 30 frames per second. The camera is a black and white camera which provides a higher contrast image than color images which further enhances the image analysis process. The camera was connected to a digital frame grabber board (Imagenation PXD1000) which was installed in a PC with a 1.0 GHz processor and 256 MByte of RAM. Real-time images were captured using ViewPXD software that was provided with the frame grabber board. Images were viewed real-time on a computer monitor which allowed for both focus and brightness to be adjusted to provide an optimum image. In short, the working real-time image after being captured and saved was also the final digital image that was used for image analysis. The microscope/camera system had a field-of-view of 3.8 mm by 3.8 mm when using the 4x microscope objective and 6.1 mm x 6.1 mm when the 2.5x microscope objective was used. These relatively large fields-of-view allowed for a single captured image to contain the entire neurite outgrowth of a DRG. This eliminated the previous need to create a collage of multiple images of larger DRGs. The image analysis process was performed using QuantIm image analysis software (Zedec Technologies, Inc., Morrisville, NC) as previously described [1]. However, an additional step, a calibration function, was added to the algorithm in order to standardize output. This calibration function produced area output results in square millimeters as opposed to the earlier method which gave areas in total number of pixels. The 4x microscope objective yielded 71,824 pixels per square millimeter and the 2.5x microscope objective yielded 27,889 pixels per square millimeter. This allows for a more consistent and comparable results between different studies and laboratories than previous methods.
References.
[1] A. J. Shah et. al.(1998) BEMS 20th Annual Meeting Abstract Book pp. 185-186.
Supported in part by the NIH Grant 2 R44 NS33033-02 to Herbst Research, Inc.

P-10

EFFECT OF COMPUTER MONITOR RADIATION ON THE ELECTRIC PROPERTIES OF THE SKIN MEASURED WITH A NEW GAS DISCHARGE IMAGING TECHNIQUE. G. Rein[1], K. Korotkov[2], P. Giacomoni[1], G. Cioca[1]. [1]New Venture Technologies, Estee Lauder Companies, Melville, NY, USA. [2]Department of Computer Science, State Technical University, St. Petersburg, Russia.

OBJECTIVE: To ascertain the linear and nonlinear effects of computer monitor radiation on the skin using a new in situ method.

INTRODUCTION: A previously unused method was chosen based on the formation of a corona discharge in the air gap between an electrode and the skin. Linear and nonlinear analysis of the resulting gas discharge images was expected to reveal new information about the sensitivity of the skin to external electromagnetic (EM) fields generated from computer monitors.

METHODS: The technique used in this study, Gas Discharge Visualization (GDV), generated gas discharge images of the air gap around the skin in response to a train of triangular electrical pulses (0.1 second duration, 1000 Hz, 3kV and 10^6 V/s). The electric field initiates electron-ion avalanches, which result in a gas discharge along the dielectric surface. The spatial distribution of discharge channels was recorded using a charge coupled optical system, digitized using a video-blaster and mathematically analyzed for several linear and nonlinear parameters including area, fractality and entropy. GDV images of each of ten fingers were obtained from 41 subjects before and after a ten minute exposure to EM fields generated by computer monitors. Before measurements were taken only when subjects remained in a computer-free environment for at least 20 minutes. Overall significance was determined using the t-test. In addition dynamic measurements were made by repeated measures (5 frames/ second) from the fourth finger on the left hand and analyzed using time series analysis.

RESULTS: The results for two parameters, area and fractality, are presented below.

Area							Fractality					
	Before	SD	After	SD	p	n	Before	SD	After	SD	p	n
All	5613	2677	5599	2991	NS	41	18.44	6.5	17.74	5.8	NS	41
Increase	5476	3080	6770	3246	NS	22	15.34	6.8	17.94	6.6	NS	18
Decrease	5773	2192	4243	1993	0.03	19	20.87	5.5	17.59	5.3	0.04	23

No significant effects were seen when pooling the data from all subjects. Examining before-after differences, two populations emerged, showing increases or decreases in GDV values. The magnitude of these responses varied from less than 10% to approximately 8-fold. Statistical significance was reached only in the population which showed decreases in both GDV image area and fractality. Control studies with the computer on and the monitor off showed no effects. No correlations with age or gender were found. Dynamic time series analysis confirmed these results showing qualitatively different patterns following EM field exposure.

CONCLUSION: The results indicate that radiation emitted from computer monitors inhibits corona discharge formation at the surface of the skin. Both linear and nonlinear measures showed statistically significant changes. The fact that only 50% of the population show a sensitivity to computer monitor radiation is of further interest.

P-11

ROTATING PERMANENT MAGNETIC FIELD EXPOSURE SYSTEM FOR IN VITRO STUDY.
T. Song, X.L. Huo, X.Y.Zhang, Z. Wang. Institute of Electrical Engineering, Chinese Academy of Sciences, Beijing 100080, China.

OBJECTIVE: To examine the influence of rotating magnetic field on cell, a rotating permanent magnetic field exposure system is designed. The system is composed of two permanent magnetic rings driven by a motor. The magnetic field is uniform, and its flux density can be adjusted from 0 to 300mT. The rotating frequency can be varied from 0 to 10Hz. We plan to find an "action window" of the rotating magnetic field on cell proliferation using the system, and assess the possible biophysical transduction mechanism.
METHODS: The rotating permanent magnetic field exposure system is composed of two permanent magnetic rings. The small magnetic ring is put in the large magnetic ring. The magnetic rings are driven by one motor through a mechanism. The inner size of the permanent magnet system is 9cm in diameter, and its outer size is 16cm in diameter. Each magnetic ring is a dipole magnet which flux density is 150mT. The rings are composed of 24 blocks of Nd-Fe-B with gradually varying magnetization direction that are placed in a circular form. So the magnetic field of each magnetic ring is very uniform. Because the magnetic rings are made of NdFeB without other magnetized material, and because the relative permeability of NdFeB is closed to the air, the magnetic field of the system is equal to the composition of the two magnetic fields of the magnetic rings. So the magnetic field of the system can be changed by adjusted the angle of two magnetic rings. According to the working modes of the motor, the system can generate four kinds of magnetic fields: (1) static permanent magnetic field whose flux density can be adjusted from 0 to 300mT (when the permanent magnetic rings are static); (2) rotating permanent magnetic field whose flux density can be adjusted from 0 to 300mT, and whose frequency can be adjusted from 0 to 10Hz (when the two magnetic rings are rotating with same velocity); (3) linearly polarized permanent magnetic field whose amplitude is 300mT, and whose frequency can be adjusted from 0 to 10Hz (when the two magnetic rings are counter turn with same speed); (4) combined static and rotating permanent magnetic field which can generate a 150mT static magnetic field and a 150mT rotating magnetic field with frequency band from 0 to 10Hz (when the large magnetic ring is stop and the small ring is driven by the motor). It is also necessary for comparative experiment to design an apparatus that is same as the rotating permanent magnetic system except that the magnetic material is not magnetized. The cell incubator is placed into the inner magnetic ring to examine the effect of the rotating magnetic field on cell.
RESULTS AND DISCUSSION: A rotating permanent magnetic field exposure system is designed for a cell exposure experiment. The system is very convenient to vary the amplitude and frequency of the rotating magnetic field. So it can be used to find the "action window" of the rotating magnetic field on cell proliferation.

P-12 Student

CONDUCTIVITY IMAGING OF THE RAT BRAIN BASED ON DIFFUSION MRI. M. Sekino*, K. Yamaguchi*, N. Iriguchi*, S. Ueno. Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan.

INTRODUCTION: Conductivity distribution in the brain is difficult to obtain by conventional methods in which currents are injected via surface electrodes, because most of the currents do not penetrate the skull. In this study, we introduce a new method of conductivity imaging based on diffusion magnetic resonance imaging (MRI).
PRINCIPLE: When an ion with a charge q moves in a solution by an applied field E, the balance of the electrostatic force and the viscous drag is expressed as $qE=6\pi r_i \eta v$ (1), where r_i is the Stokes radius of the ion, η is the viscosity of the solution, and v is the drift velocity of the ion. The current density j in the

solution of the ion density N is $j=qNv$ (2). The self diffusion coefficient of water is $D=kT/6\pi r_w\eta$ (3), where k is the Boltzmann constant, T is the temperature, and r_w is the Stokes radius of water molecules. Thus, the relationship between the conductivity σ and the self diffusion coefficient D is obtained from (1)(2)(3) as $\sigma=j/E=(r_w q^2 N/r_i kT)D$ (4).

METHODS: Diffusion MR images of the rat brain were acquired in a transversal slice. Motion probing gradients (MPGs) were applied with a b-factor of 1.4×10^9[s/m^2] in the read-out and phase-encoding directions. The self diffusion coefficient of water was calculated in each pixel as $D=(1/b)\ln(S_0/S)$, where b was the b-factor, S_0 and S was the signal intensity of the images with and without a MPG. Conductivity maps were generated by equation (4) with the following variables, $r_w/r_i=0.76$, $q=1.6\times10^{-19}$[C], $N=2.0\times10^{25}$[/m^3], $kT=4.1\times10^{-21}$[J].

RESULTS and DISCUSSION: Figure 1(a) shows a T_1-weighted image of the rat brain. Figure 1(b)(c) show the horizontal (phase-encoding) and vertical (read-out) conductivity maps. The skull does not affect this method of conductivity imaging. In addition, the anisotropy of the conductivity can be investigated by changing the direction of the MPG. Current distributions in the electromagnetic stimulation of the brain can be calculated from the conductivity maps. As an example, we calculated the current distributions in the rat brain (Figure 2) when a current was applied by a pair of electrodes placed on the upper edge and the lower edge of the model. This new method of conductivity imaging based on diffusion MRI has various potential applications in biomedical calculations such as electromagnetic stimulation of the brain, absorption of RF waves from mobile phones, and current source estimations in EEG and MEG. It provides calculation models of biological tissues with inhomogeneous and anisotropic conductance at high spatial resolutions.

This study was supported in part by a grant from the Ministry of Education, Science and Culture, Japan.

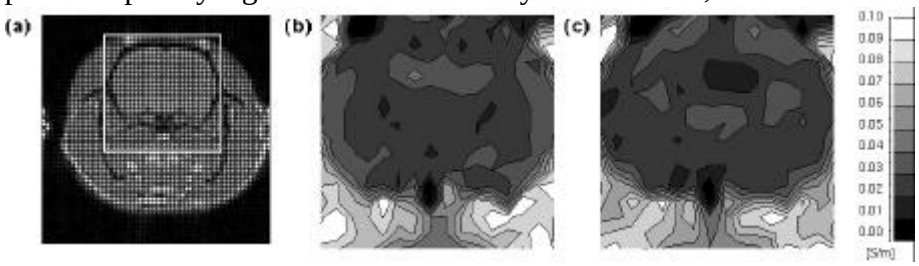


Figure 1: (a) T_1-weighted image of the rat brain, conductivity maps in (b) horizontal and (c) vertical directions.

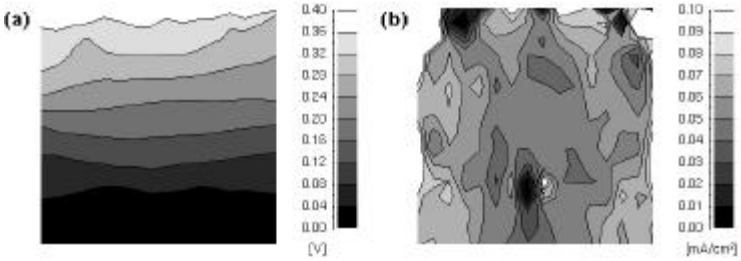


Figure 2: (a) Equipotential contour map and (b) current distributions calculated with the conductivity map.

P-13 Student

CURRENT MR IMAGING BASED ON THE RESONANT FREQUENCY SHIFT TECHNIQUE. T. Matsumoto, M. Sekino, K. Yamaguchi, N. Iriguchi, S. Ueno. Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, 113-0033, Japan.

OBJECTIVES: The molecular environment of nuclei can be investigated by the resonant frequency shift of MR signals. However, there are no applications of the frequency shift technique for the detection of electrical currents. In this paper, we propose a new method of electrical current imaging based on the frequency shift technique of MRI. Loop current and straight-line current models are introduced as electrical

sources in the brain, nervous and muscular systems. Magnetic field changes generated by the loop current and the straight-line current were detected by the resonant frequency shift.
PRINCIPLE: When a current is applied to a cable placed in a phantom, the magnetic fields that are generated around the cable are added to the main magnetic field. The main magnetic field strength increases or decreases around the electric currents, which gives rise to a shift in the resonant frequency. The component of the generated magnetic field parallel to the main magnetic field causes a detectable resonant frequency shift. The spins are not sufficiently excited at the region where the resonant frequency changes in the phantom, resulting in a decrease of the signal intensity. By detecting the decrease in the MR signal, we can observe the change of the magnetic field generated by the current.
METHOD: A 3-turn loop cable and a straight-line cable were placed in spherical phantoms filled with water. An electrical current was applied to the cable during imaging acquisition. Images were acquired with a sequence based on the spin-echo (SE-CHESS, Spin Echo Chemical Shift Selective). In the resonant frequency shift sequence, a 90-degree pulse was applied with a narrow bandwidth (160Hz) so that the spins with a shifted resonant frequency were not excited. By this method, the shift of the resonant frequency caused by the disturbance of magnetic field was precisely detected. A slice selective gradient was applied with only a 180-degree pulse rather than a 90-degree pulse. Gaussian RF pulses were used so that the decrease of the signal intensity corresponded with the amplitude of the magnetic field generated around the current. Imaging parameters were as follows: TR = 500ms, TE = 18ms, slice Thickness = 2mm, 90-degree pulse width = 6000ms, 180 degree pulse width = 2000ms.
RESULTS and DISCUSSION: The MR signal intensity inside the loop decreased as the loop currents increased. The region represented by the decreased signal intensity corresponded with the contour surface of the magnetic field component parallel to the main magnetic field. The resonant frequency shift at the center of the loop current was $\delta B/B_0 x10^6 = \mu_0 I/2rB_0 x10^6$ [ppm], where I is the current in the loop and r is the radius of the loop. By substituting $\mu_0 = 4\pi x10^{-7}$ [H/m], $r = 4x10^{-3}$ [mm], I = 100x3 [mA· turn] , $B_0 = 4.7$[T], the resonant frequency shift of this experimental condition was calculated as, $\delta B/B_0 x10^6 = 9.9$ [ppm]. The signal intensity decreased at the region where the straight current generated a magnetic field and where the magnetic field was parallel to the main magnetic field direction. In the case of the straight current, the resonant frequency shift at 4mm from the cable was calculated as $\delta B/B_0 x10^6 = \mu_0 I/2\pi r B_0 x10^6$ [ppm] = 3.2 [ppm]. Magnetic field maps are obtained by quantitatively analyzing the decrease of the signal intensity. The magnetic field map can identify the amplitude and the position of the current.
This study was supported in part by a grant from the Ministry of Education, Science and Culture, Japan.

P-14 Student

MEASUREMENT ISSUES FOR ELECTROMAGNETIC EXPOSURE ASSESSMENT AROUND UMTS BASE STATIONS. C. Olivier*, L. Martens. Department of Information Technology (INTEC). Ghent University, Ghent, Belgium.

OBJECTIVE: To address some measurement issues that will be encountered when the exposure, originating from UMTS base stations, is assessed with a frequency-selective measurement set-up.
METHODS: Because third-generation networks (UMTS) use a different radio access network than the second-generation networks (GSM), some frequency-selective measurement techniques for the exposure assessment around base stations have to be reconsidered. The main different characteristics between the UMTS signal and the GSM signal that will influence the exposure assessment are: (i) the use of WCDMA instead of TDMA/FDMA, (ii) the larger occupied signal bandwidth, and (iii) the reuse of the frequency in neighbouring cells and sectors. Because more advanced new aspects of the WCDMA radio layer, like the use of smart antennas, will not be deployed in the early phase of the UMTS network, these issues will not be considered here. In order to study the differences between the measurement of a GSM signal and a WCDMA signal, simulations were made with SimuLink and Matlab. The focus will be on the measurement

of a wide-band noise-like signal with a spectrum analyser, and on the different fast-fading of the electromagnetic field for wide-band systems.

RESULTS: When the electromagnetic field of a WCDMA frequency channel is measured with a spectrum analyser, the widest resolution filter of the spectrum analyser is often narrower than the occupied bandwidth of the WCDMA channel. Because the power of the WCDMA signal is spread in frequency, the level measured with a resolution filter narrower than the WCDMA signal, will depend on the width of this resolution filter: the measurement with a wider resolution filter will result in a higher signal level. The dependence of the measured signal level on the width of the resolution filter is shown in figure 1. In figure 1, the theoretical relationship between the measured signal level and the correct signal level is also indicated. There is a very good agreement between the simulation results and the theory. However, when the resolution filter is too narrow, the spectrum analyser sweeps too fast to enable accurate measurements. Because the power emitted by a UMTS antenna will also vary in time, the spectrum analyser should be operated in maximum-hold mode to determine the exposure in the worst-case situation. However, in maximum-hold mode, the electromagnetic field strength will be overestimated due to the noise-like properties of the WCDMA signal. In maximum-hold mode the positive-peak detector is used, which returns the maximum value that has been measured while sweeping the frequency of the spectrum analyser between two frequency points. Because the maximum occurred value of a noise-like signal, is not a good measure for the signal strength of noise, the signal strength measured with a positive-peak detector has to be adjusted. The simulated ratio between the signal strength measured with a positive-peak detector and the strength measured with a sample detector is shown in figure 2. As can be seen, the correction factor varies with the used resolution filter.

Another aspect of the wide-band nature of the signal is the protection against fast-fading arising from multipath propagation with relatively large differences in path length. The variations in signal strength due to this fast-fading will be averaged out by the signal bandwidth. This is illustrated in figure 3, where the total electric field pattern is shown for a narrow-band (200 kHz) and for a wide-band (5 MHz) signal. The scattering environment for the simulation consists of 10 scattering sources, located at randomly chosen positions and each with a random strength. One can see that the electric field is much smoother for the broadband signal, but also that the range of the values of the broadband signal is smaller, because the influence of the long paths will be averaged out over the signal bandwidth. Because the variations of the signal strength with the position, are smaller for wide-band signal, the choice of a measurement position will be less critical for wide-band signals.

A final important difference between 3G and 2G systems is the frequency reuse factor of 1 in UMTS. Because it is likely that neighbouring sectors or neighbouring base stations contribute to the total exposure in a certain point, it will not be possible anymore to determine which antenna or base station of a certain operator is responsible for the largest contribution to the exposure.

CONCLUSIONS: When the exposure around UMTS base stations is assessed, several measurement techniques have to be reconsidered: the measured electric field has to be corrected for the width of the resolution filter, and positive-peak detection, used in maximum-hold mode, will overestimate the present electromagnetic field strength. The choice of the measurement position will be less critical for a wide-band signal than for a narrow-band signal. Finally, because the frequency reuse factor in UMTS may be 1, it will not be possible to determine which antenna of a certain operator is responsible for the largest contribution to the exposure.

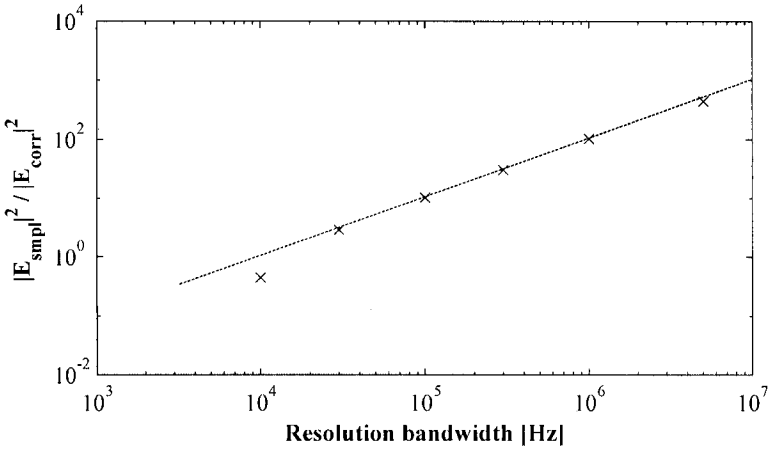


Fig. 1: Influence of the used resolution bandwidth on the measured signal strength ($|E_{smpl}|^2$) of a WCDMA signal (with signal strength $|E_{corr}|^2$). The simulation results, obtained for the different resolution bandwidths (10 kHz, 30 kHz, 100 kHz, 300 kHz, 1 MHz and 5 MHz), are indicated with ×. The theoretical relationship between the measured signal strength and the correct signal strength is shown with a dashed line. The frequency span of the spectrum analyser was 6 MHz, the sweep time 50 ms and the width of a frequency bin was 10 kHz.

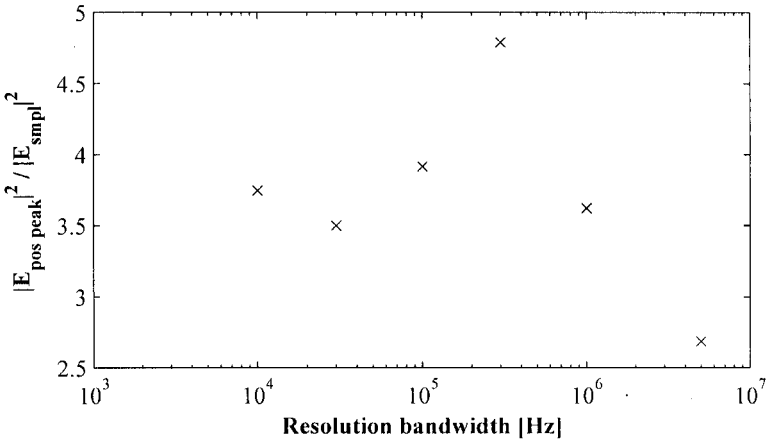


Fig. 2: Relationship between the signal strength measured with a positive-peak detector $|E_{pos\ peak}|^2$ and with sample detection $|E_{smpl}|^2$ for different resolution bandwidths.

Fig. 3: Comparison between the electromagnetic field pattern caused by 10 scattering sources with randomly chosen strength and position, for a signal with a bandwidth of 200 kHz (e.g. GSM) and a signal with a bandwidth of 5 MHz (e.g. UMTS).

Christof Olivier is research assistant of the Fund for Scientific Research Flanders (F.W.O.-Vlaanderen).

P-15 Student

THE INFLUENCE OF THE MEASUREMENT PROBE ON THE EVALUATION OF ELECTROMAGNETIC EXPOSURE AROUND A BASE STATION.

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OBJECTIVE: To check whether the exposure (public and occupational) of a base station satisfies the guidelines for electromagnetic exposure, electromagnetic field measurements around base stations must be accurate. While measuring, the electromagnetic field will be disturbed by the measurement probes

themselves. Calibration does not take into account all errors. Due to the non-negligible dimensions of the measurement probes, they will disturb the field that has to be measured. So instead of measuring the true free-space field, the disturbed field will be measured. In principle, the calibration should take into account the disturbance of the probe. However, this is only true if the disturbance is equal in the calibration set-up and during the measurement. This is certainly not the case if the measurement is done in the near field of a base station because calibration is mostly done measuring far fields of one or more electromagnetic sources. This paper will show that when a maximal disturbance of e.g. 1% is required, a suitable measurement probe, that is sensitive enough can be selected. The characterization was performed using a numerical electromagnetic computational program (NEC-Win-Pro©). No other studies up to now included the acquisition of the disturbance effect.

METHODS: Figure 1 shows a flow graph of the followed procedure. First the calibration method was simulated. The simulated calibration is a two antenna method. It is performed in two steps. In the first step, the free-space far field of a calibration antenna (e.g. a dipole) E^i is simulated. As a model for E^i the field averaged over the surface along which the measurement probe will be placed in the second simulation is determined with NEC. In the second step the configuration with two antennas – calibration antenna and measurement probe – at the same distance as in the first simulation, is simulated. By terminating the measurement probe with a 50 Ω resistance the measured voltage V^{meas} is determined. Finally the antenna factor defined as the ratio of E^i and V^{meas} is calculated. Secondly, the measurement configuration is simulated. Three orthogonal components of the electric field are "measured". The relative disturbance $\Im$ [%] of the measurement probe is determined as the relative difference between the measured field E^{meas} and the true field E^{true}. For the simulation of the free-space measurements a 900 MHz Kathrein 736863 base station is used. The far-field distance for this antenna is 22.4 m. The measurements are simulated for a frequency of 900 MHz.

RESULTS: A disturbance below 1% is desired**.** For electric far-field measurements, a dipole with length λ/2 (15 cm) and a radius of the thickness of 1.8 mm will satisfy this requirement. The used λ/2 dipole is very sensitive, with an antenna factor of 27 dB(1/m) at 900 MHz. High sensitivity is required for far-field measurements, due to the low field values. But for near-field measurements this probe will have a disturbance of more than 5% due to its dimensions. This can be solved by using a smaller probe, that has a better spatial resolution which is necessary due to fast changing field values in the near field. On the other hand the probe will be less sensitive. In order to obtain a disturbance below 1% but still have the highest sensitivity, a design of the dimension of the probe has to be made using simulations. This resulted in a 7.5 cm probe, corresponding with λ/4 at 900 MHz. The thickness was identical to the one of the λ/2 probe. The antenna factor is 44 dB(1/m) at 900 MHz, higher than the one of the λ/2 dipole but low enough to measure the higher values in the near field. Figure 2 shows the simulation of the measurements and the location of the measurement positions from 10 cm up to 100 m from the base station. The results are excellent: the disturbance is below 1%. The maximum disturbance for the λ/4 dipole and the λ/2 dipole is respectively 0.11% and 0.54%. The used procedure allowed to select the probes with minimal disturbance and sufficient sensitivity.

CONCLUSIONS: A selection of measurement probes with a disturbance required to be lower than 1% for near- and far-field exposure measurements around a 900 MHz base station is made. The design of the probes has been done on the basis of simulations of measurements. It was concluded that for far- and near-field measurements different probes have to be used. For measurements in the far field of the source antenna, measurement probes with dimensions that deliver the highest sensitivity will be used because of the lower field values. We selected a λ/2 dipole. Near-field measurements must be performed with smaller probes to lower the disturbance. Spatial resolution is more important in the near field than in the far field. The dimension of the near-field probe was determined on the basis of the highest sensitivity and a disturbance lower than 1%. This resulted in a dimension of 7.5 cm corresponding to λ/4 at 900 MHz.

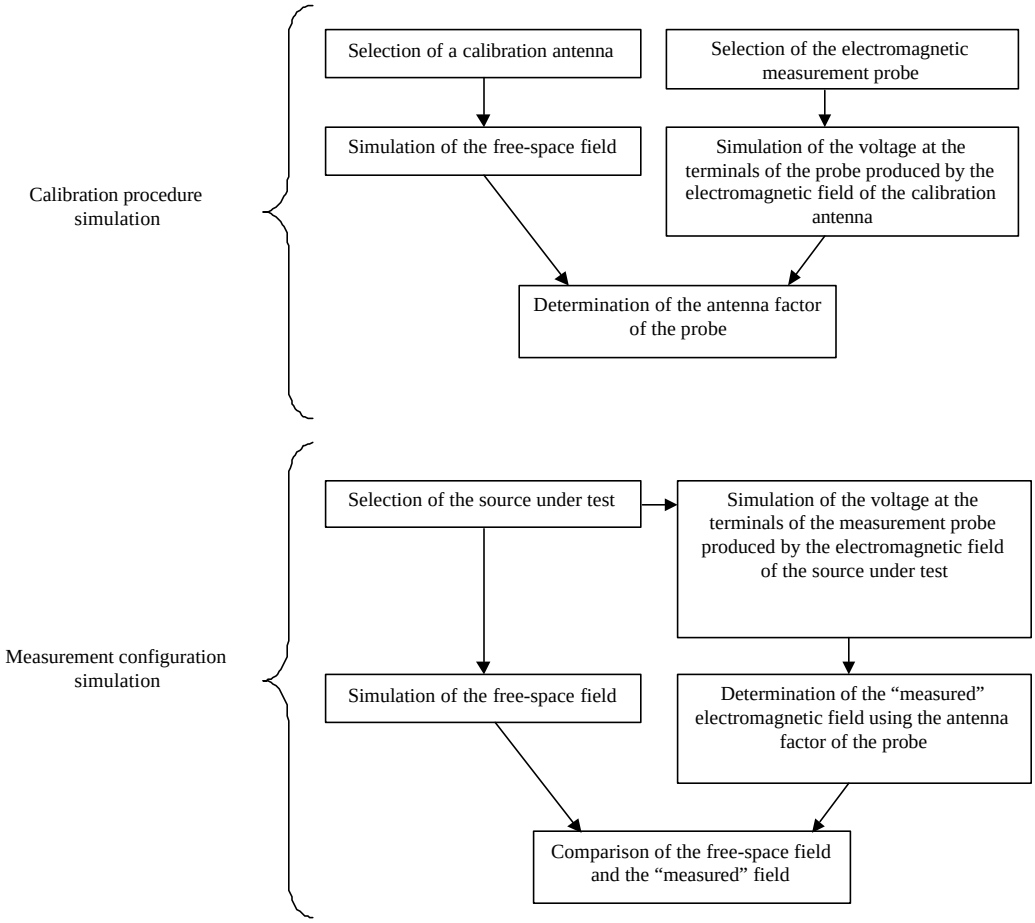
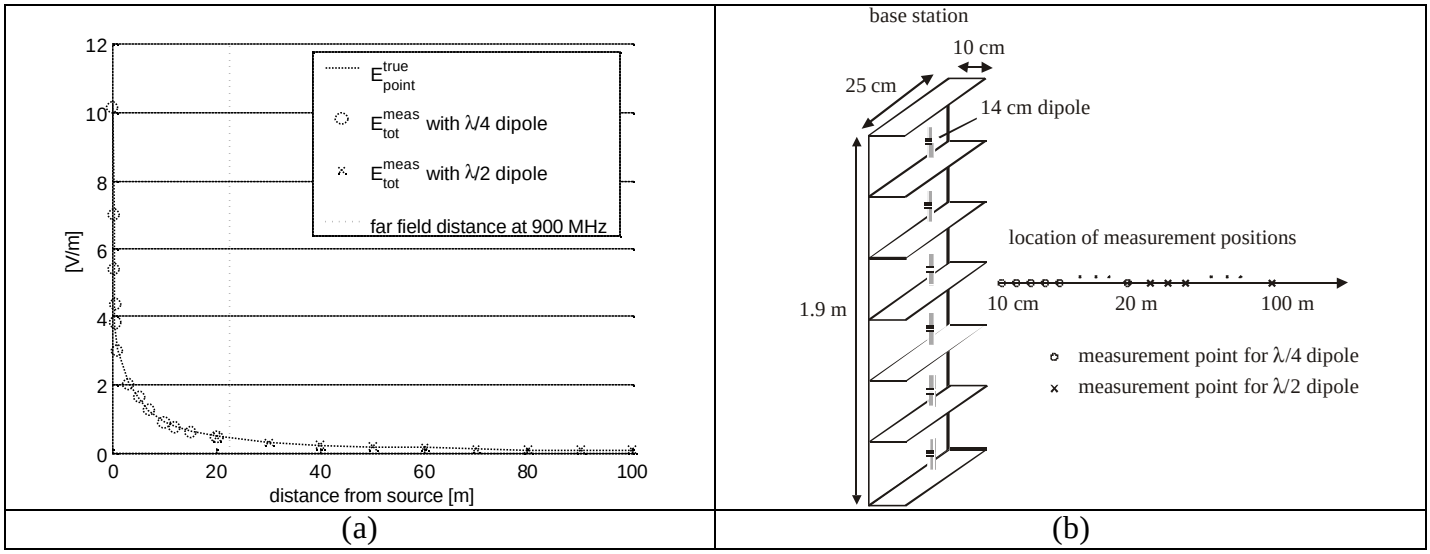


Figure 2: Flow graph of the followed procedure.



(a) (b)

Figure 3: The true electric field at 900 MHz of the base station versus the electric field measured with the λ/4 and λ/2 dipoles as a function of the distance and (b) the base station and the location of the measurement position.

P-16 Student

REQUIREMENTS FOR CONTROLLING & MONITORING SOFTWARE OF EXPOSURE SYSTEMS IN (DOUBLE-)BLINDED BIO-EXPERIMENTS. W. Oesch*, H.U. Gerber*, N. Kuster. Foundation for Research on Information Technologies in Society (IT'IS) & Laboratory for Integrated Systems, Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland, Schmid & Partner Engineering AG, 8004 Zurich.

INTRODUCTION: In order to conduct experiments in accordance with the principles of GLP, bio-experiments must be performed under highly controlled conditions with respect to the biological system, delivery of the agent and environmental parameters. In addition, experiments conducted in the framework of health risk assessment must ensure that the agent is applied relevant to the daily exposure of the exposed population. However, the application of well defined and controlled EMF exposures to biosystems is a complex task, in particular for RF experiments. It requires customized equipment optimized for the particular experiment. In addition, biological labs usually do not have the technical know-how available in-house. Furthermore, the experiments often need to be conducted in a blinded or double blinded fashion. These tasks can only be satisfactorily met with elaborate controlling & monitoring software.
OBJECTIVE: The objective of this contribution is to outline the requirements for controlling & monitoring software for bioexperiments and to present a flexible implementation allowing easy adaptation to different configurations of exposure systems and providing a user-friendly GUI.
REQUIREMENTS: The requirements may include the following tasks: 1) random selection of the exposure/sham group; 2) generation of the appropriate signal (the signal may be composed of several uncorrelated random parameters, e.g., DTX/nonDTX mode, power control, handover, frequency hopping); 3) maintenance of the desired SAR level by a feedback system (compensation for drifts, moving animals, etc.); 4) continuous monitoring of the environmental parameters; 5) provision of security against manipulation of the settings by unauthorized personnel via an intuitive GUI; 6) indication of warnings or abortion of the experiment when parameters are out of tolerance; 7) conduction of redundant checks to ensure that the system performs within its specifications; 8) generation of protocols for the entire sequence of commands and values in an encoded file to enable full reconstruction of the entire experiment for each individual run; 9) in case of malfunction, provision of a problem report and automatic transmission of the complete protocol to the remote maintenance institution for problem analysis; 10) verification that the software is still operating via a watchdog.
RESULTS: For satisfaction of GLP an exposure system typically consists of: 1) the exposure chambers, 2) an arbitrary function generator to generate the ELF signal, 3) an RF signal generator combined with an amplifier or in case of ELF a current or voltage source, 4) a data acquisition unit, 5) a switch to select which chambers are exposure and sham, 6) various sensors such as E- and H-fields, power, temperature, oxygen, humidity, etc. and 7) the controlling & monitoring computer. For more complex modulation schemes that may occur for mobile communications systems, additional devices may be necessary, such as frame generators, etc. An example of software implementation will be presented which meets all the above requirements and is currently being used in various experiments. It is written in Visual C++, and the selected communication protocol between the devices is GPIB. Maximum reuse of source code for different exposure systems is achieved by application of software design patterns such as Observer-, State-, Decorator and Template-Patterns.

P-17

REDESIGN OF THE SELF-REFERENCING PROBE FOR USE IN BIOEFFECTS EXPERIMENTS. J. Adams. Merrimack College, North Andover, MA, 01845, USA.

OBJECTIVE AND MOTIVATION: The goal of this work has been to modernize the self-referencing probe used to measure weak biocurrents, originally employed by Jaffe et. al. back in the mid 1970s [1]. This was the probe used to demonstrate the presence of weak currents around developing chick embryos and a number of other organisms. The motivations of the present work are twofold: first to modernize a useful probe which has become somewhat dated, and second to have it available to the author for real time, in vivo bioeffects experiments.
METHODS: The self-referencing probe uses as its "head" a thin, insulated wire that vibrates in a circle of diameter 50 mm or so depending on the application. The tip, which is electrically exposed, is moving in a weak potential field. The signal from the tip is amplified at the base of the wire, then amplified again, then processed. The original probe utilized a lock-in amplifier, which although it worked well was a setup that required a technician on hand throughout any experiment. The requirement of a full time technician effectively made it an expensive instrument to use. The present design is a robust, modern version that does not require the attention of a technician. The components include the self-referencing probe head unit, which is a wire vibrated with the use of two magnets, first stage amplification, second stage amplification, detection of the signal with a Data Translations A/D card, and the processing of the signal via software.
SUMMARY: The various components of the instrument work well, and prototypes have been completed. These include a prototype driver for the vibration, integration of the Data Translations A/D card into the system, and development of a Visual Basic program that takes care of all aspects of running the probe. The VB program has a front end to set the probe up, it runs the A/D card, processes the data acquired by the A/D card, and presents the results to the user.
CONCLUSIONS: The components of the redesign are complete in the form of prototypes, and will be discussed in this poster. An integrated, working version is planned for testing during spring/summer of 2002. The probe holds promise for doing real time, in vivo bioeffects experiments. For example, the endogenous currents around a chick embryo could be measured before, during, and after a wide variety of ELF waveforms, to see if there are any real time effects of such exposures.
[1] Jaffe, LF, Nuccitelli R (1974). "An ultrasensitive vibrating probe for measuring steady extracellular currents." J. Cell. Biol. 63:614-628

MECHANISMS OF INTERACTION: BIOLOGICAL TRANSDUCTION – ELF

P-18 Student

STATIC MAGNETIC FIELDS AFFECT REFRACTORY CHARACTERISTICS IN BULLFROG SCIATIC NERVE EXCITATION. Y. Eguchi[1*], H. Tatsuoka[2], and S. Ueno[1]. [1]Department of Biomedical Engineering , Graduate School of Medicine, University of Tokyo. [2]Department of Neurobiology and Neuroanatomy, Graduate School of Medicine, University of Chiba.

OBJECTIVE: The effects of static magnetic fields on nerve excitation processes are poorly understood. This study focuses on the dynamic membrane excitation and refractory characteristics of nerve fibers exposed to static magnetic fields of 8 T. The experimental results show that the compound action potentials (CAP) in the relative refractory period are affected by the magnetic fields (Fig.1). The recovery process during excitation was enhanced by long-term static magnetic field exposure after the inactivated state in the refractory period.
METHODS: The dissected sciatic nerve bundle of a bullfrog, *Rana catesbeiana*, was placed on platinum electrodes in an acrylic moist nerve chamber. The chamber was positioned at the center of a superconducting magnet (Oxford, U.K.),100 mm in diameter and 700 mm long, which produced magnetic

fields of up to 8T at its center. The ambient temperature in the magnet was maintained at 24°C ± 0.2 °C by circulating temperature-regulated water in a coiled tube. The nerve bundle was electrically stimulated and then exposed to 8 T static magnetic fields for the long axis of the nerve bundle parallel to magnetic field during the nerve excitation processes. To investigate the possible magnetic field effects on the nerve membrane excitation and refractory processes, double pulse stimulation at varying intervals between the two pulses was applied to the nerve bundle, and the CAPs were measured during magnetic field exposure. The CAPs were measured for two groups, the control group (without magnetic field exposure) and the exposed group (with magnetic field exposure for 3 hours), for 10 hours each.

RESULTS AND DISCUSSION: Figures 2a and 2b show the amplitudes of the CAP during the refractory period, which were normalized to the maximal peak of CAP after double pulse stimulation at varying intervals (1.0 ms, 1.1 ms, 1.5 ms, 2.0 ms) was applied between the two pulses. The peak of the CAP during the relative refractory period was gradually enhanced by 8 T magnetic field exposure for 3 hours compared to the control group. Although the mechanisms responsible for this acceleration have not yet been clarified, we hypothesize that the recovery process of membrane excitation was immediately accelerated by the magnetic fields just after Na^+ ion channels were inactivated. These results reveal the possible effects of static magnetic fields on the central and peripheral nervous systems, particularly the behavior of ion channels associated with nerve fibers.

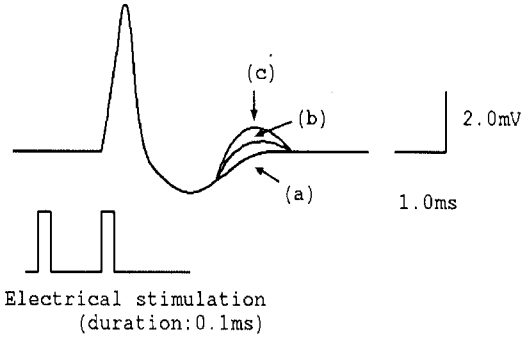


Fig.1. Compound action potentials (CAP) during a relative refractory period with and without magnetic field exposure.
(a) CAP responded to single pulse stimulation.
(b) CAP responded to double pulse stimulation without magnetic field exposure.
(c) CAP responded to double pulse stimulation with 8T magnetic field exposure.

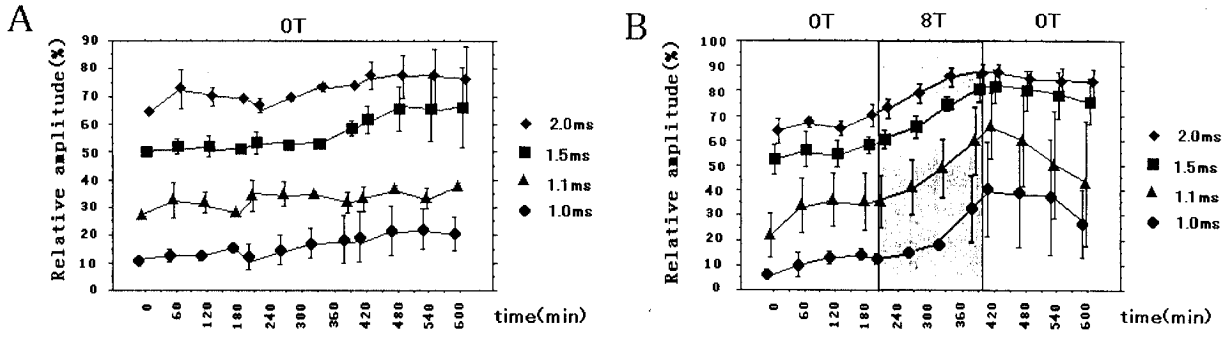


Fig. 2. The amplitudes of the CAP during refractory period, which were normalized to the maximal peak of CAP after double pulse stimulation at varying intervals (1.0 ms, 1.1 ms, 1.5 ms, 2.0 ms) was applied between the two pulses.
2a : Control group. N=2. Error bar is ±1SE. 2b : Exposed group. N=3. Error bar is ±1SE. The peak of the CAP during the relative refractory period was gradually enhanced by 8 T magnetic field exposure for 3 hours.

P-19 Student

EFFECTS OF MAGNETIC FIELDS ON CHAOTIC CALCIUM OSCILLATIONS IN MOUSE PANCRETIC BETA CELLS. F. Madec[1*], B. Billaudel[1], R. Charlet de Sauvage[1*], P. Sartor[2*] and B. Veyret[1]. [1]PIOM Laboratory, ENSCPB, University of Bordeaux 1, 33607 PESSAC Cedex, France. [2]Laboratory of Neurophysiology, UMR 5543, University of Bordeaux 2, 33077 BORDEAUX Cedex, France.

INTRODUCTION: Islets of Langerhans contain beta cells that secrete insulin in response to an increase in glucose concentration. This secretion is correlated with cytosolic calcium oscillations, synchronized in all of the islet's beta cells. These oscillations are very regular as long as the glucose concentration is sufficient. These properties of Langerhans islets are very useful in the study of biological effects of magnetic fields (MFs) since calcium processes have been suggested to be one site of interaction of MFs. We have previously applied magnetic fields on these very regular oscillations (at 50 Hz or at 0.5, 1 or 2 times the biological frequency of oscillations, with a field-strength of 0.1 mT, or static fields at 1 mT). There was no alteration of oscillation amplitude, frequency or system's organization under any of the exposure conditions.
OBJECTIVES: The aim of the present study was thus to compare these results with those obtained on chaotic oscillations, likely to be more sensitive to MFs. Therefore, we applied the MFs on chaotic calcium oscillations obtained at lower glucose concentrations. The long-term objective is to tell whether biological systems with altered functions (e.g., illness), are more easily perturbed by MFs.
METHODS: Islets were isolated from mice pancreas by the collagenase method. Islets were cultured at 37°C inside an incubator (95 % O_2, 5% CO_2) for 48 hours in RPMI-1640 medium supplemented with 10% Fetal Calf Serum, 100-IU/ml penicillin and 100-mg/ml streptomycin. Islets were loaded with the fluorescent dye Indo-1/AM (10 mM) and pluronic acid (0.03 %). After loading, islets were allowed to attach onto circular 30-mm coverslip, coated with polyornithine, inserted at the bottom of a perforated Petri dish. The 35°C-thermostated Petri dish was placed on the stage of an inverted microscope and the islets were superfused with 11.1-mM glucose KRB medium. Variable glucose concentrations in the medium were obtained using two pumps with different flow rates, connected to two bottles containing either zero or 11.1-mM glucose. At the end of the experiment, the glucose concentration was increased to 11.1 mM in order to verify that regular oscillations recovered (Figure 1 shows data from an experiment with exposure to sinusoidal magnetic field with a frequency equal to twice the biological frequency, and an intensity of 1 mT). The dye was excited in the UV at 355 nm and the ratio of the fluorescence emitted at two wavelengths (480 and 405 nm) was recorded using computer software (Axotape). The MF was applied during 7.5 min, following a 7.5-min control period, and before another 7.5-min control period. Exposure was performed using Helmoltz coils, connected to a generator with controlled frequency, phase, intensity, shape (sinusoidal or DC) and duration.

CONCLUSION: Several studies have given contradictory results on the biological effects of MFs, mainly on isolated cells. In this study, we have used islets of Langerhans, a very reliable biological model, able to give very regular oscillations over a long period. These oscillations, sustained by a process that involves inter- and intra-cellular communication, were not perturbed by MFs at low intensity and low frequency. Our objective is to determine whether less-organized system have the same properties. Moreover, it will be very useful to know whether chaotic systems if induced in diseases such as diabetes, are more sensitive to weak

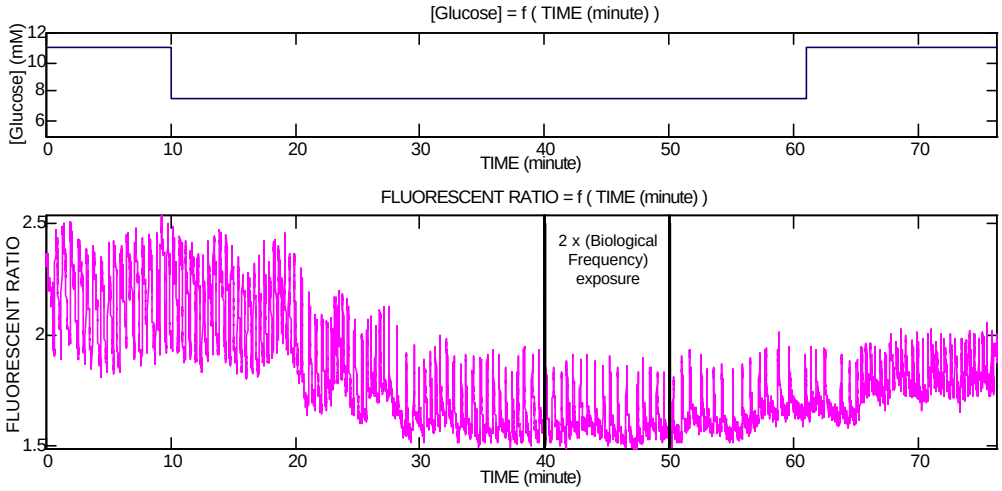


Figure 1 : Decreased of glucose concentration (up) and effect on islet calcium oscillation (down) of exposure to sinusoidal magnetic field, twice the biological frequency with an intensity of 1 mT, during the 30-60 minutes chaotic state.

perturbations. Experiments are in progress, the main difficulty being that islets have various sensitivities to glucose concentration. Therefore, we have to find the optimal glucose concentration for each islet (Figure 1).

P-20 Student

TEMPERATURE-DEPENDENT EFFECT OF 18Hz MAGNETIC FIELDS ON GAP-JUNCTION CONNEXIN-32 CHANNELS RECONSTITUTED IN LIPOSOMES. I. De Sena[*1], R. Schiavo,[*2] M. Liberti,[*2] G. D'Inzeo[2] and A. Ramundo-Orlando[*1]. [1]Institute of Neurobiology and Molecular Medicine-CNR, Via Fosso del Cavaliere, 00133-Rome, [2]Department of Electronic Engineering, ICEmB @ University "La Sapienza", Via Eudossiana, 18 00198-Rome, Italy.

OBJECTIVE: Gap junction channels mediate the communication between adjacent cells by the open-closed gating of an aqueous pore permeable to ions and small molecules in response to external stimuli and intracellular signals. 50 Hz magnetic field exposures have been postulated to suppress gap junctional intercellular communication in cells. Recently further investigation on this channelling activity has been claimed to understand how human exposure to electromagnetic fields could contribute to diseases such as cancer (1). We previously showed that hemichannel activity of gap junction connexin-32 reconstituted in liposomes could be enhanced by exposure to magnetic fields at frequency of 18 Hz selected by our previous proposed model based on Larmor precession theory (2). The objective of the presentation is to examine the temperature-dependence of this enhancement in gap junction.

METHODS: We have purified gap junction plaques from rat liver plasma membrane fractions and an *n*-octyl-β–D-glucopyranoside dialysis method was used to incorporate functional gap junction channels into the liposomes (3). Cytochrome c was loaded inside the proteoliposomes and its reduction upon addition of ascorbate in the bulk aqueous phase was taken as an index of channel opening. Aliquots of proteoliposomes (0.15 ml) were exposed for 60 minutes at dynamic B_{ac} and static B_{dc} 70 μT/18 Hz magnetic field at 16, 18, 22 and 24°C. Sham samples were also maintained at the above temperatures for 60 minutes. At the end of

the test time the changes of absorbance at 417 nm upon addition of ascorbate (0.3 mM) to the suspension of cytocrome-c loaded proteoliposomes were monitored.

RESULTS AND DISCUSSION: The opening of the gap junction hemichannel allows the diffusion of the ascorbate and a fast reduction of a significant fraction (44% and 33% at 24 and 22°C, respectively) of the intraluminal cytochrome c resulted. The later addition of Triton X-100, which permeabilizes the liposomes, further reduces the cytochrome c sequestered in the lumen of liposomes. No significant differences have been observed between 18Hz magnetic field exposed (A) and sham (B) proteoliposomes (Fig. 1) either at 22°C or 24°C. By decreasing the incubation temperature to 18°C and 16°C the opening of the gap junction channel in sham proteoliposomes was reduced to a 13% and 11%, respectively. Under these later experimental conditions a significant differences between exposed (18Hz) and sham proteoliposomes was observed (Fig. 2). Our results indicate that changes in the phase transition of proteoliposomes may influence the gating activity of gap junction channel more than may do ELF-EMFs exposure itself. On the other hand, ELF-EMFs observed effect at the lower temperatures could be direct more on the lipid membrane than on the gap junction connexin-32 channel reconstituted in.

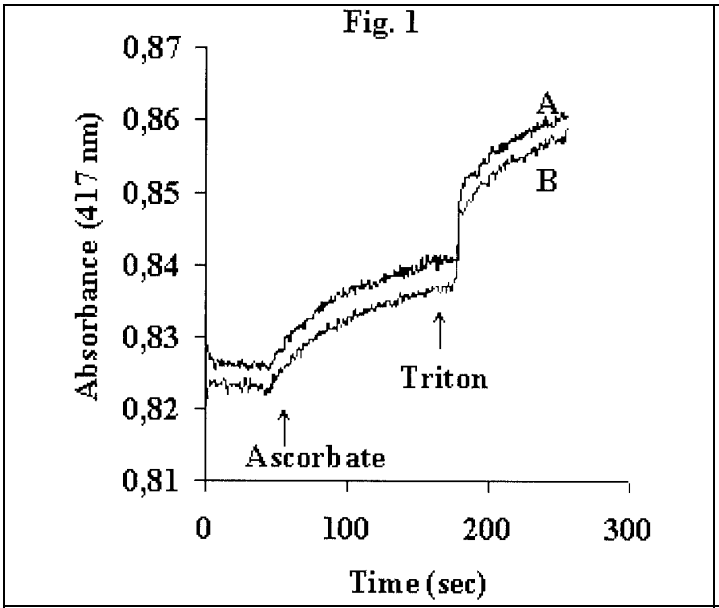


Figure1: Absorbance versus time of exposed to 18 Hz (A) and sham (B) proteoliposomes at temperature of 22 °C.

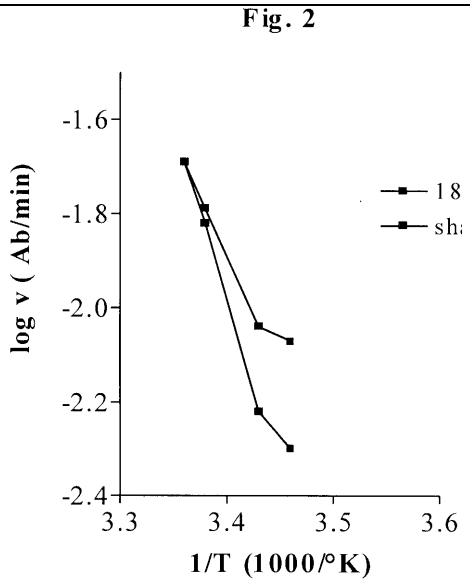


Figure 2: Absorbance versus time of exposed to 18 Hz (A) and sham (B) proteoliposomes at temperature of 18 °C.

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P-21

MAGNETO-OPTICAL EFFECTS IN ADHERENT CELLS AND INTRACELLULAR COMPONENT'S BEHAVIOR CHANGES. M. Iwasaka, S. Ueno. Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan.

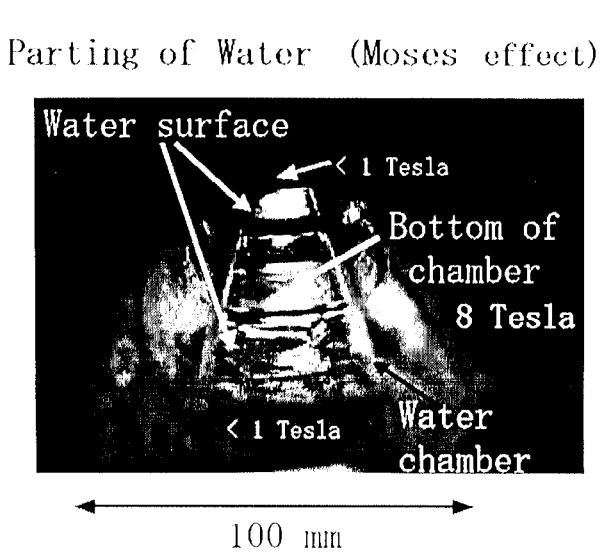
INTRODUCTION AND OBJECTIVE: Weak magnetic materials behave as non-magnetic materials under ambient magnetic fields, but diamagnetic properties such as the anisotropy of diamagnetic macromolecules exhibit a significant phenomenon under strong magnetic fields of the Tesla order. Previous studies reported the magnetic orientations of macromolecules such as actin fiber in a cell free system. In the present study, the magnetic orientations of intracellular macromolecules involving actin fiber were suggested by optical transmission changes under magnetic fields of over 10 Tesla. The purpose of the study is to investigate the possibility that strong magnetic fields affect cell component motions.
METHODS: Smooth muscle cells of rats (A7r5) were cultured in a glass petridish in a medium (D-MEM, 10% FBS) at 37 degrees centigrade. A linearly polarized light was utilized to detect the displacement of intracellular macromolecules under static magnetic fields. The smooth muscle cells were kept in the glass dish 30 mm in diameter, which was filled to more than 90% with the medium. Two polarizing plates enclosed the top and bottom of the glass dish, and the polarizing direction of the plates was normal to each other. Also, each polarizing direction was inclined 45 degree to the magnetic field direction. The light of the halogen lamp was introduced normal to the surface of both the polarizing plate (polarizer) and the petridish, and the light output from another polarizing plate (analyzer) on the top of the petridish was introduced to the photon counting system, which dispersed the light at 500 nm. The optical measurement system was set in the bore of a superconducting magnet, which produced static magnetic fields of up to 14 T.
RESULTS AND DISCUSSION: During the onset of magnetic field at 14 T, magneto-optical effects (Cotton-Mouton effect and Faraday effect) was observed, however after the onset, a gradual increase in the optical transmission was occurred and the transmission reached a plateau in two hours. In the case of smooth muscle cells orienting perpendicular to magnetic fields, cells showed nearly a 100% change in optical transmission; and cells being exposed parallel to magnetic fields showed about a 50% change. We observed that no distinct changes in cell morphology including cell membrane components occurred during three hours of exposure to the magnetic fields. Some possible mechanisms explaining the birefringence changes of cells were studied, and conclusive evidence was obtained when cells aligned in the same direction were exposed to parallel and perpendicular magnetic fields of 14 T. Based on the results, we concluded that intracellular component motion had a major impact in polarized light changes under a 14 T magnetic field. We speculated that unconstrained intracellular macromolecules, such as actin fiber and microtubules, rotated due to diamagnetic torque forces during the three hours of magnetic field exposure at 14 T.

P-22

MOSES EFFECT AND ITS INTERFACE BETWEEN BIOLOGY. M. Iwasaka, S. Ueno. Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan.

Moses effect, parting of water under high gradient magnetic fields, was observed when water was exposed to strong static magnetic fields of up to 10 Tesla order. The detailed mechanism was well explained by the diamagnetic property of water molecules in external magnetic fields. The discovery of both Moses effect and magnetic levitation of water drops stimulated physicochemical researches and resulted in reconstructing

the magneto-sciences. In the next stage, it is expected to apply the Moses effect to biology for the purpose of producing new medical applications and unique approaches to biology.
Interesting points of Moses effect from the point of view of biology and medicine can be indicated as follow.
Changes in shape of tissue boundaries can be induced by high gradient magnetic fields.
Wide differences of diamagnetic susceptibility and small differences of density will enhance the changes in tissue boundary shape.
Diamagnetic liquids, such as water and whole blood, change its surface profile when they are exposed to a magnetic field of 6 T with a gradient of 60 T/m. Fig. 1 shows the water surface under gradient magnetic fields of up to 8 T. The surface was parted by magnetic fields and the bottom of chamber was exposed to air. Oxygen molecules in air, which is a paramagnetic material, support the formation of parting water surface because paramagnetic oxygen gas increase the gas pressure in the point with a maximum magnetic flux density. The change in boundary profile of two kinds of liquid is described by Equation (1). K and ρ represents the diamagnetic susceptibility and density of liquid, respectively. Both increasing KA-KB and decreasing ρA-ρB results in an enhancement of the boundary profile change. Strong magnetic fields of Tesla order pass the living body, however, gradients of magnetic flux density in living tissues possibly transform the tissue-tissue and tissue-liquid/gel boundaries. Blood thrombus formation/dissolution process is a candidate for Moses effect in living tissues.



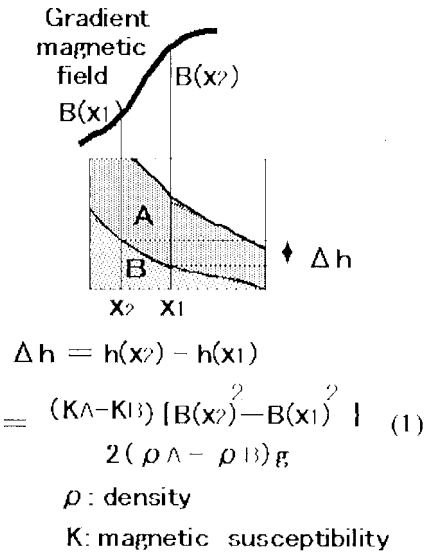


Figure 1. Boundary profile changes of liquids under high gradient magnetic fields. Left: Parting of water under magnetic fields of up to 8 T with a maximum gradient of 60 T/m. Right: boundary profile of two kinds of liquids (A and B) under gradient magnetic fields.

P-23

GRADIENT MAGNETIC FORCES ON WATER-CO_2-O_2 SYSTEM AFFECTED YEAST PROLIFERATION. M. Iwasaka[1], S. Ueno[1], M. Ikehata[2] and J. Miyakoshi[3]. [1]Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Tokyo 113, Japan. [2]Environmental Biotechnology Laboratory, Environmental Engineering Division, Railway Technical Research Institute, Tokyo 185-8540, Japan. [3]Department of Radiation Genetics, Graduate School of Medicine, Kyoto University, Yoshida-cho, Sakyo-ku, Kyoto 606-8501, Japan.

INTRODUCTION AND OBJECTIVE: Effects of oxygen concentration in both air and medium on the yeast proliferation under magnetic fields were discussed based on physical mechanisms of static magnetic fields. Yeast growth depends on dissolved oxygen. Strong and high gradient magnetic fields have an effect on the oxygen absorption in aqueous solutions. The effect of oxygen concentration changes on yeast growth under magnetic fields has not been investigated in past studies. The present study focused on the effects of gradient magnetic fields on yeast behavior, particularly, proliferation.
METHODS: Yeast, *Saccharomyces cerevisiae*, was incubated in a liquid medium at 30 degree centigrade. A sterile 15 ml cylindrical tube containing the yeast suspension was exposed to 7 ~14 T magnetic fields with a maximum gradient of 94 T/m. The cylindrical tube was filled 25% to 80%, and involved paramagnetic air in an upper part. The yeast density of the suspension was counted after dispersing the yeast homogeneously in the suspension. Light absorption at 600 nm of the suspension was measured after incubations with and without the magnetic field exposures. The numbers of cell in suspensions were counted to confirm the dependence of light absorption on cell density.
RESULTS AND DISCUSSION: When the yeast in the tube was not exposed to the air inside the magnet's bore, the proliferation rate of yeast under magnetic fields decreased by 29% compared to the control group with air-exposure after 16 hours of incubation. In contrast, when the yeast suspension was exposed to the air inside the magnet's bore, the proliferation rate under magnetic fields accelerated by 5%. After the yeast settled to the bottom of the tube, the yeast generated carbon dioxide gas in the suspension, and a concentration gradient of oxygen molecules occurred in the suspension. The yeast generated carbon dioxide molecules, and consumed paramagnetic oxygen molecules in the suspension. The result indicated that gradient magnetic fields decelerated the proliferation of yeast. It is considered that in the case of oxygen deposited solution, a concentration gradient of oxygen molecules occurred in the solution because the oxygen concentration beneath the air-solution boundary was higher than in the bottom part. When there was a gradient of magnetic susceptibility in a vertical line, magnetic field gradient in a horizontal direction causes a convectional flow. The induced convectional flow increased the oxygen concentration around yeast, and the yeast's proliferation was enhanced.

P-24

Abstract moved to 18-6.

P-25

LOCAL VARIATIONS IN CELL SURFACE CHARGE IN TISSUE. B. Greenebaum. Dept. of Physics, University of Wisconsin-Parkside, Kenosha, Wisconsin 53141-2000, USA.

INTRODUCTION: When electric or magnetic fields induce currents in tissue, it is easy to see that the fields and currents, which are essentially confined to the intercellular space by the low conductivity of the cell membrane, twist and turn while passing through the tissue (Barnes, 1992; Wachtel, 1992). However, models for the mechanism of interaction between applied low frequency fields and cells have generally

looked at either single cells or macroscopic averages, not at the level of cells in tissue. Basic electromagnetic theory requires changes in charge density on the boundaries of regions in which the field and current density change direction. In this case the changes occur in the charge density on or near the exterior surface of the cell membrane. These changes presumably come about as ions or electrons move between the body of the intercellular fluid and the neighborhood of the double layer at the membrane. If these local nonuniformities in cell surface charge, which are too small to alter overall cell membrane potentials, sufficiently exceed the normal local fluctuations in surface charge as charge carriers move in and out of the double layer region, their local fields may be strong enough to influence the functioning of ligand binding sites, channels and other cell membrane constituents and hence affect cell functions.

OBJECTIVES: We calculated local fields, potentials and charge densities in the space between cells in two simplified, but realistic models. We find that the number of additional local charges in the region of highest field intensity between two cells approximates v*n*, whre *n* is the expected fluctuation in the number of charges needed to produce the normal transmembrane potential in the same region, when the externally applied field in the tissue is ~1 mV/m for circular cross-section cells and at lower fields for cells with square cross-sections and rounded corners.

METHODS: The calculation modeled unpolarizable cells with non-conducting walls using the partial differential equation-solving package (FlexPDE, PDE Solutions, Inc., Antioch CA USA). Results agreed with approximate analytical calculations. We modeled two-dimensional arrays of very long cells, as in muscle, for example. Intercellular distances were 5 μm , cell cross sectional dimensions were 50 μm and dielectric constants and conductivity were 100 and 2, respectively, characteristic of physiological fluids. Normal cell membrane surface charge concentration was taken to be s_{av} = ~ 6 x 10^{-5} C/m^2 for V_m = -50 mV across a 7 nm membrane.

RESULTS: DC and 60 Hz results are similar. For closely packed circular cross-sections and $E_{applied}$ =1 mV/m, the calculated local surface charge variation (s (x) - s_{av}) has opposing maxima and minima of about ± 2 x 10^{-8} C/m^2 and K 0.7 x 10^{-8} C/m^2. Since these concentrations are only 3 or 1 x 10^{-4} of s_{av}, respectively, V_m would change by only that small fraction. However, the local charge signal-to-noise due to the altered charge, measured as S/N = [number of extra charges causing (s (x)- s_{av})]/[v(average number of charges causing s_{av})] is 0.4-0.2, respectively, very close to unity. For a 50 μm square cross-section with rounded corners (2.5 μm radius), arranged in offset layers like bricks in a masonry wall, (s (x) - s_{av}) has values that peak where the straight side ends and the curved corner begins; at these points (s (x) - s_{av}) = ~ 0.1-1.0 s_{av} and S/N >1 in that narrow region. In general, S/N, calculated here for $E_{applied}$ = 1 mV/m, increases linearly with applied field strength.

DISCUSSION: The variation in local charge appears to represent a significant local fluctuation in the arrangement of counterions and/or water molecules at the membrane surface and hence in the local environment of membrane channels, receptors and other proteins. For alternating fields, the variation would be cyclical. This possible mechanism for field interaction with biological organisms requires further modeling and experimental investigation. Modeling continues using polarizable cells. One possible experimental test, using higher applied fields than those discussed here, would be to investigate whether electroporation in tissue (or tissue culture) occurs most significantly at locations where intercellular distances are smallest.

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P-26

APPLICATION OF THE TURBULENT MAGNETIC FIELD (TMF)-INFLUENCE FOR DECREASE OF THE POSTOPERATIVE LYMPHORRHEA AFTER SURGICAL TREATMENT OF THE LOCALLY SPREAD BREAST CANCER. V.P. Letiagin*, Ya.V. Dobrynin*, Yu.L. Rybakov*, N.V. Protchenko*. Russian Cancer Research Center of the Russian of Medical Sciences, Research-Production Center "Zdorovye", Moscow, Russia.

OBJECTIVE: This investigations was study of effect TMF - influence on the patients with the postoperative lymphorrhea after radical operations (mastectomy, resection) of the mammary gland. TMF-influence carried out by magnetotherapeutic clinical unit “Magnetoturbotron”. As an object of treatment and examination it was decided to take a group of 106 patients of different age (32-68 years) with locally spread breast cancer. From them in control group there were 56 patients, in group with TMF-influence - 50 patients. At the majority of the patients (87 cases) alongside with the basic tumour took place the metastasis of regional lymph nodes. In the preoperative period all patients received the radiation exposure of mammary gland and adjacent regions in total dose of 40 - 46 Gy combined with chemotherapy according to Cooper’s modified scheme. To the majority of the patients the operation radical mastectomy with muscles pectoral preservation was made. During operations on the mammary gland to the patients the regional lymph nodes were deleted. The formed during operation cavity was drained. After the termination of the outflow of both blood and clots and starting conglutination of the inside surfaces of the postoperative cavity a drainage was removed. Next in a zone of operational intervention the hypodermic accumulation of a liquid (lymph) was observed. The collected liquid periodically was deleted by means of the puncture. Every day the accumulation of the lymph could achieve the level of 200 - 600 ml. The treatment of the postoperative cavity was accompanied by a fading away lymphorrhea.
METHODS: The treated patients after the specified drainage for the purpose of reduction of the postoperative lymphorrhea were exposed to TMF-influence. Within the period of treatment of 5 - 12 procedures the patients were receiving daily TMT-influence (3,0 mT, 100 Hz) with the duration of 40 - 60 minutes. All the patients standed the procedures well. The control group of the patients did not receive the TMF-influence. In this group the duration of the postoperative lymphorrhea (within a distance of a drainage before the termination of the hypodermic accumulation of a liquid) lasted from about of 6 up to 34 days. The average duration of the lymphorrhea in control group made 13 days. In group of the patients with TMF-influence the general duration of the lymphorrhea had been changed from 3 up to 33 days that made on average 8 days. To the majority of the patients lymphorrhea by the application of TMF-influence during 24 hours had been decreased from 200 - 300 ml to 50 ml or even absolutely stopped within 2 weeks. After 5 - 6 procedures of TMF-influence on 8 patients the lymphorrhea with 600 ml for one day stopped completely.
CONCLUSIONS: The carried out investigations demonstrated the certain efficiency and utility of application of TMF-influence with the purpose of reduction of volumes and terms of the postoperative lymphorrhea after extensive operations on the mammary gland.

P-27

TREATMENT OF LATE LUNG CANCER PATIENTS USING MICROWAVE COMBINE WITH LOCAL CHEMOTHERAPY VIA FIBROBRONCHOSCOPE. J. Fa-Guang[1], L. Tong-Gang[1], F. En-Qing[1], C. Dong-Ling[1], X. Yong-Hong[1] and H. Gian[2]. [1]Department of Respiratory, Tongdu Hospital, Fourth Military Medical University. Xi'an 710038; [2]Nanjing Great Microwave Electronics Lab. 210013.

To improve the treatment effect of bronchus obstructive late lung cancer, we had cure 25 patients using microwave combine with local chemotherapy via fibrobronchoscope and total body chemotherapy. The effect is perfect.
MATERIALS: Observing group (microwave+local chemotherapy+total chemotherapy) 25 patients, 20 male, 5 female. Range 42-78 yr of age (mean 52yr), small cell carcinoma of lung (7 cases), Non-small (18 cases). Control group(only total chemotherapy) 25 patients, 19 male, 6 female, range 38-72yr of age (mean, 53yr), small cell carcinoma of lung (8 cases). The two groups didn't significantly in terms of age, sex cytological classificantion and staged.
METHODS: The two groups were treated by ICE chemotherapy method, (Iphhosphamide 1.5-2.0g/m2 in first days, adriamycin 40-60mg/m2 in first days, cisplatin 400-600mg/m2 in first days), and repeated after 3 weeks. A course of treatment include 3 cycles. The observing group were treated by microwave combine with local chemotherapy via fibrobronchoscope in 7th day after total body chemotherapy. Microwave probe through biopsy to scorch the tumour for times, then Cisplatin 30mg was injected in tumour's center. The treatment effect was estimated after a course.
RESULT: l. The near future curative effects. We valued all fifty patients after a treatment course (three cycles). As the results, in observing group, 8 cases were the apparent effective (32%), 13 cases were effective (52%), 4 cases were non-effective (16%), the total effective is 84%. In control group, 3 cases were the apparent effective (12%), 6 cases were effective (24%), 16 cases were non-effective, the total effective is 36%. There were obvious imparity in statisic ($P<0.01$)
2. Existence rate, All existing patients follow-up survived over two years. Follow-up survived patients was treated by total body chemotherapy a course (3 cycles) every half year. Every patients was treated by total body chemotherapy a course (3 cycles) every half year. Every patients was treated 1-3 course in different level. The result is existence rate: observing group 92%, control group 60% in a half year; Observing group 60%,control group 48% in one year; observing group 32%, control group 12% in two years. The two group' existence rate were obvious imparity in statisitic ($P<0.05$).
DISCUSSION: l. This group brief on microwave combine chemotherapy can diminish or disapear tumour in bronch-bumen, expand atelectasis. It improves clinical symptom lengthen life cycle. 2. It is a kind of effective new method of treatment with development prospects, it has clinical practice and popularize value.

P-28

USING RF HYPERTHERMIA TO AVOID STRESS RESPONSE. N. Szasz[1], O. Szasz[2*] and A. Szasz[2,3]. [1]Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. [2]Szent Istvan University, Godollo, H2103, Hungary. [3]University of Strathclyde, Glasgow, Scotland G1 IXQ, UK.

INTRODUCTION: The use of electro-therapeutic and electro-diagnostic methods employing DC through microwave frequencies is becoming widespread in cancer therapy. Most of the therapies involve focused energy deposition for hyperthermia treatment. Hyperthermia is coupled with the synthesis of heat shock proteins or stress proteins (SPs), which aid the adaptation of tumor cells to stress, including radiotherapy and chemotherapy, mainly by suppressing apoptosis and stabilizing proteins[1]. The results of hyperthermia are therefore often unpredictable and can be harmful to the patient.

OBJECTIVE: Design an electro-magnetic "hyperthermia" (EMHT) treatment of tumors that avoids stress response and adaptation by targeting the extra-cellular matrix (ECM) of the tissue, thus disrupting the cellular membrane before heat diffusion induces SP synthesis.
Theory: Intracellular SPs impede hyperthermia treatment efficacy by developing stress tolerance and suppressing apoptosis of the tumor cells. Since SP synthesis is the greatest hindrance to hyperthermia efficacy, blocking SP production during hyperthermia is our main goal. Heating the ECM increases ion-mobility and intensifies the metabolic rate of tumor cells. Heat-flow also alters cell membrane permeability, generating extra ionic influx, which decreases membrane potential and increases cellular osmotic pressure through the Onsager-relation. These effects promote cell membrane damage and lead to cell death before the activation of undesired intracellular SP synthesis. On the other hand, SPs secreted into the ECM enhance apoptosis[2], support tumor-specific antigens[3], and stimulate an inflammatory response[4]. Therefore, despite the negative effects of SPs intracellularly, their release into the ECM is desired for pro-inflammatory stimulation. After membrane damage, any already synthesized SPs are released into the ECM and initiate the desired immune response against tumor cells. The use of an electric field to rapidly heat the ECM (but not the cells) is therefore desired. Penetration of the electric field into the cells at frequencies below 15 MHz is negligible, with the energy absorbed mainly by the membrane and the ECM. Generally, the lower the frequency used, the greater the penetration depth, and also the lower the energy deposition. Near certain frequencies, the inhomogeneity of the complex dielectric constant and vascular properties further aids in focusing the energy to malignant areas.
METHODS: Several EMHT devices have been developed to target a wide range of malignant sites with great success: DC, low RF (240 kHz), and high RF (13.6MHz) capacitively coupled systems are used for surface, intracavital, and deep-seated tumor treatments, respectively. These devices use a sinusoidal carrier frequency modulated by 1/f noise and amplitude feedback.
Results and Discussion: Clinical results show a significant synergy between EMHT and other treatments (e.g. chemotherapy, radiotherapy, and surgery) in effectively destroying tumor cells and improving patient survival rates. For example, the expected survival rate for colo-rectal carcinoma using chemotherapy alone is 48-58% and 14-19% for one and three years, respectively. Using EMHT[5] along with chemotherapy increases survival rates to 91±3% and 31±6%, respectively. Unlike EMHT, a combination of classical treatments and traditional hyperthermia has not been shown to improve survival rates consistently[6]. Even brain malignancies (astrocytoma, glioblastoma), where other treatments fail, can be successfully treated with EMHT. Results show drastic edema decrease due to the non-thermal effects of EMHT, as well as a significant regression of the tumor in most cases. This treatment is highly selective, safe, and provides all of the benefits[7] of conventional hyperthermia without initiating harmful stress adaptation.
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I would like to thank P. Siparsky for all his help.

DIAGNOSTIC – ELF

P-29 Student

Abstract not accepted for publication in the Abstract Book.

THERAPEUTIC – RF

P-30

FIBROPTIC BRONCHOSCOPY INTERVENING MICROWAVE TREATMENT ON INFLAMMATORY TRACHEA STENOSIS. X.M. Sun, X.Y. Chen, X.H. Wei, X.Q. Liu, Department of Respiratory, The First Hospital of Xi'an Jiaotong University, P. R. Chian, 710061.

To treat inflammatory trachea stenosis associated with any factors such as infection is one of the difficulties to internal physicians. In order to investigate new non-operative treatments for the disease, we have tried treating 21 cases with microwave and bronchus perfusion intervened with fibroptic bronchoscopy (FB), and obtained satisfying results as well as some useful experience.
MATERIAL AND METHODS: FB vision and pathologic findings- Observed with FB, 21 patients had different symptoms: bronchus mucous congestion, edema, ulcer, necrosis, unsmooth lumen, nodosity of polyp eminence or luminal stenosis of occlusion. The pathologic findings were bronchus mucous inflammation, inflammatory granuloma or tuberculosis inflammation with tyroid necrosis. One to three mecobacteria tuberculosis were found in seven patients.
THERAPEUTIC METHOD: Before the procedure ,we gave patients some medicine and performed according to the routine of operating FB. Meanwhile about 55ml lidocaine was given to perform thyrocricoid puncture for intra-bronchus anaethesia. First of all, we sucked up the secretion on the affected parts with FB, or poured 50ml normal saline into the parts and sucked it out later. Then using microwave probe through the biopsy to scorch the narrow bronchus or inflammatory granuloma. The microwave power was 60-100w, scorching times was for 5-20, each time was 8-10 seconds. To the patients with granuloma with granuloma and necrosis, we removed the granuloma and necrosis tissue with crocodilian aperture tongs. At last poured some gentamycin, amikacin or rimifon into the affected parts. The procedure was performed one or two times a week. We observed whether the patients had hemoptysis or stethocatharsis 3 or 5 days later.
RESULT: 21 patients were treated differently from 1-9 times. Most of them for 4-5 times. FB showed that local bronchus pathological changes took a great turn for the better and the stricture lumen didn't exist any longer. One patient suffering from right middle and lower lobe atelectasis recovered completely after he was treat twice. 6 patients with right middle lobe atelectasis recovered within the next 3 months.
DISCUSSION: It is a new and safe way to cure local bronchus congestion, edema and inflammatory granuloma with FB intervening microwave to relieve inflammatory bronchus atenosis. Its effect is satisfied.

RISK, SAFETY STANDARDS AND PUBLIC POLICY-RF

P-31

PUBLIC EDUCATION AND RISK COMMUNICATION REGARDING THE SAFETY OF WIRELESS TELECOMMUNICATIONS. R.F. Cleveland, Jr.[1], J.T. Bushberg[2], C.K. Chou[3], J.A. Elder[3], J.C. Lin[4], and R.D. Owen[5]. [1]Federal Communications Commission, Washington, DC 20554, USA. [2]University of California, Davis, Sacramento, California 95817, USA. [3]Motorola Florida Research Laboratories, Plantation, Florida 33322, USA. [4]University of Illinois, Chicago, Illinois 60607, USA. [5]Food and Drug Administration, Rockville, Maryland 20852, USA.

With the increasing use of telecommunications technology there has also been a concurrent increase in public awareness and concern over the safety of radio frequency (RF) electromagnetic energy. For example, in the United States, the US General Accounting Office, in a recent report, directed the Federal

Communications Commission (FCC) and the Food and Drug Administration (FDA), to devote significant resources to providing more comprehensive and readily available information to the public on this topic. In general, factors relevant to educating the public regarding potential risk from telecommunications and other RF technologies deserve careful analysis and study. In preparing for a recent international workshop on the safety of wireless technology, members of the US delegation spent a significant amount of time considering these factors. We have found that clear, concise and unbiased information is not always readily available for the public. In addition, controversy is often created by such things as the existence of conflicting scientific data, inaccurate or distorted media reports, differences in safety standards from country to country, and by confusion over the meaning of scientific terms such as "radiation." Also, it is generally true that people and the media tend to focus more on adverse effects than on beneficial (e.g., medical uses) effects. Therefore, a single study showing a potentially adverse RF biological effect may not easily be offset by numerous studies that fail to show an association. It is important, first of all, to adequately explain to the non-scientist how science "works." There are many experimental issues that members of the public typically do not understand and would not normally consider in deciding about relative risk. Examples are the complexity of RF dosimetry, possible inattention to significant engineering or biological details in experimental procedures, and situations where "positive" effects are later shown to be artifacts. In addition, the public usually does not understand that no individual research report can definitely answer most scientific questions and that conclusions must be based on consensus drawn from cumulative evidence. Various sources of information on RF safety are available from governmental and private sources. For all of these sources we believe the following principles should be followed: (1) simplify technical information, (2) establish trust in sources of information – the independence of a source of information is important, (3) use appropriate analogies when discussing risk, (4) explain the scientific process, and (5) address concerns of the public honestly and openly. Information on RF safety can be provided through Web sites, special consumer phone lines and through printed material. In the USA, the FCC and the FDA are developing a joint Web site on the safety of wireless communication technology and special telephone numbers have also been made available for this purpose. This paper will provide further details and discussion on these topics, and it will provide examples of risk communication and public education currently underway in the United States.

This paper is based on a presentation made in October 2001 at the International Scientific Workshop on Health Aspects of Mobile Telephony held in Brussels, Belgium. The views expressed in this presentation are those of the authors and do not necessarily reflect the official views of the Federal Communications Commission or the U.S. Food and Drug Administration.

P-32

IMPLEMENTATION OF THE NEW IEEE STANDARD FOR COMPUTATIONAL DOSIMETRY.

J. Fröhlich*, E. Cherubini*, N. Kuster. Foundation for Research on Information Technologies in Society (IT'IS), Swiss Federal Institute of Technology (ETH), Zurich, Switzerland.

INTRODUCTION: An IEEE standard was evaluated by the working group SCC – 34, subcommittee 2 [1]. This standard is meant to take all situations into account arising from compliance testing of mobile phones. Body tissue and tissue belonging to extremities are evaluated separately. The evaluation of the peak average SAR over 1 and 10g is a demanding task. Therefore the implementation of such a standard has to be very efficient in terms of the computational language used and in terms of algorithmic aspects.

OBJECTIVE: The algorithm shall be implemented in order to be able to evaluate the peak average SAR within arbitrary highly inhomogeneous structures over a cube containing a given mass. The distribution of the average SAR at every location can be visualized. The implementation is validated using different canonical examples that can be compared to measurements.

METHODS: The standard is implemented into the post-processing unit of the simulation platform SEMCAD [2]. The implementation in C++ uses updating schemes that allow the iterative as well as the

direct solution of the arising equations for the different situations. For iteratively searching the dimension of the resulting cube containing the required mass an inverse polynomial approximation is used. The direct solution is based on the cardanian formulas for cubic equations.
RESULTS: The new standard of the IEEE was implemented for general non-homogeneous grids. An iterative approach based on inverse polynomial approximation is compared to the direct solution for the resulting extensions of the cube containing the required mass. The algorithm is applied to the SCC34 benchmark problem as well as to simulations of a standardized measurement phantom. For these two applications a variety of measurements and simulations exist. Finally an example of numerical compliance testing of a real mobile phone at the human head is presented.
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P-33
A STATUS REPORT ON THE INTERNATIONAL SAR INTER-COMPARISON FOR WIRELESS PHONE CERTIFICATION. C. Davis, E. Merideth, N. Vakili, B. Beard. Department of Electrical and Computer Engineering, University of Maryland, College Park, MD 20742, USA.

INTRODUCTION: Several national and international organizations have established guidelines for human exposure to radio frequency energy. In the USA the Federal Communications Commission (FCC) has promulgated exposure guidelines with which wireless communication devices must comply [1]. The FCC guidelines specify maximum SAR values that are permitted over spatially averaged regions within the body. Specifically, these guidelines require hand-held wireless phones not to exceed an SAR of 1.6 W/kg averaged over any one centimeter cube. Other countries have their own specific guidelines.
SAR Assessment: It is not possible to place probes in the head of a wireless phone user to determine the actual spatial SAR distribution. Instead what is done is to make measurements on absorbing phantom models of various degrees of complexity, and by comparing theory and experiment obtain conservative estimates of the SAR in the human head [2]. The most complex models of the head that are used in this way are based on MRI images, subdivided into volume pixels (voxels) by tissue type, with a specified complex dielectric constant for each voxel. Theoretical analysis of SAR distribution is carried out by modeling the antenna of a source placed close to the head and solving Maxwell's equations by various numerical techniques. The two most important phantom models that are used for SAR experiment and comparison with theory are (1) a flat phantom, designed to constitute an infinite absorbing half-space adjacent to a standard dipole antenna or wireless phone, and (2) an anthropometric phantom model of the human head and torso. Both phantoms are generally fabricated as thin shells of low-loss material, which is filled with a dielectric liquid whose dielectric properties are an average value for the values measured for the tissues in the head (especially brain tissue).
SAR Inter-Laboratory Comparison: The University of Maryland and the United States Food and Drug Administration are currently coordinating an international effort to standardize the test procedures used for assessing the SAR values that result from the use of commercial wireless phones. The first phase of this inter-laboratory comparison is currently underway. A flat phantom and mounting assembly, complete with standard dipole antennas for both the 900MHz and 1800MHz wireless phone bands, power meters, couplers and cabling, is currently circulating among the laboratories of the various wireless phone manufacturers who are members of the MMF (the Mobile Manufacturers Forum). Each laboratory will mix its own simulant fluid according to a prescription developed by the IEEE, and whose dielectric properties will be verified by local measurement. Each laboratory will then map the SAR within the phantom on a specified grid, with the results reported as W/kg/W. Each laboratory will also calculate a $1cm^3$ average maximum

SAR. The results will be reported back to the University of Maryland/FDA, who will independently be making SAR measurements on identical phantoms, with identical antennas, couplers, cabling, and power meters. The variables among the various laboratories include (a) the E-field probes used within the phantom, (b) their method of calibration, (c) the locally produced simulant fluid, (d) the scanning system, and (e) the measurement electronics. This inter-laboratory comparison will serve to verify the degree of reproducibility that can be obtained in relatively complex exposure assessments of this kind. It will also provide a framework for establishing the measurement procedures required for wireless phone certification. We will report on the procedures used for independent E-field calibration, simulant fluid verification, and SAR determination, and provide an updated report on the status of the MMF SAR inter-laboratory comparison project. This work follows closely the emerging measurement protocol being developed by an IEEE Standards Coordinating Committee.

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THEORETICAL AND PRACTICAL MODELING -ELF/RF

P-34

MODELLING OF ELECTRIC POTENTIAL BAND FORMATION ALONG THE CELL MEMBRANE. A.I. Lavrova, T.Yu. Plusnina, G.Yu. Riznichenko. Department of Biophysics, Biological Faculty, Moscow State University, Vorobjovy Gory, Moscow State University, 119899, Moscow, Russia.

The physiological role of some electric phenomena such as propagation of nerve impulse, oscillations of membrane potential is well studied. But there are some processes that have no clear physiological sense. In particular, the non-uniform distribution of electric potential along the surface of cell of some species of green alga *Chara* and *Nitella*, an asymmetric distribution of ? $?^{2+}$ channels over zygote of marine brown alga *Fucus*. As a rule potential distribution combines with ions distribution along cell membrane. The consequence is an asymmetric ?? distribution along plasmalemma of green alga *Chara* and *Nitella* that is responsible for ions currents between «alkaline» and «acid» bands and ? $?^{2+}$ currents between the apical and rhizoid poles of *Fucus* zygote. Apparently such distributions can play some role in morphogenesis.

The purpose of this work is to propose the mechanism of non-uniform potential distribution considering as an example cell membrane of algae *Chara*.

To construct the model we used partial differential equations, describing the system dynamics in time and in space. Elongated construction of the cell allowed to research one-dimensional problem. We supposed that considering nonlinear processes are connected with acting of proton ATPase, providing active transport, and proton channels, providing passive transport.

Offered mechanism is based on positive feed-back loop between proton flux and transmembrane potential. It is supposed that the band formation takes place due to nonlinear character of ion fluxes across the membrane. This flux consists of active and passive components. Active flux is provided by the ATPase pump activity. We obtain equations for proton concentration changes on the external side of the membrane in accordance to ATPase kinetic scheme. Firstly the scheme with six enzyme conformational states was considered. The hierarchy of time processes allowed us to reduce the scheme to two enzyme conformational states due to the existence of fast and slow transitions between the different conformation states of ATPase. Slow transitions are connected with the ? $^{+}$ transfer from internal to external membrane side and transitions

of free enzyme. The proton transfer constants depend on the membrane potential. Fast processes are connected with interactions of the protons and ATP with enzyme. To describe electric potential behavior we use the cable properties of the system. The total membrane current was consisted of the capacity current and ionic current. Ionic current was consisted of ATPase ionic current and leak current. The last includes all the other ionic fluxes.
Analytical and numerical study of the above system showed the existence of the oscillative regimes damped and undamped at the certain parameters of the system, that in agreement with the experimental data. Having studied the reaction-diffusion system we found dissipative structures at some values of parameters. Increasing the parameter corresponded to light intensity results in increasing of the number of structures, which is conformed by experiments.
The model adequately describes the threshold character of banding formation of transmembrane potential and pH, depending of this of light intensity and ionic composition of media.

P-35

Abstract withdrawn by author.

P-36

DEVELOPMENT OF AN ANTHROPOMORPHIC NUMERICAL MODEL OF DIELECTRIC ANATOMY BY MRI. R. Pontalti[1*], L. Cristoforetti[1*], L. Sandrini[1,2*], M. Mazzurana[1,3*], C. Malacarne[1,4]* and A. Vaccari[1]. [1]ITC-irst - I-38050 Trento – Italy. [2]ICEmB - Genova – Italy. [3]University of Padova – Italy. [4]CET - Trento – Italy.

OBJECTIVE: Permittivity values have a dominant role in the overall considerations of interaction between electromagnetic fields and matter and in related applications such as electromagnetic radiofrequency dosimetry. There are still some concerns about the accuracy of published data and about their variability due to the heterogeneous nature of biological tissues.
Objective of this study was to provide an alternative method by which numerical dielectric phantom for dosimetric studies can be obtained. It makes use of a Magnetic Resonance Imaging (MRI) tomographer to directly map water content and consequently complex permittivities.
It is different by the commonly used techniques which are based on diagnostic images processing to identify different regions/organs and assignment of dielectric values to them. The proposed method provides a phantom in which permittivity varies with continuity even throughout the same organ and presents a wide spectrum of values which reflects the intrinsic realistic spatial dispersion of such parameter.
METHOD: Body acquisition was performed by a commercial clinical tomographer with two different interslice distances: 2 mm for the head region and 5 mm for the remainder of the body. The better resolution for the head was chosen because many dosimetric studies are devoted to the evaluation of the SAR deposited by personal communication devices such as cellular phone. In a second phase the images was interpolated to obtain a phantom inscribed in a volume of 900 × 255 × 130 voxels, 2 × 2 × 2 mm^3 each. Images were further slightly post-processed to improve the erected posture of the body particularly concerning the feet. Indeed it is important to have a realistic contact surface with the ground in case of dosimetric experiments in which this aspect may be crucial (e.g. low frequencies).
Water content was obtained by a T1 spin-echo sequence (TE = 15 msec) with two different repetition times (TR_{long} = 2400 msec and TR_{short} = 450 msec). The ratio between images at different TR allowed us to compensate for field inhomogeneity artifacts. While a linear dependence between T1 and water content has been determined with the exception of fat tissue, a clear monotonic relationship which would give permittivity (ε) and conductivity (σ) as a function of water content was not found. For this reason two

functions was semi-empirically determined ε (T1) and σ (T1) to match at best the average literature reported values, but still allowing the spatial dispersion throughout the same tissue to be modeled. Tissues providing low MRI signal (cortical bone and lung) were separately treated. It is important to underline that this method is able to distinguish the differences among bone regions, depending on whether they are cortical, trabecular or marrow.
Frequency dependence of ε and σ was modeled by the appropriate multiple relaxations functions with parameters provided by Gabriel [Gabriel S et al.].
CONCLUSIONS: Our results suggest that the use of a diagnostic MRI tomographer to automatically obtain digital complex permittivity maps of the human body may be a valid alternative to the organs and anatomical structures segmentation. Furthermore the proposed technique allows the model to take into account a wide range of permittivity values inside the same anatomical region or organ.
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P-37

10 mW/cm^2 LEVEL OF EMF IN STANDARDS OF RUSSIA AND 1000 mW/cm^2 LEVEL IN ICNIRP'S RECOMMENDATIONS (experimental studies for the characterising of these differences).
Yu. Grigoriev, V.S. Stepanov, L.A. Tomashevskaya, A.V. Shafirkin, A.L. Vasin. State Research Center-Institute of Biophysics, Moscow, Russia.

INTRODUCTION: Discussion of EMF standards' levels for the population in GHz range at 10 μW/cm^2 level (Russia) and 1000 μW/cm^2 (ICNIRP, European countries and USA) have been continuing more than 30 years. There are two points of view on the MPE, the first – 10 μW/cm^2 level is more stringent, another one – exposure with 1000 μW/cm^2 power density may be harmful for population.
In 1957 in USSR the MPE was established at 10 μW/cm^2 level. Since that time until today this level was not changed and no pathology occurred among populations. ICNIRP and USA have already reduced MPE from 10 000 to 1 000 μW/cm^2.
Existing differences in MPE were caused by absence of complete information about results of base experiments held in USSR (Russia) as well as by different approaches to their motivation (only thermal or additional non-thermal mechanisms were considered). Criteria of the risk assessment are also different.
OBJECTIVES and METHODS: Presented results of two groups of base studies were used for standards establishing in the USSR
The first group: five experiments with chronic exposure of EMF 850-1750 MHz in anechoic chamber with the frequency modulation of 400 Hz and with continuos wave (CW) were held. Power density was 10, 20, 50, 100, 140 and 500 μW/cm^2. Time of exposure was 6, 12 and 16 hours in a day during 4 months. 1150 rats were used in the experiment. Physiological, biochemical, immunological methods were applied in the experiments; generative function was evaluated.
The second group: eleven experiments with chronic exposure of EMF 2375 - 2750 MHz in anechoic chamber with the modulation frequency of 400 Hz and with CW were held. Power density was 1, 10, 50, 100, 500, 1000 and 2500 μW/cm^2. Time of exposure was 6 -16 hours in a day during 1-4 months. Exposure has continued from 1 to 45 days for the evaluation of possible cumulative effects. The series of experiments was conducted to investigate EMF influence in rats' foetus development which were exposed during the whole period of pregnancy (from 1 to 19 days). 2170 rats were used in experiments. Methods used were the same as in the first group. Cytology results and data of progeny study were presented.
RESULTS: «Dose-effect» ratio was obtained in both groups. In September 2002 in Russia (Moscow and Saint Petersburg) within the framework of the 3^d International Conference on "WHO EMF Biological Effects and Standards Harmonisation» a round table would be hold, where results (with protocols) will be presented for additional chronic experiments of bioeffects of 10 - 10000 μW/cm^2 power density range.

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Abstract not accepted for publication in the Abstract Book.

P-39

Abstract not accepted for publication in the Abstract Book.

CLINICAL DEVICES – RF

P-40

DO CURRENT RECOMMENDED PROCEDURES FOR ELECTROMAGNETIC IMMUNITY TESTING OF MEDICAL DEVICES SUFFICIENTLY ADDRESS DIGITAL RF EMISSIONS FROM CELL PHONES. J.J. Morrissey, M.L. Swicord. Motorola Florida Research Labs, 8000 West Sunrise Blvd., Ft. Lauderdale, Florida, 33322, USA.

OBJECTIVE: To perform initial tests on representative medical devices to see if current recommended procedures for medical device electromagnetic immunity adequately address exposure from TDMA-type digital cell phones emissions.

BACKGROUND: As cell phones continue to increase in popularity, use by doctors, nurses, staff, and patients within hospitals has also become increasingly common. In response to concerns over possible electromagnetic interference with sensitive medical devices, many hospitals initially introduced precautionary policies banning the use of cell phones in specific areas where critical medical devices were located. As wireless technology has evolved to offer increasing communication benefits for the healthcare sector, however, many hospitals have turned to better characterization, device management, and system engineering to control EMI issues to allow cell phones to be used by staff throughout their facility. Minimizing EMI has also been facilitated by new IEC recommendations in the revised Collateral Standard 60601-1-2 raising the level of recommended RF immunity for critical medical devices from 3 V/m to 10 V/m in the frequency range of 150 kHz – 2.5 GHz. Current testing to determine if medical devices meet these recommended immunity levels, however, has traditionally been performed using a frequency sweep with an 80% sine wave signal modulated at either 2 Hz or 1 kHz. The majority of TDMA-type digital cell phone signals, in contrast, are 100% modulated with more rapid rise times. The question has been raised that TDMA-type modulation might affect the threshold level for interference effects more than the modulation currently employed.

METHODS: We performed comparative testing in well-characterized anechoic chambers located at two different medical device manufacturer facilities. In each case, we used carrier frequencies of 835, 1850, and 2450 MHz for testing. We chose a limited number of devices from each manufacturer with easily observable endpoints for EMI. Testing was first performed at the three carrier frequencies using the standard 80% sine wave signal modulated at 2 Hz. The threshold for observable EMI was recorded and compared to previous internal compliance reports to confirm the accuracy of the test. Testing was then performed at the same carrier frequencies but with a 100% square wave signal (while digital communication signals are not true square waves, ever increasing rapid rise times allow a square wave to represent a worst-case scenario). An 11 Hz modulated square wave approximated iDEN TDMA technology and a 50 Hz modulated square wave signal approximated NADC TDMA technology. The threshold for observable interference effects were compared.

RESULTS & DISCUSSION: No obvious differences were observed using different signal modulations at any of the carrier frequencies (835, 1850, or 2450 MHz). Although the study is certianly not comprehensive, the results do not offer evidence to suggest that current recommended test procedures as outlined in IEC 60601-1-2 should be reconsidered to better address immunity against TDMA-type digital cell phone signals.

DOSIMETRY – RF

P-41

A MEASUREMENT OF FIELD DISTRIBUTION OF MOBILE PHONES HANDS-FREE KITS IN PLASTIC SPHERICAL AND FLAT PHANTOMS. K. Ahlskog*, V. Santomaa*, A. Toropainen*. Nokia Research Center, 00180 Helsinki, Finland.

OBJECTIVES: Purpose of this study was to investigate effect of personal hands-free kits to the human exposure of a GSM phone. Another objective for this study was to investigate the suitability of commonly used EMC leakage E-field probes for SAR measurements.
METHODS: The measurements were done at 900MHz GSM band with an EMC E-field probe and normal SAR -probes. Field exposure measurements were done with three different types of plastic phantoms: spherical, flat and human shaped twin-phantom commonly used in RF dosimetry. The phantoms were filled by brain tissue equivalent liquid (dielectric parameters $\varepsilon_r = 42.4$ and $\sigma = 0.83$ S/m). The field distributions inside the phantoms were measured with GSM phone alone and with hands-free kit connected. During the tests the phones were adjusted to transmit at maximum power level (2W). The measurement setup consisted of an E-field probe, spectrum analyzer, probe positioner and a PC controlling the positioner. The SAR measurements were made with commercially available SAR test system. The GSM phones were positioned to different distances from the outer surface of the phantoms and moved along the hands-free kits wire. The test setups were built on the polystyrene supports.
CONCLUSIONS: The EMC leakage probe was found to be unsuitable for measurement of SAR equivalent exposure in a phantom. Some of the results of E-field measurements with EMC probe were comparable than those reported in References [1] and [2]. However, the results were quite different from those obtained with the SAR test system. This is due to the high uncertainty of EMC probe measurement due to large size and unisotropy of the probe, and the poor EM interference immunity of the probe cable. The measurement with SAR test system seems to give repeatable results, if the position of the phone and hands-free kit relative to the phantom are accurately defined. The SAR values measured for hands-free kits were considerably lower than those measured for a mobile phone in the same position at the phantom surface.
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P-42

CHANGES IN DIELECTRIC PROPERTIES (800 - 1900 MHz) OF PIG BRAIN TISSUE IN THE TRANSITION FROM LIFE TO DEATH. G. Schmid[1], G. Neubauer[1], F. Alesch[2*]. [1]ARC Seibersdorf Research, Austria, [2]Institute of Neuro Surgery, University of Vienna, Austria.

OBJECTIVE: We have reported preliminary results of six animal experiments using pigs as test animals to examine possible changes of dielectric properties of brain tissue in the transition from life to death (1, 2). These experiments indicated a noticeable decrease in conductivity after death. This project was extended to collect data on four additional pigs in order to validate the preliminary data.
METHODS: The animals were anaesthetised by a procedure which was designed to have minimum influence on relevant brain tissue characteristics such as water content and blood perfusion (Thiopental only for inducing and Isoflurane for maintaining anaesthesia). To provide access for the probe head of the dielectric property measurement system (HP8722C vector analyser in combination with the HP85070B dielectric probe kit), a trephine hole (diameter: 3 cm) was made in the pigs' skull about 3 cm off the head midline and about 5 cm behind the eye-to-eye line. The dura mater was removed and the calibrated measurement probe was carefully placed onto the brain cortex (the arachnoidea was left intact). Brain tissue temperature was monitored and recorded during the experiments using the fibre optic temperature measurement system LUXTRON 790. Important vital parameters (aortic blood pressure, venous blood pressure, heart rate, electrocardiogram, rectal temperature, artificial respiration pressure and end tidal CO_2 pressure) were monitored and recorded during each experiment by a medical recording system. Dielectric property measurements in the frequency range from 800 to 1,900 MHz were performed periodically about once per minute. After measuring the dielectric properties of the brain tissue for about one hour during stable anaesthesia the pigs were sacrificed using a potassium chloride solution (applied intravenously) which caused death by cardiac arrest within 3 minutes. Continuous measurements of dielectric brain tissue properties, brain tissue temperature and intracranial pressure were made from 1 hour prior to death to about 18 hours after death.
RESULTS: The additional animal experiments performed so far confirmed the indication that conductivity decreased after death. At 900 MHz, the mean value of conductivity dropped from 1.29 S/m immediately before sacrificing the animal to 1.07 S/m at 2.5 hours after death. Correcting the measured data for the drop of tissue temperature after death with a temperature coefficients of 1 %/°C, partly compensates the drop in conductivity. However a drop of about 10 % still remains, which probably is due to biochemical processes in the tissue after death.
References.
Neubauer et al., *23rd Annual BEMS Meeting*, St. Paul, Minnesota, USA, June 2001, Abstract Book p. 41
Schmid et al., Proceedings of International Millennium Workshop on Biological Effects of Electromagnetic Fields, Crete/Greece, Oct. 2000, pp. 215-222.
The entire project was submitted to and permitted by the local animal investigations committee of Lower Austria.
This work was sponsored by Motorola Inc., Fort Lauderdale, Florida, USA

P-43

DESIGN, CONSTRUCTION, AND TESTING OF THE IN VITRO EXPOSURE SYSTEM FOR THE EUROPEAN "CEMFEC" PROJECT. G. d'Ambrosio*, G. De Prisco*, R. Massa*. Università di Napoli Federico II, Department of Electronic and Telecomunication Engineering, Via Claudio, 80125 Napoli, Italy.

INTRODUCTION: The european CEMFEC project (Combined effects of ElectroMagnetic Fields and Environmental Carcinogens) is based on both *in vitro* and *in vivo* laboratory 900 MHz exposures. For the *in*

vitro exposure device the design procedure had as a starting point the choice of the rectangular waveguide as the basic structure. Commercially available components were preferred, by reducing to a minimum custom made parts; thus the construction reduced to the assembly of few simple devices. The preliminary design was based on the results of a series of measurements, while numerical tests (simulations) played the key role in defining the final configuration of the exposure system. A thermostating system for the waveguide chamber was provided by external circulation of water (30 m of plastic tube -8 mm inner diameter- winding about the waveguide). A Colora WK 5 DS water bath (± 0.1 °C) was used.
External measurements under matched load and short circuit conditions: The exposure chamber was made of rectangular waveguides (248 mm x 124 mm; SAIREM), the two sample-holders first tested were rectangular plastic flasks (91 mm x 83 mm x 37 mm, inner dimensions), the samples were a 15 ml (tap water, or D-mem) liquid layer (≈2 mm thick) in each flask, and the flasks were kept in place by means of polystyrene foam blocks. The waveguide chamber and the flasks inside were kept in such a way that the E field was either horizontal (parallel to the liquid layer) or vertical (perpendicular to the layer). Under matched load conditions the overall power absorption efficiency of the applicator, (absorbed power) / (incident power), was evaluated by measuring the amplitude of the reflection (s_{11}) and transmission (s_{21}) coefficients of the exposure chamber, under different loading conditions. Measurements were carried out over the band 0.8 – 1.0 GHz, by means of a microwave vector network analyzer (Wiltron-Anritsu 37269B). As expected it was found that a maximum of absorbed power occurs when the E-field is horizontal (parallel to the liquid layer) and the samples are both kept where the incident electric field $E = E_y$ and the transverse magnetic field (H_x) are maximum. Also four-flask (8 ml each) configurations were examined. Under short circuit loading conditions the absorption efficiency evaluations were based on the measurement of the reflection coefficient (s_{11}) alone.
Thermal evaluations of the Specific Absorption Rate (W/kg): Experimental evaluations of the Specific Absorption Rate (SAR) were carried out at high (1 W) incident power level, by measuring (fiber-optic probes FISO, and computer controlled thermometer, FISO UMI 4) the heating curves at nine points in the liquid sample (15 ml).
NUMERICAL EVALUATIONS AND CONCLUSIONS: Numerical evaluations were performed by using a commercial code, based on the Finite Integration Technique, and the comparison with the experimental SAR pattern was found satisfactory. Both a two-flask loading (15 ml each), and a four-flask loading (8 ml each) were analized. The best trade-off between efficiency (SAR per 1 W incident power) and SAR uniformity, was found under horizontal E-field polarization and matched load conditions. Power efficiency was found to be 13.6 W/kg/W in the two-flask case, and 5.6 W/kg/W in the four-flask case; SAR standard deviation over the mean value was found to be sligthly higher than 30% in the former case and 15% in the latter. Statistical analysis of the SAR distribution was also performed.

P-44

SAR VARIATION DUE TO DIFFERENT FREQUENCIES IN ONE CHANNEL BANDWIDTH OF CELLULAR TELEPHONE DEVICES. S. Watanabe[1], K. Fukunaga[1*], M. Taki[2], Y. Yamanaka[1*].
[1]Communications Research Laboratory, Tokyo 184-8795, Japan. [2]Tokyo Metropolitan University, Tokyo 192-0397, Japan.

OBJECTIVE: To quantify the variation of specific absorption rate (SAR) values to be determined for compliance with safety standards for mobile phones such as cellular telephones. EU and Japanese authorities recently issued new standard procedures for SAR measurement (CENELEC, 2001 and TTC/MPT Japan, 2000). IEEE and IEC are also drafting similar documents and will be issued in near future. Those standards all describe some examples of uncertainty evaluations for typical cases in their documents. It is however necessary to investigate characteristics of SAR variation in order to improve the accuracy and repeatability of those standard methods. It is especially important to quantify the variation of SARs measured at different frequencies in one channel bandwidth where tested cellular telephones operate

because future broadband communication systems require wider bandwidth. We therefore numerically quantified the SAR variation due to different frequencies in one channel bandwidth and compared it with other variation factors.
METHODS: SARs are numerically evaluated by an FDTD simulator with two numerical head phantom models and one cellular telephone model (Watanabe et. al., 2001). The cellular telephone model was positioned at the "touch position" described in the standard measurement methods with the left side of the head phantoms. Three typical channel bandwidths of Japanese digital cellular telephone (TDMA and W-CDMA), i.e., 38 MHz for 900-MHz band, 24 MHz for 1.5-GHz, and 10 MHz for 2 GHz, were assumed. SARs were calculated at the lowest, middle, and highest frequencies in each channel bandwidth. It was also simulated that the electrical properties of phantom liquid were changed slightly (within 5% of target values, which is allowed by the standard procedures).
RESULTS AND DISCUSSION: It is shown that the variations of the calculated maximum values of both 1-g and 10-g averaged SARs calculated at different frequencies in each channel bandwidth are quite small (about 1% or less). The variations due to change of the electrical properties of phantom liquid are, on the other hand, 4~6% and 2~3% for 1-g and 10-g averaged SAR, respectively. Actual variation due to electrical properties could be larger because of measurement errors of electrical properties (5~10%). Consequently, our simulation suggests that the difference of frequencies in each channel bandwidth can be negligible to typical uncertainty (about 30%) of standard measurement procedures.
References.
CENELEC (2001), EN50361.
TTC/MPT Japan, (2000), Partial Report to the 118th Inquiry.
Watanabe, et. al., (2001), Asia Pacific Radio Science Conference, K7-03, p.272.

P-45

STATISTICAL CHARACTERIZATION OF SAR IN THE HUMAN BRAIN CAUSED BY CELLULAR PHONE DEVICES. K. Wake[1], S. Watanabe[1], Y. Yamanaka[1], M. Taki[2]. [1]Communications Research Laboratory, Tokyo 184-8795, Japan. [2]Tokyo Metropolitan University, Tokyo 192-0397, Japan.

OBJECTIVE: The health effects of exposure to microwaves from cellular phones have become great concern. There have been many *in vivo*, *in vitro* and epidemiological studies for that issue. Recently, IARC (International Agency of Research on Cancer) has conducted the international study on health effects (especially brain cancer) of cellular phones [1]. For these study, it is important to identify the exposure from cellular phones precisely. It is known that SAR (Specific Absorption Rate) inside the human head caused by a cellular phone is very localized. Therefore, for the epidemiological study on the relation between brain cancer and cellular phone, it is important to evaluate the SAR at the position where brain cancers were found. However, to identify the position of each brain cancer for all cases with high resolution is difficult. The purpose of this study is to evaluate the effects of characterizing the SAR with coarse resolutions based on the results of calculations with fine resolution.
METHODS AND MODELS: Head models [2] were placed close to phone models with $\lambda/4$ monopole antenna on a metallic box. The resolution of the head model was 2 mm. We calculated the SAR in heterogeneous and homogeneous models at 900, 1500 and 2000 MHz. The FDTD method was used for calculations. In all calculations, we re-calculated SAR by averaging over 1-cm or 2-cm cubes from the results of the calculation with the 2-mm resolution model.
RESULTS AND DISCUSSION: The difference between maximum SAR of the heterogeneous head model and the homogeneous head model were within 30 % in all cases. The maximum SAR with 1-cm resolution and 2-cm resolution differed from that with 2-mm resolution by the factor of about 50 % and 75 %, respectively. The characteristics of the SAR distributions with these resolutions were similar to each other (Figure 1). Histograms in Figure 2 showed the distribution of cubes in each range of the SAR value with various resolutions. SAR values were normalized with 10-g peak SAR. Although the ratio of cubes in higher

SAR regions decreased as larger resolution was applied, the shape of the histogram for 1-cm resolution was similar to that of 2-mm. From these results, the SARs averaged over 1-cm cubes provided appropriate approximation of statistical characteristics obtained by the calculation with fine resolution except maximum SAR value.

[1] IARC, http://www.iarc.fr/pageroot/PRELEASES/pr127e.html.
[2] Visible Human Project, http://www.nlm.nih.gov/research/visible/visible_human.html.

Fig. 1 SAR distributions with various resolutions (left 2 mm, center 1 cm, right 2 cm). SAR was calculated with the heterogeneous model at 900 MHz.

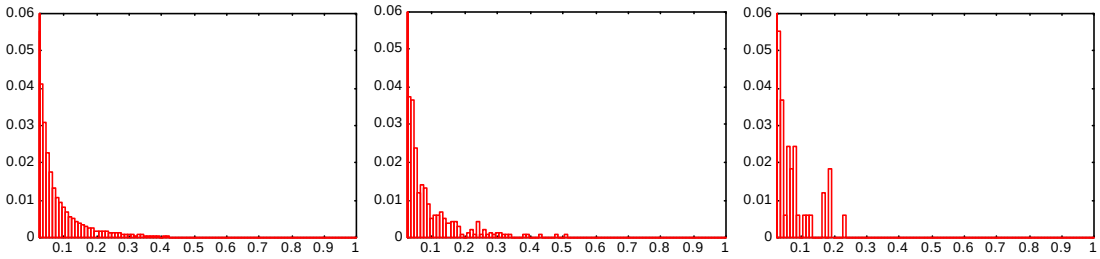

Fig. 2 Ratio of cubes in each range of SAR values with various resolutions (left 2 mm, center 1 cm, right 2 cm). Ordinate is the ratio of cubes and abscissa is SAR value normalized with 10-g peak SAR value. SAR was calculated with the heterogeneous model at 900 MHz.

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Abstract withdrawn by author.

P-47

DOSIMETRY FOR *IN VITRO* CELL CULTURES DURING 35-GHz EXPOSURE. N.J. Millenbaugh[1,2*], P.A. Mason[1], W.D. Hurt[1], C.T. Kuhnel[1], L.R. Johnson[1], J.E. Kalns[1,2*], R.V. Blystone[3*], and J.L. Kiel[1]. [1]Air Force Laboratory, Directed Energy Bioeffects Division, Brooks AFB, Texas, 78235; [2]Veridian Engineering, San Antonio, Texas; [3]Trinity University, San Antonio, Texas 78212, USA.

INTRODUCTION: Deposition of microwave energy and subsequent heating in an *in vitro* culture vessel is non-uniform and greatly influenced by such characteristics of the system as the polarization of the E-field, shape and size of the vessels, and presence of abrupt changes from culture medium to air. Thus, careful estimation of the specific absorption rate (SAR) distribution within the culture vessel is necessary to properly describe and optimize an exposure system.

OBJECTIVES: Our laboratory is developing a system to expose adherent cell cultures to millimeter waves for the purpose of studying induced biological responses to thermal and non-thermal exposures. The goal of the dosimetry work is to determine the predicted distribution of SAR in commonly available culture vessels of various sizes and shapes and with different E-field polarizations. This will identify which vessel gives

the most homogeneous SAR pattern and will define a sampling area within the vessel. Secondly, the work will validate the SAR prediction by comparing the estimation results to actual experimental heating profiles within the culture vessels.
METHODS: Commercially available software employing the finite-difference time-domain method (X-FDTD®, REMCOM) was used to calculate SAR distributions on the bottom layer of culture medium in rectangular culture flasks and petri dishes of two different sizes and with two different E-field orientations. The model was setup to simulate 35-GHz exposure through the bottom of the vessels. The dielectric properties of all exposed materials were obtained from the literature. Initial simulations revealed that the results were significantly dependent upon the volume of free air space around the culture vessel model and that not including sufficient free space gave artifactual SAR patterns. However, there was a practical limit to the amount of free space that could be used due to increasing computer run time and required memory. Results also indicated that SAR distributions were significantly influenced by the culture vessel size and shape and orientation of E-field, thus, in agreement with previous reports. The rectangle flasks gave a more convenient pattern for sampling cells than the petri dishes. Infrared thermography of the surface of the culture medium exposed to 1 W/cm^2 was used to verify the predicted SAR values. In addition, temperature probes were used in a separate experiment to verify the temperature measurements of the infrared camera.
CONCLUSIONS: The results of these investigations provide a means for characterizing and optimizing the *in vitro* exposure system so that artifacts and confounding results may be better avoided and to better ensure reproducibility of experimental data.
Research was funded, in part, by the Air Force Office of Scientific Research.

P-48

FINITE DIFFERENCE TIME DOMAIN (FDTD) MODELS PREDICT ORGAN RESONANCES IN THE 1-MM MAN MODEL. J.M. Ziriax[1], J.A. D'Andrea[1], W.D. Hurt[2], D. Verrett[2], P.A. Mason[2], D. Hatcher[1], and D. Cox[1]. [1]Naval Health Research Center Detachment, Brooks Air Force Base, Texas 78235, USA; [2]Air Force Research Laboratory, Directed Energy Bioeffects Division, Brooks Air Force Base, Texas 78235, USA.

INTRODUCTION: Empirical dosimetry of even a single frequency is time consuming and labor intensive, and as such, is typically limited to critical dosimetric data. Computer models can be used to explore a wide range of frequencies and exposure conditions in anatomically realistic models without the expense and effort of empirical measurements.
OBJECTIVES: Localized SARs vary dramatically with frequency and exposure orientation. Here we report on an ongoing exploration of localized and whole body SARs in our 1-mm human model across a range of exposure frequencies and orientations.
METHODS: NHRC-DET and AFRL/HEDR have jointly developed models of mouse, rat, goat, and rhesus monkey in addition to the human model (Mason et al., 1995, 1999). The 1-mm man model was developed from images provided by the National Library of Medicine as part of the Visible Human Project (http://www.nlm.nih.gov/research/visible/visible_human.html). Our FDTD program, based on code originally developed by Kunz and Luebbers (1993), was used to calculate SARs in the 1-mm man model. The electrical properties of each of tissue were set according to data and fits published by Gabriel (1996). The code has been parallelized using the MPI message-passing library. The advantage of using the MPI is that the code can run on parallel computer systems composed of networks of computers. These may be networked workstations, or massively parallel systems such as Linux-based Beowulf systems. These systems are easily constructed of relatively inexpensive PC-hardware. The figure shows sample results for whole body, skin and eye structures for 1700 to 4000 MHz in the PEKH orientation (RF source is to right of then man with a vertical E-field).
CONCLUSIONS: In this frequency range, SARs for whole body and skin change monotonically, while SARs for the eye structures change non-monotonically. We will expand the graph to lower frequencies

where we have seen eye (900 MHz) and whole body (70 MHz) resonance in coarser or head-only models. As the FDTD predicts SARs with the same resolution as the anatomical model, in this case in 1-mm volumes, then this resolution easily exceeds that of typical empirical methods. The thermal and biological significance of these highly localized SARs will have to be determined experimentally.

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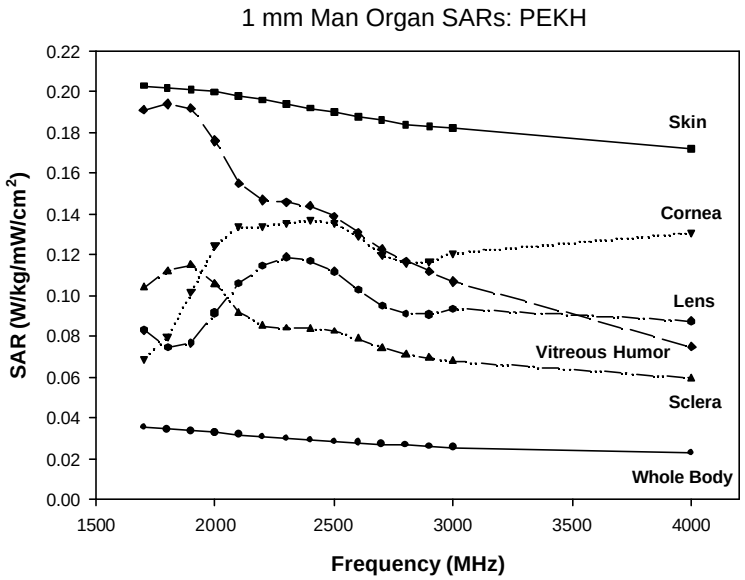
The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of the Air Force, Department of Defense, or the U.S. Government.



P-49

SENSITIVITY AND ACCURACY OF EXTRAPOLATION AND INTERPOLATION METHODS USED TO DETERMINE AVERAGED SAR IN CONFORMITY TESTS OF CELLULAR PHONES.

M. Francavilla*, A. Schiavoni. TILab - Telecom Italia Lab, Via Guglielmo Reiss Romoli 274, 10148 Torino, Italy.

OBJECTIVE: The CENELEC standard EN50361 [1] (and other draft standard), requires the use of extrapolation and interpolation techniques to determine the value of the Specific Absorption Rate (SAR) close to the internal surface of a phantom. These procedures are used to evaluate the averaged SAR, on a cubic mass, to be compared with basic limits of the standard. Furthermore the standard requires the value of the uncertainty associated to the interpolation and extrapolation methods to be included in the global uncertainty associated to the averaged SAR value [1]. The objective of this work is to apply the procedures proposed in the standard to real cases and to determine the significance of the uncertainty value associated to the interpolation and extrapolation methods.

METHODS: Averaged SAR is evaluated by means of integration techniques applied to local SAR values measured on a volumetric grid of points. Since is not possible to get SAR values on the internal surface of the phantom, but at a fixed distance, and the dimension of the measurement step on the volume depends on

the scanning system, generally it is necessary to perform interpolation and extrapolation of the measured data in order to get missing values or to increase the definition in the volume [2]. The standard [1] proposes analytical functions and data to be used as Reference to determine the uncertainty associated to the methods of interpolation and extrapolation. Two methods have been applied to the analytical functions in order to determine the sensitivity of the implemented procedures with respect to: 1) the distance of the points on the measured volume; 2) the extension of the measured volume; 3) the number of points between measured points, generated with interpolation; 4) the dimension of the extrapolation region. A sensitivity analysis with respect to variation in SAR distribution has been performed to determine the confidence level on the uncertainty values associated to the interpolation and extrapolation techniques.

RESULTS AND DISCUSSION: Figure 1 shows the sensitivity of the implemented interpolation method, in terms of relative error with respect to the Reference function [1 – f1 Annex C], versus the number of interpolated points included between "measured" points. Figure 2 shows the same example as in fig. 1 for the other Reference function reported in [1 – f2 Annex C]. Even if standard requires considering the maximum of the uncertainty derived by the different Reference data, it has been shown a different behavior of the interpolation and extrapolation method on input data, leading to conclude that the uncertainty of the method depends on the input data and the proposed test could be not representative for every measured SAR pattern.

CONCLUSIONS: The uncertainty associated to the interpolation and extrapolation method depends on the Reference data and on the dimension of the volume considered for its determination. An extension of the uncertainty value determined as proposed in standard could or could not be applied to whatever pattern of measured data.

References

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[2] Brishoual, Dale, Wiart, Citerne, "Methodology to interpolate and extrapolate SAR measurements in a volume in dosimetric experiments", IEEE Trans. on Electromagnetic Compatibility, vol43, no.3, 08/2001.

Acknowledgements: This work has been fully supported by TIM – Telecom Italia Mobile.

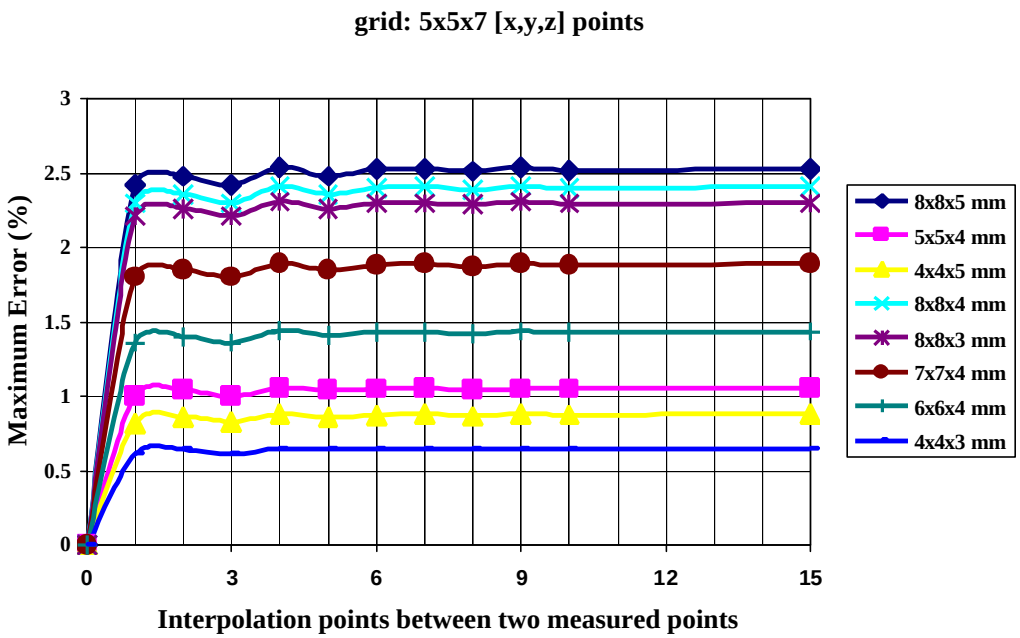

Figure 1: Maximum of the relative error for the analytical function f1 [1] versus the number of interpolated points used to increase the definition. The legend reports the distance between "measured" points on the volumetric grid.

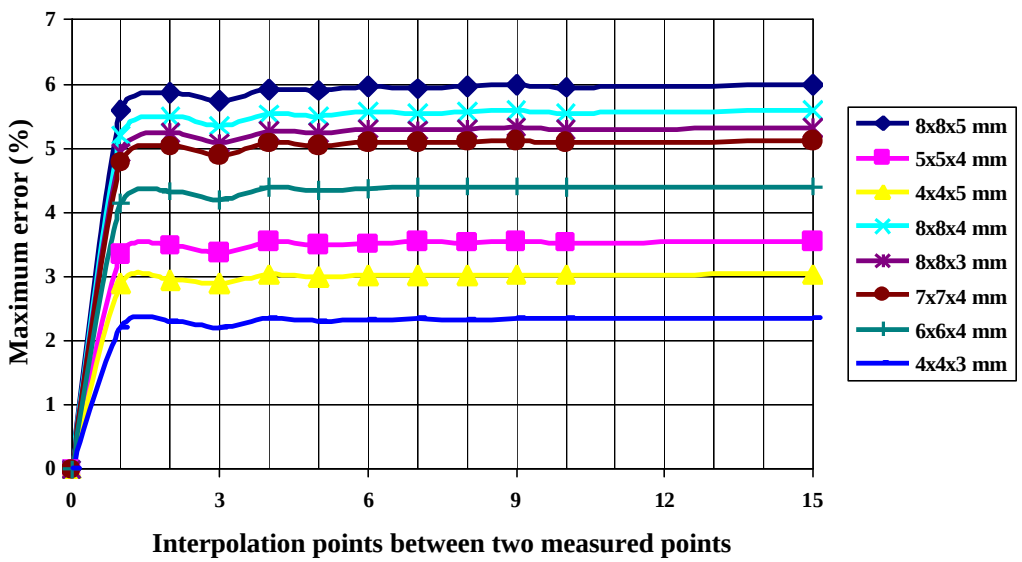

Figure 2: Maximum of the relative error for the analytical function f2 [1] versus the number of interpolated points used to increase the definition. The legend reports the distance between "measured" points on the volumetric grid.

P-50

COMPLIANCE DISTANCE OF BYSTANDERS FROM MOBILE ANTENNAS AT FREQUENCIES FROM 30 MHZ TO 900 MHZ. G. Bit-Babik*, A. Faraone*, and C.K. Chou. Motorola Labs, Corporate EMF Research Laboratory, Fort Lauderdale, FL 33322, USA.

INTRODUCTION: This study addresses the compliance of bystanders exposed to the RF energy emitted by mobile antennas mounted on the trunk or roof of vehicles. The IEEE C95.1-1999 *Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields from to 3kHz to 300 GHz* defines the Maximum Permissible Exposure (MPE) in terms of *rms* E, H field strengths and equivalent plane-wave power densityThe International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines follows essentially the same approach, by defining "Reference levels" for the incident field quantities. Field strength limits can be exceeded if compliance can be shown with respect to the *specific absorption rate* (SAR) average over the body and the spatial peak SAR average over 1 g (IEEE) or 10 g (ICNIRP) limits. Due to the lower IEEE limit, SAR compliance with IEEE standard implies compliance with the ICNIRP guidelines. Both standards implement a two-tier approach, with five-fold lower exposure limits for the uncontrolled environment (general population) than for the controlled environment (occupational exposure).

OBJECTIVES: The goal of this work was to determine compliance distances of bystanders from mobile antennas at frequency from 30 MHz to 900 MHz based on the IEEE peak 1-g average and whole-body average SAR limits for uncontrolled environments. The method of assessment introduces intentionally a wide compliance margin by overestimating deliberately the exposure, thus allowing to generate a frequency-independent compliance table, which in turn allows to implement simple guidelines for mounting mobile antennas on vehicles.

METHODS: The physical model used for this study was chosen to cover all possible mobile antenna mounting configurations and environment by intentionally overestimating the exposure. SAR calculations

induced at the surface of bystander model are based on a very convenient approximate formula proposed by Kuster and Balzano ("Energy Absorption Mechanism by Biological Bodies in the Near Field of Dipole Antennas above 300MHz." IEEE Trans. on Veh. Technol., vol. 41, No.1, Feb. 1992), modified for the case of a monopole antenna.

RESULTS: Table I presents compliance distances based on the IEEE C95.1-1999 SAR limits (both peak 1-g average and whole-body average) computed by the above mentioned approach. Conveniently, the compliance distances are frequency independent; they are related to the RF power of the mobile unit only. This was achieved by taking the larger between the compliance distances for the peak 1-g and the whole-body average SAR separately for different frequencies and power levels. Computations have showed that there is no big variation between compliance distances for different frequencies at the same power. This particular future yields a table based only on power level, which is convenient since it enables the formulation of simple installation guidelines for mobile antennas on vehicles.

Less than 7 W	**0.14**
7 to 15 W	**0.20**
16 to 50 W	**0.36**
51 to 110 W	**0.53**

Table I. Compliance distance vs. power levels of the mobile

CONCLUSIONS: This study takes into account the wide variation of vehicle-mounted mobile antennas and operation environments by overestimating the exposure, and enables to differentiate the compliance distances based solely on power radiated by antenna regardless of operating frequency. The table is very simple and yields simple guidelines for mobile antenna installation.

P-51

GSM MOBILE PHONE OUTPUT POWER DISTRIBUTION BY NETWORK ANALYSIS OF ALL CALLS IN SOME URBAN, RURAL AND IN-OFFICE NETWORKS, COMPLEMENTED BY TEST PHONE MEASUREMENTS. T. Persson[1], C. Törnevik[1], L.E. Larsson[2], J. Lovén[3*]. [1]Ericsson Research, Ericsson Radio Systems AB, SE-164 80, Stockholm, Sweden. [2]Telia Mobile AB, SE-651 15 Karlstad, Sweden. [3]Telia Mobile AB, Research and Development, SE-131 86 Stockholm, Sweden.

INTRODUCTION: A GSM mobile phone adjusts the output power to provide sufficient signal strength at the base station for acceptable quality of connection, while at the same time keeping the output power as low as possible in order to minimize interference and increase battery life. Depending on the predominant location of the user the average mobile phone output power will vary significantly. In the GSM system the peak output power ranges from 2W down to 1mW (33 dBm to 0 dBm).

OBJECTIVE: To analyze the mobile phone output power distribution in various environments and evaluate typical average mobile phone output power.

METHODS: It is possible to characterize the output power used by all the mobile phones in a network using Ericsson basestations by means of a network software feature called MRR (Measurement Result Recording). Telia has performed network measurements in different locations in Sweden using this MRR software feature. In this way comparisons can for example be made between urban and rural areas as well as with company indoor GSM office networks.

A customized software has been developed at Ericsson which registers the output power of a T28 mobile phone during normal use. The information is stored in non-volatile memory and can be downloaded to a PC for subsequent analysis. In addition to output power information the number of calls, frequency band and timing information as well as DTX (discontinous transmission) information is stored. Some users within the Stockholm area were used to characterize typical mobile phone output power distribution. The DTX feature in GSM reduces the RF output substantially while not talking and in the sample measurements using test phones this is taken into account, in contrast to the network statistical analysis where this information is not available.

RESULTS: In order to compare urban and rural areas a number of communities with large differences in density of inhabitants as well as indoor office networks have been selected for comparison. As can be seen from figure 1: Stockholm City region – a metropolitan area, figure 2: Sunne Municipality – a rural village including countryside surroundings and figure 3: A typical indoor office network, there are noticable differences in the output power distribution. The network analysis is based on all calls in the communities during one week in September 2001. In Stockholm City there is both a GSM900 and a GSM1800 network whereas in Sunne there is only a GSM900 network.
In the sample measurements using test phones primarily in the Stockholm area mixed use typically corresponded to an average output power of approximately 20 % of maximum available output power. In predominately indoor GSM office environment the corresponding typical value was noted to about 4 % of maximum available output power.
CONCLUSIONS: The average output power of a mobile phone is often well below the maximum available output power but there are significant differences depending on the location of use. In indoor GSM office network solutions the required mobile phone output power is often very low.

Mobile phone output power distributions in different locations.
PDF – Probability Density Function

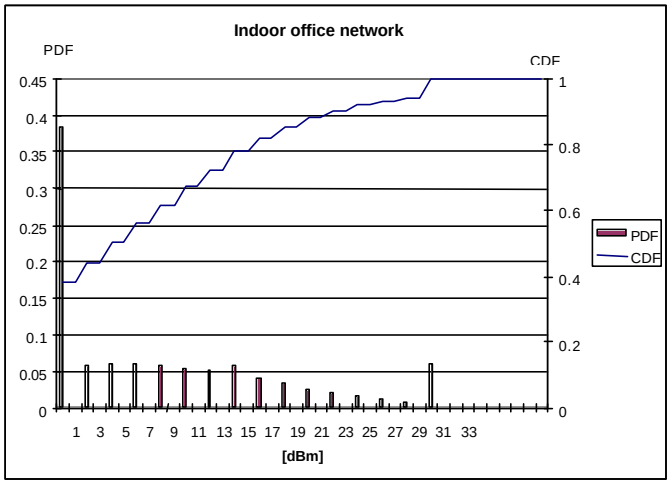

Figure 1

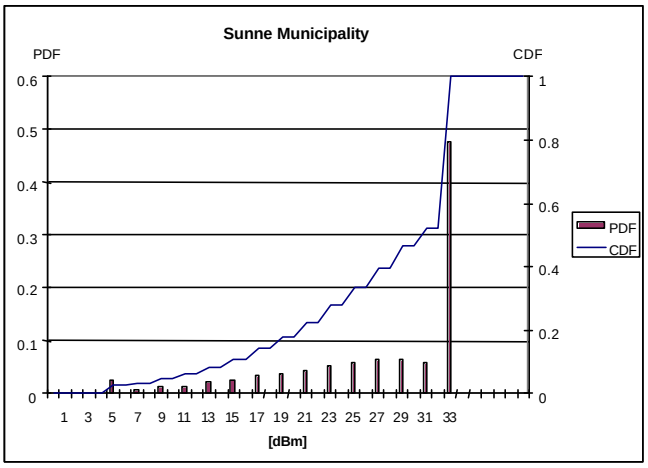

Figure 2

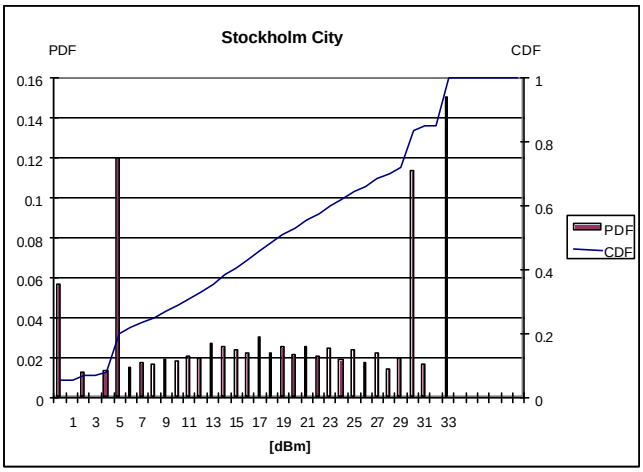

Figure 3

P-52

EVALUATION OF SAR IN BIOLOGICAL SAMPLES EXPOSED TO RF FIELDS FROM A PYRAMIDAL HORN ANTENNA. G. Gajda, A. Thansandote*, E. Lemay, J. McNamee, P. Bellier. Consumer & Clinical Radiation Protection Bureau, Health Canada, Ottawa, Ontario K1A 1C1, Canada.

INTRODUCTION: Horn antennas have been used to produce radiofrequency (RF) fields to irradiate samples for *in vitro* study of genotoxic effects. Some researchers[1] quantified sample exposures in terms of the power density (PD) of the radiation field. Such quantification may be acceptable if the sample is placed in the far-field region of the antenna. However, in order to obtain desirable exposure levels in the far-field, a high-power RF source would be required. In general, exposures of samples are in the near-field region, and therefore the specific absorption rate (*SAR*) in the exposed sample should be determined in order to obtain a meaningful and reliable study outcome.

OBJECTIVE: The purpose of this work was to evaluate *SARs* produced in 2 ml samples of whole blood by RF fields from a pyramidal horn antenna (Narda, Model 645) and relate them to measured equivalent far-field power density. Measurements were at the nominal frequency used by PCS mobile telephony (1.9 GHz) in Canada.

METHODS: The SAR was measured using a thermistor probe (BSD Medical Corp., Model 21-10104-002) while PD was measured with a Narda 8623D/8616 E-field probe and meter. The sample container was a 2 ml plastic vial (42 mm long x 10 mm dia.). The thermistor was held in place in the center of the vial by a 2mm plastic tube inserted through the lid. Whole blood was simulated with a mixture of de-ionized (DI) water, ethylene glycol and salt in proportions of 100g, 95g and 0.9g, respectively. This gave the same dielectric constant and conductivity ($\varepsilon_r = 60$, $\sigma = 2.1$ S/m) of whole blood at 1.9 GHz. SAR measurements were carried out, as a function of distance from the aperture plane, for two orientations of the sample: parallel to the incident E field (**E**-orientation) and parallel to the antenna axis (**k**-orientation). The horn and sample and/or E-field probe were located in an anechoic chamber while all other instrumentation was in an adjacent shielded room. Positioning of the sample or E-field probe was achieved with a servo-positioner under PC control.

RESULTS AND DISCUSSION: The specific heat capacity of the sample mixture was determined calorimetrically to be 3.75 J/g^{o}C with an estimated uncertainty of ± 5%. SARs of DI water were also measured for comparison with simulated whole blood. SAR variation as a function of distance showed a broad peak at distances of 10 to 20 cm, beyond which the SAR decayed roughly at the same rate as the equivalent power density. At their peak, the normalized SARs for **E**-orientation were 3.3 and 1.1W/kg per W for whole blood and DI water, respectively. For **k**-orientation, they were 0.32 and 0.20 W/kg per W. For whole blood there was a ten-fold difference between **E** and **k** orientations at all distances measured (out to

36 cm). The equivalent power density, which is more appropriately described as the square of the E-field, displays a slight oscillatory behavior close to the aperture. This is mirrored by the **E**-orientation SARs for both whole blood and DI water. At distances of 50 to 60 cm or more the equivalent power density begins to assume the inverse R^2 behavior of the far field (calculated to be 150 cm for the 350mm x 260mm aperture). More detailed results will be presented at the meeting.

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

P-53

TISSUE EQUIVALENT LIQUIDS FOR SAR MEASUREMENTS AT MICROWAVE FREQUENCIES. A. Peyman, C. Gabriel. Microwave Consultant Ltd. Woodford Road, London E18 2EL, United Kingdom.

INTRODUCTION: The basic restriction on human exposure to electromagnetic fields is given terms of specific absorption rate (SAR). The experimental evaluation of the exposure of people in the near-field of sources requires knowledge of the SAR which in turn calls for electric field measurements in tissue equivalent liquid contained in models (phantoms) representing the part of the body exposed to the radiation. Recently developed SAR measurement standards have specified the morphological details of a head phantom to be used for the assessment of exposure from handheld radiotelephones. Also specified were the dielectric properties of tissue equivalent liquids in the frequency range 300 - 3000 MHz. A number of different recipes for such liquids have been developed and included in an informative annex in EN50361, the recently published standard by CENELEC. These recipes contain organic compounds, some of which have undesirable properties. For example, Diethylene Glycol Butyl Ether (DGBE) which is severely irritant to eyes and skin and is quite harmful if inhaled or swallowed. It is also sensitive to air and could result to the formation of peroxides. Another factor to consider is the stability of the compound. Therefore it is necessary to find alternative recipes, which make use of materials that are relatively more stable and safer to use.
OBJECTIVE: To develop new recipes for tissue equivalent material, using organic compounds with relatively high stability and safe to use. The liquid will have the required permittivity and conductivity at certain frequencies.
METHODS: In order to find the best suitable material for the purposed recipe, the following criteria have been considered. First, the material must have a good degree of solubility in water, such as compounds that hydrogen-bond with water. Secondly, in making a solution, the organic material must reduce the permittivity of water significantly, which means that the material should have a relatively large non-polar organic content. The third criterion is the safety of the material of the interest. Although DGBE fulfils the first two criteria, it fails in on the third. This paper will present aqueous mixtures with the required properties. The data will be presented in the permittivity-conductivity plane at the mobile communication frequencies. By way of an example, the dielectric properties of a 50% aqueous organic solution in given in Figures 1 and 2 together with a similar concentration DGBE in water at 20°C.
RESULTS AND DISCUSSION: The organic compound in Figure 1 and 2 is relatively non-hazardous and is often used as a food additive. It is also noted that the new mixture is more stable and safer to handle and use at the laboratory.

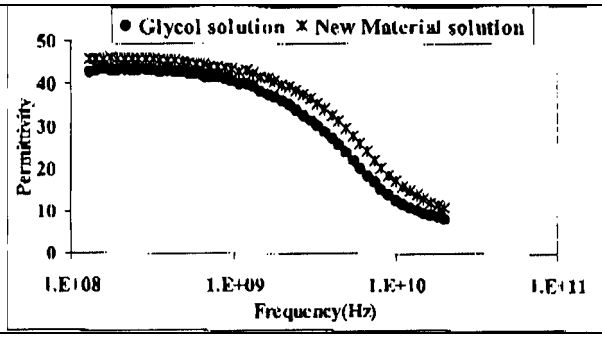

Figure 1- Permittivity of Glycol and the new material solution (50%) at 20°C.

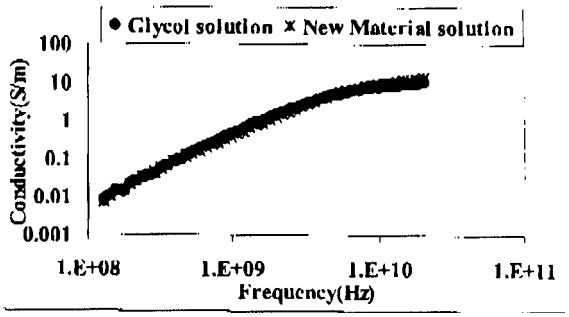

Figure 2- Conductivity of Glycol and the new material solution (50%) at 20°C.

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THE NUMERICAL EVALUATION OF THE SAR DISTRIBUTION AND TEMPERATURE INCREASE AROUND METALLIC IMPLANTS IN RF EXPOSED WORKERS. R. McIntosh, R. McKenzie, V. Anderson. Telstra Research Laboratories, 770 Blackburn Rd, Clayton 3168 VIC, Australia.

OBJECTIVES: To develop a computational modeling environment for determining both the Specific Energy Absorption Rate (SAR) and accompanying temperature increase around metallic implants in persons exposed to radiofrequency (RF) fields.
BACKGROUND: There are many people who by accident or for medical reasons carry metallic items of various shapes inside their bodies. Whenever a RF field impinges on such metallic implants, the field in scattered around the conductor and may redistribute the energy of the incident field to produce significant peak SAR concentrations around certain parts of the implant. A number of RF safety guidelines [1] and standards [2] require that such persons occupationally exposed to high fields should be assessed for the potential to exceed allowable 1ocalised SAR limits. A more fundamental consideration is whether the resultant temperature increase arising from the redistributed SAR would exceed acceptable levels.
METHODS: The SAR is calculated with the commercially available finite-difference time-domain software, XFDTD [3], using an anatomically accurate human body model developed from the 'visible human' [4]. A finite difference model based upon Penne's bio-heat equation has been developed to determine the 1ocalised temperature increase in the human model due to the SAR [5].
RESULTS: The figure displays a two-dimensional cross-section of an XFDTD model of a thigh. A 'nail' has been inserted down the middle of the femur to investigate the RF effects on an RF worker who has such an implant. The nail is 450 mm in length and
14 mm in diameter. Analysis indicated that the worker could still operate within occupational guidelines.
CONCLUSIONS: The output of this SAR/thermal modeling environment will provide quantitative estimates for evaluating SAR compliance around implants. It is conceivable that the thermal analysis will indicate that the heat sink *characteristics* may protect against a potentially harmful temperature rise, even if localised SAR limits are exceeded.

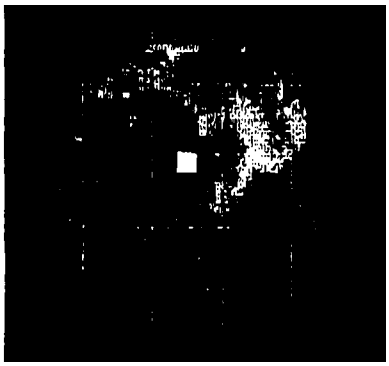

Figure: XFDTD plot of 1 g average SAR in cross-section of thigh with 'nail' inserted in the femur

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DOSIMETRY – ELF

P-55

Abstract not accepted for publication in the Abstract Book.

P-56

Abstract not accepted for publication in the Abstract Book.

P-57

Abstract not accepted for publication in the Abstract Book.

P-58

OCCUPATIONAL EXPOSURE TO MAGNETIC FIELDS FROM INDUSTRIAL DEVICES. A. Thansandote[1], W. Gorman[1], D. Lecuyer[2], G. Gajda[1], E. Lemay[1]. [1]Consumer & Clinical Radiation Protection Bureau, Health Canada, Ottawa, Ontario K1A 1C1, Canada. [2]Richard Brancker Research Ltd, Ottawa, Ontario K1S 3Y7, Canada.

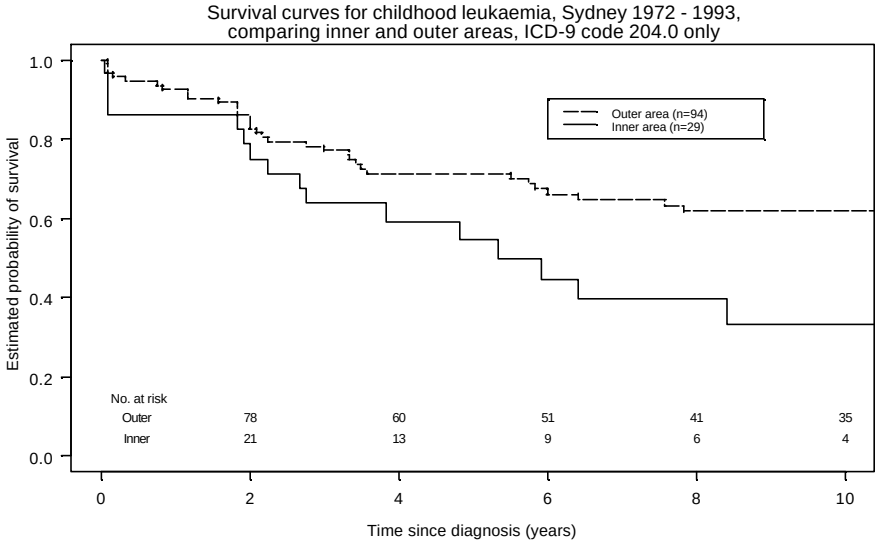
INTRODUCTION: High-power industrial devices such as induction furnaces, welders and plasma arc cutters are used in material processing laboratories and foundries. During the operation, workers are often exposed to the magnetic fields produced by these devices, while also encountering high temperature and splashes of hot metal particles. Recent epidemiological studies suggesting a possible association between magnetic field exposure and certain types of cancer have raised concerns among workers of such devices. To provide guidance, the Canadian Consumer and Clinical Radiation Protection Bureau has, on several occasions, been requested by various government agencies to assess the magnetic field exposure of the workers.
OBJECTIVE: The purpose of this work was to evaluate the magnetic fields produced by typical induction furnaces, welders and electric plasma arc cutters.
METHODS: The instrumentation consisted of a custom-made, triaxial magnetic field probe, a scope meter (Fluke, Model 97), two magnetic field measurement systems (Enertech, EMDEX II), an ELF magnetic field meter (Holaday, HI-3627) and a VLF magnetic field meter (Holaday, HI-3637). Investigations were first made with the scope meter and the magnetic field probe to determine the frequency of operation of the device. Measurements were then carried out to determine the magnetic field intensity around the device at several locations on the operator's body (head, chest, waist and gonads), using an appropriate magnetic field meter. It was also determined how the field intensity varied with distance from the device surfaces. This procedure was used on 13 different models of induction furnaces, one spot welder and one plasma arc cutter.
RESULTS AND DISCUSSION: The frequencies of the magnetic fields produced by the tested induction furnaces were found to be in a range from 60 Hz to 9.2 kHz, while that of the spot welder and plasma cutter was 60 Hz. Depending on the device, the maximum magnetic field intensity at operator positions varied from 1.4 μT to 400 μT. The corresponding levels for the plasma arc cutter and the spot welder were 1830 μT and 2.2 μT, respectively. In many cases, operators of these devices were at times exposed to magnetic fields at levels exceeding the limits given in a number of exposure guidelines. Due to potentially high magnetic field intensity, pacemaker wearers should be restricted from the vicinity of these industrial devices. More detailed results will be presented at the meeting.

P-59

DECREASED SURVIVAL FOR CHILDHOOD LEUKAEMIA PROXIMITY TO TV TOWERS. B. Hocking[1*], I. Gordon[2*]. [1]Camberwell, Vic, Australia 3124. [2]Statistical Consulting Centre, University of Melbourne Parkville Vic Australia 3052.

OBJECTIVE: In a previous study we reported an increased risk of childhood leukaemia in municipalities proximate to TV towers in north Sydney compared with more distant ones (Hocking B Gordon I Hatfield G Grain H. Cancer incidence and proximity to TV towers Med J Aust 1996; 165: 601-605). The rate ratio for incidence, comparing the inner ring of municipalities to the outer ring, was 1.55 (95% confidence interval 1.00 – 2.41) and for mortality the rate ratio was 2.74 (95% confidence interval 1.42 – 5.27). The objective

of the current study was to analyse the survival experience of the cases in detail, to determine whether there are differences between the two populations.
METHODS: Survival data on cases diagnosed from 1972-93 were analysed. Data on all cases who survived for less than one month were verified by the NSW cancer registry and one case diagnosed at autopsy excluded. Data were described by Kaplan-Meier curves. The log-rank and Wilcoxon tests were used to compare the two groups. Cox's proportional hazards model was used to adjust for confounders.
RESULTS: There were 123 diagnosed cases of acute lymphatic leukaemia (ICD-9 204.0) of which 29 (16 deaths) were in the inner ring of municipalities and 94 (34 deaths) were in the outer ring. We found a significant difference in survival (log rank: $P = 0.03$; Wilcoxon: $P = 0.05$). The 5 year survival in the inner ring of municipalities was 55% and in the outer ring 71% (inner 23% worse); at 10 years the survival was 33% and 62% respectively (inner 47% worse). After adjustment for the potential confounders using Cox's model, the mortality rate ratio comparing the inner ring with the outer ring was found to be 2.1 (95% confidence interval: 1.1 – 4.0). We were not able to control for cytogenetic abnormalities.
CONCLUSION: There was an association between proximity to the TV towers and decreased survival, among cases of childhood leukaemia.



P-60

THE CHILDHOOD CANCER, LEUKAEMIA AND ELECTROMAGNETIC FIELD STUDY (CCLEF): INITIAL ANALYSIS OF RECORDED FIELD MEASUREMENTS. E.J. Dunn*, M.G. Wright, J. Eavis*, P. Grainger* and A.W. Preece. Division of Medical Physics and Oncology, University of Bristol, Bristol Haematology & Oncology Centre, Bristol BS2 8ED, United Kingdom.

INTRODUCTION: The debate surrounding the possible link between exposure to electromagnetic (EM) fields and childhood cancer has provided a focus for research for many years. Whilst the findings of individual epidemiological studies have on the whole proved inconclusive and contradictory, recent meta-analyses have suggested that an association between EM field exposure and childhood leukaemia does exist (Angelillo et al[1] 1999, Greenland et al 2000[2], Ahlbom et al 2000[3]). The recent Advisory Group on Non-Ionising Radiation (AGNIR) [4] commissioned by the National Radiological Protection Board (NRPB) in the UK concluded that there appears to be a doubling of the incidence of childhood leukaemia in 50Hz magnetic fields of 0.4μT or greater. Whilst the IARC followed the U.S NIEHS when stating in June 2001 that power frequency magnetic fields are a possible human carcinogen. However debate now surrounds the possible consequences of this fact and the likely mechanism underlying it.

OBJECTIVE: The CCLEF study commenced in 1997. Its purpose was to assess the role of magnetic and electric field exposure in childhood cancer and thus derive further information on the aetiology of cancer. This would add to the debate on whether a policy of "prudent avoidance" is desirable for EM fields.
METHODS: The study adopted a case - control format. All children (under 16 years) living in the South-West of England, with a diagnosis of leukaemia or a brain tumour and under the care of one of the two main Paediatric Oncology Centres were eligible for inclusion. Each case recruited to the study was matched with a control on the basis of gender, ethnicity and age.
Data was collected during a visit to the family home using two separate instruments:

1. *An Interview / Questionnaire* conducted with the parents. This focused on activities in the home that may affect exposure to EM fields, including the use and location of electrical appliances. In addition data was collected concerning established confounding factors such as maternal therapeutic drug use and exposure to x-rays.
2. *A field survey* comprising detailed measurements of the electromagnetic fields in the home. A comprehensive series of measurements were made in order to assess the EM environment to which the child (and the Mother during pregnancy if diagnosis was before 5 years old) had been exposed.

Magnetic field measurements were conducted throughout the family home, and where possible, a personal measurement was obtained through the child wearing a meter and completing a diary of movements and activities. This study incorporated extensive measurements of the electric field. This component of electromagnetic fields has often been neglected because of a supposed lack of evidence for physical effects, but more likely, because of the difficulty in making accurate measurements. We measured the unperturbed electric field and also made a direct measurement of the perturbed field. In order to achieve this, we designed and constructed a phantom that models the perturbing action of a child when placed in a field. This phantom allowed us to assess the field actually encountered by the child and so provide a considerably more accurate measure for exposure determination.
RESULTS & DISCUSSION: 128 cases and 102 controls were recruited on to the study overall. Analysis to date has been concerned with the Time Weighted Average (TWA) field values for the matched case – control pairs. Table 1 provides a complete breakdown of results at present. There appears to be no association between personal exposure to magnetic fields and leukaemia and cancer. For TWA magnetic field based on the location of the child's bed again there appears to be no association with tumours, but the leukaemia pairs reach a significant level, and possibly a higher relative risk for an A.L.L subset, which is approaching significance.
No association between tumours or leukaemia could be detected by measurement of the perturbed electric field at the location of the child's bed.
Since these results for magnetic field were based on the bed location at diagnosis, whereas the personal exposure was by definition very much post-diagnosis. This may account for the association being apparent with the magnetic fields at the child's bed but not personal exposure.
These results suggest that data collection over a longer period would be needed. However since the protocols followed are similar to those of other studies they may be of value to add to relevant meta-analyses. Some measurement parameters in this study remain to be analysed, as do the potential confounders.
However these results support the hypotheses that A.L.L could be associated with elevated magnetic fields.
References.

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2. Greenland et al. Epidemiology 11(6): 624-634. 2000.
3. Ahlbom et al. B J Cancer 83(5): 692-698. 2000.
4. AGNIR. Doc. NRPB 12(1): 1-179. 2001.

This work was funded by Children with Leukaemia. We are grateful for the assistance of Dr. W. Kaune in developing the measurement protocols.

Table One:

TWA Magnetic Field Exposures			
Meter Location	**Match**	**R.R**	**C.I**
Personal	All pairs	1.11	0.44 – 2.76
	All leukaemia pairs	1	0.35 – 2.84
	All A.L.L pairs	0.81	0.23 – 2.86
	All tumour pairs	1.57	0.24 – 10.30
Child's Bed	All pairs	2.61	0.88 – 7.74
	All leukaemia pairs	**5.05**	**1.04 – 24.36**
	All A.L.L pairs	7.98	0.94 – 67.46
	All tumour pairs	1	0.18 – 5.50
Mother's Bed	All pairs	0.57	0.13 – 2.54
	All leukaemia pairs	1.54	0.24 – 9.71
	All A.L.L pairs	1.55	0.24 – 9.97
	All tumour pairs	N/A	N/A
**Comparisons based on high v low exposure <0.099μT >0.100μT*			
TWA Perturbed Electric Field Exposures			
Meter Location	**Match**	**R.R**	**C.I**
Child's Bed (vicinity)	All pairs	1.28	0.57 – 2.87
	All leukaemia pairs	1.23	0.50 – 3.03
	All A.L.L pairs	1	0.35 - 2.80
	All tumour pairs	1.61	0.23 - 11.26
**Comparisons based on high v low exposure <9.9Vm (perturbed) >10.0Vm (perturbed)*			

P-61

A DOSIMETER PHONE TO ASSESS CELL PHONE USE IN SUPPORT OF RELEVENT EPIDEMIOLOGIC STUDIES. J.J. Morrissey, *G. Bit-Babik, *A. Gessner, *A. Dietrich, M.L. Swicord. Motorola Florida Research Labs, Ft. Lauderdale, Florida, USA 33322.

OBJECTIVE: To gather detailed data on dose, position, and pattern of radiofrequency (RF) exposure to the head of cell phone users.

BACKGROUND: The development of a Motorola 900 MHz (GSM) StarTac dosimeter phone was presented at the 2001 BEMS meeting in St. Paul. The phone was described as containing multiple electric sensors to allow measure of distance (angle of tilt), accelerometers to measure position relative to gravity (angle of twist), and additional components to measure net output power and position of the antenna

(extended or retracted). These dosimeter phones will be used to collect information on RF exposure (in terms of peak SAR in the head) during use. Such information should strengthen the interpretation of recently published epidemiologic studies (Rothman et al. 1996; Hardell et al. 1999 and in press; Morgan et al. 2000; Muscat et al. 2001, Inskip et al 2001) as well as offer stronger dose assessment information for ongoing epidemiologic studies that investigate whether any correlation exists between various health effects and cell phone use. To date, such epidemiologic studies have relied on billing records, questionnaires, and job titles to evaluate RF exposure.

METHODS, RESULTS & DISCUSSION: Ten dosimeter phones have been assembled and calibrated in our laboratory during full power transmission to obtain peak SAR values in the head when held at incremental positions. These calibration values are used to convert data from the dosimeter phones into meaningful information on dose, position, and pattern of RF exposure during cell phone use. Preliminary data collected from a limited number of dosimeter phones will be presented.

P-62

EXPOSURE TO ELECTRIC AND MAGNETIC FIELDS FOR PEOPLE LIVING NEAR HIGH POWER LINES.

D. Gauvin[1*], P. Levallois[1], S. Gingras[1*], M. Bourdages[2]. [1]Unité de recherche en santé publique, Centre Hospitalier Universitaire de Québec, Québec (Québec) Canada, G1E7G9. [2]Institut de recherche d'Hydro-Québec, Varennes (Québec) Canada, J3X 1S1.

OBJECTIVES: We studied recently the effects of electromagnetic fields urinary excretion of 6-sulfatoxymelatonin for women living near and far away from a 735 kV transmission line (Levallois et al 2001). Detailed exposure assessments were carried out during this study. The objectives of this communication is to describe the exposure to low frequency electric and magnetic fields, using different indexes of exposure, for people living near and far from high power lines.

METHODS: 215 women, aged between 20 and 74, and living within 200 meters of a 735 kV line (Group 1) were compared to 196 women living at least 400 meters from any power line (Group 2). Only women living in single-family or semidetached house were selected. Each participant had to carry an Emdex Lite monitor (Enertech Consultants, Campbell, California) during the day and put it under the bed during the night. Measures of magnetic field were done every 30 seconds for 36 hours. Indoor electric field was evaluated using a Holaday HI-3604 ELF/power frequency survey meter (Holaday Industries Inc., Minnesota) which measures the vertical component in the 50-1000 Hz range. One measure during each of two visits was taken in the center of the kitchen, living room and bedroom.

SUMMARY of the RESULTS: Mean magnetic field intensity for 24h was much higher for those living near the lines (GM = 0.33 μT Vs 0.13 μT, $p<0.001$). The ratio of magnetic field intensity between group 1 and group 2 was the highest when considering only night exposure (Intensity ratio= 3.5). This difference of exposure between the two groups was broader when considering only the time spent inside the residence (GM=0.38 μT Vs 0.12 μT, $p<0.001$). The same pattern was found when considering the percentage of time above 0.2 or 0.5 μT. When considering the standardized rate of change metric (RCMS), variability of exposure was significantly lower for those living near the line (≤0.01). No difference in variability was found when considering the Jag5. Electric field exposure was also found higher inside the houses located near the line (GM = 10.5 Vs 5.8 V/m, $p<0.001$) .

CONCLUSIONS: High power lines contribute significantly to personal exposure to electric and magnetic field. The intensity of exposure to magnetic field is 2 to 3 times higher for those living near but the variability of exposure measured by the RCMS appears to be lower.

Reference.

Levallois P. et al. *Am J Epi* 2001;154:601-609.

This study was supported by Hydro-Québec and Electricité de France.

P-63

HEAT SHOCK PROTEIN EXPRESSION IN CHROMAFFIN CELLS STIMULATED WITH EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS. L. Verdugo-Díaz[1], M. Martínez-Vargas[1]*, L. Navarro[1]*, R. Drucker-Colín[2]. [1]Depto. De fisiología, Fac. De Medicina. [2]Depto. De Neurociencias, Instituto de Fisiología Celular, UNAM, 04510, México, D. F. México.

INTRODUCTION: Previous results showed that 0.7 mT, 60 Hz electromagnetics fields (ELF EMF) induces biochemical and morphological changes on rat chromaffin cells in culture. Differentiation induced by ELF EMF was similar to that produced by nerve growth factor (NGF) [1]. Some authors showed ELF EMF induction of specific gene and proteins. In particular, induction of stress proteins had been demonstrated [2]. Previously, we are reported differential gene expression induced by ELF EMF [3].
OBJECTIVE: The aim of this study was to determine the Hsp70 expression in the chromaffin cell exposed to ELF EMF and compared the results with NGF-differentiated chromaffin cells.
METHODS: Chromaffin cells were obtained from neonatal Wistar rats. Cultures were divided in 3 groups: control (without any stimulation), NGF group (with 20 ng/ml of NGF al time of plating) and ELF EMF group (60 Hz, 0.7 mT, daily 2 hours in the morning and 2 hours in the afternoon). After 7 days in vitro cells were scrapped, homogenized and centrifuged at 39 000xg for 15 min at 4oC. Precipitated fragment and supernatant liquid were collected. Western blot and immunocitochemistry with polyclonal antibody anti-Hsp70 were carried out.
RESULTS AND DISCUSSION: Protein levels of Hsp70 obtained from the membrane fraction in the three experimental groups were similar. The analysis of soluble fraction showed that: 1) No Hsp70 expression on control cultures of chromaffin cells, 2) ELF EMF exposure induced significant increases in protein levels for Hsp70 and 3) NGF-induced Hsp70 expression is higher than ELF EMF induction. These results showed that both differentiation stimuli (NGF and ELF EMF) induce Hsp70 expression on cultures of chromaffin cells. However, the differences observed on Hsp70 expression could suggest differential mechanism of induction. Our results confirm that the electromagnetic field stimulation induces stress protein expression.
References.

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Supported by DGAPA-UNAM IN208799 and Fideicomiso UNAM to RD-C.

P-64

EFFECT OF FIBRIN FIBER ORIENTATION ON T2 RELAXATION OF FIBRIN GEL. M. Takeuchi*, M. Sekino*, K. Yamaguchi*, N. Iriguchi*, S. Ueno. Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo.

OBJECTIVES: The spin-spin relaxation time T_2 of protons in water molecules varies between tissues depending on its molecular structure and surrounding conditions. However, the detailed mechanisms responsible for the different T_2 values are still unknown. To investigate the effect of surrounding fiber structures as a possible mechanism, we compared the T_2 relaxation times of a fibrin gel containing magnetically oriented fibrin fibers and a fibrin gel containing randomly oriented fibrin fibers.
METHODS: Fibrin gels were polymerized from fibrinogen solutions (3.0 mg/ml) in 50 minutes by adding thrombin (1.0 U/ml) and $CaCl_2$ (200 mM). Two fibrin gels were prepared: one was polymerized in a 7.05 T

static magnetic field so that fibrin fibers were oriented parallel to the magnetic field because of the anisotropic magnetic susceptibility of fibrin fibers, and the other was polymerized without a magnetic field. The T_2 relaxation times of the fibrin gels were measured by the Carr-Purcell-Meiboom-Gill (CPMG) method using a Varian 7.05 T MR imaging system equipped with a 300 MHz volume RF coil.
RESULTS: Water molecules in the fibrin gel that was polymerized in the magnetic field exhibited only one relaxation time $T_2 = 0.35$ s, whereas, water molecules in the fibrin gel that was not exposed to a magnetic field during polymerization had at least two components in the T_2 relaxation time. The long component, $T_2 = 0.35$ s, was the same order as the T_2 of the fibrinogen solution (= 0.41 s) and the fibrin gel polymerized in the magnetic field. The short component was $T_2 = 0.01$-0.15 s.
DISCUSSION: In the fibrin gel polymerized without a magnetic field, fibrin fibers were randomly oriented. Each fibrin monomer generated microscopic magnetic fields. The water protons far from the fibrin fibers were not exposed to the microscopic fields. However, the water protons near the fibrin fibers were exposed to the microscopic fields, which gave rise to the short component of the T_2 relaxation time. In the fibrin gel that was exposed to a static magnetic field, the fibrin fibers aligned parallel to the magnetic field during the polymerization. All the magnetic characteristics of the fibers are consequently eliminated. Thus, the water protons are no longer exposed to the microscopic magnetic fields from the fibrin fibers. Cell structural transformations such as tumors causes a change in the T_2 relaxation time. The mechanism of the change is partially understood by these results.
This study was supported in part by a grant from the Ministry of Education, Science and Culture, Japan.

P-65 Student

EFFECT OF A STATIC NON-UNIFORM MAGNETIC FIELD ON THE SURFACE PROPERTIES OF ACRYLIC RESIN. A. Gasparetto[1], I. Hibler[2], A.J. Palangana[2], C.R. Paula[3], R. Oliveira[4]. [1]Department of Dentistry Universidade Estadual de Maringá, Maringá, Paraná 87080-310 Brazil. [2]Department of Physics, Universidade Estadual de Maringá, Maringá Paraná, 87020-000 Brazil. [3]Department of Microbiology, Universidade de Sao Paulo, Sao Paulo 05508-730 Brazil. [4]Center of Biological Engineering, Universidade do Minho, Braga, 4710-057 Portugal.

INTRODUCTION: The acrylic resin is a polymeric material with several applications in different scientific and technological fields, especially in medicine and biotechnology. Its physical characteristics or their possible modifications can imply new ways of utilization and applicability.
OBJECTIVE: To study the effect of a magnetic field on the surface physico-chemical properties usually implied in bacterial adhesion, especially surface hydrophobicity.
METHODS: the hydrophobicity of the resin surface was determined by sessile drop contact angle measurements, using van Oss (1994) methodology. Accordingly, a substance (i) is considered hydrophobic when the variation of the free energy of interaction between two entities of substance (i) immersed in water is negative ($\Delta G_{iwi}<0$). That is to say, the two entities of substance (i) interact preferentially between them then with water. On the contrary, if $\Delta G_{iwi}>0$, substance (i) is hydrophilic.
Two types of resin samples were used: hydrated and non-hydrated ones. The hydrated samples were obtained by autoclaving at 121ºC. Before contact angle measurements, the samples submitted to the magnetic field were exposed during 24 hours to a field of 500gauss generated between to parallel magnetite plates.
RESULTS AND DISCUSSION: The principal results are summarized in Table 1. As could be expected the hydrated resin is hydrophilic, while the dehydrated is hydrophobic. However, when the hydrated resin is submitted to the magnetic field it becomes even more hydrophobic than when dehydrated. This can be explained by the effect of the magnetic field on the orientation of the water molecules of hydration. Consequently, there is an evident alteration of surface properties promoted by the magnetic field.

Table 1 – Acrylic resin degree of hydrophobicity expressed as $D\mathbf{G_{iwi}}$

Acrylic Resin Condition	$D\mathbf{G_{iwi}}$
dehydrated	-9.232704494
hydrated	0.371220915
hydrated and under magnetic field exposition	-22.06587279

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Van Oss, C.J., (1994). Interfacial Forces in Aqueous Media, Marcel Dekker, Inc., New York.
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P-66 Student

EFFECTS OF STATIC MAGNETIC FIELDS ON FIBRINOLYSIS. *R. Emura[1], *J. Hashimoto[1], T. Higashi[1], T. Takeuchi[2]. [1]School of Allied Health Sciences, Faculty of Medicine, Osaka University, Suita, Osaka 565-0871, Japan, [2]Low Temperature Center, Osaka University, Toyonaka, Osaka 560-0043, Japan.

OBJECTIVE: Since orientation of fibrin fibers was shown by Torbet et al in 1981 [1], many studies have been reported in relation to the phenomenon [2, 3]. Fibrinogen is a rod-like protein, and has an anisotropic diamagnetic susceptibility because of its alfa-helix. As they are polymerized orderly by thrombin, the anisotropic diamagnetic susceptibility of fibrin fibers increases additively. When the reaction was advanced in a static magnetic field, the magnetic energy becomes larger than the thermal energy, and the growing polymers are rotated by the magnetic moment. Fibrin fibers were oriented with their long axis parallel to the magnetic field direction.
What are the effects of static magnetic fields on fibrinolysis? After completing the clot of fibrin fibers outside the magnetic field, it is putted into the field. The small fragments must be oriented as the fibrin fibers are lyzed by plasmin. We investigated the effects of their shaking and mixing powers on the fibrinolysis time.
METHODS: Experiment 1 (Effects of continuous exposure): The fibrinogen solution (700mg/dl) was mixed with plasminogen ((0.42u/ml) and urokinase (0.83u/ml) in advance. Five minutes after adding thrombin (0.025u/ml) and forming the clot, the sample was putted into the sample space of superconducting magnet (8T in max.). The laser light was introduced into and out of the sample and the change of optical transmissivity was monitored among about 60 minutes.
Experiment 2 (Effects of periodic exposure): The process of fibrinolysis was monitored in the same as Experiment 1 except the condition of magnetic exposure. The sample was exposed for 15 seconds at 15 seconds' interval.
RESULTS AND DESCUSSION: At both of Experiment 1 and 2, there was no difference in the fibrinolysis time between the clots in and outside the magnetic field. It is considered that the higher intensity of magnetic field, the higher frequency of exposure period and the higher viscosity of sample solution will bring the positive results.
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SPECIFIC SINGLE PULSED ELECTROMAGNETIC FIELDS INHIBIT OSTEOCLAST-LIKE CELLS FORMATION IN OVARIECTOMIZED ANIMALS. W.H. Chang[1], K.T. Chang[1], Y.S. Yu[1], and C. Shih[2]. [1]Department of Biomedical Engineering, Chung-Yuan Christian University, Chung-Li, Tao-Yuan 32023 Taiwan, ROC. [2]Institute of Biology and Anatomy, National Defense Medical Center, Taipei 11472 Taiwan, ROC.

OBJECTIVE: With use of specially engineered single pulsed electromagnetic fields (sPEMF) stimulators, the effect of extremely low frequency electric fields on osteoclast-like cells (OCLs) recruitment can be investigated from bone marrow cells of ovariectomized rats.
BACKGROUND: Withdrawal of estrogen in women is associated with an increase in bone turnover with bone resorption exceeding bone formation leading to bone loss. This so-called postmenopausal osteoporosis (PMO) has become a serious issue worldwide and need to be tackle immediately. Alternatives such as sPEMF have provided an avenue to deal with PMO and acquired some positive results both in animal and clinical experiments. However, the underlying mechanisms of how sPEMF prevent and treat PMO still remain equivocal. The effects of sPEMF have been examined on bone cells but with a focus on the osteoblast. In this study reported here we intended to find out whether our own sPEMF parameters, which had been previously proved to be effective in preventing PMO in animal model, could suppress the formation of OCLs derived from bone marrow cells of ovariectomized rats.
METHODS: Eighteen 8-month-old female Wistar rats were involved in this study. The experimental rats were randomly divided into four groups: intact (INT), sham-ovariectomy (SHAM), ovariectomy (OVX), and PEMF stimulation (STI). Each group was further divided into two subgroups: Subgroup I (four days after surgery) and Subgroup II (seven days after surgery). All rats (except INT group) were subjected to bilateral ovariectomy. Ovaries were exteriorized and replaced for rats subjected to sham ovariectomy. Bone marrow cells harvested from both femora and tibiae of STI groups received the PEMF signals for 1 hour per day, which consist of a single pulse with pulse width of 300 μs, frequency of 7.5 Hz and induced electric fields intensity of 2 mv/cm. After 9 days of stimulation, recruitment of OCLs and associated cytokines (tumor necrosis factor-a, interleukin-6 and interleukin-1ß) were determined by tartrate-resistant acid phosphotase (TRAP), pit formation and enzyme-linked immunosorbent assays (ELISA).
RESULTS: Authentication of OCLs were confirmed in this in vitro bone marrow cells culture model by using TRAP stain and scanning electron microscope observation of pit formation on porcine bone slices. Formation of OCLs had no differences among INT, SHAM and OVX groups both in Subgroup I and II. However, the nuclei distribution of OCLs of OVX Subgroup I were significantly increased as compared to INT Subgroup I. Cytokines concentrations of these groups also had no differences. After stimulation by sPEMF (i.e. STI group), the proliferation of OCLs was inhibited significantly both in Subgroup I and II. The corresponding concentrations of cytokines were also decreased significantly when compared to OVX group.
DISCUSSION: In the work presented here, the formation of OCLs in this osteoporotic murine marrow culture model was suppressed when exposed to sPEMF. OCLs associated cytokines assayed in this study also demonstrated consistent variations. The dynamic homeostasis between bone formation and bone resorption was damaged once the gender steroids in both women and men were removed. In this study the playing role of sPEMF on OCLs, which are responsible for bone resorption, was our major interest. These results allow us to propose that the inhibition of bone resorption and the subsequently bone loss by sPEMF in vivo osteoporotic animal model can be elucidated partly by field attenuation of the recruitment of OCLs. Cytokines like tumor necrosis factor-a, interleukin-6 and interleukin-1ß might play a pivotal role between sPEMF and OCLs formation. Further exploration of this efficacious sPEMF on bone cells such as osteoclast and osteoblast might result in the promise for clinical applications on other skeletal disorders.

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Abstract withdrawn by author.

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CELL CYCLE DISTURBANCES, INTERLEUKIN PRODUCTION AND GENERATION OF SUPEROXIDE RADICALS AFTER EXPOSURE TO 50 Hz EMF IN MOUSE MACROPHAGES. M. Simkó, J. Rollwitz*. Division of Environmental Physiology, Institute of Cell Biology and Biosystems Technology, University of Rostock, Albert-Einstein-Str. 3, D-18059 Rostock, Germany.

OBJECTIVES: In a previous study (Simko et al., 2001), we described the activating capacity of sinusoidal 50 Hz electromagnetic fields (EMF) on the phagocytic activity and the production of free radicals in mouse bone marrow-derived (MBM) macrophages. We present here data on the production of interleukin-1ß (IL-1ß), nitrogen oxide radicals (NO) and superoxide radicals (O_2) after exposure to EMF for 24 h. Furthermore, we investigated the influence of 50 Hz EMF during DNA-synthesis.
METHODS: Mononuclear precursor cells were prepared from tibial and femural mouse bones and kept 7-10 days in cell culture for differentiation to mouse bone marrow-derived macrophages. Cultures were exposed to 50 Hz EMF at 1.0 mT for 45 min for phagocytotis assay, and free radical production or for up to 24 h for IL- ß production and BrdU assay, MBM-cells were cultivated on coverslips, incubated for 30 min in fresh culture medium containing 1 µm latex beads in the presence or absence of EMF or TPA (as positive control). The numbers of internalised beads per MBM-cells were determined with Nomaraki differential interference contrast (DIC) microscopy in 100 cells per cover slip. For O_2 determination the reduction of NBT to formazan within the cells was assayed by measuring the optical density at 550 nm using a microplate reader. The production of NO was measured by a colorimetric method based on the Griess nitrite assay. The generation of IL-1ß was determined by ELISA. To detect the influence of 50 Hz EMF on the S-phase of MBM-cells a colorimetric immunoassay, based on the incorporation of 5-bromo-2'-deoxyuridine (BrdU) into the DNA of proliferating cells was applied.
RESULTS AND DISCUSSION: It can be concluded, that EMF induced a significantly increased phagocytic activity in MBM cells (158 ±66%). Furthermore, our data indicate significant differences in the production of superoxide radicals but no differences in NO after stimulation with EMF compared to control cells. Also a significant increase of IL-1ß production could be observed after 24 h EMF-exposure, from 19,7±5,1 pg/ml to 71,5±35,5 pg/ml in the cell culture medium (CI: 2,42 for control and 15,96 for exposed cells, respectively). After 12 h exposure to 1 mT up to 24 h a significant increase in the uptake of BrdU was observed after, when compared to controls.
In summary, it can be supposed, that 1 mT magnetic fields induced a cell activation process, which could be shown through the significant increase in phagocytic uptake, in the production of superoxide radicals and IL-1ß, as well as through the changes in the DNA-synthesis. Further studies about the mechanisms of the cell activating process and EMF as a possible mediator are in progress.
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P-70

EFFECT OF LOW FREQUENCY (50 Hz) ELECTROMAGNETIC FIELD ON PRIMARY HUMAN ORAL KERATINOCYTES (HOK). V. Manni[1]*, A. Lisi[1]*, D. Pozzi[2]*, S. Rieti[1]*, L. Giuliani[3]*, S. Grimaldi[1]. [1]Instituto di Neurobiologia e Medicina Molecolare CNR- Rome, Italy. [2]Dipartimento di Medicina Sperimentale e Patologin Universita La Sapienza Rome, Italy. [3]ISPESL- DIPIA Rome, Italy.

OBJECTIVE: In this work we analysed the effect of ELF on a primary normal human oral epithelial cell line (HOK). Epithelial cells are an interesting model to study the biological effect of the interaction with non-ionising radiations, are not shielded by any other stratum of cells in the impact with electromagnetic radiation, and so they are totally available to the field.
METHODS: HOK cells were exposed in a solenoid placed in a cells incubator to maintain temperature at 36.5 °C and CO_2 at 5% with 90% humidity. Primary human keratinocytes cells are also a very good model for studying of epithelial switch between proliferation and differentiation . The effect of 50 Hz 2mT electric and magnetic field exposure on HOK cells resulted in both a decrease in cells proliferation and a minor clonogenic capacity . As compared to control unexposed cells the 96 hours exposure to a 50 Hz field caused HOK cells to grow at lower values. By ultra microscopy, at 72 hours, exposed cells showed a modified morphological aspect: they are bigger and more elonged than control ones. Exposed cells lost filopodia, and show a big number of lamellipodia, specialized structures for cell-cell contact. The augment of cell junctions as of cell adhesion, is also supported by a major expression in β-Catenin. β-Catenin is a protein implicated in cell-cell-adhesion, binding cytoplasmic domain of cadherin, and in signal transduction . The adhesion marker β catenin in 72 hours exposed cells is clearly more dense in spots around the cytoplasm, while in non exposed cells is just visible, distributed throughout the whole cells . Cell adhesion molecules and their association with actin cytoskeleton play an important role not only in the manteinance of tissue integrity, but also in proliferation and differentiation . Exposure to the field also causes rearranging of actin filaments, leading to an increase in actin expression in the formation of stress fibres that cross parallel to the elongated cells. In human epidermis, involucrin is first observed in the cytoplasm of spinous and granular layer cells. In transition cells, it is equally distributed between the cytoplasm and the nascent corneified envelope, while in the corneocytes it is largely corneified envelope associated. In our experiments involucrin expression in the exposed cells, is increased compared to control. This observation may suggest that the exposed cells are at a major differentiation status than controls, also confirmed by the increase in cell-cell adhesion and by the decrease in of cellular growth rate of exposed samples. These interpretation also agree with data about the decrease of the expression of EGF receptor. The EGF receptor plays a central role in numerous aspects of keratinocytes biology. In normal epidermis the EGF receptor is important for autocrine growth of this renewing tissue, suppression of terminal differentiation, promotion of cell survival, and regulation of cell migration during epidermal morphogenesis and wound healing. We report a decrease of expression of EGF receptor in 50 Hz, 2 mT exposed cells, compared to controls. These data confirm that 50 Hz 2 mT electromagnetic field, carries human keratinocytes, to an upper differentiation level.
SUMMARY AND CONCLUSION. 2 mT electromagnetic field induces an alteration of growth and differentiation pattern on HOK cells, throughout a decrease of EGF receptor expression. The modification of morphology, cytoskeletal assept, and expression of adhesion markers confirm that exposed cells are at an upper differentiation level.

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ENHANCEMENT OF UV-INDUCED CHROMOSOMAL RECOMBINATION IN YEAST BY EXPOSURE TO ELF MAGNETIC FIELDS. Y. Takashima[1*], M. Ikehata[2*], T. Koana[1,2*], J. Miyakoshi[3]. [1]Department of Built Environment, Tokyo Institute of Technology, Nagatsuta 4259 Midori-ku Yokohama, 226-8502, Japan. [2]Railway Technical Research Institute Hikaricho 2-8-38 Kokubunji Tokyo, 185-8540, Japan. [3]Department of Radiation Genetics, Graduate School of Medicine, Kyoto University. [3]Yoshida-Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan.

INTRODUCTION: We have previously reported that exposure to a 50Hz, 20mT magnetic field increased the frequency of mitotic recombination in post replication repair defective mutants of *Drosophila melanogaster* [1]. In this study, we examined whether ELF magnetic fields affected the frequency of the gene conversion/recombination in diploid budding yeast.

METHODS: Gene conversion/recombination at the *ARG4* heteroallelic locus was measured in a *Saccharomyces cerevisiae* DNA repair proficient strain XD83 and a nucleotide excision repair deficient (*rad3*) strain MLD39. The late log phase cells were washed and plated on to SD plates with amino acids except arginine. Cells were irradiated with UV or sham irradiated. Then, plates were randomly divided into two groups. One group was exposed to a 30mT, 50Hz magnetic field for 3 days at 30±0.5°C. The other group was incubated in conventional incubator as control. Number of colony on each plate was scored as revertant that restored the functional allele of *ARG4* by gene conversion/recombination.

RESULTS: There was no significant difference in revertation rate in cells with magnetic field exposure alone, either in repair proficient and deficient strains (Fig.1). However, magnetic field exposure after UV irradiation, the gene conversion rate increased in repair proficient strain (Fig.1 A), whereas the increase was not observed in repair deficient strain (Fig.1 B). These results suggested that the magnetic field affected the chromosomal recombination via nucleotide excision repair process that is induced by UV-irradiation.

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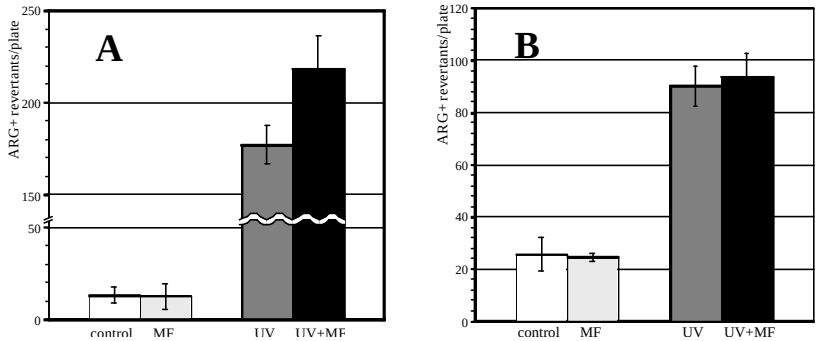

Fig.1 Results of 50Hz magnetic field exposure on *ARG+* gene conversion rate. A: XD83, UV=100J/m^2. B: MLD39, UV=3.3J/m^2

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MIGRATORY BEHAVIOR OF PERIPHERAL HUMAN LYMPHOCYTES (CD4) UNDER 50HZ MAGNETIC FLUX DENSITIES OF 10mT. E. David[1], J. Reißenweber[1], F. Gholamrezaei[1], A. Wojtysiak[1], M. Pfotenhauer[*1], B. Niggemann[*2], K.S. Zänker[*2]. [1]Electropathological Research Center, Witten/Herdecke University, D-58453 Witten, Germany. [2]Institute of Immunology, Witten/Herdecke University, D-58453 Witten, Germany.

INTRODUCTION AND OBJECTIVES: Lymphocyte locomotion under the influence of rather strong magnetic fields in comparison to that without field should be investigated. Extremely-low-frequency (ELF) and low-frequency (LF) electric and magnetic fields and high-frequency (HF) electromagnetic fields of our everyday life are discussed to provoke various impairments of well-being in some few percent of the general population. In this context the term of electromagnetic hypersensitivity must not be neglected. Up to now investigations using magnetic flux densities of up to 100 µT (50 Hz) in human and animal experiments normally did not result in significant biological effects. Nevertheless public discussions about biological or medical effects of electromagnetic fields are more intensive than ever. Therefore we elucidated the question whether lymphocyte migration and velocity – important parameters of the body´s immune surveillance – might be modified by magnetic flux densities in the intermediate intensity range. So magnetic 50 Hz flux densities of 10 mT were given. This value represents the 100 fold of the German threshold value of 100 µT (50 Hz) concerning extremely low frequency (ELF) electric and magnetic fields implemented in the 26th Ordinance of the German Federal Immission Control Act. Thus a major objective of this preliminary study was to test if magnetic flux densities above that threshold value could cause significant effects in terms of enhanced or diminished cell velocity or motility.

METHODS: Peripheral human lymphocytes (CD4) were examined in regard of average velocity and percentage of moving lymphocytes under and without exposure in extremely low frequency magnetic fields. Lymphocytes were taken from blood samples drawn from healthy humans by antecubital venipuncture. Cluster of differentiation (CD4) cells were chosen which represent T-helper cells or T-inducer cells. Homogeneous sinusoidal flux densities of 10 mT at a frequency of 50 Hz were applied by a magnet. By time-lapse video microscopy groups of exposed and sham-exposed (control) lymphocytes were filmed during migration for 120 minutes each. 30 single cells were selected within the dish in each trial and subsequently analysed as regards their locomotory behavior. For assessment of the moving percentage of 30 lymphocytes the individual pathway (track) was recorded and plotted. Finally the velocity of all lymphocytes could be determined. A temperature of 37 °C was exactly maintained throughout the experiments independently from status field on/off.

SUMMARY OF THE RESULTS AND DISCUSSION: A clear, however small, difference between mean percentages of moving lymphocytes (cells moving in percent of 30 cells observed) under and without field exposure could be demonstrated: the mean values with field were more elevated than those without field. However, no significant difference in cell velocities appeared between status field on/field off. Possibly lymphocytes´ locomotion in vivo through the tissue and even in vitro is influenced by adhesion phenomena between cells and cells or between cells and connective tissue or matrix, respectively. It is conceivable that adhesion forces might be weakened by the external magnetic field. Thus the average percentage of moving lymphocytes could indirectly be increased by the magnetic field. Another explanation: a quite small not measurable enhancement of temperature (heating effect) eventually caused by the magnetic coil could enhance lymphocyte metabolism. This would be a microthermic effect.

CONCLUSIONS: The present preliminary results point towards a visible difference between curves representing the lymphocytes´ migratory behavior with and without magnetic field influence which can be detected only as concerns the percentage of moving cells and not in terms of cell velocities. However, heating effects of magnetic coils cannot completely be excluded from the first. These new findings should be verified in future experiments where larger numbers of cells should be integrated and beyond the percentage of moving cells and lymphocyte velocity further even mathematical parameters of cell locomotion under and without field influence should be examined.

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TIME DEPENDENCE OF STATIC MAGNETIC FIELD EFFECTS ON ERYTHROCYTE CELL AGGREGATION, SURFACE ELECTRIC CHARGE DENSITY AND MICRO-VISCOSITY IN MODEL SYSTEMS *IN VITRO.* L. Traikov, M.S. Markov*. Medical University-Sofia, Department of Medical Physics and Biophysics, Sofia-1431, Bulgaria; *EMF Therapeutics, Inc., Chattanooga, Tennessee 37405, USA.

INTRODUCTION: Several studies on the identification and characterization of the membrane surface electrical charge cell aggregation parameter and micro-viscosity under magnetic field (MF) action were published during last decade. They attempted to elucidate physical or physicochemical parameters, which are responsible for membrane alterations. Membrane surface is one of the main targets for MF action, conformational and configurational changes in structural components of erythrocyte membrane are the reason for changes of relative interactions between the cells in the suspension. Respectively these changes can be used as an indicator for evaluation of static magnetic field action on the erythrocyte membrane *in vitro*.

MATERIALS AND METHODS: Blood taken from healthy volunteers and prepared for blood transfusion was used in this investigation. The experiments were performed up to 24 h after donation. After plasma removing, the blood cells were washed twice in Phosphate Buffer Solution by a centrifugation at 2000 g for 10 min. The relative cell concentration (Ht%) under this preparation was 0.70±0.02 %. Static MF of 5 mT was created by the system MS-2 (Sofia University, Bulgaria). The electrophoretic mobility (EPM) test was used as a screening method. The experiments were performed in the same day after the blood was taken. Exposition time (5, 10, 15, 20, 25, 30, 40, 50 and 60 min.) at constant temperature 22^{o}C. A Cytopherometer (OPTON, Germany) was used to evaluate the EPM of red blood cells. The method is based on applying a weak electric field to a very diluted cell suspension as described elsewhere. The time of migration was the parameter monitored in the experiment. The migration time of each particular cell was measured 30 times. The pH and conductivity of the suspension were controlled by a digital pH meter (Zeibold) and a conductivity meter (Zeibold). A Dzeta-crit metter (Medical University-Sofia, Bulgaria) is used for evaluation of the rate of cell aggregation.
Oswald-Microviscosity metter is used for evaluation of the microviscosity of erythrocyte cell suspension. A paired Student t-test was used to evaluate the statistical significance of the results.

RESULTS AND DISCUSSION: The results suggest that MF can induce formation of specific kind of lipid domains, which mainly depend on the electric charge distribution. These changes are most probably related to the electric charge redistribution as a result of MF action. The changes in the EPM are most pronounced at 30 min exposure time against the control samples. EPM and related parameter-surface electric charge density (σ) are increased at these conditions (σ=2.1419 ± 0.0189.10^{-8} m^2.V^{-1}.s^{-1}), this means that raise content of charged group. Parameters cell aggregation (ς-crit up to 66.567 rel.u.) and microviscosity (up to η_{rel} =15.5 mPa.s), in vitro are increased also, and they are evidence for decreasing of stability of erythrocyte cell suspension. The effects demonstrated time dependence of MF action and confirmed other result obtained by us for 30 min specific time window. For other exposure times the differences we have not obtained significant results against the control (at the same conditions).

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EFFECTS OF ELLIPTICALLY POLARIZED MAGNETIC FIELDS ON MUTAGENIC ACTIVITY OF DOXORUBICIN USING GENE-LOCUS MUTATION ASSAY IN HUMAN LYMPHOBLASTOID CELLS. K. Tamura[1*]. K. Yasunaga[2*] and M. Sekijima[2*]. [1]Technical Research Center, The Kansai Electric Power Company, Inc. Hyoho 661-0974, Japan. [2]Mitsubishi Chemical Safety Institute Ltd., Ibaraki 314-0255, Japan

OBJECTIVE: We have previously investigated the biological effects of 60 Hz elliptically polarized magnetic fields (EPMFs), the environmental magnetic fields under power lines. Gene expression [1, 2] and triggers apoptosis [3] have been examined with human cancer cells, although no direct effects have been identified. In this study we investigated the possible synergistic effects of 60 Hz EPMFs on the gene-locus mutations that triggers point mutation, deletion, and/or translocation of the thymidine kinase (TK) locus causing the anti-cancer drug to damage DNA in the human lymphoblastoid cell lines, WTK-1 cells ($tk^{+/-}$, *p53* mutation).

METHOD: We used the EES-002, the multifunctional exposure system, manufactured by Electric Research and Management [4]. WTK-1 cells, mid log-phase growth (5×10^5 cells / ml, grown in RPMI medium 1640 supplemented with 10 % v/v heat inactivated FBS) were treated for 1 h with doxorubicin (Dox: anti-cancer drug, 0, 0.02, 0.05 μg / ml). Then, during the phenotypic expression period, 3 days for the TK locus, the treated cell concentrations were determined daily and the cells were diluted with medium to 7.5×10^6 cells per culture. The cell cultures were plated to determine the mutation fraction on the third day after treatment. Cells were plated in a 96-well microtiter plates at 2000 cells per well in medium containing 3 μg / ml trifluorothymidine (TFT) (4 plates per culture) for mutant selection and 1.6 cells per well in medium (2 plates per culture) for cell viability. The number of wells containing colonies was counted on day 14 and 28 after plating, and large and small colonies were scored, respectively. The large colonies were formed from the normal growing cells with point mutation of TK locus and the small colonies were the slow growing cells with deletion and/or translocation of TK locus. The 60 Hz EPMFs (0.5 mT) were exposed at two different periods. These periods were first, co-exposure of EPMFs with Dox for 1 h, and, second, during the phenotypic expression period, post Dox treatment for 72h.

SUMMARY: Initial DNA damage caused by the Dox treatments for 1h increase in dose-dependent induction of growth arrest, resulting in an increase in the mutant fraction (MF), TK locus mutation, could be demonstrated in WTK-1 cells. Survival rates were unaffected by EPMFs exposure either during initiation or in the phenotypic expression period. Co-exposing WTK-1 cells to 60-Hz EPMFs (0.5mT) and Dox for 1 h caused no statistical difference ($p>0.05$) in the rate of MF with TK-locus mutation. After exposure to 0.5 mT EPMFs in the phenotypic expression period, the mutation frequency of TK-locus were also not markedly changed after 28 days culturing presence of TFT when compared with the sham-exposure. There was no significant difference ($p>0.05$) in the genotoxic events in WTK-1 cells between EPMFs exposed and sham exposed cultures. These results suggest that EPMFs could not affect the progression that triggers gene mutations causing anti-cancer drug that damages DNA. On going now, we will be evaluating the long time cultures, during the mutant colony formation period in presence of TFT for 28 days, exposed to EPMFs being synergistic or having additive effects on Dox induced TK-locus mutation of human lymphoblastoid cell lines using this system.

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P-75

HUMAN GLIOBLASTOMA A172 CELLS EMBEDDED IN TYPE I COLLAGEN GEL ARE ORIENTED UNDER 10T STATIC MAGNETIC FIELDS. H. Hirose[1,2*], T. Nakahara[1*], M. Yoshida[1*], J. Miyakoshi[1]. [1]Dept of Radiation Genetics, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan. [2]Kashima Laboratory, Mitsubishi Chemical Safety Institute Ltd., Ibaraki 314-0255, Japan.

OBJECTIVE: It was reported that neurite elongation was oriented with magnetically aligned collagen, and that osteoblast and endothelial cells were oriented without collagen under static magnetic fields (SMF). In this study, we investigated the preferred orientation of human glioblastoma A172 cells under exposure to SMF with and without collagen to approach molecular orientation of intracellular proteins such as cytoskeletal filaments.
METHODS: We used an exposure system to strong SMF up to 10T. The temperature of the incubator could be set at 20-37? ±0.2? . A172 cells were cultured in the Dulbecco's modified minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The cells were seeded in the culture dishes or on the cover glasses in a dish with type I collagen solution. The dishes were exposed to 10T SMF for 1 hour, and then transferred to a conventional CO_2 incubator. After the incubation without the field for 2 hours, we added a fresh DMEM supplemented with 10% FBS on the gel embedding the cells. The cells were observed using a microscope everyday for 7 days. Immunostaining of cellular β-tubulin was done at the 3rd day.
RESULTS AND DISCUSSION: A172 cells embedded in collagen gel were oriented perpendicular to the direction of the SMF at 10T, while the cells were not oriented without SMF exposure. The growth of A172 cells embedded in collagen gel showed no significant difference between SMF- and sham-exposure groups. A172 cells cultured without collagen were not orientated after 7 days of incubation under exposure to 10T SMF. These results suggest that A172 cells might be oriented with magnetically aligned collagen. The orientation of A172 cells embedded in collagen gel could be observed by immunostaining cellular β-tubulin. There was no difference in distribution of cellular β-tubulin between SMF-exposed and sham-exposed cells.
This work was supported in part by a Grant-in-Aid from the Research for the Future Program, Japanese Society for the Promotion Science.

P-76

EFFECTS OF INTERMITTENT MAGNETIC FIELDS ON TRANSIENT INCREASE IN INTRACELLULAR Ca^{2+} CONCENTRATION IN PHEOCHROMOCYTOMA CELLS. K.H. Park[1], T. Ikehara[2], H. Houchi[3*], H. Yamaguchi[2], Y. Kinouchi[1], K. Yoshizaki[*2], H. Miyamoto[4]. [1]Department of Electrical & Electronic Engineering, Faculty of Engineering. [2]Department of Physiology, School of Medicine. [3]Department of Pharmacy, School of Medicine, The University of Tokushima, Tokushima 770-8506, Japan. [4]Department of Life, Environmental and Information, Faculty of Domestic Science, Tokushima Bunri University, Tokushima 770-8514, Japan.

OBJECTIVE: Studying on effects of magnetic fields for various cellular functions will lead to new applications to frontier medical treatment, e.g., regenerative medicine, biomaterial, etc. The purpose of this study is to find mechanism that the magnetic fields inhibited Ca^{2+} release from intracellular Ca^{2+} store induced by addition of several stimulant in pheochromocytoma cells (PC12 cells).
MATERIAL and METHODS: Rat pheochromocytoma PC12 cells were cultured in RPMI 1640 medium containing 5 % fetal bovine serum and 10 % horse serum in plastic culture flask. Cells were dispersed in the same culture dishes of 35 mm in diameter for measuring the Ca^{2+} concentration. After the cells were attached to the dishes or cover glasses in the dishes, they were maintained for 2-3 days in a CO_2 incubator.

Magnetic fields are produced by an electromagnet designed and set up by Hitachi Metal Indust. Co. (Tokyo, Japan)[1]. This system produced intermittent magnetic fields varied between 0.07 to 1.5 T at an interval of 3 seconds. The cover glasses (13 mm in diameter) in the edge of plastic culture dishes (35 mm in diameter) were put in special incubator to keep their temperature at 37±0.2°C, and then the incubator was placed horizontally in the gap between two poles of electromagnet. The medium was changed to balanced salt solution (BSS) at this time. The concentration of intracellular Ca^{2+} was measured with Fura 2 by ARGUS 50/CA (Hamamatsu Photonics, Hamamatsu , Japan).

RESULTS and DISCUSSION: We tested the effects of the intermittent magnetic fields on increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) of PC12 cells by addition of 30mM caffeine. The increase in $[Ca^{2+}]i$ was inhibited by 2 hours-exposure under balanced salt solution excluding Ca^{2+} (Ca^{2+}-free BSS), but was unaffected under BSS. When the medium was changed to BSS with 1μM thapsigargin after the cells were incubated in Ca^{2+}-free BSS for two minutes, the $[Ca^{2+}]i$ was increase strongly. But the increase was not influenced by the exposure. Therefore these results suggest that the intermittent magnetic fields inhibit the Ca^{2+} release from intracellular Ca^{2+} stores of PC12 cells but not Ca^{2+} influx via cell membrane.

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P-77

EXPOSURE TO 50Hz MAGNETIC FIELD: INDUCED THE STRESS-ACTIVATED PROTEIN KINASE AND P38 MAPK SIGNAL PATHWAYS IN CULTURED CELLS. W.J. Sun[1], H. Chiang[1], Y.T. Fu[1], Y.N. Yu[2], H.Y. Xie[2] and D.Q. Lu[1]. [1]Bioelectromagnetics Lab. [2]Department of Pathophysiology, Zhejiang University School of Medicine, Hangzhou, 310031, P.R. China.

INTRODUCTION: Signal transduction pathway may mediate electromagnetic field (EMF) signals to generate bioeffects, and protein phosphorylation is one of the important processes of cell signal transduction pathways. Some reports showed that exposure cells to extremely low-frequency electromagnetic field (ELF-EMF) induced protein phosphorylation and stimulated activation of protein kinases [1, 2]. Kie et al. [3] found that ELF-EMF could induce the mitogen-activated protein (MAP) kinase (Erk1/2) activity. Stress-activated protein kinase (SAPK) and P38 MAPK belong to subgroups of mitogen-activated protein kinase (MAPK) superfamily. SAPK and its upper activators (SEK1/MKK4 etc.), P38 MAPK and its upper activators (MKK3/MKK6 etc.), and their substrates (c-Jun, Elk-1, etc.) make up signal transduction pathways—stress-activated protein kinase (SAPK) pathway and P38 MAPK pathway. In mammalian cells, these pathways are activated in response to a variety of stresses, especially physical stresses (such as ultraviolet ray, high osmosence and heat shock etc.), and regulate cell growth, proliferation, apoptosis, etc. We suppose that the 50Hz magnetic field (MF) may induce SAPK and P38 MAPK pathways.

OBJECTIVE: To investigate the possible effects of exposure to 50Hz MF on phosphorylation and activation of SAPK and P38 MAPK pathways in cultured cells.

METHODS: The exposure system consists of Helmholtz coils, two power regulators and a set of CO_2 incubators. A very uniform 50Hz MF was generated in the center of the coils, and the magnetic flux densities could be regulated from 0 to 0.8mT. Exposure and sham-exposure cells were cultured in the central area of the coils at 37±0.5? with 95% air and 5% CO_2, and the MF was perpendicular to the bottles. The exposure cells were exposed to 0.4mT for various times (3, 5, 10, 15, 30min, 1, 12, 24h, and a sham exposure). Cells was exposed to 50Hz MF for its corresponding time before harvested, and all subgroup cells were harvested at the same time (4 days). Following MF or sham-exposure for various times, the cells were suspended in lysis buffers, and whole cell extracts were extracted by centrifugation. The aim proteins were measured by western blotting analysis using corresponding antibodies. The phosphorylated form of SAPK/JNK was recognized from the shifted JNK band, and the enzymatic activity was confirmed using the solid-phase kinase assay. The activated forms of SEK1, P38 MAPK, and MKK3/MKK6 were recognized by Phospho-SEK1/MKK4 (Thr261) Antibody, Phospho-P38 MAPK kinase (Thr180/Tyr182) Antibody, and

Phospho-MKK3/MKK6 (Ser189/207) Antibody (BioLabs), respectively. The antibody-antigen complex was visualized by the ECL (Amersham). The percentage of optical density (O.D.) was calculated and compared. Experiments were repeated three times.

RESULTS: After exposure to 0.4mT MF for 3min, the phosphorylation of SAPK started enhancement, then appeared a slight dephosphorylation course. At 15min point, the phosphorylation of SAPK reached the maximum extent, which was 1.20 times that of the sham exposure. Following that, the phosphorylation of SAPK began to dephosphorylate, and the phosphorylation was lower than sham exposure. In order to explore the correlation between the phosphorylation and enzymatic activity of SAPK. The 15min was selected as the exposure time to detect the enzymatic activity of SAPK with solid-phase kinase assay. The results showed that after treated by 0.4mT MF for 15min the enzymatic activity of SAPK was 2.91±0.4 times that of the sham exposure. But 0.4mT MF exposure did not activate the SEK1 within 24h. Exposure to 0.4 mT MF for various times transitorily induced the activation of P38 MAPK at 10 to 15 min. Like the SEK1, 0.4mT MF exposure also didn't phosphorylate/activate the MKK3/MKK6 within 24h, which is a general upstream kinase of P38 MAPK.

DISCUSSION: The results of the present study showed that 0.4mT MF exposure of CHL cells could enhance SAPK phosphorylation. The solid-phase kinase assay demonstrated that SAPK phosphorylation was positive-correlation with its enzymatic activity. In the study for P38 MAKP pathway, the results also showed the activation of MF to P38 MAPK. It indicates that both SAPK and P38 MAPK pathways are concerned with the signal transduction of 50 Hz MF. SAPK and P38 MAPK activation reached maximum extent before 30min, which corresponded to the results of tyrosine phosphorylation of cellular proteins[2]. These activations belong to the early reaction to 50Hz MF exposure. However, the MF with the same condition did not activate their general upstream activators: SEK1 and MKK3/MKK6. It is suggested that 50Hz MF may activate the SAPK and P38 MAPK through the kinases other than SEK1/MKK4 and MKK3/MKK6.

CONCLUSION: 50Hz MF could activated SAPK and P38 MAPK via time-dependent manner, the biological effects caused by 50Hz MF maybe related with these signal transduction pathways. However, MF activates the SAPK and P38 MAPK signal transduction pathways through the kinases other than SEK1 and MKK3/MKK6. The activation mechanism needs to be identified.

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P-78

ELECTROMAGNETIC NOISE BLOCKS THE GAP-JUNCTIONAL INTERCELLULAE COMMUNI-CATION SUPPRESSION INDUCED BY 50Hz MAGNETIC FIELD.

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OBJECTIVE: Gap junctions are membrane channels that permit the transfer of ions and small molecules between contiguous cells. They play an important role in the maintenance of cell proliferation and differentiation. Disruption of GJIC may be a significant step in cancer promotion and provides an important index for identification of suspected cancer promoters. In our previous studies, we found that 50Hz ELF magnetic field (MF) inhibited gap-junctional intercellular communication (GJIC) at magnetic flux densities

of 0.4mT and 0.8mT[1,2]. Litovitz et al[3] and a number of papers reported that the presence of noise, comparable in magnitude to the MF signal, can nullify the MF bioeffects. In the present study, we explore whether the superposition of an incoherent (noise) magnetic field can block GJIC suppression induced by 50Hz 0.4mT MF.

METHODS: 1. ELF and noise exposure system consist of Helmholtz coils which set in a CO_2 incubator. The dishes containing mouse fibroblast cells (NIH3T3) were placed coaxially with the center line in the central area of the coils. Three treatment groups were conducted: a) sham exposure, b)0.4mT MF, c) 0.4mT MF conbined with 0.4mT noise (noise: MF=1:1). And the exposure time was 24hr.

2. The GJIC was determined using fluorescence recovery after photobleaching (FRAP) analysis, which performed with a laser-scanning confocal microscope (Leica, Germany). Cells were first photobleached to 30-50% of their original fluorescence intensity. After bleaching, we determined the fluorescence recovery of the bleached cells for 10min. The percentage of fluorescence recovery was used as the index of GJIC function.

RESULTS: The results in Table 1 show that the 0.4mT exposure group significantly inhibited GJIC as compared with the other groups ($P<0.01$). But there is no significant difference between the control group and MF-plus-noise group ($P>0.05$).

CONCLUSION: The function of GJIC is inhibited by 0.4mT 50Hz MF, and the electromagnetic noise with same intensity of MF can block the suppression induced by 0.4mT MF. The results demonstrated that ELF MF noise could inhibit the bioeffects produced by coherent ELF MF.

Table 1. Effect of noise and/or 0.4mT MF on GJIC in NIH3T3

Treatment groups	n	FRAP(% recovery)
Control	20	45.57±9.72
0.4mT MF	20	27.67±5.12*
MF-plus-noise	20	52.61±8.30

n, number of the bleached cells from the repeated experiments. Values are the means ± standard deviation. Student'T-text.

- $P<0.01$ vs. control or MF-plus-noise.

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P-79 Student

Abstract not accepted for publication in the Abstract Book.

P-80 Student

Abstract withdrawn by author.

P-81 Student

ELECTRIC FIELD REGULATION OF CHONDROCYTE BIOSYNTHESIS IN AGAROSE GEL CONSTRUCTS. N. Szasz[1], H. Hung[1*], and A. Grodzinsky[1*]. [1]Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

INTRODUCTION: The effects of physical factors on chondrocyte biosynthesis of extracellular matrix (ECM) macromolecules are important in cartilage degeneration and repair[1,2]. Electric currents are known to be coupled to mechanical deformation and fluid transport within cartilage due to the charged proteoglycan constituents of the ECM. Previous studies have shown that externally applied electric and electromagnetic fields can increase DNA synthesis[3], glycosaminoglycan (GAG) content[4] and protein synthesis[5] by isolated chondrocytes[3] or chondrocytes in explant organ culture[4,5]. Chondrocyte mechanotransduction has also been studied in 3-D agarose gel culture[6,7], a system in which the effects of a chondrocyte-synthesized ECM on the cellular response to physical forces can be studied experimentally over time in culture. Based on these studies, we hypothesized that electric fields could modulate ECM biosynthesis by chondrocytes in agarose gel culture, a useful system for further study of physical transduction mechanisms.

OBJECTIVES: (1) Quantify the effects of applied electric fields over a range of frequencies on proteoglycan (GAG) and protein synthesis by chondrocytes. (2) Correlate the observed changes in biosynthesis with changes in the production of stress response proteins.

METHODS: Chondrocytes were isolated from calf femoral condyle cartilage by established methods. Cells were cast at 1.5×10^7 cells/ml in 2% agarose and cultured at 37ºC in DMEM + 10%FBS. After several days, samples (5-mm X 5-mm X 1-mm-thick) were mounted in teflon holders that fit within test and control chambers (Fig 1). Samples were separated from platinum electrodes by 1" salt-bridges, to isolate the specimens from any electrode reaction products. 20μCi/ml ^{3}H-proline and 10μCi/ml ^{35}S-sulfate were added to the culture media as measures of protein and GAG synthesis, respectively. A 0.01-10kHz, 25 mA/cm^2 electric current density was applied across test specimens mounted in series for 20hrs at 37ºC, using a feedback-controlled constant current source. pH and temperature remained constant under these conditions. Specimens were then washed, weighed wet, and digested. Aliquots were taken for scintillation counting, GAG and DNA measurements. HSP70 levels in selected samples were measured by Western blotting.

RESULTS: Electric fields applied at 1kHz significantly upregulated ^{3}H-proline on day 5 ($p<0.01$) and day 8 ($p<0.001$) and increased ^{35}S-sulfate incorporation on day 8 ($p<0.001$) relative to controls in 3 independent experiments (Fig 2, ANOVA + post-hoc analysis). Studies at 10kHz showed no significant change in radiolabel incorporation compared to controls. Preliminary results at 100Hz show an increase in ^{3}H-proline incorporation but no effect on ^{35}S-sulfate incorporation; further studies at lower frequency are ongoing. HSP70 levels (Fig 3) were not elevated in samples subjected to electric fields compared to controls and, indeed, were significantly lower than the levels in positive controls cultured at 43°C for 3 hours.

DISCUSSION: An increase in proline incorporation as a result of a 1kHz, 25mA/cm^2 current density stimulus revealed an upregulation of protein biosynthesis, and further studies showed that this upregulation was not associated with the appearance of stress response proteins. Nevertheless, the exact nature of these proteins requires further investigation. Since 75-80% of proline incorporation within these same calf chondrocytes in the parent cartilage explants is associated with synthesis of collagen molecules[1], it can be hypothesized that the upregulated protein synthesis seen here corresponds in part to increased collagen production. The increase in sulfate incorporation is typically associated with production of proteoglycans such as aggrecan.

CONCLUSIONS: Electric fields can regulate the synthesis of ECM macromolecules by chondrocytes in agarose gel culture. It remains to be shown whether endogenous streaming potentials caused by compression of cartilage are related to these observations. In the future, we plan to combine these results with compression and transport studies in agarose gel and native cartilage explant systems to evaluate their relative and combined effects.

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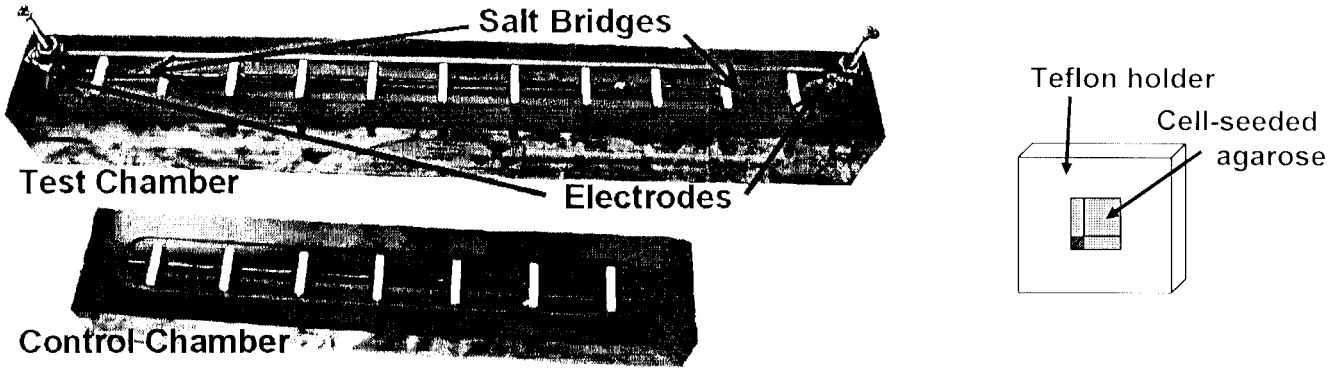

Figure 1. Test and control chambers. Each chamber holds 7 teflon holders with cell-seeded agarose in series. The test chamber has platinum electrodes at each end with salt bridges separating the samples from possible electrode reaction products.

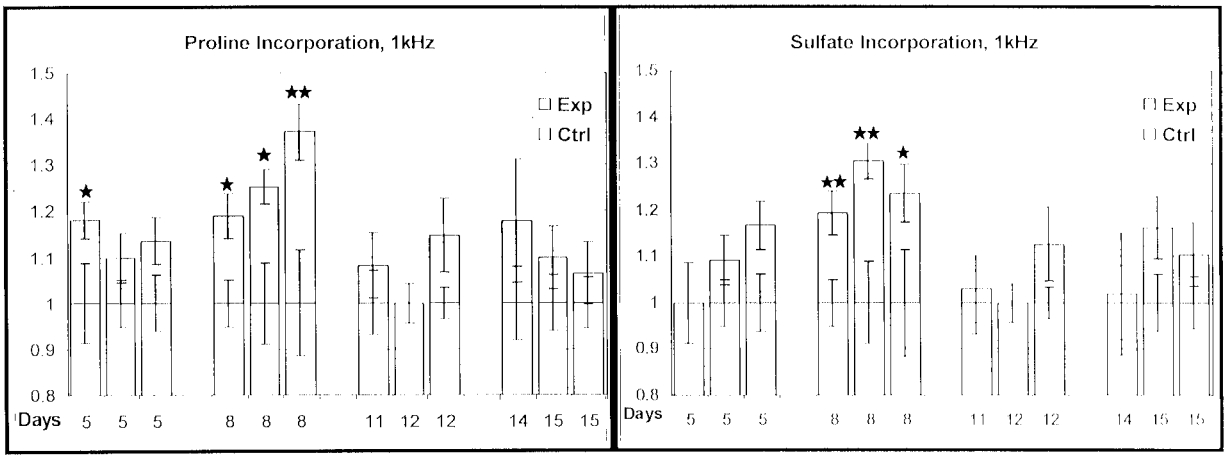

Figure 2. [3]H-proline incorporation at 1kHz after 5-15 days in culture. Results are normalized to non-stimulated controls. ANOVA revealed a significant difference between controls and experimental results on day 5 and 8 (*p<0.05, **p<0.01, n=7 for each group).

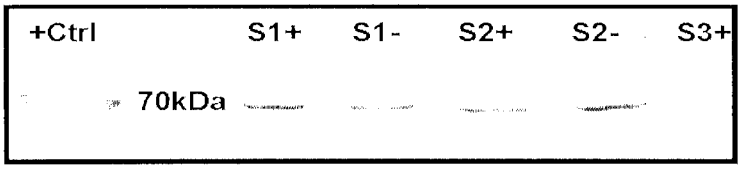

Figure 3. Western analysis for HSP70. The first lane contains the positive control (+Ctrl): selected samples were incubated at 43ºC for 3hrs, then frozen until later analysis. S1+, S2+, and S3+ were samples from day 8, 1kHz experiments. S1- and S2- were non-stimulated controls from the same experiment. (The empty lane between +Ctrl and S1+ contains a rainbow standard, which is too faint to see on the scan.)

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P-82 Student

Abstract withdrawn by author.

P-83 Student

EFFECT OF ELF MAGNETIC FIELDS ON THE MARKERS OF DIFFERENTIATION IN CULTURED OSTEOBLASTIC CELLS. Y. Nakano[1], K. Hosokawa[2], Y. Yamaguchi[2], K.H. Park[1], T. Ikehara[2], M. Kitamura[2], Y. Kinouchi[1], K. Yoshizaki[2], H. Miyamoto[3]. [1]Department of Electrical & Electronic Engineering, Faculty of Engineering, 2 Department of Physiology, School of Medicine, The University of Tokushima, Tokushima 770-8506, Japan. 3 Department of Life, Environmental and Information, Faculty of Domestic Science, Tokushima Bunri University, Tokushima, Japan.

OBJECTIVE: The objective of this study is to examine the physiological effects of exposure to ELF magnetic fields on induction of differentiation in culutured osteoblasts.We had obtained the preliminary results that treatment of triiodothyronine(T3) as a regulator of osteoblastic differentiation and exposure to ELF magnetic fields resulted in same effects of osteocalcin expression and inductive pathways of differentiation in osteoblasts seemed to be quite differed in both T3 and ELF conditions.In this study, we tried a variety of approaches to reveal more about the effects of exposure to the ELF magnetic fields.
MATERIALS and METHODS: Osteoblast-like cells of mouse(MC3T3-E1) were cultured with a alpha-modified minimum essential medium (H.Miyamoto et al,. 1976) supplemented by 10 % fetal bovine serum in plastic culuture dish of 35 mm in diameter. The culuture dishes were placed in two special incubators to keep the temperature of the cultures constant (37±0.2?). Magnetic fields produced by the coils were sinusoidal(60 Hz) and their rms values were from 1.25 to 3 mT. Induced current density in the medium was about 10 mA/m2 in average.Duration of exposing to magnetic fields was 24 hours about 3 days and 7 or 8 days cultures, respectively. Cultures were maintained to grow with or without 10-6M phorbol myristate acetate(PMA) and 10-3M cAMP. Intracellular alkaline phosphatase (ALP) activities were measured by colorimetric assay kit as the indicators of the differentiation of the osteoblasts in vitro.
RESULTS and DISCUSSION: Treatment of PMA for 24 hours to cultured MC3T3-E1 cells had not effected to the ALP activities in the cells. However, the treatment of cAMP for 24 hours to the cells showed results of enhancement on ALP activity in these cells.These results indicate that the effect of exposure to ELF magnetic fields seems to promote the effect of reagents as the inducer of differentiation. Also, we are trying to measure of activity on mRNA of osteocalcin as the marker of differentiation of the osteoblasts in order to examine with precisely the effects of ELF magnetic fields on the gene level.

P-84 Student

EFFECTS OF ELECTRICAL STIMULATION ON PC12 CELLS. M. Ogiue-Ikeda*, M. Sekino*, S. Ueno. Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan.

OBJECTIVE: Electrical stimulation promotes the regeneration of neurons and is effective in the treatment of neural diseases. However, the behavior of neurons exposed to electrical stimulation is not yet understood. In the present study, we investigated the effects of electrical stimulation on the growth of PC12 cells (model cells of neurons).

METHODS: PC12 cells were used as model cells of neurons since they experience enhanced axon outgrowth with the addition of NGF (nerve growth factor). Cells were prepared in Dulbecco's modified Eagle's medium containing 10 % fetal bovine serum, 10 % horse serum and 1 % antibiotic-antimycotic solution. Two platinum wires (diameter=200 μm) were placed parallel to each other at the bottom of a dish (diameter=60 mm) (Figure 1). Electrical currents were applied to the PC12 cells at different frequencies (0.1 Hz, 1 Hz, 10 Hz, 20 Hz) through the pair of platinum wires for 24 hours. NGF was added to the medium at a density of 100 ng/ml. The cells were stimulated at 37 degrees centigrade in 5 % carbon dioxide. Except for the stimulation condition, the control group was treated under the same conditions.

RESULTS and DISCUSSION: The PC12 cells gradually died under electrical stimulation of 10 Hz, 2200 V/m, 24 hours, whereas normal axonal outgrowth occurred in the cells under electrical stimulation of 10 Hz, 1800 V/m, 24 hours. Both dead cells and living cells were intermingled under electrical stimulation of 10 Hz, 2000 V/m, 24 hours (Figure 2). Thus, 10 Hz, 2000 V/m, 24 hours electrical stimulation is the threshold of survival for PC12 cells. As the stimulus frequency increased, the threshold decreased. The orientation of axonal growth of PC12 cells was not affected by the homogeneous, uniform electric fields used in this experiment. These results are useful for determining the appropriate safety standards of electrical stimulation on the human body, and for the control of neural growth.

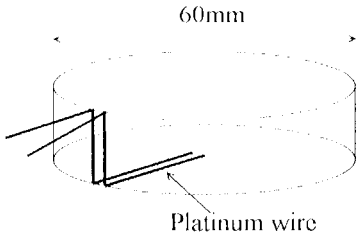

Figure 1: The position of the platinum wires at the bottom of the dish

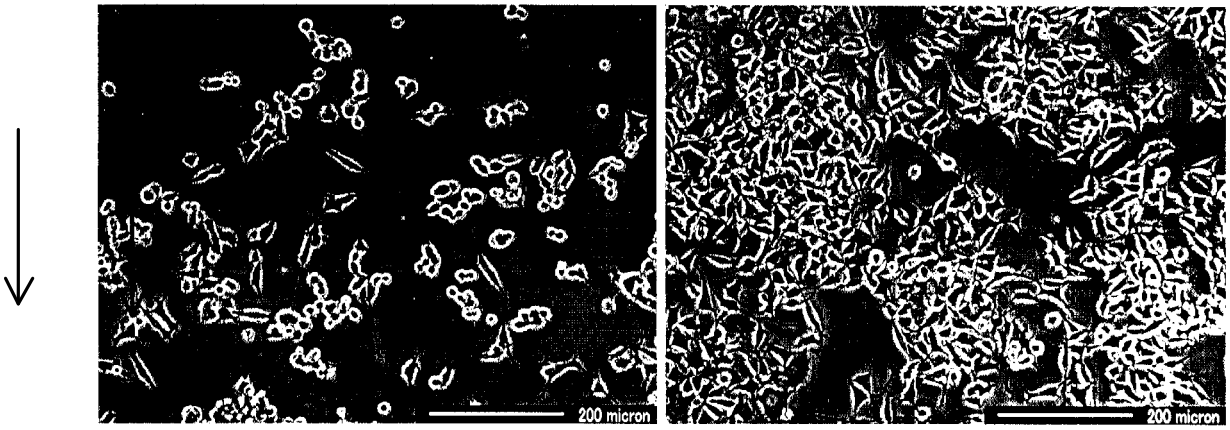

Figure 2: PC12 cells under 10 Hz, 2000 V/m, 24 hours electrical stimulation (left), and the control group (right). The arrow represents the direction of the electric field.

P-85 Student

EXPRESSION OF HEAT SHOCK PROTEINS IN CHROMAFFIN CELLS ELF MF DIFFERENTIATED. T. Olivares-Bañuelos[1,2], L. Verdugo-Díaz[1], L. Navarro[*1], and R. Drucker-Colín[2]. [1]Depto. De fisiología, Fac. De Medicina, [2] Depto. De Neurociencias, Instituto de Fisiología Celular, UNAM, 04510, México, D. F. México.

INTRODUCTION: Electromagnetic Fields have precipitated a cadre of research describing the interaction and alteration of biological substances and systems. They include modulation of ion and protein flow across membranes. Less information on protein synthesis regulated by Extremely Low Frequency Magnetic Fields (ELF MF) has been published with no direct evidence on synthesis and phosphorylation of heat shock proteins (Hsp).
OBJECTIVE: In this way, the aim of this work is analyze the expression of Hsp70 in chromaffin cells ELF MF differentiated.
METHODS: Three chromaffin cell groups were used: control (without any stimuli), NGF (20 ng/ml) and ELF MF (60 Hz, 0.7 mT 4 h/day for 7 days. Total RNA was obtained using TRIzol method. RT-PCR was made using a 17-bases primer named HsPAS (AGAGTCGATCTCCAGGC) specific for the Hsp70 messenger. The PCR reaction included a second 17-bases primer named HsPS (TGCTGACCAAGATGAAG) specific to the same messenger. PCR reaction was carried out at 94ºC for 1 minute, 55ºC for1.5 minutes and at 72ºC for 1 minute by 30 cycles. The samples were run into 2% agarose gels and were observed with ethidium bromide.
CONCLUSIONS: The gels were analyzed and the groups were compared. We observed a 450-bp band corresponding to the expected size in control and NGF groups; meanwhile in the ELF MF GROUP, the band did not appear. The results suggest us that the ELF MF suppresses the expression level of an Hsp70 type in differentiated chromaffin cells.
Supported by DGAPA-UNAM IN208799 and Fideicomiso UNAM to RD-C.

P-86

GENOME-SCALE GENE EXPRESSION AND BIOINFORMATICS IN MORPHOLOGICAL DIFFERENTIATION OF NORMAL HUMAN EPITHELIAL CELL LINE, MCF-10A. S. Nakasono[*#%], C. Laramee[*#], K. McLeod[#]. [#]Program in Biomedical Engineering, SUNY at Stony Brook, Stony Brook, NY, 11794, [%] Bio-Science Department, CRIEPI, 1646 Abiko, Abiko-shi, Chiba 270-1194, Japan.

BACKGROUNDS: Genome-scale analysis for toxicology has been developing as a most powerful technique to find and classify toxicity of chemical and physical perturbation. We have previously investigated the biological effects of high flux density (10-300 mT) power frequency magnetic fields (MF). Genome-scale gene expression have been studied with bacteria (prokaryote), yeast (eukaryote) as simple model systems, and whole brain of mouse, although no effects have been identified. It may be possible that the biological system previously used is not sensitive since the cells were a mixture in different cell cycle (yeast and bacteria) or of different cell type (mouse brain). A subtle expression difference in a type of cell in the mixture will be impossible to find.
OBJECTIVE: The aim of this study is to construct a experimental model to find subtle effect of environmental perturbation by using normal human epithelial call line MCF-10A, which can differentiate morphologically in extracellular matrix [1].
METHOD: MCF-10A was obtained from ATCC. The cell line was cultured by modified H14 medium which does not contain serum. For morphological study, we used matrigel (BD Labware) as extracellular matrix. The cells could differentiate in extracellular matrix (10micro-g/mL) to make acinus structure at an initial cell density of 90K cells/cm^2. Phenotypic change in morphology was found by using alteration of gel

matrix, chemicals in medium, etc. For positive control, we chose collagen as matrix, high initial cell density (8 times) or no hydrocortisone in the medium, which condition would make spindle, branching and big colony, respectively. As a negative control, cells grown on plastic surface for 2 days (log phase) were used. The total RNA sample was took by using TRIzol, and was cleaned by RNeasy kit (Qiagen). Quality of the samples was checked by gel electrophoresis and A260/280, and only samples which have 10 to 15 micro-g total RNA, concentration of 1 micro-g/micro-L or greater, OD 260/280 ratio of 1.9 or greater or no degradation on gel picture, were used for GeneChip analysis. The labeled cRNA for hybridization was synthesized by amplification by RNA polymerase from promoter attached cDNA GeneChip HU95A, which has over 12,000 known genes (ca. 1/3 of expected total human genes), was used for each sample. For a bioinformatic analysis, we first used GeneSpring software (Silicon Genetics).

RESULTS AND DISCUSSION: At first, we checked reproducibility of gene expression in differentiation stage. Two different passages at five different stages (day 2, 4, 6, 8. 10) were used. In phenotypic view, the cells could be seen making acinus structure in matrigel during day 0-3, with dividing cells. The making structure was completed after day 4. The structure could be observed by general or confocal microscopy. No significant difference between passages was observed in visually. In preliminary gene expression analysis, 30-36 % of genes were estimated as present in passage A, and 5-16 % of all genes were estimated as changed genes in expression vs. day 2 on plastic. Only day 2 in matrigel was 5% and others were 13-16%. In passage B, 23-31% of the genes were estimated as present, and 7-14 % of all genes were estimated as changed genes. Only day 2 was 7% and others were 12-14%. Most of changed genes were same between passage, but expression level in many genes was too different between passage to get good cluster. While the PCA analysis of expression data, not ratio data, gives reproducible value, in first and second PCA, between differentiation stage. We are trying to make a good cluster by ratio data because the passage difference in gene expression could be neglected, and by using RNA from positive controls and static MF exposure (10-1000G). We also developing an advanced mathematical protocol to find subtle and reproduced effect of environmental perturbation.

Reference:

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CELLULAR – RF

P-87

EFFECTS OF CONTINUOUS OR MODULATED 2.45 GHZ ELECTROMAGNETIC FIELD ON MUTAGENESIS IN SALMONELLA TYPHYMURIUM.

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OBJECTIVE: During the last ten years, the works carried out at the frequency of 2.45 GHz did not show a direct carcinogenic activity of microwaves but suggested a possible contribution at the level of promotion or co-promotion. The aim of the present investigation was to test *in vitro* the indirect mutagenic effect of low power microwaves in combination with known carcinogens. The experiments were performed with procaryotic cells (*Salmonella typhimurium*) using the Ames II™ assay. This is a short-term genetic toxicity test used to detect mutagenicity of biological mixtures or complex environment. It allows large samples to be studied rapidly.

METHOD: Ames assay is based on the use of specially selected strains of bacteria *Salmonella typhymurium* containing different types of point mutations in the histidine operon. These resulting his^- organisms cannot grow in a medium unless histidine is supplied. When a mutagenic event occurs, it causes the reversion of the his^- strains to their wild type.
The bacteria were exposed to the electromagnetic field during their growing phase (16 h) and/or during the incubation time with the mutagen agent mixture (4-nitroquinoline-N-oxide, 125 ng/ml and 2-nitrofluorene, 195 ng/ml) (90 min.). The carrier frequency was 2.45 GHz, wave was either continuous (CW) or 217 Hz (square wave) amplitude-modulated (PW) with a power density of 8 mW/cm^2 corresponding to an average specific absorption rate (SAR) of 3.33 W/kg. For each experiment, a non exposed (sham) and an exposed Ames assay were carried out simultaneously in two identical incubators, under shaking, at 37°C. The waveguide antenna (rectangular horn) was placed alternatively above one incubator and the other at a distance allowing far field exposure. An assay corresponded to the repetition of the same culture conditions in 22 wells. Each experiment was reproducibly repeated 10 times. Growth of the bacteria was controlled under and after microwave exposure. Statistical analysis of the data was realized with the Student's *t* test and/or the Mann-Withney U-test.
RESULTS: The number of revertants remained unchanged when the bacteria were exposed to CW 2.45 GHz radiation during the growing and the mutagen treatment phases (16 h + 90 min). It decreased significantly when the cultures were exposed to PW 2.45 GHz radiation during the same incubation time ($p = 0.003$ for Students *t* test and $p< 0.001$ for Mann-Withney U-test). No significant effect was detected when irradiation took place during the period of incubation with the mutagen agents (90 min.). No difference were detected between sham and exposed bacteria density after irradiation, neither in their growing capacity after exposure.
CONCLUSION: The present work shows an influence of 217 MHz pulsed 2.45 GHz electromagnetic field on *Salmonella typhymurium* mutation level while no changes are observed after continuous microwaves exposure. In these experimental conditions and contrary to expectations, the data suggests a low "protective" effect of electromagnetic field exposure related to the modulation.
Research supported by the DGA (Direction Générale de l'Armement).

P-88

NON THERMAL EFFECTS OF 900 MHZ GSM SIGNAL RADIOFREQUENCIES ON HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS) *IN VITRO*. M. Capri[1*], C. Fumelli[1*], E. Scarcella[1*], S. Salvioli[1*], E. Bianchi[1*], M.P. Gianninoni[1*], P. Mesirca[2*], A. Antolini[3*], A. Schiavoni[3], F. Bersani[2] and C. Franceschi [1*]. [1]Department of Experimental Pathology, Section of Immunology, University of Bologna, 40126 Bologna, Italy. [2]Department of Physics, University of Bologna, 40126 Bologna, Italy. [3]TILAB, Telecom Italia Laboratories, Torino, Italy.

OBJECTIVE: The increasing spreading of mobile communication requires a better understanding of the interactions between radiofrequencies (RF) and biological systems, since non thermal effects are still largely unknown. In particular, studies on RF effects on human immune system are scanty. Immune system plays a central role in defensive mechanisms and in contrasting cancerogenesis. Alterations of a correct functionality of immune system cells can lead to the development of cancer. The aim of this study is to evaluate the effects of 900 MHz GSM signal RF on the functionality of human PBMCs *in vitro*. The following biological endpoints were evaluated: 1) cell proliferation, 2) cell cycle, 3) apoptosis, 4) mitochondrial functionality.
METHODS: PBMCs from 25 human healthy young donors cultured up to 72 hours were either exposed to RF in a TEM cell (SAR=76 mW/Kg) or sham exposed, 1 hour per day for 3 days. TEM cell was connected to a generator, an amplifier and a personal computer. TEM cell and sham system were placed in a thermostatic chamber at constant temperature of 37 °C. Dosimetry and control of temperature were technically taken into account.

The first hour of exposure was performed immediately after cell seeding and treatment (mitogens or apoptosis inducer). The following biological endpoints were evaluated 24 hours after last exposure:
cell proliferation was determined by ^{3}H-thymidine incorporation test after mitogenic stimulation with different concentrations of PHA (0.1, 1 and 5 µl/ml) or anti-CD3 (10 and 20 ng/ml).
Cell cycle was measured in stimulated lymphocytes by flow cytometry after 5-bromo 2'-deoxyuridine (BrdU), anti-BrdU monoclonal antibody and propidium iodide (PI) incorporations.
Spontaneous and 2-deoxy-D-ribose induced apoptosis (Barbieri et al, 1994) was evaluated by flow cytometry using Annexin-V / PI staining, in order to detect phosphatidylserine on the outer leaflet of the plasma membrane (early apoptotic stage).
Mitochondrial membrane potential modifications were measured by flow cytometry using the specific probe JC-1 (Cossarizza et al, 1993).
RESULTS: A small, but significant decrease in cell proliferation was observed after RF exposure in comparison with sham exposure, when the lowest dose of mitogen (0.1 µl/ml PHA) was used to stimulate cells, even if no alteration of cell cycle was found. Moreover, a slight increase of phosphatidylserine on the outer leaflet of the plasma membrane was observed in 10 mM dRib treated and exposed cells but no changes of mitochondrial membrane potential were revealed in spontaneous or induced apoptosis.
References.
Barbieri D. et al, Biochem Biophys Res Commun 201: 1109-1116, 1994
Cossarizza A.et al, Biochem Biophys Res Commun 197: 40-45, 1993
This study was fully supported by TIM (Telecom Italia Mobile).

P-89

EFFECTS OF GSM-900 EXPOSURE ON 70 KDA HEAT SHOCK PROTEINS IN NEURONAL AND GLIAL CELLS. F. Poulletier de Gannes, I. Lagroye, E. Haro, P. Dulou, B. Billaudel, B. Veyret.
PIOM/Bioelectromagnetics Laboratory, ENSCPB/EPHE, Pessac, France.

INTRODUCTION AND OBJECTIVE: In response to environmental disturbances, cells respond by expressing heat shock proteins. In this study, we focused on the 70-kDa family which is the major form of stress proteins found in the brain. Expression of the constitutive Hsc70 and the inducible Hsp70 forms were followed. The objective was to determine whether exposure to GSM-900 microwaves could change or induce the expression of the Hsc70 and Hsp70 proteins in neuronal and glial cells.
MATERIAL AND METHODS: Human neuronal (SH-SY5Y) and rat (C6) or human (U87) astrocytic cell lines were used as models. Three days before the experiment, cells were plated on glass coverslips in 24-well plates at a density of $0.5x10^{5}$ cells/well. The day before the experiment, coverslips were transferred to 35-mm diameter Petri dishes, and these were placed in the incubator in a wire-patch antenna for an 18-hour temperature stabilization.
In vitro exposure to GSM-900 was performed using a wire-patch antenna linked to a RF generator which generated the GSM-900 signal at 0.2 or 2 W/kg during 48 or 1 hours, respectively. Sham-exposed samples were run in the same way in a non-activated wire-patch antenna placed in a second identical incubator.
Following RF or sham exposure, the cells were fixed in PBS-paraformaldehyde (4%) for immunocytochemistry. Antibodies anti-Hsc70 and anti-Hsp70 were obtained from Stressgen®. The first antibody was revealed using an FITC-labelled antibody. Coverslips were mounted on slides with Mowiol® before microscopy observation. For each exposure condition, two to three independent experiments for each conditions have been performed in a blind manner.
Positive controls for heat shock proteins induction were performed by exposing the different cell lines to a heat shock at 45°C for 20 min.
RESULTS: Heat shock increased expression of the Hsc70 and Hsp70 in all cell lines.

Exposure to GSM signals at 0.2 W/kg for 48 hours and 2 W/kg for 1 hour increased slightly Hsp70 expression in C6 and U87 cell lines. No difference was observed in the SH-SY5Y cell line compared to sham exposure.
Regarding Hsc70 expression, no clear conclusion can be drawn from microscopy observation, because of the high basal level of Hsc70 expression in all cell lines.
DISCUSSION AND CONCLUSIONS: Our data show that exposure to GSM-900 microwaves are able to induce Hsp70 expression in rat and human glial cells. These results must be confirmed by Western blotting. If confirmed, this finding could mean that glial cells can sense RF as a stress factor, but also that they are able to react to it.
This work was supported by the European Union (Reflex Project of the 5th frame Work Programme), the Aquitaine Council for Research and the CNRS.

P-90

DNA DAMAGE AND MICRONUCLEUS INDUCTION IN HUMAN LEUKOCYTES AFTER ACUTE IN VITRO EXPOSURE TO A 1.9 GHZ CONTINUOUS WAVE (CW) RADIOFREQUENCY FIELD. J.P. McNamee, P.V. Bellier, G.B. Gajda, E.P. Lemay*, S.M. Miller* and A. Thansandote. Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa K1A 1C1, Canada

The current study was undertaken to investigate whether acute exposure of human blood to 1.9 GHz radiofrequency radiation, as emitted from digital PCS mobile phones, could elicit DNA damage and/or induce the formation of micronuclei in phytohemaglutinin (PHA)-stimulated leukocytes. Human blood cultures were exposed to a 1.9 GHz continuous wave (CW) radiofrequency field (RF) using a series of six circularly polarized, cylindrical waveguides. Mean specific absorbtion rates (SAR's) of 0.0, 0.1, 0.26, 0.92, 2.4, and 10 W/kg were achieved and the temperature within the cultures during exposure was maintained at 37.0 ±0.5 °C. Concurrent negative- and positive- (1.5 Gy ^{137}Cs ?-irradiation) control cultures were run for each experiment. DNA damage was quantitated immediately after RF exposure using the alkaline comet assay and four parameters (Tail Ratio, Tail Moment, Comet Length and Tail Length) were used to assess DNA damage for each comet. No evidence of increased DNA damage was detected by any parameter for RF-exposed cultures at any SAR tested. The formation of micronuclei in the RF-exposed blood cell cultures was assessed using the cytokinesis-block micronucleus assay. There was no significant difference in either the binucleate frequency, incidence of micronucleated binucleate cells or total incidence of micronuclei between any of the RF-exposed cultures and either the sham- or incubator negative controls at any SAR tested. These results do not support the hypothesis that acute, non-thermalizing 1.9 GHz CW RF field exposure causes DNA damage in cultured human leukocytes.

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***IN VITRO* EFFECTS OF 1800 MHz RADIOFREQUENCY ON HUMAN PERIPHERAL MONONUCLEAR CELLS (PBMCs) FROM YOUNG AND OLD DONORS.** M. Capri[1*], E. Scarcella[1*], C. Fumelli[1*], E. Bianchi[1*], M.P. Gianninoni[1*], P. Mesirca[2*], C. Agostini[2*], C. Franceschi[1*] and F. Bersani[2]. [1]Department of Experimental Pathology, Section of Immunology, University of Bologna, 40126 Bologna, Italy. [2]Department of Physics, University of Bologna, 40126 Bologna, Italy.

OBJECTIVE: Within the frame of the European Reflex Project, Bologna's group (Workpackage 4) has studied the possible effects of radiofrequency (RF) on human PBMCs *in vitro*. The cells from young and old donors were exposed to1800 MHz RF, modulated at different signals (GSM basic, TALK and DTX) and analysed for the following biological parameters:1) cell cycle; 2) spontaneous and induced-apoptosis; 3)

mitochondrial membrane potential (MMP) modifications in induced and spontaneous apoptosis; 4) expression of membrane receptors such as CD25, CD95 (or FAS or APO-1) and CD28 in CD4+ helper and CD8+ cytotoxic T lymphocytes, respectively. In specific, CD25 is the p55 chain of interleukin-2 receptor; CD95 is the receptor activating the pathway of programme cell death and CD28 is a membrane protein that transduces signals delivered by T cell receptor complex to active naïve T cells.
METHODS: 35 donors were studied until now (all of them gave their informed consent).
The exposure system, provided by Prof. Kuster's team, consist in two wave guides localised inside the incubator (atmosphere at 37 °C and 5% of CO_2) and connected to a generator, an amplifier and a PC by which is randomly determined the operating wave guide. All experiments are performed in blind. The exposure time was 10 min ON and 20 min OFF for 44 hours. PBMCs from 8 young donors were exposed or sham exposed to GSM basic signal (SAR= 2W/Kg), PBMCs from 10 young and 9 elderly to TALK signal (SAR= 2W/Kg) and PBMCs from 8 young were exposed or sham exposed to DTX signal (SAR= 1.3 W/Kg). Immediately after the exposure, biological parameters were evaluated by flow cytometry techniques, using a FACScalibur cytometer (Becton Dickinson). In specific: 1. cell cycle was evaluated in unstimulated and anti-CD3 stimulated lymphocytes after 5-bromo 2'-deoxyuridine (BrdU) and propidium iodide (PI) incorporation. 2. Spontaneous and 2-deoxy-D-ribose-induced apoptosis was analyzed using Annexin-V/PI staining. 3. MMP modifications were measured using a specific probe, JC-1, which switches its fluorescent emission from green to greenish-orange depending on mitochondrial membrane polarization. 4. CD25, CD95 and CD28 in CD4+ e CD8+ T cells were analysed using triple staining with monoclonal antibodies.
RESULTS: Data obtained until now, clearly indicated that no statistically significant differences were found between sham or field exposed PBMCs in cell cycle, apoptosis and MMP in all the enrolled donors. On the contrary, very small, but significant increases or decreases on membrane markers were found when the cells were exposed to TALK or DTX RF signals, both in young and old subjects. The observed effects were largely within the range of the physiological inter-individual variability. Actually, further experiments are in progress in order to increase the number of the subjects studied and to perform experimental repetitions, using cells from the same donor.
This study was fully supported by REFLEX project (5th Framework Programme of the European Union) "**R**isk **E**valuation of Potential Enviromental Hazards **F**rom **L**ow Energy **E**lectromagnetic Field (EMFs) e**X**posure Using Sensitive *in vitro* Methods"

P-92

LACK OF CANCER-RELATED CELLULAR EFFECTS IN MURINE CELL LINES FOLLOWING CO-EXPOSURES TO 900 MHz RADIOFREQUENCY RADIATION AND MX. O. Zeni[1], R. Di Pietro[1], M. Capri[2], E. Scarcella[2], E. Bianchi[2], C. Franceschi[2], G. d'Ambrosio[3], R. Massa[3], M.R. Scarfi[1]. ICEmB at: [1]CNR-Institute for Electromagnetic Sensing of Environment, Via Diocleziano, 328 – 80124 Naples; [2]Dept. of Experimental Pathology, Section of Immunology, University of Bologna, 40126; [3]Dept. of Electronic Engineering and Telecommunications, University Federico II, Via Claudio, 21 – 80125, Naples, Italy.

OBJECTIVE: NIH 3T3 and L929 murine cell lines were co-exposed to 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and 900 MHz radiofrequency (RF) radiation. The following endpoints were evaluated: cell growth; cell cycle; mitochondrial membrane potential (MMP) changes; induction of oxidative stress by means of Glutathione (GSH) content and Reactive Oxygen Species (ROS) formation.
METHODS: cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS), 1% L-Glutamine and 0.5% Penicillin-Streptomycin at 37°C in humidified 5% CO_2 atmosphere. Cell growth was evaluated by counting cells at light microscopy; cell cycle and MMP changes were investigated by flow cytometry techniques (FACScalibur; Becton Dickinson), by using BrdU, anti-BrdU monoclonal antibody and propidium iodide incorporation and a specific probe (JC-1),

respectively. Intracellular GSH content was measured spectrophotometrically, by using a commercial enzymatic kit (Calbiochem) and ROS formation was determined by measuring the conversion of 2'7'-dichloroflureseine diacetate to dichlorofluoresceine by ROS mediated oxidation (LS50 Fluorimeter, Perkin-Elmer).
Cell cultures were exposed for 30 minutes to RF (900 MHz, continuous wave; exposure in waveguide), at two SAR values (1 and 0,3 W/Kg). To investigate cell growth, cell cycle and MMP changes, cell cultures were exposed immediately after 24 hours of cell treatment with MX 50 µM final concentration. In the case of oxidative stress evaluation, cells were treated for 1 hour with MX (500 and 200 µM final concentration for L929 and NIH3T3, respectively) and exposed during the first 30 minutes of MX treatment.
For each biological endpoint 3 conditions were tested: sham exposures (controls), exposures to RF at SAR of 0.3 W/kg, exposures to RF at SAR of 1 W/kg. For each condition an MX-treated culture was also set up.
RESULTS: Preliminary results seem to indicate absence of effects following the RF exposure, alone and in combination with MX treatment, except for L929 cells where a slightly increase in the depolarisation of mitochondrial membrane was detected in co-exposed samples (SAR 1 W/Kg). Experiments are in progress to evaluate the effects of GSM signal in the same experimental conditions. Research supported by EU -Fifth Framework Programme- CEMFEC, Grant No. QLRT-1999-01129.

P-93

CANDIDA ALBICANS ENHANCED PHOSPHOLIPASE PRODUCTION AFTER EXPOSITION TO A STATIC NON-UNIFORM MAGNETIC FIELD. A. Gasparetto[1*], T.E. Svidzinsky[2*], C.R. Paula[3*], R. Oliveira[4*], J. Azeredo[4*]. [1]Department of Dentistry Universidade Estadual de Maringá, Maringá, Paraná 87080-310 Brazil. [2]Department of Physics, Universidade Estadual de Maringá, Maringá Paraná, 87020-000 Brazil. [3]Department of Microbiology, Universidade de Sao Paulo, Sao Paulo 05508-730 Brazil. [4]Center of Biological Engineering, Universidade do Minho, Braga, 4710-057 Portugal.

INTRODUCTION: Microbial virulence factors are responsible for tissue damage in hosts. *Candida albicans* is an opportunistic pathogen that constitutes an increasing risk of infection, especially for immunosuppressed or immunocompromised patients.
OBJECTIVE: The objective of this study was to determine the effect of a static non-uniform magnetic field on the phenotype expression of different strains of *Candida albicans*.
METHODS: The strains of *Candida albicans* were grown on phospholipase-agar, according to Shimizu et al. (1996) and incubated at 37 ºC inside a magnetic field (except the assays used as blank). The magnetic field was generated by two magnetite plates (Figure 1) and standardized as a function of distance versus number of magnetic plates (Figure 2). The magnetic field was of 500 gauss in the central part between the two magnetic plates.
RESULTS: The preliminary results show a visible increase in the halo formed due to phospholipase production, suggesting that the exposition to a magnetic field can enhance the expression of this virulence factor.

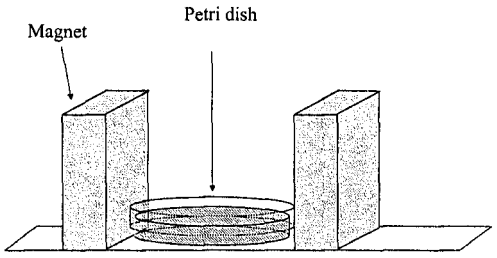

Figure 1- Schematic representation of the experimental set-up to generate the magnetic field.

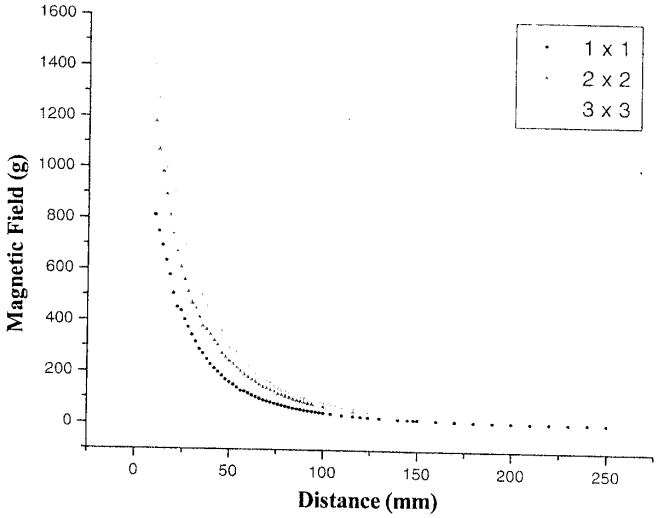

Figure 2 - Magnetic field (gauss) versus distance between magnetic plates (one, two or three in each side).

Reference.
Shimizu MT, Almeida NQ, Fantinato V, Unterkircher CS. Studies on hyaluronidase, chodroitin sulphatase, proteinase and phospholipase secreted by *Candida* species. *Mycoses* 1996; 39: 161-167.
We acknowledge the grants of A. Gasparetto by CAPES Proc. BEX Nº0103/012 and FAPESP 2000/13380-6.

P-94

STUDY OF MICROORGANISM STERILIZATION BY INSTANT MICROWAVE AND ELCTROMAGNETIC PULSE. Z.Y. Lu*, W.F. Wang*, L.F. Kong*, Z.Q. Niu* and S.H. Zhu*.
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Sterilization effects of constant electromagnetic wave and instant pulse acting on foods and traditional Chinese medicines pills are introduced in the paper. Their effects of sterilization are compared and discussed. But mechanism of action and biological effects are analyzed.
OBJECTIVE AND METHODS: The frequency of constant microwave in study is 2450MHz, the average power density is 520 w/cm^2. The instant pulse used is produced by pulse discharge tube, the rising time of the pulse is 0.33ns, the peak voltage is 2420v, the average light intensity is 10 candela. The pulse with high peak value and narrow width is produced when the discharge tube is punctured, then energy is radiated into the sterilizer by the antenna. "Yang Rong pill", "Bao He pill" and normal eating sauce were selected as experiment objects. They were sampled weighed (reserved some for reference) and placed into the sterilizer respectively. The samples which have been sterilized by constant microwave and electromagnetic pulse for some time and referential sample were paved on the board in the culture medium prepared previously. Eight paved boards for every sample. At last, culture medium were put into constant temperature of 37°C to culture for 48 hours. Germs will arise ,we made then numerical statistics of the germs number of all the samples.
RESULTS: Now, sterilized results of constant microwave and electromagnetic pulse (EMP) acting on several objects discussed upward are listed in the table below.

Germ contents/g	Electromagnetic pulse	Constant Microwave F=2450MHz		
Radiation time	Sauce	Sauce	Yang rong pill	Bao he pill
0s	$2.4*10^6$	$2.4*10^6$	$1.7*10^6$	$1.5*10^6$

30s	$1.6*10^3$	$1.2*10^5$		
100s		$2.1*10^3$	$1.71*10^4$	$5.29*10^4$
160s		$1.8*10^3$	1.45'10^4	$1.0*10^4$

DISCUSSION: From data in the table, we can find that no matter it is constant microwave or electromagnetic pulse, all have very good sterilization effects. But sterilization effects of them are different from each other for different samples when the constant microwave has the same radiation energy and the same acting time. Major objective may have two: Firstly, sterilization effects of microwave have relation to electric parameters of all material (dielectric e, conductivity s). Polarization of electric medium can not catch up with the variation of high frequency electromagnetic fields. The medium also appears energy loss which can be explained for damping force. The dielectric constant is a complex (e (?)= e'(?)-j e"(?)). True part e'(?) has same means with formerly dielectric constant, while imaginary part e" as s have the same function. When microwave energy be absorbed by the sample, the loss energy change into Joule's heat, in the hand, because the double polarity that is polarize by electromagnetic wave change fast, the heat is made through interaction and collision between double polarity and located nearby molecule. Both heat is cause of killing germs. Thus, we think that killing germs by constant microwave is base on thermal bioeffects. As table shows, the number of eating sance's s and e" is bigger than Chinese medical pills on the same condition, sterilization effect to eating sauce is better than to "Yang Rong pill and Bao He pill". Secondly, kinds of germ are different in the material. General, kinds of out of tens in them, the most kind germs can be killed by microwave thermal effects for tens seconds, but for some germ, such as fungus, viruses and spore, which can not be killed through microwave radiation in a short time. The more bacteria of this kind that sample owns, the more long time of killing germ are required. For example "Yang Rong pill and Bao life pill" have this character. Obviously, germ number of goods has not incline at same scale on a certain time. There is best killing germ time and electromagnetism dose for different sample. As the table shows, it seems that sterilization effect of instant pulse is better than that of constant microwave. The germs number of eating sauce can be decreased from 24000 to 16000 per gram in 30s, while it needs 100s to complete the process using constant microwave. It is a very complex course that EMP act on biology body base on the data. EMP can destroy bacteria's cell, tissue and lead them to stop breathe and finally dive out. In the course, sample temperature has not rise obviously. We think that the sterilization mechanism of electromagnetic pulse is base on nonthermal bioeffects of electromagnetism biology.

P-95

THE SPECTRUM OF CALCIUM OSCILLATIONS OF CANCER CELLS AND EFFECTS OF ELECTROMAGNETIC WAVES ON THE SPECTRUM.** Z.Q. Niu[*1], J.Q. Hou[*1], H.B. Wang[*1], J. Yan[*1], Z.Y. Lu[*1] and H. Zhang[*2]. [1]School of Electronic Engineering, Xidian University, [2]Northwestern Polytechnical University, [1]Xi'an 710071, P.R. China.

INTRODUCTION: The periodic variation of [Ca^{2+}] (free calcium ion concentration) of intracellular plasma with time is called the calcium oscillations, which represents a change law in time domain. In fact, in order to study the law of a oscillation, the two respects works need to be done, the first work is study in time domain that is study in the character of the oscillation with the lapse of time, the second work is study in frequency domain, that is study in the what components of frequencies contained in the oscillation and ratio of the components each other.
OBJECTIVE: The spectrum of the calcium oscillations in cancer cells and the effects of EMW (electromagnetic wave) on the spectrum were studied based on experiments and Fourier transformation.
METHODS: In the study, the Fluo-3 fluorescent reagent, was used for probe detecting [Ca^{2+}], the laser scanning confocal microscope was used for detecting the fluorescent intensity of the plasma in live cells, and the fluorescent intensity was used as basic data describing [Ca^{2+}], the cells are liven liver cancer cells. After cell sample were loaded by Fluo-3 fluorescent reagent, the fluorescent intensity of plasma in the cells

and one of background out the cells were detected by the laser scanning confocal microscope respectively, and the carves and data of their fluorescent intensity were obtained; the carves and data are the expression in time domain, which describes the variation law of the calcium oscillations with time. The significance change in [Ca^{2+}] was produced by some EMW with specific frequencies and intensities, that is, the window effects are present in the [Ca^{2+}] (see: BEMS 23rd Annual Meeting Abstract Book, P83,15-1). The spectrum analysis of calcium oscillations is based on the carves and data. It is known that any complex and irregular oscillation can be decomposed by Fourier transformation into many simple harmonic oscillations with different frequency and amplitude. The expression in time domain represents the variation in size of an oscillation, and the expression in frequency domain represents the frequency composition of the oscillation. The two expressions are similar important. For a cell in the sample, the amount of the data recorded by the microscope is 60-80, and there is an interval of 1s between two adjacent data. Based on aforementioned detected condition, the width of the spectrum analysis is 0,5 Hz.

RESULTS AND DISCUSSION: It is indicated from preliminary results of the spectrum analysis that: (1) There are some main frequency components in the spectrum of cell calcium oscillation of the liver cancer cells, the main frequency components are 0.14, 0.19, 0.23; 0.33 and 0.45Hz; (2) Certain EMW with specific parameters can cause [Ca^{2+}] to change, also cause the-spectrum to change, such as the EMW with f=16Hz, Epp=42.5V/m cause the spectrum to change from containing aforementioned frequency components into containing the components of 0.1, 0.23,and 0.47Hz.

** The Project Supported by National Nature Science Foundation of China and by Nature Science Foundation of Shaanxi province.

P-96 Student

EFFECT OF PULSED MICROWAVES (2.45 GHz) ON ESCHERIA COLI AT 37C. C. Rougier, J. Rabaste[2]*, F. Solano-Serena[1]*, P. Leveque[2]*, P. Leprat *[1]. Institut de Recherche en Communication Optique et Microondes (UMR CNRS 6615), Universite de Limoges, France.

OBJECTIVES: The aim of this study is to investigate the interactions between *Escherichia coli* and an electromagnetic field in non-thermal conditions using pulsed microwave (PW) irradiation. Suspensions of bacterial cells were subjected to either conventional heating (CH) or PW irradiation at 37°C.

METHODS: The PW irradiation system consisted of a function generator (Agilent, model 33120 A, USA), a 2.45 GHz microwave generator (SAIREM, model GMP 20 KE/D, France) and a cylindrical applicator (Figure 1). The function generator was programmed to deliver repetitive square pulses. 5 ml of bacterial cells were irradiated in glass tubes (diameter 14 mm ? length 400 mm). The programs for PW irradiation have been defined according to the microwave power and final temperature. They involve the following stages : an initial exposure phase (I) to rise from room temperature to 37°C and repetitive pulse trains of exposure (E) and non exposure (NE) phases to maintain the temperature at 37°C. The durations of I and E phases were determined for each microwave power ranging from 200 to 2000 W. PW irradiations were performed over different periods of time, up to 30 min. The temperature was sequentially measured by a fluoroptic thermometer (Luxtron, model 501, USA) during irradiation at different places in the test tube including the top, the middle and the bottom of the cell suspension.

The effects on cell membrane integrity were evaluated by flow cytometry (FCM) using propidium iodide (PI) staining. The leakage of protein in the liquid medium was also investigated. In order to determine if injury on membrane permeability was lethal or not, PI staining cells were sorted by FCM and incubated on an agar plate medium under optimal growth conditions.

RESULTS: The numeration of culturable cells on nutrient solid media after treatment did not show any measurable cell mortality. However, PW irradiation induced effects on membrane permeability for approximately 8 % of cells at 400 and 800 W. For *E. coli* suspensions exposed to 1400 or 2000 W, the percentage of permeabilized cells was less important and no effect was detected for 200 W and CH.

Increasing exposure times did not induce an increase of the percentage of permeabilized cells. The initial exposure phase (I) appeared to be responsible for most of the measured effects on membrane integrity. As compared to CH, PW irradiation induced no statistical effects on protein leakage whatever the microwave powers and the time of exposure were. Only, a part of permeabilized cells induced by PW irradiation was able to form colonies on solid medium.
CONCLUSIONS: Comparative study on *Escherichia coli* using CH and PW irradiation at 37°C indicated no effect on cell mortality but significant effects on cell membrane permeability. However, a fraction of permeabilized cells was able to form colonies. In order to determine if such effects are due to thermal (small localized hot spots) or non-thermal effects of microwave irradiation, a more detailed thermal characterization is in progress.
Moreover, the investigations of the effect of microwave powers ranging from 2 to 20 kW on bacterial cells is one of the future developments of this work.

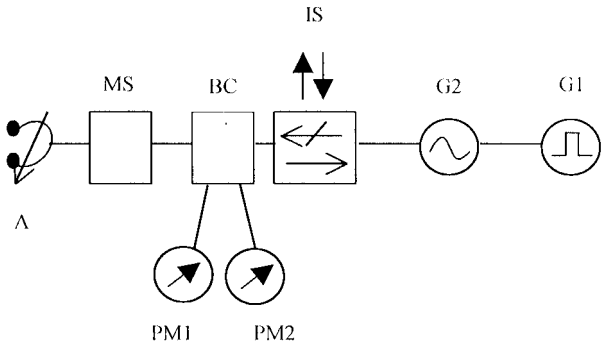

Figure 1 : Schematic of experimental PW irradiation system : function generator (G1), 2.45 GHz microwave generator (G2), isolator (IS), bi-directional coupler (BC), power meters (PM), matching system (MS) and cylindrical applicator (A).

P-97 Student

TRANSIENT POTASSIUM CURRENTS AFFECTED BY NONTHERMAL EFFECTS OF 1.9 GHz ELECTROMAGNETIC FIELDS IN ASTROCYTES. A. Wojtysiak[1*], U. Kullnick[2]. [1]Electropathological Research Center, University of Witten-Herdecke, D-58453 Witten; [2]Electromagnetic Fields Management, SIEMENS AG, D-81667 München; Germany.

Control of ionic current through the membrane is one of the most important mechanisms for correct cellular responses to environmental factors. Former experimental studies on this system with weak electromagnetic fields delivered contradictory outcomes, especially regarding the evidence for nonthermal effects due to high frequency fields. The presented results were achieved with a specially designed attempt to identify nonthermal effects. In this assay, the principally unachievable precondition of no temperature effects even with RF field exposure is bypassed by provoking the same temperature effects in the controls. The new data complete the results of a pilot study on outward potassium current in glial cells.
METHODS: Astrocytes from neonatal rats were grown on glass cover slips in DME medium with 10% FCS, Glutamin and Penicillin/Streptomycin. Experiments were conducted in a temperature controlled and superfused chamber laying in a rectangular waveguide with the cells located in the center and close to the bottom wall (superfusate solution: 140 mM Na^+, 3 mM K^+, 2 mM Ca^{2+}, 1 mM Mg^{2+}, 149 mM Cl^-, 10 mM Hepes, 11 mM Glucose, pH 7,4). Cells are visible through a fine wire mesh at this site. A continuous wave 1.9 GHz field is adjusted to cause a temperature rise in the bathing medium of 1°C or 3°C. The corresponding SAR values were 5.7 W/kg and 17.8 W/kg respectively, determined by FDTD calculation. The conventional heating is done by a stirred water bath connected with the tempering system of the

chamber. Temperature in the vicinity of the measured cell is permanently monitored with small fiberoptic probes. Transient outward potassium currents were measured with whole cell patch clamp under both (HF-EMF and conventional heating) conditions. Two connected clamp protocols were run for each current measurement to assess transient potassium currents. They were started when same temperature levels under both exposure conditions were reached. Current to voltage relations were determined before and during exposure and normalised to enable comparison between the cells.
RESULTS: Transient whole cell potassium currents rise temperature dependent with pure thermal and with RF field exposure. The Q_{10} for current increase is in all experiments around 1.4 (n=60). The current increase over the value at 37 °C is in most cases slightly reduced when the temperature level is reached with field exposure compared to conventional heated samples. This is consistent with previous reported results for the delayed rectifier potassium channel (see BEMS 2001). The maximum difference is reached at high exposure levels when temperature shifts from 37°C to 40°C. Differences in current change were up to 25% and statistifically significant ($p<0.01$, t-test). In the experiments series with lower field and ther-mal energy the reduction in current is less obvious and does not reach statistical significance.
DISCUSSION: The assay is a useful tool to identify nonthermal effects of high frequency electromagnetic fields in a biological system. The biological system shows a clear temperature related dose response. The 1.9 GHz field leads to a lesser increase in outward potassium current in astrocytes than would be expected from temperature elevation due to electromagnetic field absorption. This can not be explained by differences in general heating of the sample. The results on transient potassium current are consistent with previously shown data on delayed rectifier potassium channel. In combination with these former data, an effect on open probability of the channel gate is more likely than on diffusion behaviour of ions in the membrane channel molecule.
We thank for support from Ministerium für Schule und Weiterbildung, Wissenschaft und Forschung of Nordrhein-Westfalen and A. Bitz and V. Hansen, University of Wuppertal for exposure and dosimetrical support.

P-98 Student

IN VITRO EXPOSURE TO 0.6-MHz CURRENTS AFFECTS DIFFERENTLY CELL GROWTH AND CELL VIABILITY IN HUMAN CANCER LINES AND PRIMARY LYMPHOCYTES. M.L. Hernández-Bule[1*], M.A. Trillo[1], J. Matilla[2*], M.A. Martinez[1*], J. Leal[1], T. Montero[2*] and A. Ubeda[1]. Serv. Bioelectromagnetica[1] y Bioquimica[2], Hosp. Ramon y Cajal, 28034 Madrid, SPAIN.

OBJECTIVE: Sine wave electric currents at 0.6 MHz have been successfully used in Capacitive-Resistive Electric Transfer (CRET) therapy for muscle and tendon injuries. Preliminary data have shown that *in vitro* exposure to sub-thermal to thermal (10 to 150 $\mu A/mm^2$) levels of the currents can provoke cytotoxic or cytostatic effects in human cancer cell lines (Ubeda et al., BEMS 2000; Trillo et al., BEMS 2000). The aim of the present study was 1) to confirm the response of the cancer lines to the exposure to CRET signals at a non-thermal dose: 50 $\mu A/mm^2$, and 2) to compare such a response to that from human, primary lymphocytes obtained from healthy donors.
METHODOLOGY: Human cancer cell lines NB69 (neuroblastoma) and HepG2 (hepatocarcinoma), and human lymphocytes isolated from blood samples were seeded separately in plastic petri dishes and growth at 75% confluence. At that moment, pairs of sterile electrodes were inserted in the dishes. The electrodes in the experimental dishes (n = 5 per cell type) were stimulated during 24 hours with 5 minute trains of CRET currents at 50 $\mu A/mm^2$. During that time, the electrodes in the corresponding control dishes (n=5) remained unstimulated. A total of 19 experimental replicates were carried out: 5 with the NB69 line, 5 with the HepG2 cells and 9 with the primary lymphocytes. At the end of the 24 h of exposure and incubation, the cultures were examined for cell viability and proliferation and for DNA and protein content.

RESULTS AND CONCLUSIONS: Fig 1 shows the growth curves of control cells during the 24 h of sham-exposure. As can be seen, the cancer lines NB69 and HepG2 express a significant proliferative activity, whereas the primary cultures of normal lymphocytes do not proliferate but tend to decline in cell number. As shown in Fig. 2, the 24-hour intermittent treatment with a 50-$\mu A/mm^2$ current significantly increased the proportion of dead cells in the NB69 line ($p<0.05$, Student's t-test) and reduced the number of viable HepG2 cells ($p<0.01$). In both of the cell types, the described responses were accompanied with significant increases in the DNA/protein rates ($p<0.05$), confirming previously reported indications that the observed cytotoxic / cytostatic effects could be mediated by a deregulation of the cell cycle (Trillo et al., BEMS 2000). In contrast, the primary lymphocytes from human donors responded to the treatment with a modest though significant increase in the percent of viable cells ($p<0.05$) and no changes in the DNA or protein contents, which could be indicative of a potential slowing or recovering from the normal decline in the cell number observed in the control primary cultures (Fig. 1).
In sum, the present results show that: 1) Different cell types respond differently to a non-thermal dose of electric stimuli currently used in CRET therapy; 2) the data confirm preliminary observations that the treatment provokes cytotoxic or cytostatic effects (increased cell death or reduced cell proliferation) in human cancer cell lines NB69 and HepG2, respectively, and 3) in contrast, the same treatment seems to favour cell survival in primary cultures of human lymphocytes. Such a specificity in the cellular response to the in vivo treatment with CRET currents may have clinical implications that are worth of being investigated.

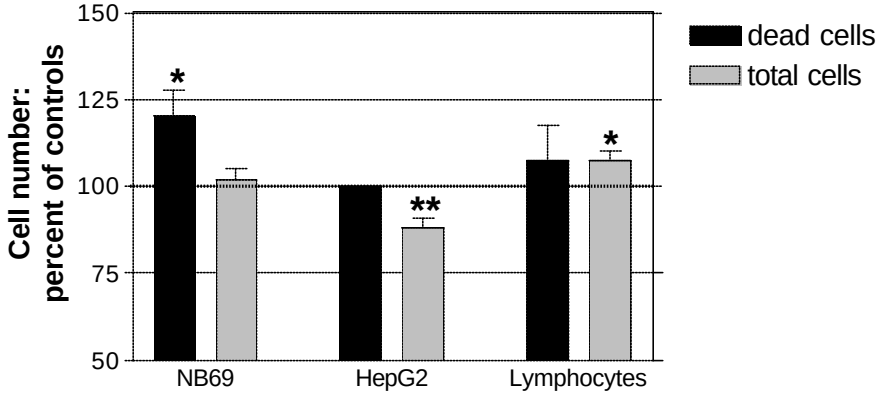

Fig. 1: Cell growth in sham-exposed controls. See text for details.

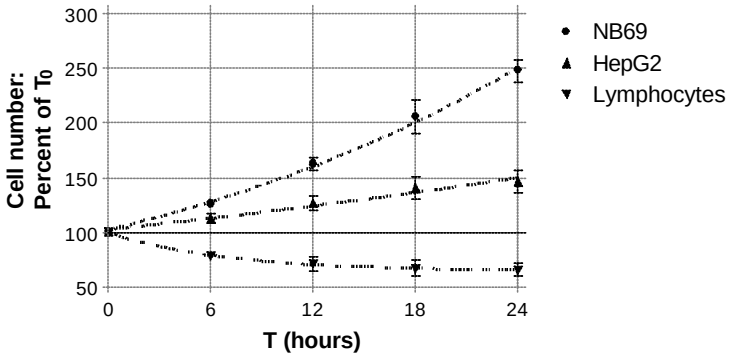

Fig. 2: Cell response after 24-h exposure to 0.6 MHz, 50 $\mu A/mm^2$. See text.

Study commissioned by Direccion General de Productos Sanitarios (NIH, Spain) and supported by INDIBA, S.A., Barcelona (Spain).

P-99

GAP JUNCTION COMMUNICATION IN TRANSFECTED HUMAN CELL LINE: ACTION OF MELATONIN AND MAGNETIC FIELDS. X. Wang[1*], D.E. House[1*], J. Page[2], C.F. Blackman[1]. [1]National Health and Environmental Effects Research Laboratory, USEPA, Research Triangle Park, North Carolina 27711 USA, [2]Oakland, California.

OBJECTIVE: We previously showed that functional gap junction communication (GJC), as monitored by dye transfer (DT), could be enhanced in mouse C3H 10T1/2 cells and in mouse primary hepatocytes by treatment with physiological levels of melatonin (Mel), and that magnetic fields could reduce the Mel-induced effect. The objective of this study is to test the ability of physiological levels of Mel to enhance DT in human-derived HeLa cells that had been transfected with the connexin 32 (Cx 32) gene, thereby rendering them DT-competent. A pilot test was performed to determine if the GJC enhancement caused by Mel could be affected by exposure to magnetic fields as we had previously observed.
METHODS: HeLa cells transfected with the Cx 32 gene were obtained from Mesnil et al. (IARC, Lyon, France). The cells were maintained as per methods described by those investigators. Confluent monolayers of cells were treated with 0, 0.1, 0.4, 1 or 4 nM Mel in complete growth medium for 24 hours. In a pilot experiment, cells were exposed to 4 nM Mel for 24 hours then exposed to sinusoidal magnetic fields, 38.4 μT DC and parallel 45-Hz AC at 24.4 μTrms, for 3hr. Following all treatments, the cells were microinjected with Lucifer yellow dye and the percentage of nearest neighbor cells to which the DT was determined.
RESULTS: The amount of DT was statistically enhanced ($p<.05$) for cells treated with 0.1 nM Mel (88.0% ±3.8 SE) and with 4 nM Mel (90.3% ±3.4 SE) compared to the untreated controls (73.2% ±4.7 SE). DT in cells treated with 0.4 and 1 nM Mel (76.4% ±4.8 SE and 68.9% ±5.5 SE, respectively) was not different from the untreated cells. Fifty cells were tested in each treatment category. In the pilot study, cells treated with 4 nM Mel and subsequently with magnetic fields showed no change in DT compared to similar cells not exposed to magnetic fields. DISCUSSION: Connexin genes have recently been shown to display tumor suppression properties, presumably through the activity of their protein products that form gap junctions, allowing GJC between adjacent cells. One measure of GJC is the transfer of Lucifer yellow fluorescent dye among cells. Putative and known tumor promoting chemicals can reduce GJC, and it has been hypothesized that such reduction is a significant event in the tumor promotion process. We have shown that melatonin, a hormone with oncostatic properties, can enhance GJC in a cell line (10T1/2 cells) and in mouse primary hepatocytes, and that magnetic fields can remove that GJC enhancement as measured by DT. In this presentation we show that human cells transfected with connexin 32 gene so as to perform DT, can also respond to Mel with increased DT at physiological levels of Mel. However, unlike the other cells showing this property, this Cx 32-transfected, human cell line does not respond to magnetic field exposure by reducing the increased DT caused by Mel. This result could be interpreted as demonstrating that cells requiring a transfected gene to express GJC, although responsive to Mel, may not have all the regulatory elements other cells have to control the DT. An alternative possibility is that only specific connexins are responsive to magnetic field treatment. Since 10T1/2 cells express Cx 43, whereas mouse hepatocytes express Cx 26 and Cx 32, it is possible that only Cx 26 and Cx 43 are sensitive to magnetic field treatment. Although more study is needed to establish the basis for this finding, it is apparent that magnetic fields can be used to explore the biochemical control features of GJC regulation.
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P-100

ABSENCE OF MICRONUCLEUS INDUCTION IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES FOLLOWING EXPOSURES TO 900 MHz RADIOFREQUENCY RADIATION.

M.R. Scarfì[1], P. Olla[1], A. Sannino[1], F. Bersani[2], A. Schiavoni[3], A. Antolini[3], O. Zeni[1]. [1]ICEmB at CNR-Institute for Electromagnetic Sensing of Environment, Via Diocleziano, 328 – 80124 Naples; [2]ICEmB at Dept. of Experimental Pathology, Section of Immunology, University of Bologna, 40126; [3]TILab MANCA INDIRIZZO.

OBJECTIVE: Aim of this study was to evaluate the induction of micronuclei (MN) in human peripheral blood lymphocytes exposed to 900 MHz radiofrequency (RF) radiation, both continuous wave (CW) and GSM signal, at SAR level of 1.59 W/kg. In the same experimental conditions cell cycle kinetics has been also investigated.

METHODS: Peripheral blood from 14 healthy donors, aged between 24 and 39 years, was employed in this study. Cell cultures were set up following standard methods and at 44 hours of growth 6µg/ml of cytochalasin-B was added to block cytokinesis. Cells were harvested following 72 hours of growth, as previously described [1]. 900 MHz RF exposure (SAR = 1.59 W/kg) was performed in a TEM cell during the first 44 hours of growth: it was 6 minutes long with 3 hours of interval, to obtain 14 on/off cycles. The induction of MN was evaluated on 1000 cytokinesis-blocked cells and expressed as percentage, while cell proliferation was estimated as cytokinesis-block proliferation index (CBPI), by classifying 500 cells according to the number of nuclei. For each donors an exposed culture and a control one were set up and located in the same incubator where TEM cell was placed.

RESULTS AND CONCLUSIONS: Following exposure of human peripheral blood lymphocyte cultures to 900 MHz, both CW and GSM signal, no statistically significant differences (two tailed paired Student's t test) were found when exposed cultures were compared with the respective controls, either in terms of MN induction and cell cycle kinetics (Table 1).

In conclusion, in the experimental conditions here adopted, the results obtained indicate absence of genotoxic and cytotoxic effects.

Table 1 - MN frequency and cell cycle kinetics in cultured human peripheral blood lymphocytes following exposures to 900 MHz RF radiation (GSM and CW). Results are expressed as means ± standard deviations.

Endpoint	**N° of subjects**	**GSM signal**		**CW**	
		control	**RF exposed**	**control**	**RF exposed**
% MN	7	0.54 ± 0.33	0.57 ± 0.25	0.39 ± 0.13	0.46 ± 0.01
CBPI	7	1.64 ± 0.12	1.69 ± 0.14	1.68 ± 0.15	1.67 ± 0.18

Reference.

[1] Scarfi' MR, Lioi MB, Zeni O, Della Noce M, Franceschi C, Bersani F: *Health Physics*, 76(3), 244-250 1999.

Research supported by TIM – Telecom Italia Mobile.

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References
[1] Scarfi' MR, Lioi MB, Zeni O, Della Noce M, Franceschi C, Bersani F: *Health Physics*, 76(3), 244-250 1999.
Research supported by MANCA CONTRATTO

P-101

RADIOFREQUENCY EMF AND DNA STRAND BREAKS. K. Schlatterer[*1]. R. Tauber[*1], R. Fitzner[1]. University Hospital Benjamin Franklin, Free University of Berlin, Hindenburgdamm 30, D - 12200 Berlin, Germany.

INTRODUCTION: A DNA damaging effect of radiofrequency radiation on different cell lines has been discussed controversely so far. Lai and Sigh had described DNA strand breakage in rat brain cells directly after exposure to microwaves (2450 MHz) in 1995. Using different cell lines and different radiofrequency signals in other model systems, no such effect could be reproduced by other groups. After having analysed cell growth characteristics with different radiofrequency signals and markers in our cellular model, the human promyelocytic leukaemia cell line HL60, we focused on the evaluation of possible direct genotoxic effects, with proven relevance for cellular growth behaviour and cancerogenesis, at 1800 MHz.
OBJECTIVE: Goal of these studies was to determine if radiofrequency electromagnetic fields induce structural alterations on the genetic level with strong correlation to cell growth and cancerogenesis. Analysis was focussed the detection of DNA strand-breaks. The human promyelocytic leukaemia cell line HL60 was continuous wave-exposed to 1800 MHz at SAR 1.3 and 1.0 W/kg over 24 hours. DNA strand-breaks were examined by means of the single cell gel electrophoresis (SCGE), i.e. Comet assay. The Comet assay is used as a very suitable test system to study genotoxic effects on the single cell level with high sensitivity. Applying the alkaline Comet assay, single as well as double strand-breaks can be detected.
METHODS: HL60 cells were cultured in RPMI 1640 medium with 10% FCS under temperature- and pH-control conditions at 37°C. For radiofrequency exposure experiments the initial seeding density per 35 mm petri dish was 7.5 x 10^5 cells. Exposure and sham-exposure were performed double-blinded. Subsequently, cells were monitored for DNA breakage by use of the alkaline Comet assay. 1000 cells per slide were scored manually by fluorescence microscopy for Comet formation according to Diem et al. (1999),

generating a tail factor by a simple mathematical approach. This tail factor allows to quantitatively compare the induction capacity of different environmental noxes concerning DNA-stand-breakage.
RESULTS AND DISCUSSION: The tail factor determined in these experiments is significantly increased for exposed HL60 cells as compared to sham-exposed controls. As positive control for DNA strand-breakage the tumour initiator 7,12-dimethylbenz[a]anthracene (DMBA), was used at a concentration of 25 µg/ml culture medium (0.01 mM). As DMBA was solubilized in dimethylsulfoxide (DMSO), we also performed sovent control experiments. Evaluation of the tail length showed that treatment of cells with DMBA causes a significantly higher tail factor as compared to the DMSO solvent control, which is in accordance to previous studies performed for Hep G2 cells (Yusuf et al., 2000). Experiments are ongoing, but these results indicate, that there might be a different susceptibility for different target tissues concerning direct genotoxic effects. This increased susceptibility may be correlated with an altered DNA damage repair capacity.
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Supported by European Union (Programme “Quality of Life and Management of Living Resources”, Key Action 4 “Environment and Health”: QLK4-CT-1999-01574, REFLEX)

P-102

EXPRESSION OF DNA DAMAGE-INDUCED REPAIR ENZYMES IN RESPONSE TO 2450 MHZ MICROWAVE RADIATION. M. Natarajan[1]. S. Mohan[2*], M.L. Meltz[1]. Departments of [1]Radiation Oncology and [2]Pathology, University of Texas Health Science Center, San Antonio, Texas 78229-3900, USA.

OBJECTIVE: In this study we investigated whether exposure of $CD14^+/CD16^+$ human monocytes to radiofrequency radiation triggers the expression of repair enzymes involved in DNA damage-induced repair.
METHODS: The cells were exposed to 2450 MHz microwave radiation continuously for 90 min at a power density of 5 mW/cm^2. The mean specific absorption rate at the bottom of the flasks, calculated by Finite Difference Time Domain (FDTD) analysis, was 12.46 W/kg. The SAR ranged from The cells were harvested at 1 and 3 h post-exposure. The mRNA levels of repair genes were examined by RNase protection assay using the Multi-probe Analysis System (Pharmingen, San Diego, CA). Total cellular RNA was isolated by a modified guanidium thiocyanate-phenol-chloroform method, using the RNA Stat-60 reagent (Tel-Test Inc., Friendswood, TX). Radio-labeled [(^{32}P)-UTP] antisense ribo-probes for human double strand break repair genes (LIM15, RAD50, RAD54, RAD52, MRE11, XRCC2, XRCC3, RAD51, RAD51B, RAD51C, and RAD51D), mismatch repair genes (PMS1, PMS2, MSH2, MSH3, MSH6, MSH5, MYH, MLH1) and house keeping genes (L32 and GAPDH) were synthesized by *in vitro* transcription using template sets following the manufacturer's protocol (Pharmingen, San Diego, CA). The probes were combined with RNA samples and co-hybridized overnight at 45°C. Hybridized samples were RNase-digested at 37°C for 1 h. Denatured samples were resolved on urea-polyacrylamide gels and exposed to Hyperfilm MP (Amersham). Care was taken to compensate for potential variations in RNA loading and transfer by normalizing the levels of gene expression of the corresponding L32 and GAPDH mRNA levels.
RESULTS AND CONCLUSIONS: There was no detectable repair gene expression in sham irradiated control samples. In the microwave exposed samples, there was a slight increase in the induction of both double strand break repair and mismatch repair genes at 1 h post exposure. The induction of these repair genes was elevated considerably at 3h post-exposure. Further study is necessary to determine whether RFR

exposure stimulates signaling pathways that are responsible for DNA damage-induced repair mechanisms, without this being caused by DNA damage, or if RFR exposure cause alterations in the DNA that are not detected by conventional cytogenetic analysis, leading to the gene expression.
Supported by U.S. Air Force Office of Scientific Research Grant F49620-01-1-0349.

TISSUE & ORGAN – RF/ELF

P-103

THERMAL LIMITS FOR FUNCTIONAL DAMAGE AND RECOVERY IN BRAIN TISSUE AFTER A BRIEF (500 MS) HIGH-INTENSITY MICROWAVE EXPOSURE. A.G. Pakhomov[1,2], J. Doyle[*1], J. Ashmore[*1], and M.R. Murphy[2]. [1]McKesson BioServices, US Army Medical Research Detachment, and [2]Directed Energy Bioeffects Division, Human Effectiveness Directorate, Air Force Research Laboratory, Brooks Air Force Base, San Antonio, Texas, 78235-5324, USA.

OBJECTIVE: While thermal effects of microwaves have been studied intensively for decades, little is known about the bioeffects of brief (sub-second) heat pulses (BHP). This lack is explained, at least in part, by technical problems in producing and accurately measuring BHPs. In the absence of actual data, BHP effects can only be extrapolated from findings at longer exposures. However, extrapolation fails to acknowledge the possibility of triggering qualitatively different biophysical mechanisms by BHP, resulting in erroneous predictions. This study is the first attempt to analyze BHP functional effects in brain tissue *in vitro*.
METHODS: The experiments were performed in sagittal hippocampal slices (350-μm thick) isolated from the brain of 4- to 6-week old male Sprague-Dawley rats. The slices were held at the bottom of the exposure bath and superfused with an artificial cerebrospinal fluid (ACSF) at 34 ^{o}C. Nerve fibers of the *Stratum radiatum* area were stimulated at 10-sec intervals, which evoked excitatory postsynaptic potentials (EPSP) and population spikes (PS) in the CA1 area. A BHP was caused by a single 500-ms train of microwave pulses (9.6 GHz, 2-μs width, 800 kW/g peak SAR). Temperature reached maximum by the end of the train, which was exactly 10 ms prior to the next stimulus. Cooling back to 34 ^{o}C was complete within 1-2 s after the train. Temperature rise was controlled by the pulse repetition rate in the train, up to 24-28 ^{o}C at 160-200 p.p.s. Each brain slice was exposed to several BHPs, with time intervals sufficient for complete EPSP and PS recovery. Temperature in the slice was measured by a microthermocouple; the accuracy of this technique was justified earlier[1,2] and validated again by comparing with readings of a Nortech fiber optic sensor.
RESULTS AND DISCUSSION: We identified three temperature ranges that correspond to three different types of biological response: (1) Heating up to 48-49 ^{o}C caused instant PS suppression, followed by instant and complete recovery as soon as the temperature returned to pre-heating level. Even the most profound instant suppression (by 100%) showed no consequences for the function of the brain slice afterwards. BHP effects within this temperature range seem to be just a "passive" response, i.e., due to temperature-induced changes in physico-chemical properties and the rate of chemical reactions. (2) Heating to temperatures from 48-49 to 58-59 ^{o}C caused both instant and delayed PS suppression. The complete (by 100%) instant suppression was followed by a brief recovery (for some 20-50 sec) and then by a delayed suppression. Most remarkable, the magnitude and duration of the delayed suppression were exponentially dependent on the temperature reached during the exposure, and could be characterized by a Q_{10} index (which normally applies to changes **during** the period of elevated temperature, not after it). PS recovery after the delayed suppression usually was complete, even though it could take up to 1-2 hours. We interpret the delayed suppression as an "active" response (i.e., not just physico-chemical, but physiological). We observed no adaptation or sensitization to repeated BHPs. (3) Temperatures in excess of 59-61 ^{o}C caused instant and irreversible suppression of PS and EPSP. However, pre-synaptic potentials could recover even after this extreme heating.

It is well known that heating of brain slices for a sufficient time (e.g., for 5 sec or 5 min) has an extended effect on the PS shape: the PS becomes polyphasic, which is a manifestation of hypoxia and metabolic disarray. In contrast, a 500-ms BHP never induced polyphasic PS (regardless of the temperature), indicating the prevalence of heat damage mechanisms other than hypoxia.

SUMMARY: BHP effects proved to be markedly different from those observed with a more prolonged heating. Studying the mechanisms of BHP effects in more detail will help to refine microwave exposure safety guidelines. Besides, the possibility to affect specific cell functions by BHP prompts the possibility of using it as a novel research tool in experimental biophysics and neurophysiology.

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P-104

COMPARATIVE EFFECTS OF CONTINUOUS-WAVE AND HIGH PEAK POWER MICROWAVE EMISSIONS ON THE INDUCTION OF LONG-TERM POTENTIATION.

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INTRODUCTION: This work is a continuation of previous studies[(1,2)] which explored the effect of extremely-high power microwave pulses (EHPP) on the long-term potentiation (LTP) of the population spike (PS) in isolated rat hippocampal slices. Irradiation was completed shortly before induction of LTP by a brief tetanic stimulation[(1)]; or, conversely, the irradiation was performed when the LTP was already induced and reached its steady-state maximum[(2)]. In both situations, EHPP had either no effect on LTP, or produced a transient effect proportional to the temperature rise during irradiation; there was no indication of EHPP-specific ("nonthermal") effects. However, this conclusion remains incomplete without studying the effects of EHPP irradiation at the time of LTP induction and subsequent transition to the potentiated state. One can speculate that neurons may be more vulnerable during the transition, as compared to the steady-state conditions before LTP induction and after the LTP is already developed.

METHODS: The exposure, dosimetry, and physiological techniques were similar to described before[(1,2)]. In brief, sagittal hippocampal slices (350-μm thick) were isolated from 4- to 6-week old male Sprague-Dawley rats, fixed at the bottom of a custom-made exposure chamber, and superfused with oxygenated artificial cerebrospinal fluid at 34 ^{o}C. Refining the construction of the exposure chamber increased the efficiency of microwave absorption by about 50%, to 2 W/g per 1 W of transmitted power at 9.3 GHz. Population spikes (PS) in the CA1 neuronal area of the hippocampus were repeatedly evoked every 30 sec by stimulating *Stratum radiatum* area. LTP was induced by tetanization for 2 sec at 50 Hz.

Experiments began after a 30- to 90-min stabilization and lasted for 15 min. Each slice was used in a single experiment. In all experiments, irradiation was turned on at 1 min 45 sec, tetanization was performed at 4 min, and the radiation was turned off at 8 min 45 sec (in unexposed controls, transition to the potentiated state took about 4 min after tetanus). The main endpoints were the magnitude and time course of changes in the PS peak-to-peak amplitude after tetanization, as influenced by different regimens of microwave exposure.

The data were collected from a total of 63 successful experiments comprising 5 groups. Group 1 served as a sham-exposed control (microwaves were turned on, but routed away from the exposure chamber). Groups 2 and 3 were exposed at 0.25 W/g, groups 4 and 5 were exposed 1 W/g. This SAR was produced by a

continuous-wave (CW) radiation in the groups 2 and 4, or by EHPP in the groups 3 and 5. The EHPP pulse width was 0.5 μs in the group 3, or 2 μs in the group 5; the pulse repetition rate and peak SAR were 1 Hz and 500 kW/g in both the groups. Microwave heating reached about 0.4 ^{o}C in groups 2 and 3, and 1.6 ^{o}C the groups 4 and 5.

RESULTS: In the sham-exposed group, LTP developed within 4 min after the tetanus, and the PS amplitude stabilized at 160-170% of the initial. CW exposures slowed down the transition to the potentiated state; the effect was more pronounced in the group 4, which was exposed at the higher SAR. Potentiated amplitude of the PS after cessation of the exposure stabilized at the same level as in the sham controls. The time course of the LTP development in the EHPP-exposed groups 3 and 5 showed the same difference from the sham control group, but no difference from the CW-exposed groups. The results suggested that CW and EHPP exposures influenced LTP via some common mechanism, apparently by heating. Same as in the previous studies[1,2], we could find no indication of EHPP-specific bioeffects.

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The work was supported by the U.S. Army Medical Research and Materiel Command and the U.S. Air Force Research Laboratory (AFOSR) under U.S. Army contract DAMD17-94-C-4069 awarded to McKesson BioServices.

P-105 Student

APOPTOSIS AND MORPHOLOGICAL CHANGES IN HIPPOCAMPUS OF RAT'S BRAIN FOLLOWING EXPOSURE TO ELECTROMAGNETIC PULSE. M.L. Zhao, X.Z. Cao, D.W. Wang* and X.M. Cuei*. Institute of Radiation Medicine, Taiping 27, Department of Pathology, Beijing 100850, China.

INTRODUCTION: Basically, electromagnetic pulse (EMP) consists of a pulse of radio-frequency waves with a nearly instantaneous rise in the electric and magnetic fields and a subsequent decline in the fields. EMP radiation may be represented as a traveling wave consisting of transverse electric and magnetic oscillating fields. An analysis of an EMP showed that it contained various frequency components extending from 0Hz to 10^{9}Hz. However, the pulse configuration was such that its power was mainly confined to the longer wave-lengths (<30MHz).

OBJECTIVE: EMP, an uncommon form of electromagnetic field, has been reported to cause easy fatigability, extreme irritability, aching joints and mild frontal headaches in people. Up to now, this potential hazard to man has been a matter of concern, and safety standards have not been proposed. Because that there are not enough biological data to establish firm standards. To explore the possible injury mechanism with observing the apoptosis and morphological changes on primary culture cells and Wistar rat's brain following exposure to EMP.

METHODS: EMP simulator was designed and established by National University of Defense Technology (CHINA), which provides 2.5 pulses/min with a high electric field intensity 60 KV/m, 20-nsec rise time and 25μs pulse wide. The death and apoptosis of rat hippocampal neuronal cells in primary culture following exposure to EMP were showed by MTT and flow cytometry respectively. 65 Wistar rats (weight 180±20g) were divided into exposure groups and control (sham-exposure) groups. Then the rats were killed at 1h, 6h, 12h, 24h and 48h separately. The histological sections were examined with light and electron microscopy.

RESULTS: Results show that not only happened the death of hippocampal neuron at early stage, but occurred apoptosis following exposure to EMP. MTT examine inferred EMP can promotes necrosis and apoptosis of neurons, the MTT value was significantly decreased at 0hr, 1hr and 6hrs with exposure groups (compare with control, $P<0.05$). The highest apoptosis ratio was found by flow cytometer at 6hr with hippocampus neurons. At same time, EMP could cause disturbance of center nervous system circulation,

neuron degeneration and destruction of synapse. The morphological changes in blood vessels, neuroglia cells and neurons were found in rats.
CONCLUSION: The results indicated that EMP can promote necrosis and apoptosis of hippocampal neurons induce morphological changes in blood vessels, neuroglia cells and neurons at early stage, which may be the possible injury mechanism in rat brain induced by EMP.

P-106 Student

500 μT 50 HZ CIRCULARLY POLARISED MF DOES NOT ACUTELY SUPPRESS MELATONIN FROM CULTURED WISTAR RAT PINEAL GLANDS. H. Tripp, G. Warman and J. Arendt. Centre for Chronobiology, School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK.

INTRODUCTION: It has been proposed that 50/60 Hz electromagnetic fields (EMF) might affect melatonin synthesis in mammals, however, there is little evidence for a mechanism by which this possible interaction may occur. This research examines the acute effects of these fields on melatonin production, monitored using a novel flow-through system.
OBJECTIVES: Melatonin release from isolated Wistar rat pineal glands was examined in flow-through culture during and after a 4 h exposure to a 500 μT 50 Hz circularly polarised magnetic fields (MF).
METHODS: Individual pineals from male rats (sacrificed 2 h after lights on (ZT 2)) were cultured at 37°C in a flow-through system similar to that of Tosini and Menaker, 1996. Two experimental regimes were used:
Wash-through culture. Dissected pineals (n=6) placed immediately in culture system. Exposed to a 500 μT MF for 4 h followed by 5 h of sham exposure.
Plateau culture. Pineals (n=6) in static culture for 4 h, before flow-through culture and exposure.
Sham treated pineals (<1 μT) (n=6) were cultured as controls for each regime. The exposure system was a Merritt coil ensuring >90% field uniformity and generation of appropriate sham controls.
RESULTS: 1. Wash-through phase culture produced an initial peak in melatonin production of 240 ± 57 pg/ml/30 min, plateauing at 100 ± 1.97 pg/ml/30 min after 330 min in culture (Fig.1). Only one time point significantly altered (at 240 min) (P>0.05, un-paired t-test) in melatonin production after MF exposure. No significant differences were observed between control and exposed conditions; or during and after treatment (P<0.05, repeated measures ANOVA). 2. Plateau phase culture showed no overall significant differences between control and exposed pineals using students un-paired t-test (P<0.05) or repeated measures ANOVA (P<0.05) (Fig. 2).
CONCLUSION: Flow-through culture provides high temporal resolution to enable the analysis of MF effects. Melatonin production of isolated pineals is not suppressed by a 500 μT 50 Hz circularly polarised MF. These data suggest that MF exposure administration at this zeitgeber time does not affect pineal melatonin production, or, if it does that an intact circadian axis is necessary to mediate any effects.

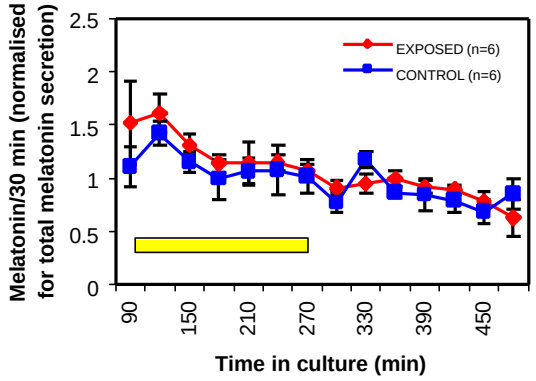

Fig.1 Effects of 4 h MF exposure on melatonin secretion from individually cultured Wistar rat pineal glands dissected ZT 2 and cultured immediately in flow-through system.

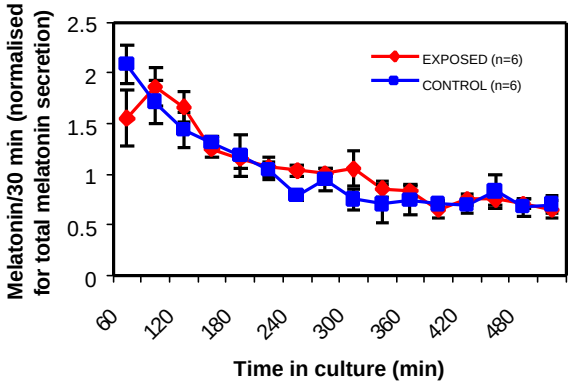

Fig. 2 Effects of a 4h MF exposure on melatonin secretion from cultured Wistar rat pineal glands dissected at ZT 2 and cultured for 4h in a static culture before placement in a flow-through system.

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This work was supported by National Grid plc and by a BBSRC CASE PhD award.

IN VIVO STUDIES: ANIMAL – ELF

P-107

A STUDY OF CHRONIC EXPOSURE TO 60 HZ EMFS AND THE POTENTIAL DEVELOPMENT OF NEURODEGENERATIVE DISEASES IN F344 RATS. R. Mandeville[1], P. Brousseau[1], A. Larrivee[1], D. Goulet[2] and K. Gibney[2]. [1]Biophage Pharma Inc., Montreal, Quebec, [2]Hydro Quebec, [2]B.C. Hydro.

INTRODUCTION: During the past several years, several studies have demonstrated a potential risk of neurodegenetive diseases associated with exposure to 60 Hz magnetic fields, more specifically with (a) Alzheimer and dimentia and (b) Amyotrophic lateral sclerosis and other motor neuron diseases.
OBJECTIVES: The major goal of the present study was to compare the patterns of protein expression in the brain of animals exposed during 2 years to ELF magnetic fields of different intensities (sham, 2, 20, 200 and 2000 uT) in order to assess any CNS damage induced by this type of exposure. For a more detailed analysis of the exposure parameters see Mandeville et al (FASEB Journal: 11: 1127-1136,1997).
METHODS: An extensive immunohistochemical analysis of the three most important structures of the brain (glial cells; neurons and synapses) and we studied the mechanism of active plasticity and apoptosis. Paraffin-embedded brains collected at the sacrifice of animals exposed to 60 Hz linearly polarized, sinusoidal, continuous-wave magnetic fields (MFs) were used. Each animal was exposed for 2 years (20h/day) to magnetic fields of different intensities, i.e., sham exposure (< 0.02 µT) and 2, 20, 200, and 2000 µT. Another group of animals was housed in a separate room and serve as negative control.. positive Controls included a group of 5 two-years old female Fischer rats in which damage to the brain was induced by physical trauma (i.e., insertion of a syringe in the parietal region in 5 to 6 adjacent sites). Animals were sacrificed 3 to 7 days after the trauma. Antibodies used included : GFAP (Glial fibrillary acidic protein); MBP (Myelin Basic Protein); MAP-2 (Microtubule Associated Protein); GAP (Growth Associated Protein) Anti-Synapsin-1.
CONCLUSIONS: Data generated demonstrate the importance of the use of well-selected controls because controls are essential for interpreting data obtained. Also the use of the appropriate controls allows the optimization of the conditions required for successful *in situ* detection of immunohistochemical staining

without expending valuable test samples. Our results also demonstrate a statistically significant increase of GFAP antigens in the brain of animals exposed for 2 years to 2, 20, 200 and 2000 µT magnetic fields. This increase in immunohistochemical staining was however seen in our sham-exposed animals. It is important to note that there was no statistically significant difference between our negative controls as far as the number of neural cells undergoing apoptosis (programmed cell death). This is an important observation since Fanelli et al., (FASEB Journal 13: 95-102,1999) reported that static magnetic fields with intensities starting at 600 uT exert a strong and reproducible effect of reducing apoptosis in several different human cell systems. Our data are in agreement with earlier studies published by Reipert et al., (Life Sci. 61: 1571-1582, 1997) reporting that ELF magnetic fields do not disturb or interfere with the regulation of apoptosis in multipotent hemopoietic progenitor cells.
IN BRIEF, data generated from this study demonstrate that chronic exposure of animals to ELF magnetic fields does not induce any significant trauma to the brain cells and could not be the cause of neurodegenerative diseases as suggested by epidemiological studies.

P-108

EFFECTS OF WHOLE BODY EXPOSURE TO 50 HZ ELECTROMAGNETIC FIELDS ON THE LEUKOCYTE BEHAVIOR IN THE MICROCIRCULATION IN MICE. A. Ushiyama, C. Ohkubo. Department of Physiological Hygiene, National Institute of Public Health, Tokyo 108-8638, Japan.

OBJECTIVES: Effects of 50Hz electromagnetic fields(EMF) on leukocyte are mainly evaluated *in vitro* system, however, little information is available from *in vivo* experiments. In order to investigate the exposure effects of 50Hz EMF on leukocyte behavior in *in vivo,* we used a newly-developed dorsal skinfold chamber(DSC) as well as a cranial window(CW) for measuring the behavior of intra-microvascular leukocytes in the cutaneous and cerebral microcirculation in mice, respectively(Figs. 1-2).
METHOD: Cutaneous Microcirculation: Male mice (BALB/c) having the DSC were subjected to intravital-microscopic study. We have developed the DSC with non-metal materials of Duracon resin, which could not be physically affected by EMF exposure. In order to evaluate the behavior of intra-microvascular leukocytes, fluorescent dye (rhodamine 6G; 0.3mg/kg, iv) was used. The magnetic flux densities used for the acute exposure (30 minutes) were controlled at 3, 10, 30 mT at the center of animal body (n=10 each), respectively. For sub-chronic exposure study, mice were divided into 2 groups (n=10-12 each), i.e., exposure group with 50 Hz EMF at 3 mT and control group with sham exposure. The 50Hz EMF exposure was intermittently performed everyday from 14:00 to 12:00(22hours/day) for 15 days. The numbers of the leukocyte rolling or adhering to the venular walls in the cutaneous tissue were measured from video images. At the end of sub-chronic exposure experiment, several kinds of cytokine level in plasma were measured. Cerebral Microciruclation: Male SCID mice having CW were subjected to evaluate the effects of 50 Hz EMF on leukocyte behavior in the pial microcirculation. Mouse CW is a closed glass window system installed into the pariental region of the mouse. Concurrent with usual fluorescent microscopy, real time confocal laser fluorescent microscopy was also used for the vital study. For acute exposure study, mice having the CW were divided 2 groups, i.e., exposure group with 50 Hz EMF controlled at 30mT for 30 minutes and control group with sham exposure for 30 minutes. For sub-chronic exposure study, mice were divided into 2 groups (n=10-12 each), i.e., exposure group with 50 Hz EMF at 3 mT for 20 days and control group with sham exposure. Adherent leukocyte counts to the venular walls were compared between values of before exposure and those of after exposure.
RESULTS: Cutaneous microcirculation: Acute Effects: A tendency to increase the adherent cell count of leukocytes due to the 50 Hz EMF exposure toward higher magnetic field intensity was recognized. Following the exposure at 30 mT, the counts of adherent cells were significantly higher than those obtained before exposure ($p<0.05$). Sub-chronic Effects: Following sub-chronic exposure to 50Hz EMF, statistically significant increases($p<0.05$) in adherent leukocyte counts were noticed, however, there was no change in IL-1ßplasma levels. No noticeable changes in the adherent cell counts were observed between before and after sham exposures in control group. Cerebral microcirculation: After EMF exposure, adherent cell counts

also tended to increase compared with those of before EMF exposure, however, no statistical changes were recognized in the both acute and sub-chronic exposure experiments.
CONCLUSION: The results indicated that 50 Hz EMF exposure influences cell to cell interaction between venular endothelial cells and leukocytes. Previous observations using human monocyte in *in vitro* system indicated that changes in cytokine profile were induced by exposures of 50 Hz EMF[1-2] may be involved in this phenomenon. Further studies will be required.
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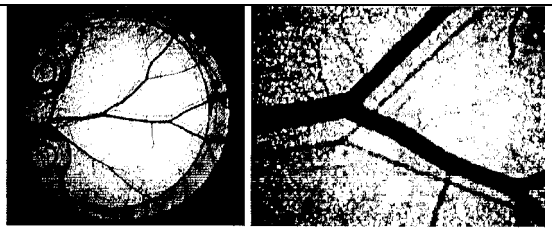

Figure 1. Overviews of dorsal skinfold chamber with low(left) and high(right) magnification

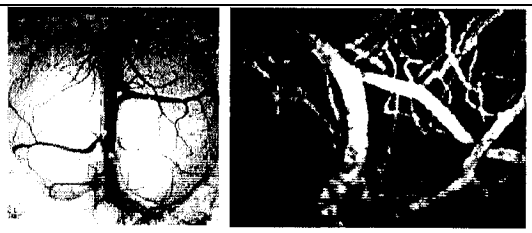

Figure 2. Overviews of Cranial Window with low(left) and high(right) magnification

P-109

EFFECT OF THE EXPOSURE TO LOW FREQUENCY FIELD ON THE MATURATION OF CENTRAL NERVOUS SYSTEM IN NEWBORN RATS. A. Lisi[1]*, V. Manni[1]*, D. Pozzi[2]*, S. Rieti[1]*, L. Giuliani[3]*, S. Grimaldi[1]. [1]Istituto di Neurobiologia e Medicina Molecolare CNR- Rome, Italy; [2]Dipartimento di Medicina Sperimentale e Patologia Universita La Sapienza Rome, Italy; [3]ISPESL-DIPIA Rome, Italy.

OBJECTIVE: The aim of study is to verify if the exposure to electric and magnetic field at extremely low frequency (EMF) can alter the evolution of newborn rat central nervous system.
Cultured cerebellar granule cells and primary cell cultures were exposed to EMF at various intensity, in orther to characterize their morphology, cellular and molecular biochemistry under exposure conditions.
METHODS: Cell were exposed in a solenoid placed in a cell incubator enable to generate field with magnetic induction 1 mT at a frequency of 50 Hz. Experiment were performed on granule, neurons explant from the cortex of newborn rats.
Nervous cell primary cultures represent one of more diffused systems for the study of a lot of neuronal parameters. A wide scientific literature documents the use of this system for the study of both cellular development and functions and for morphological, biochemical and molecular modifications. In our primary culture system, cells are prepared by newborn rat cerebellum (5-8 days after birth). Nervous tissue is dissociated with mechanical and enzymatic actions that produce a cellular suspensions containing granules. Cerebellar granules use glutamate as neurotransmitter and their differentiation in vitro can be compared to in vivo one. Dissociated cells are cultured in growth-medium plus foetal calf serum in umidified atmosphere at 37°C and 5% Co2. An antimitotic agent for non-neural cells is used (AraC). In this culture cerebellar granules represent 95% of total cells, the 5% is represented by GABA neurons, Purkinjie cells, glial cells and oligodendrocytes. Culture longevity essentially depends by medium composition and supplements (growth factors as insulin, glucose) and can vary from 10 to 30 days. Coltures are used 48 hours after plate-seeding.
RESULTS AND DISCUSSION: After explant cerebellar granule cells had been exposed to the field for five days while control non exposed granule were cultured *in vitro* for the same period of time than both expose and non exposed cells were subjected to a cytotoxic glutamate pulse (100µM, 30 min incubation)

and tested for their glutamate sensitivity. Glutamate showed 20% of cells death on exposed cells while 95% cells survival was reported for unexposed granule. It is known that the life span of rat cerebellar granule in culture is limited and after 8 days culture cells became sensitive to glutamate showing excitotoxicity. It is evident from our experiment that exposure to the field is reducing the life span of granule inducing differentiation and a faster expression of glutamate acid receptor (maturation).
By indirect immunofluorescence we could also detect, in the exposed neurons an accumulation of neuronal filament rather than neuron control.
Our results suggest that low frequency field can be able to induce differentiation and maturation in cultured cerebellar granule cells.

P-110 Student

COMBINED EFFECTS OF ELECTROMAGNETIC FIELDS WITH CARCINOGENIC AGENTS.

P. Heikkinen[1], T. Kumlin[1], A. Markkanen[1*], A. Ruotsalainen[1*], J. Naarala[1*], J. Juutilainen[1], V.-M. Kosma [2*], H. Huuskonen[3*], H. Komulainen[3*], L. Puranen[4]. [1]Department of Environmental Sciences and [2]Department of Pathology and Forensic Medicine, University of Kuopio, FIN-70211 Kuopio, Finland. [3]National Public Health Institute, 70211 Kuopio, Finland. [4]STUK Radiation and Nuclear Safety Authority, FIN-00881 Helsinki, Finland.

OBJECTIVE: This is a summary of several *in vivo* and *in vitro* studies that our group has conducted to study possible combined effects of electromagnetic fields (ELF and RF) with known physical or chemical carcinogenic agents.
METHODS: Effects of 50-Hz magnetic field (MF) exposure on UV-induced skin tumorigenesis were studied in mice (1). Further studies with mice evaluated effects of MFs and UV exposures on epidermal ornithine decarboxylase (ODC) and polyamine levels (2), and on apoptosis in mouse skin (unpublished). Combined effects of 50-Hz MFs and UV radiation on cell cycle kinetics and growth were studied in yeast (*Saccharomyces cerevisiae*) cells (3). In addition, the effects of 50-Hz MF on ionizing-radiation-induced carcinogenesis were studied in mice (4). Combined effects of mobile phone-type radiofrequency (RF) radiation with ionizing radiation (5) or UV radiation (unpublished) have been investigated in two long-term carcinogenesis studies with mice. Samples for studying genotoxicity (micronuclei) were taken in these two mouse studies (unpublished). Effect of RF exposure on UV-induced apoptosis was studied in yeast (unpublished). An ongoing project (www.uku.fi/cemfec) evaluates combined effects of RF exposure with the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) *in vivo* and *in vitro*.
RESULTS AND DISCUSSION: Exposure to 50 Hz MFs did not promote carcinogenesis initiated by ionizing radiation (4), but seemed to enhance skin tumor development induced by repeated UV exposure (1). The latter experiment was a cocarcinogenesis rather than a promotion study, which is one possible explanation for the different results (6). 50-Hz MF exposure also seemed to increase the response of yeast cells to UV radiation (3) and enhanced the effects of UV radiation on apoptosis in mouse skin. The combined effects of UV radiation and 50-Hz MFs might be explained by the radical pair mechanism (the MF flux density was of the order of 0.1 mT in all experiments). Exposure to mobile phone-type RF radiation did not promote carcinogenesis initiated by ionizing radiation (5), and did not statistically significantly enhance skin tumors induced by repeated UV exposure. In a mutant yeast strain that shows apoptotic responses to stress, UV-induced apoptosis was significantly enhanced by pulse-modulated RF radiation similar to that emitted by GSM mobile phones, but not by unmodulated RF radiation at identical specific absorption rates (0.6 or 5 W/kg). There is little evidence that RF radiation enhances the effects of genotoxic carcinogens, but the suggestive positive findings warrant further study.
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Work Environment Fund, Elisa Communications Corporation, Nokia Mobile Phones, and Sonera Corporation.

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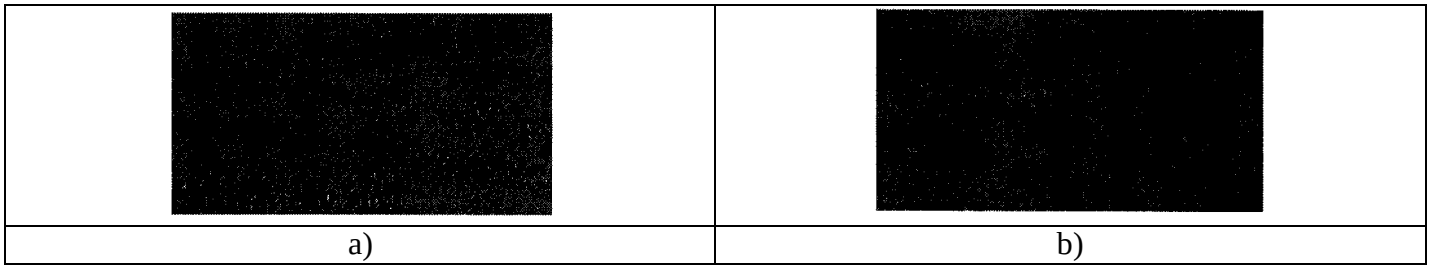
P-111 Student

EFFECTS OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION RAT HIPPOCAMPAL NEURONS. H. Funamizu[1], M. Ogiue-Ikeda[1], S. Kawato[2] and S. Ueno[1]. 7-3-1 Hongo, Bunkyo-ku, Tokyo,1 Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, 153 Komaba, Meguro-ku, Tokyo,2 Department of Biophysics and Life Sciences, Graduate School of Arts and Sciences, University of Tokyo.

OBJECTIVE: Repetitive transcranial magnetic stimulation (rTMS) has a potential therapeutic application for the treatment of Parkinson's disease. However, the mechanisms responsible for the effects of rTMS on the brain have yet to be clarified. The aim of this study is to investigate the effects of rTMS on rat hippocampal neurons.

METHODS: Male Wistar rats received four MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) injections per day to induce the degeneration of neurons prior to stimulation. RTMS was administered for 48 hours with the following stimulation parameters, 1.2 T, biphasic pulses 365 µsec, 25 pulses/sec ×8 sec×10 trains = 2000 pulses. After 72 hours of observation, the rats were sacrificed, and tissues were collected and prepared for nissl staining. The control group did not receive rTMS. Histochemical analyses were performed to determine the effect of rTMS on rat hippocampal CA3 cells.

RESULTS AND DISCUSSION: As shown in Figure 1a, the hippocampal CA3 cells of the control group remained damaged without rTMS, whereas the rat hippocampal CA3 cells of the stimulated group (Figure 1b) appeared to be repaired or normal. These results indicate that rTMS can protect or repair the injured cells of the rat hippocampal CA3 cells. We hypothesize that rTMS stimulates BDNF (brain-derived neurotrophic factor) expression, which may have contributed to the recovery process. However, further research must be carried out to clarify the mechanisms responsible for the effects of rTMS on the brain before integration into routine clinical treatments.



a) b)

Figure 1: Histochemical images of hippocampal CA3 lesions.

a) without rTMS
b) with rTMS, Scale bar = 50 µm.? = pyramidal cell layer ? = granule cell layer

P-112 Student

STATIC MAGNETIC FIELD EFFECTS ON MICROVESSEL TONE IN SKELETAL MUSCLE.

C.E. Morris*, T.C. Skalak*. Department of Biomedical Engineering, Health Sciences Center, University of Virginia, Charlottesville, Virginia 22908.

OBJECTIVE: To examine the effect of a localized static magnetic field (SMF) on the diameter of microvessels in skeletal muscle *in vivo.* Research has shown that exposure to SMF can modulate vascular tone in cutaneous tissue (Ohkubo & Xu, 1997), and whole body SMF exposure can increase blood flow in skeletal muscle (Xu, Okanao & Ohkubo, 2000), but direct measurements of vessel diameter changes in response to SMF exposure have not been obtained. Our primary objective is to directly quantify the effect of localized SMF exposure on the diameter of microvessels in adult rat skeletal muscle.

METHODS: Female Sprague-Dawley rats were anesthetized and the spinotrapezius muscle was exteriorized, maintaining all feeder vessels. The muscle was then stretched to approximate in vivo length and continuously suffused with a Ringers solution maintained at 35°C and pH 7.4. Arteriolar vessel networks were then exposed to a localized, uniform -700G SMF for 15 minutes. The magnet was positioned above the muscle with a micro-positioning apparatus to ensure consistent separation distance from the muscle surface. Uniformity of the SMF at this separation distance was determined by three-dimensional mapping of the magnetic flux density. Following a 30-minute recovery, the procedure was repeated for each animal. A sham magnet was used for control experiments. Videotape was acquired prior to SMF exposure, immediately following exposure, and 15-30 minutes post-exposure utilizing a Zeiss intravital microscope under transillumination. Vessel diameters were measured manually with an image-shearing device.

RESULTS: Preliminary data shows that vessels with initial diameters in the ranges 25-35µm, 45-55µm and 55-65µm have increased reactivity to SMF exposure, resulting in a 65% average reduction in flow resistance (30% average diameter increase) following the second SMF exposure. Conversely, vessels in the initial diameter ranges of 17-25µm, 35-45µm and 65-75µm were less reactive, leading to no appreciable change in flow resistance of these vessels. We calculated the resulting overall network resistance using a simple hierarchical model, and it was found to decrease by 22% given the individual vessel resistance changes. This corresponds to a 22% increase in the overall network blood flow, assuming that arterial pressure remains constant.

CONCLUSIONS: Our preliminary findings show that different vessels in the hierarchy of the vascular network may respond differently to static magnetic field application, but the combined effect on network resistance is measurable, and yields an overall reduction in flow resistance.

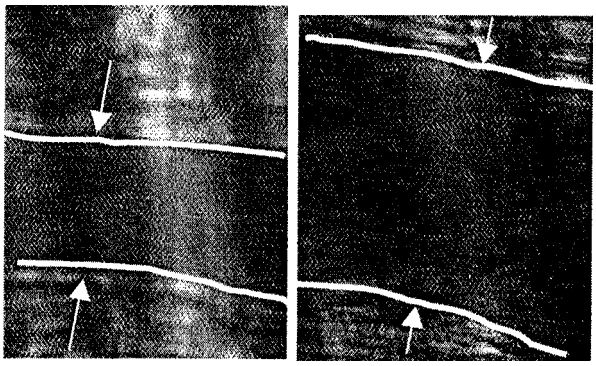


Figure l: Vessel diameters A. before field exposure (58.95um) and B. following 30-minute recovery from application #2 (94.52um).

P-113 Student

THE SENSITIVITY OF BLOOD PARAMETERS IN INFLUENCING A WEAK MAGNETIC FIELD. V. Kostenkova, G. Andreenko, K. Nikolskaya, L. Podorolskaya, T. Serebrayakova. Dept. of High Nervous Activity, Moscow State University.

INTRODUCTION: It is well known that information overload is one of factors provoking diseases in blood, cardiovascular and nervous systems of humans. As a rule, cognitive activity of modern humans is realized in conditions of increased level of MF background produced by electronics. The aim was to study the state of fibrinolysis and haemostasis parameters of blood of Wistar rats being learnt on a background of a weak static MF.

METHODS: Information overload was a food-getting problem situation in a multialternative maze where rats had to ascertain structure of cognitive task and autoshape corresponding to that operant habit was used for estimation of animal cognition activity and various behavioral manifestations. Three experiments were carried out in Wistar rats: 1) free behavior was observed in conditions of "living room" (control, Exp.1), where magnitude of MF induction varied from 12 μT to 210 μT. Learning was observed in two situations: on the background of natural MF, B=37 ± 2 μT (NMF, the Exp. 2) and on a background of static MF (up to 300 μT) modulated by three magnets placed under the maze (SMF, the Exp. 3). The state of 12 main biochemical parameters of fibrinolysis (ELT-euglobulin lysis time; FA- fibrinolytic activity; PA- plasmin activity; PAA- plasminogen activator activity; PAI - plasminogen activator inhibitor; $\propto_2$-AP – antiplasmin; NF – nonenzymatic fibrinolysis; FF – fermentative fibrinolysis activity; SF – summary fibrinolysis) and haemostasis (RT- recalcification time; Fb-fibrinogen concentration; AT-III- antithrombin III activity) of blood was studied before and after learning.

RESULTS: It was revealed that only 40 % in Exp. 2 was able to form food operant habit, while rats trained on SMF (Exp. 3) did not form by themselves goal-getting behavior in view of complete suppression of explorative activity by the MF factor in all animals. Only after a brief external stimulation, 50% of SMF-induced rats as well as in the Exp. 2 were learnt. The formed habit in these rats was very unstable and was accompanied by active stress and neurotic-like reactions while these signs have not been observed in NMF-learned animals (Fig.1). Biochemical analysis showed that both fibrinolysis and haemostasis systems in Exp. 2 were activated; however these changes were balanced and had adaptive character. The information overload provoked significant decreasing ELT (Fig. 2, 3) and increasing of the content $\propto_2$-AP, FA, PA and Fb (Fig. 2). Norm reaction of most of fibrinolysis and haemostasis parameters was enlarged significantly (Fig. 3, 4). This state was preserved during one month after abolishment of the overload. Unlike the ???. 2, all observed parameters of fibrinolysis and haemostasis reacted on information overload if the learning was on background of SMF (Fig.2-4). Compared with the ???. 2, the activity of RT and AT-III was decreased sharply on the background increased content Fb in Exp. 3 that has testified the development of hypercoagulation (Fig. 2). Coincidental with this, the activity of such inhibitors as $\propto_2$-AP and PAA were suppressed, while PA was increased in 5 time. Specific effect of SMF consisted in narrowing norm reaction in most of fibrinolytic and haemostasis parameters (Fig. 4). These effects were preserved also long as in the Exp. 2.

DISCUSSION: The data obtained testifies that weak MF fluctuations (not more 300μT) modulated by magnets increased a sensitivity of fibrinolytic system on different influences including information load. It had a negative effect since expressed hypercoagulation has developed if the animals were involved in cognitive process. Heightened tensity of fibrinolysis and haemostasis conditioned by MF factor has been preserved during 30 days after learning. It is discussed that SMF increases the risk to develop thrombosis state as a result of overstrain of fibrinolysis system. The latter was conditioned by a stable stress state, which

was provoked by unfavorable external magnetic conditions in realizing cognitive activity, although these MF fluctuations were small.

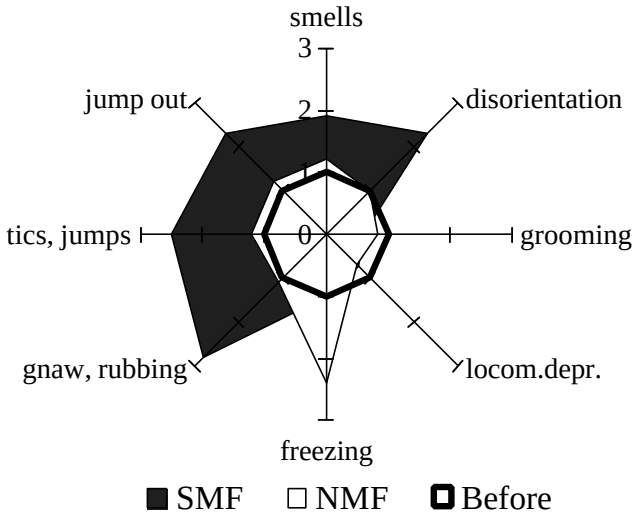


Figure 1. Psycho-emotional manifestations in learning the rats under different experiments.
Before – prelearning values were taken as 1.

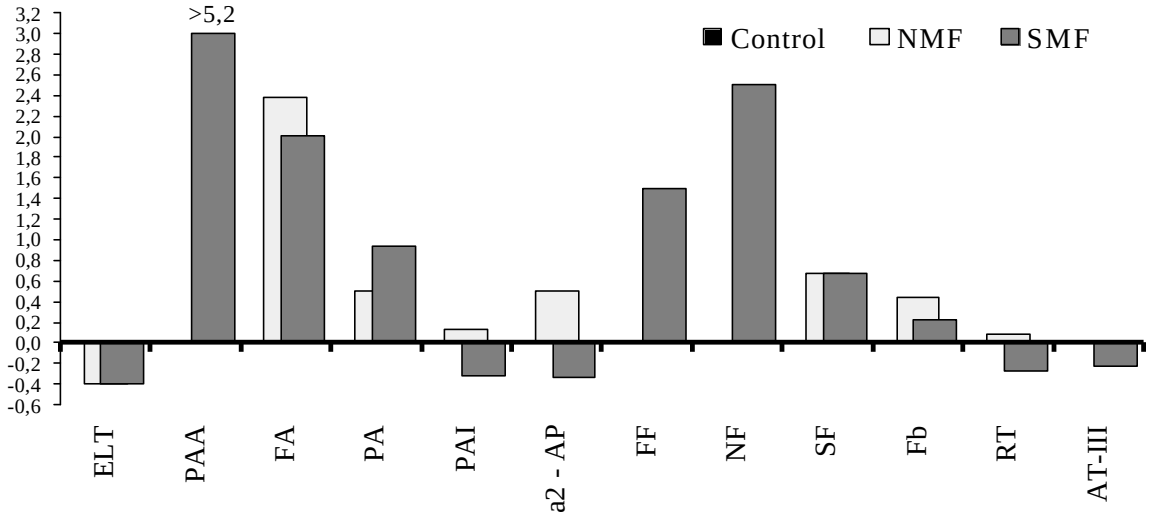


Figure 2. Fibrinolysis and haemostasis parameters of blood in learning the rats under different experiments.
Control values were taken as 0.

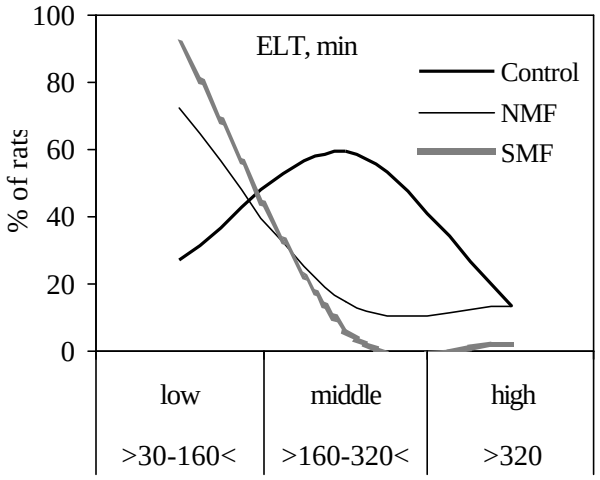


Figure 3. The time of euglobulin lysis in influencing of information overload.

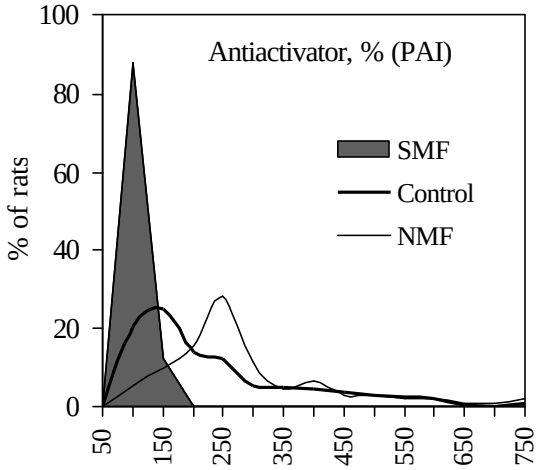


Figure 4. The norm reaction change of PAI under different experiments.

ANIMAL – RF

P-114

EFFECT OF GSM-900 MICROWAVES ON THE SKIN OF HAIRLESS RATS (A PRELIMINARY STUDY). B. Billaudel, S. Sanchez*, H. Masuda*, P.E. Dulou*, E. Haro*, R. Anane, B. Veyret. PIOM Laboratory CNRS-ENSCPB, University of Bordeaux, 33607, Pessac-France.

OBJECTIVE: The most important predisposing factors for skin cancer is excessive exposure to UV and ionizing radiation. However, the influence of other types of radiation such as radiofrequency (RF) used in mobile telephony, has not been widely studied to date. The purpose of this pilot work is to determine whether the cellular structures of Hairless-rat skin is affected by a sub-chronic local exposure to GSM-900 at different specific absorption rates (SARs).
METHODS: Hairless female rats (four-week old) were progressively habituated to the exposure setup (rocket-type) over two weeks. Thereafter, the animals were exposed or sham exposed for two hours per day, 5 days per week, during four weeks to GSM-900 signals using a loop-antenna located at a selected location on the right part of the rat backside. The local SARs were 1, 2 and 4 W/kg at the skin level. A simulation was done using a Vitek temperature probe to estimate the SAR distribution in the skin of the animals using a phantom placed inside the rocket. Three to four rats were randomly distributed in each group. At the end of the experiment, the animals were sacrificed and a skin biopsy was done not only at the location of exposure, but also on the symmetrical part of the back in order to get an internal control. The parameters studied were the rat body weight, skin thickness, cutaneous irritation, and histological analysis with various biomarkers (Hematoxylin Eosine Safran (HES), Ki-67, filaggrin, collagen and elastin). The analysis was performed in a blind manner on skin biopsies and sections.
RESULTS: For an incident power yielding a 2-W/kg SAR level at 3-mm depth, the measured SAR at 0 and 3 mm were respectively 2.7 and 2.05 W/kg. Thus, the incident power was set to induce SAR levels of 1, 2 and 4 W/kg (Exp1W, Exp2W, and Exp4W, respectively) at skin level. The body-weight time profile was not different among the various groups. Analysis of skin sections using HES coloration showed no difference of skin thickness or apparent cell toxicity (with no sign of cellular necrosis) among the animal groups. Histological analysis of the epidermis showed that the ratio between cells expressing Ki-67 (cellular proliferation marker) and total cell number remained in the range of normal proliferation ratio (<5%): Control: 1.66%, Sham: 0.33%, Exp1W: 0.75%, Exp2W: 1.25%, Exp4W: 2.25% (range 0 to 6%) for the

exposed side of the animal. On the non-exposed side, a similar observation was made (respectively 0.33, 1, 1.25, 0.75 and 0.5%). At the dermis level no Ki-67 labeling was observed. Results on filaggrin, collagen and elastin levels are in progress.

CONCLUSIONS: These preliminary results of a sub-chronicle study do not demonstrate any major physical and histological variations in the skin. Other immuno-histological studies have to be completed. A chronic study allowing measurements during a skin-cell regenerating cycle, i.e. at least 3 months, is in progress.

This work is supported by France Telecom R & D (France) under grant N° 01 1B and by the CNRS.

P-115

USE OF GENE ARRAY TECHNOLOGY TO SURVEY GENE EXPRESSION CHANGES IN TISSUES OF RATS EXPOSED TO 35-GHz OR INFRARED HEATING. N.J. Millenbaugh[1,2*], J.E. Kalns[1,2*], R.V. Blystone[3*], P.A. Mason[1], J.S. Eggers[1*], J.M. Frazier[4*], K.L. Ryan[5*], W.S. Lawrence[3*], L.L. Soza[3*] and J.L. Kiel[1]. [1]Air Force Laboratory, Directed Energy Bioeffects Division, Brooks AFB, Texas, 78235; [2]Veridian Engineering, San Antonio, Texas; [3]Department of Biology, Trinity University, San Antonio, Texas; [4]Air Force Research Laboratory, Deployment and Sustainment Division, Wright-Patterson AFB, Ohio 45433; [5]Institute of Surgical Research, Ft. Sam, Houston, Texas 78234, USA.

INTRODUCTION: It is widely accepted that thousands of genes function in a very complex and orderly fashion in normal and disease states in a living organism. Currently, a major transition in biological and biomedical research is occurring that involves global, genome-wide investigations of these biological phenomona. High-throughput, state-of-the-art technologies have been developed that use the vast amount of genetic information now available to systematically study complicated biological systems for the purpose of understanding diseases and searching for effective targets for diagnostics and therapeutics. One powerful technology that has been designed is the gene array chip. This is a solid-state platform that contains probes to measure the level of expression of thousands of genes simultaneously. This offers enormous advantage over traditional, more time-consuming methods that study only one or a few genes at a time. Thus, use of the gene array chip is an extremely efficient way to survey gene expression changes and allows the investigator to more quickly focus in on important molecular targets for subsequent study.

OBJECTIVES: Our laboratory has been studying thermal-induced biological changes and is interested in determining if the changes produced by prolonged millimeter wave (MMW) exposure are different than those produced by a more common modality of heating - infrared exposure. Because little is known about the bioeffects of prolonged MMW exposure, gene array chip technology was selected to survey a large number of genes for expression changes.

METHODS: Sprague-Dawley rats were anesthetized with a mixture of ketamine and xylazine at a dose of 50 and 10 mg/kg, respectively. Rats were sham exposed or exposed to MMWs (35 GHz, 90 mW/cm^2, >40 min) or infrared heating. Skin surface temperature was measured with an infrared camera and exposures were adjusted so that skin surface temperature profiles were the same for MMW and infrared exposures. Rats were allowed to recover and tissues were harvested 24 hr after the end of exposure. Gene expression levels were quantified using an Affymetrix rat genome GeneChip® and changes were determined by comparing data from MMW- and infrared-exposed rats to data from sham-exposed rats.

RESULTS: In lung tissue, data showed changes in expression for 28 genes in MMW-exposed rats and for 12 genes in infrared-exposed rats. Only three of these genes were the same for MMW versus infrared exposures, suggesting that MMW- and infrared-induced biological responses are different. This may be due to the difference in depth of penetration of the energy. Several of the changes that were detected point to a thermal-induced systemic immune response. These include an acute phase protein, a pro-inflammatory cytokine, chemotactic factors, and mediators of extracellular matrix turnover.

CONCLUSIONS: The results indicate the differential expression of a number of genes after prolonged MMW and infrared heating, some of which may provide information about molecular events involved in the systemic response to thermal exposures. As this is only an initial screening methodology, further validation of molecular pathways is needed and will include additional time points for more complete kinetic analysis and confirmation of changes of selected specific targets using other technologies. Research was funded, in part, by Air Force Office of Scientific Research.

P-116

EXTENSION OF THE SINGLE-PULSE, CONTACT-STIMULATION STRENGTH-DURATION CURVE DOWN TO 5 NANOSECONDS. W. Rogers[1]. J. Merritt[2] and M. Murphy[2], with T. Barker[1], C. Kuhnel[2], and L. Johnson[2]. [1]Veridian, San Antonio, TX, [2]Radio Frequency Radiation Bioeffects Research Laboratory, USAF; San Antonio, TX, 78235-5324.

OBJECTIVES: As part of the health and safety assessment of ultrawide band (UWB) sources with nsec pulses, it is useful to determine the stimulation threshold for electrically excitable tissue with nsec pulses. Pearce *et al*. (1982) describe strength-duration (S-D) curves down to 1 μsec.
METHODS: Simulation thresholds were measured using isolated frog gastrocnemius muscles bathed in Ringer solution. Stimulation was delivered through a pair of gold-plated electrodes spaced 10 mm apart. Three different sources were used: a Grass S88 stimulator (minimum duration = 10 μsec and maximum voltage = 150 V), a Velonex pulser (minimum duration = c. 50 nsec and maximum voltage = 1 kV), and a Bournlea pulser (duration = c. 5 nsec and maximum voltage = 11 kV). To assess current, voltage across a known series resistance was determined. Tektronix oscilloscopes were used to capture representative waveforms and to measure voltage. S-D curves can be described using the formulation of Blair (1932): threshold = $b/(1-e^{-d/t})$, where b = rheobase, which is the minimum threshold (V or A) for long-duration stimuli; τ = an experimentally derived time constant (Reilly, 1998); and d = duration. For nerve, τ = c. 0.2 msec; for muscle, τ = c. 2.0 msec. The isolated muscle includes a distal portion of nerve.
RESULTS: For a 500 msec pulse, the mean contact threshold was 0.195 V; this value can be used as rheobase. At 300 nsec, the threshold was 338 V. For these data, τ is 159 μsec. With a pulse of c. 5 nsec, the threshold was 6,205 V. The S-D curve for voltage has the classic form (Fig. 1). The curve appears to "flatten" slightly for pulses of 150 nsec or less. It is possible that this is artifactual because ringing became appreciable for such short pulses (Fig. 2). Longer pulses better approximated square waves, with some signs of capacitive effects (Fig. 3).
CONCLUSIONS: It is clear that a single pulse of c. 5 nsec can elicit contraction; there are few positive reports of effects from UWB pulses. There is some uncertainty about the threshold estimates; presumably accuracy can be increased through improved stimulus delivery and recording. Although the possibility of novel mechanisms with very-short, high-voltage pulses cannot be excluded, a standard S-D formulation can be extended downwards to a few nsec.

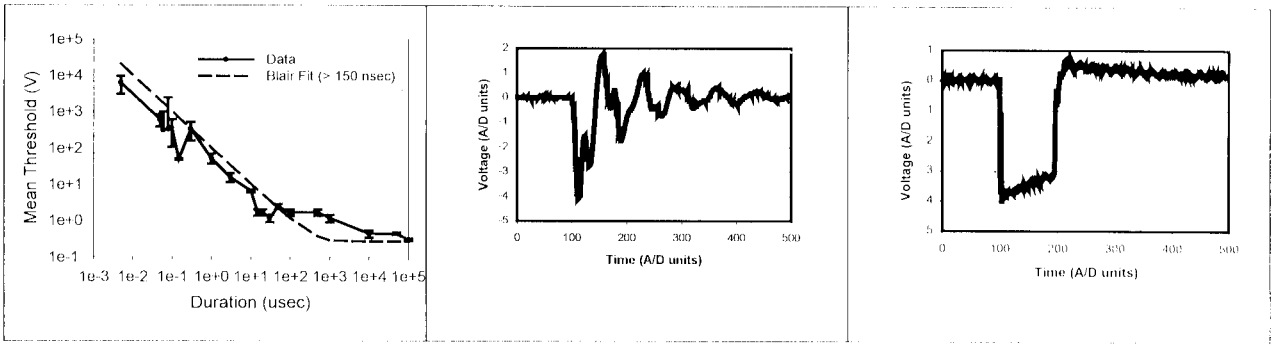


Figure 1. Voltage S-D curve with 95% CIs, plus Blair.

Figure 2. A waveform for a pulse of 50 nsec.

Figure 3. A waveform for a pulse of 1 μsec.

Funded by the United States Air Force. The opinions are solely those of the authors.

P-117

PROTEOMICS ANALYSIS OF PLASMA REVEALS THAT PROLONGED 35-GHZ EXPOSURE CAUSES UPREGULATION OF ACUTE PHASE PROTEINS. J. Kalns[1,2*], N. Millenbaugh[1,2*], R. Blystone[3*], J. Eggers[1*], W. Lawrence[3*], L. Soza[3*], J. Kiel[1], K. Ryan[5*], P. Mason[1], and F. Witzmann[4*]. [1]Air Force Research Laboratory, Directed Energy Bioeffects Division, Brooks AFB, Texas, 78235; [2]Veridian Engineering, Inc., San Antonio, Texas; [3]Department of Biology, Trinity University, San Antonio, Texas; [4]Indiana University School of Medicine, Indianapolis, Indiana; [5]Institute of Surgical Research, Ft. Sam Houston, Texas, USA.

INTRODUCTION: The biological effects of prolonged 35-GHz exposure (90 mW/cm^2, > 40 min) are not yet fully elucidated. Due to the penetration depth of millimeter waves (MMWs), we hypothesized that exposure may invoke biological responses that are different than those produced by more common heating modalities (e.g., infrared). Traditional approaches to understanding the effects of biological stressors are based on testing of a specific hypothesis. Hypotheses are frequently tested by measuring selected biological mediators (e.g., cytokines) in plasma and then determining if the observed changes are consistent with the hypothesis. While this approach can be useful, it focuses on a small subset of the information available in a biological sample. Because of this, the relevance of conclusions reached by this approach is limited. A better approach is to measure the levels of all biological mediators at the same time. While this approach is not yet feasible, recent technological advances have enabled the measurement of several hundred protein mediators simultaneously from a biological sample. This approach is called proteomics and we are using it extensively to understand the biological effects of MMW exposure. The information that is obtained from a proteomics analysis can rapidly give a clear and comprehensive understanding of the biology associated with MMW heating. Subsequent studies will use the information obtained from proteomics analysis to design hypothesis-driven experiments.

OBJECTIVES: The specific goal of this study was to determine how the expression of proteins in plasma is affected by prolonged exposure to MMW or infrared heating.

METHODS: Sprague-Dawley rats were anesthetized with a mixture of ketamine and xylazine at a dose of 50 and 10 mg/kg respectively. Rats were exposed to MMWs (35 GHz, 90 mW/cm^2) for 40 - 45 min. Other rats were exposed to infrared heating so as to mimic the heating profile produced in the sub-dermis during the MMW exposure. Animals were allowed to recover and plasma samples were collected 0, 24, 48, or 72 hr after exposure. Plasma proteins were separated on a 2-dimensional electrophoretic gel, stained, and the stained image of the gel was digitally captured. Comparisons were made to both identify proteins that are upregulated, as well as identify proteins that may be uniquely associated with prolonged MMW exposure.

RESULTS: More than 40 acute phase proteins were upregulated in both MMW- and infrared-heated animals at 24, 48 and 72 hr post-exposure. Approximately 5 proteins were upregulated following MMW exposure, but not infrared exposure. This finding suggests that prolonged MMW exposure produces some biological responses that are different than those produced during prolonged infrared exposure.

CONCLUSION: Our findings demonstrate that the heating produced by prolonged MMW exposure causes upregulation of numerous proteins; some of which were not upregulated during prolonged infrared heating. Identification of these proteins is expected to provide an indication of how the thermal stress produced by prolonged 35-GHz exposure might affect the skin and internal organs.

Research was funded, in part, by the Air Force Office of Scientific Research.

P-118

COMPARISON OF CHANGES IN COLONIC AND SKIN TEMPERATURES DURING PROLONGED EXPOSURE TO MILLIMETER WAVES, ENVIRONMENTAL HEAT, OR INFRARED HEAT LAMPS. J. Kalns[1,2], N. Millenbaugh[1,2], R. Blystone[3], J. Eggers[1], W. Lawrence[2], L. Soza[2], J. Kiel[1] and P. Mason[1]. [1]Air Force Research Laboratory, Directed Energy Bioeffects Division, Brooks AFB, Texas, 78235; [2]Veridian Engineering, Inc., San Antonio, Texas; [3]Department of Biology, Trinity University, San Antonio, Texas, USA.

INTRODUCTION: New communication, weapon detection, and non-lethal weapon technologies are being developed that make use of the millimeter wave (MMW) range of the electromagnetic spectrum. Experiments are being conducted by scientists from Trinity University and Brooks AFB to determine the biological effects of MMW exposure and to determine which endogenous substances (e.g., proteins, lipids) could be used as clinical biomarkers of overexposure. An important concern for these studies is the selection of the thermal control for MMW exposures. Possibilities include exposure to infrared heat lamps or a warm ambient temperature. In order to determine if the biological response to these forms of heating is different then that produced by 35-GHz exposure, we must first establish methods of iheating that produce heating rates in skin that are similar to those produced during MMW exposure. Once these methods are established, the biological responses of the skin and internal organs of animals exposed to MMWs and these other forms of heating will be compared.
OBJECTIVES: Establish methods for infrared or environmental heating that mimic heating profiles produced during prolonged MMW exposure. The specific goal of the experiments was to determine if these heating modes produce core, subcutaneous, and skin surface heating profiles similar to those produced during prolonged 35-GHz exposure.
METHODS: Sprague-Dawley rats were anesthetized with a mixture of ketamine and xylazine at a dose of 50 and 10 mg/kg respectively. Rats were exposed to MMWs (35 GHz, 90 mW/cm^2, >40 min), infrared heating, or a combination of infrared heating and environmental heating. Skin surface, sub-dermis, and colonic temperatures were measured with an infrared camera, thermal microprobe, and rectal probe, respectively. Since heating of the dermis during either MMW or infrared exposure is not uniform, the highest temperature at the center of irradiation was reported.
RESULTS: Environmental heating and 35-GHz exposure produce similar rates-of-increase in colonic temperatures. However subcutaneous temperature was substantially lower during environmental heating as compared to during MMW heating. Infrared- or MMW-heating can produce similar surface and sub-cutaneous temperature profiles, however, there was only a small change in colonic temperature with infrared heating.
CONCLUSION: Our findings suggest that the skin and colonic heating produced during prolonged MMW exposure can be mimicked by a combination of environmental heating and infrared heating.
Research was funded, in part, by the Air Force Office of Scientific Research.

P-119

ACETYLCHOLINE RELEASE IN HIPPOCAMPUS OF FREE MOVING RATS EXPOSED TO LOW ENERGY 915 MHZ RADIOFREQUENCY FIELDS EFFECT OF LOW FREQUENCY AMPLITUDE MODULATION. G. Testylier[1]*, M. Hugon[2]*, J.C. Debouzy[1]. [1]C.R.S.S.A., La Tronche - Laboratoire Neuropharmacologie. Centre de Recherches du Service de Santé des Armées. - BP 87. - 38702 La Tronche cedex - FRANCE [2]DGA. Département Technique des Sciences de I'homme - 00460 Paris – France.

It has been reported some central cholinergic effects in animals after acute exposure to radiofrequency electromagnetic field at low energy (Lai 1987, Kunjilwar 1993, Testylier 2001). In this work, we studied the

acetylcholine (ACh) release in the brain of free moving rats exposed to a continuous wave (CW) 915 MHz field or to a 400 Hz (square wave) modulated 915 MHz field.
Measurements were performed by microdialysis using a membrane implanted through the upper CA1 region of the hippocampus. The rats were exposed for 14 hr to fields with a mean power density of 500 $\mu W/cm^2$.
Exposure to the 915 MHz CW or AM 400 Hz RF induced a significant 25 % decrease of ACh release during the period 11 PM - 4 AM compared to sham exposed rats. But no significant effects could be observed between animals submitted to CW or AM radio frequency field.
This work corroborates our previous studies showing that modifications of the hippocampal cholinergic system can be observed during exposure to low energy RF. No effect of the modulation was observed suggesting that the cholinergic disturbance was resulting from the interaction between brain tissue and the 915 MHz carrying frequency itself.

P-120

Abstract withdrawn by author.

P-121

PREVENTATIVE ANTI-STRESS OF LOW INTENSITY ELECTROMAGNETIC FIELD. N.A. Temuryants, E. Chuyan*, V.S. Martynyuk*, N. Verko*, E.N. Tumanyants*, E. Shishko*. Tavrida National University, Simferopol, Crimea 95007, Ukraine.

OBJECTIVE: Many studies have confirmed high therapeutic efficacy of low intensity electromagnetic fields (low intensity EMF). It has been shown that this effect is conferred through low intensity EMF's ability to limit the development of stress reaction. These data were obtained when EMF and the stress factor acted on subjects simultaneously. This study aimed at studying preliminary activity of EMF on the development of the hypokinetic stress.
METHODS: EMF parameters were as follows: wavelength - 7.1mm, flow density - 0.1 mV/cm2. Field was created with "Luch Ramed-Expert-01" generator. Stress reaction was modulated through housing of animals in magazines that limited movements in all directions.
All experiments were conducted on white male rats of the same age, weight and individual characteristics determined by the "open field" test. All animals were divided into three groups, 8-10 rats in each group. Animals in the 1st group remained in the normal conditions at the vivarium (control group). Animals in the second group were exposed to EMF with the following regimen: daily 30 minutes exposures of the occipital area for 9 days. After such exposure regimen had been completed, animals were housed in transparent plastic magazines that considerably limited mobility. The animals were kept in such magazines for 9 more days, 20 hours per day. The 3rd group of animals was maintained in hypokinetic conditions along with the animals in the second group, except they had no EMF exposure.
The status of stress reaction was monitored through the cytochemical status of neutrophiles (peroxidase, PO; lipid profile; cation proteins, CP; succinate- and a-glycerophosphatedehydrogenase, SDG, a-GPDG) and of lymphocytes (SDG, a-GPDG). Both statuses reflect non-specific resistance. We also studied behavioral criteria of adaptation.
RESULTS: In the intact animals, EMF lead to an increase of indicators of the non-specific resistance. The cytochemical indicators (CCI) of peroxidase content increased by 15%, of lipids - by 19%, of cation proteins - by 23%. Average content of SDG in neutrophiles increased by 22%, in lymphocytes - by 12%. Hypokinetic conditions of the animals in the 3rd group lead to a progressive decrease of all measured parameters that was especially noticeable on the 9th day of the experiment. During that time of observation, SDG in lymphocytes declined by 48%, PO CCI declined by 23%, CP CCI declined by 44%, lipid CCI -

declined by 23%. Such dynamics of the parameters we studied characterized the development of a stress reaction. Animals exposed to EMF prior to hypo-dynamic confinement did not exhibit a decline of parameters of functional activity of neutrophiles and lymphocytes. On the contrary, some increase of CP CCI took place, by 4%, lipid CCI increased by 11%, CCI of average SDG activity in lymphocytes increased by 8%. The same dynamics was noted in behavioral indicators. Pre-exposure to EMF inhibits CNS excitability, as indicated by a decrease in the motor activity of the 2nd group animals, as compared to the ones in the 3rd group.

CONCLUSION: Results of this study indicate that pre-exposure to low intensity EMF limits the development of the hypokinetic stress. It seems that this could be a consequence of the EMF's ability to boost non-specific resistance. As our data indicates, such influence may also be conferred on the intact animals. Clinical observation we have conducted to-date show that low intensity EMF may be successfully employed in not only treatment, but also in prophylaxis of a number of illnesses in adult and pediatric patients.

P-122 Student

EFFECTS OF 900 AND 1800 MHz GSM RADIO FREQUENCY FIELDS ON THE INNER AUDITORY SYSTEM OF RATS. M. Parazzini[1,3*], C. Marino[2], P. Galloni[2*], M. Piscitelli[2*], G. Tognola[1*], F. Grandori[1*], P. Ravazzani[1*]. [1]Centro di Ingegneria Biomedica CNR, 20133 Milano, Italy; [2]Unit of toxicology and biomedical science, ENEA Casaccia, 00060 Roma, Italy; [3]Politecnico di Milano, Dipartimento di bioingegneria, 20133 Milano, Italy.

OBJECTIVE: Aim of this work is the evaluation of possible effects on cochlea functionality of rats exposed to electromagnetic fields at the frequencies of mobile communications (900 and 1800 MHz).
METHODS: The cochlear functionality and the micromechanical activity of the outer hair cells of the cochlea before and after the exposure to GSM microwaves are tested by Distortion-Product Otoacoustic Emissions (DPOAE) that are known as indicator of the cochlea status: they are present in all healthy cochleae of humans and many species of animals, whereas they are not generally observed or are greatly reduced in ears with mild to profound hearing loss. DPOAE can therefore provide indirect information on the inner auditory system functionality. As a first step, in order to define the DPOAE experimental protocol, DPOAE were recorded from a population of 8 “normal” unexposed rats (Sprague-Dawley) to identify the best recording parameters and the reliability and repeatability of the measurements. For this purpose, we tested the 16 ears of the 8 rats with equal level primary tones (F1=F2=70 dB SPL, F1=F2=65 dB SPL, F1=F2=60 dB SPL, F1=F2=55 dB SPL) and with unequal level primary tones (F1=70 dB SPL F2=65 dB SPL, F1=65 dB SPL F2=60 dB SPL, F1=60 dB SPL F2=55 dB SPL, F1=65 dB SPL F2=55 dB SPL). Three recording sessions were performed at time 0, after 2 days and 3 weeks. In each recording session F1/F2 (ratio of frequency) was set to1.22 and the frequency range tested was 0-12 kHz. Statistical analysis on these data was performed to identify the best recording condition.
After the definition of the audiological protocol, a population of Sprague-Dawley rats are subjected to a localized exposure (in the vicinity of the ear), simulating the use of a cellular phone, by 3 different sets of 4 loop antennas, one for sham and two for exposed animals (changing loops for 900 and 1800 MHz). Four animals (male Sprague-Dawley rats weighted about 250 gr.) per set are exposed 3 hours/day, 5 days/week, for 4 weeks, 2 W/kg of SAR. In the end, 16 rats are exposed to 900 MHz EM field, 16 to 1800 MHz and 16 sham. DPOAE tests are carried out before exposure and immediately, one day, two days, and one week later; experimental tests, the collecting of data and the statistical evaluation are performed in blind mode respect to the exposure conditions (sham or 2 W/kg).
RESULTS: The best recording parameters was identified by the statistical analysis in F1=65 dB SPL F2=55 dB SPL because the data collected with these parameters show the best SNR ratio both in high (6-8 KHz) and in low frequency region (2-4 KHz) and the best repeatability between recordings from the same ear in different test sessions. The exposure experiments and results evaluation are currently in progress.

CONCLUSION: The results of this study could provide objective evidence of potential adverse effects of GSM cellular phones on hearing: statistically significant differences of the DPOAE recorded before and after the exposure could be interpreted by an influence of the exposure itself on the cochlear outer hair cells. To this purpose, the definition of the optimal audiological protocol for recordings Sprague-Dawley rats should be considered crucial. The DPOAE experimental protocol identified in this study is the best for reliability and repeatability of the measurements.
This study is performed in the framework of the European Project GUARD "Potential adverse effects of GSM cellular phones on hearing" (FP5,QLK4-CT-2001-00150, 2002-2005).

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EM FIELD-INDUCED MARKERS AS DELINEATORS OF INTERACTION MECHANISM. R. Goodman[1], H. Lin[2], L. Ye[1], D. Weisbrot[1]. Departments of Pathology[1] & Anatomy[2], Columbia University Health Sciences, 630 West 168 St, NYC, NY 10032, USA.

INTRODUCTION: There is, as yet, no complete understanding of the EM field-interaction mechanisms. Efforts toward this end have been largely confined to prevailing paradigms, focusing on the initial physical transduction step at the membrane level, on the signal transduction pathway from membrane to nucleus, and on the effects of the waveform and wave shape. However, the induction of stress response proteins (i.e., molecular chaperones) in cells and tissues exposed to weak EM-fields offers clues to the cell response and how they cope with this exogenous stress at the molecular level.
There are significant changes in stress protein hsp70 levels and in binding-activity of several transcription factors (AP1, HSF1) in cells exposed to EM fields long before changes due to increased temperature become evident. EM fields induce stress response proteins through two pathways (both different from the heat shock pathway). In one, a specific DNA sequence on the HSP70 promoter is EM responsive and acts as a sensor for EM field exposures. The second involves HSF1 phosphorylation by members of the MAPK subfamilies (ERK1, JNK/SAPK and p38 protein kinase), that results in increased protein levels for hsp70, c-Fos, AP-1 binding activity and increased MAPK/ERK1/2 phosphorylation.
OBJECTIVE: Our experiments were designed to determine whether levels of stress response protein hsp70 and binding-activities of specific signal transduction factors, AP1, SRE and MAPKinase, are affected by a magnetic oscillator designed to block or reduce nonthermal effects emitted by active cell phones. This device, producing ultra low magnetic signals, is an aluminum antenna (attached to the phone), containing an aqueous saline solution structured by an electromagnetic charge.
MATERIALS AND METHODS: Six vials each containing 6 females and 3 males *Drosophila melanogaster* were placed next to the antenna of an active cell phone with the attached aluminum antenna device. A second group of 6 vials were placed next to the antenna of an active cell phone without the aluminum antenna device. A third set of 6 vials were placed next to the antenna of an inactive cell phone (control). All the vials were exposed for one hour in the morning and one hour in the afternoon for ten days. Eggs, larvae/ pupae and adult flies were counted, protein extracted for Western blot analyses of hsp70 levels and binding-activation of AP1, SRE and, MAPKinase (methods in Reference below).
RESULTS AND CONCLUSIONS: The difference in the number of offspring from flies exposed to active cell phone, with the attached aluminum antenna and the number of offspring from controls, was statistically insignificant at the 2% level, as were hsp70 levels and binding-activation of AP1, MAPKinase, and SRE. In contrast, offspring from flies exposed to active cell phone, *without* the attached aluminum antenna, had significantly increased numbers of offspring, higher levels of hsp70 and increased binding activation of

transcription factors AP1, SRE and MAPKinase than flies exposed to cell phones *with* the aluminum antenna. We conclude that in flies exposed to cell phones without the aluminum antenna, cell phone exposure interfered with normal egg divisions within the females and normal regulatory mechanisms for developmental control were disrupted..

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P-125

INFLUENCE OF EXTREMELY LOW FREQUENCY (ELF) MAGNETIC FIELD ON RAT PAIN THRESHOLD BY ENDOGENOUS OPIATES. Z. Wang, X.L. Huo, X.Y.Zhang, T. Song. Institute of Electrical Engineering, Chinese Academy of Sciences, Beijing 100080, China.

OBJECTIVE: We have designed series experiments to examine the effects of ELF magnetic fields on rat's pain threshold (PT). The effects of ELF magnetic fields on rat's PT with different wave types, frequencies, amplitudes and action times, can be positive, passive or non-obvious. Our objective is to find the "action window" of an effective combination of magnetic field frequency, amplitude and time, which can significantly enhance the rat's PT. Then we discussed the possible biophysical transduction mechanisms by which the rat can have a response to the magnetic fields and have a result of PT's change. Especially, we assessed the effects of endogenous opioid peptides system.

METHOD: Two sorts of magnetic field generators were designed and applied to the experiments. The first is the cylindrical solenoid which can generate square waves or triangular waves. The amplitude of the first field is in the range from 5mT to 20mT and it's frequency is from 10Hz to 200Hz. The solenoid inner size is 19cm in diameter and 42cm in length. The second field is composed of three pairs of mutual orthogonal Helmholtz coils (120-cm-diameter coils control the vertical field, 106-cm-diameter and 92-cm-diameter coils control the horizontal field), which is used for the compensation of the geomagnetic field.

After the rats were raised at least three days for adapting environment, the latency of individual rat tail-lifting response to a radiation thermal stimulus was measured as basic PT. Then the rats were exposed separately in 4 rats of one group to the two sorts of fields. We recorded individual rat's PT per three days. Both sham and ELF magnetic field exposures were carried out in the same apparatus. After the rats were exposed a week, 1mg/kg naloxone was subcutaneously injected into rats. We calculated individual rat PT change percentage compared with the basic PT. These data of sham groups and control groups were statistically analyzed by T test .

RESULTS AND DISCUSSION: The control rats' PT showed distinct increase after a week exposure(8 hours every day, triangular wave, 0-8mT and 56Hz magnetic fields, the direction parallel to geomagnetic field). But after two weeks the PT relieved to base. This indicated that the effects of magnetic field had a time characteristic. PT of sham exposure groups had no distinct change through two weeks exposure. The statistic significance between rats PT of post- and of pre-naloxone treatment was gained. This indicated that endogenous opioid peptides contributed ELF magnetic analgesia. PT of rats in the second apparatus through two weeks showed significant increase compared with control group. This indicated that geomagnetic field also affected rats PT. So we will plan to observe the effects of ELF magnetic fields on rats PT in weak geomagnetic field with the second apparatus.

P-126 Student

SHORT-TERM EFFECTS ON MELATONIN SYNTHESIS IN RATS AFTER EXPOSURE TO A 1439 MHz TDMA ELECTROMAGNETIC FIELD. K. Hata[1,2], H. Nagawa[1], H. Yamaguchi[1], G. Tsurita[1], and S. Ueno[2]. [1]Department of Surgical Oncology, University of Tokyo, Tokyo, Japan. [2]Department of Biomedical Engineering, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

OBJECTIVES: Melatonin is a sleep-related hormone synthesized by the pineal body. The light dark cycle causes blood and pineal body melatonin levels to increase during the dark cycle and decrease during the light cycle. Although it was reported that low frequency electromagnetic fields influence melatonin synthesis [1,2], it is uncertain whether high frequency electromagnetic fields influence melatonin synthesis. We investigated the effects of exposure to a 1439 MHz TDMA (time division multiple access) electromagnetic field, as used in cellular phones in Japan, on melatonin synthesis in rats.
MATERIAL AND METHODS: Male Sprague-Dawley (SD) rats were acclimatized to a 12 h light-dark cycle for at least one week. The light cycle began at 8 p.m. and ended at 8 a.m. (400 lux at the cage level), while the dark cycle (only dim red light) began at 8 a.m. and ended at 8 p.m. (less than 1 lux).
The rats were divided into three groups, the electromagnetic field (EM) group, the sham group, and the cage control group. The EM group was exposed to a 1439 MHz TDMA field for 4 hours for one day in the dark condition (8 a.m. to noon), the sham group was placed in the exposure system without TDMA exposure, and the cage control group was not placed in the exposure system.
A carousel type exposure system was used. The calculation of the specific absorption rate (SAR) was described by Watanabe et al [3]. The peak SAR of the brain was 7.5 W/kg, and the average SAR of the whole body was 1.7 W/kg.
After the rats were anesthetized, blood was collected by cardiac puncture and serum was frozen at –20 °C until assay. After the rats were decapitated by guillotine, the pineal body was collected and homogenized in 1 ml of phosphate buffer (pH 6.0). After debris was removed by centrifugation, the supernatant was frozen at –20 °C until assay. The melatonin concentrations were measured by radioimmunoassay. These procedures were performed under a dim red light (less than 1 lux) until the pineal body was collected.
RESULTS AND CONCLUSIONS: The pineal melatonin level per body weight had a tendency to decrease with short-term TDMA exposure, although the difference was not statistically significant (P=0.11). On the other hand, the pineal melatonin level and the serum melatonin level were unchanged. Short-term TDMA exposure may slightly inhibit pineal melatonin synthesis, but no significant effects were observed in this limited experimental setting.
We thank the members of the Committee to Promote Research on the Possible Biological Effects of Electromagnetic Fields in Ministry of Public Management, Home Affairs, Posts and Telecommunications in Japan.
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P-127 Student

B-16 F10 MELANOMA GROWTH IN MICE EXPOSED TO ELECTROMAGNETIC MILLIMETER WAVES. A. Radzievsky, Jr.*, A.A. Radzievsky, I. Szabo*, O. Gordiienko*, M.C. Ziskin. Richard J. Fox Center for Biomedical Physics, Temple University Medical School, Philadelphia, Pennsylvania 19140, USA.

According to the numerous experimental and clinical reports, mostly from Eastern European countries, exposure to low power electromagnetic millimeter waves can suppress and, some times, even completely

prevent tumor development. However, in virtually all of these reports the results were presented qualitatively rather then quantitatively. Thus, the main aim of our present study was to quantitatively analyze the influence of multiple Millimeter Wave Treatments (MWT) on subcutaneous B-16 F10 melanoma growth when applied during different stages of the tumor development.
60 male Swiss Webster mice were used. $0.25x10^6$cells in 0.2ml of phosphate buffer were injected subcutaneously in the inner surface of the right thigh. The melanoma growth was monitored daily by measuring its diameter for 21 days following tumor cells injection. All animals were subdivided into 5 experimental groups. Group I mice (N=15;cage control) were only injected with melanoma cells; Group II animals (N=15; sham control) were restrained but not exposed to MW; Mice of the groups III, IV, and V (N=10 for each group) received MWT on each of 5 consecutive days starting from the day of melanoma cells injection (group III), on the fifth day after the tumor inoculation (group IV), or on the tenth day after the tumor cells injection (group V). Millimeter waves were applied to the nose area of unanesthetized restrained mice. MW exposure parameters were: frequency = 61.22 GHz; incident power density = 15 mW/cm^2; duration = 15 min.
Conducted experiments have shown that the course of 5 MWT cannot prevent a development or cause a complete regression of the melanoma after subcutaneous injection of B16 F10 cells in mice. Tumor developed in all animals except 2 mice (one from the cage control group and one from the group IV). By the end of the experiment the tumor sizes were variable, ranging from 5mm in diameter to 25 mm. In one group (group IV), when MWT was started on day 5 after the melanoma cells injection, on the days 19, 20, and 21 the average tumor size of the treated animals was statistically significantly smaller ($p<0.05$) than that of the sham control and cage control groups (16.1±1.2mm; 21.2±1.4mm; and 19.9±1.1mm respectively on day 21). The average tumor sizes of all other experimental groups were not statistically significantly different from the tumor sizes of the control groups.
We can only speculate about the possible explanations of why in the experimental group IV, when the treatment has started on day 5 following the tumor cells injection, MWT has caused significant suppression of B16 F10 melanoma growth. Probably, the stage of the tumor growth and the host immune response to the tumor cells is important for when the MWT is applied. Additional immunologic tests are necessary to define what exact mechanisms are involved.
This work was sponsored by Richard J. Fox Foundation

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NONLINEAR DEPENDENCE OF NEURONAL EFFECTS OF MICROWAVE EXPOSURES UPON INTENSITY OF IRRADIATION. R.A. Chizhenkova. Institute of Cell Biophysics, Russian Academy of Sciences; Pushchino, Moscow region, 142290, Russia.

INTRODUCTION and OBJECTIVE: In our previous investigations it was found that 1-min. microwave exposure affected little on the mean frequency of background activity of single cortical neurons but produced significant shifts in neuronal evoked activity. The purpose of the present study was to consider influence of microwaves of different intensities on characteristics of interspike intervals in background activity of population of cortical neurons. The consideration of pulse flows of neuronal populations was more desirable than the activity of single neurons. The point is that, individual accidental fluctuations are levelled and dominant changes are emphasised in neuronal populations. Moreover, interspike intervals are more informative than mean spike frequency.
MATERIALS and METHODS: Experiments ware carried out on unanesthetized nonimmobilized rabbits with electrodes preimplanted under barbitural narcosis into sensorimotor region of the cortex for recording the neuronal spike activity. Pulse flows of populations of cortical neurons were investigated prior, during, and after 1-min microwave irradiation (wavelength 37.5 cm, power density from 0.2 till 40 mW/cm^2).

RESULTS: Changes of interspikes intervals occurred through these exposures. Shifts as the decrease of mean values of interspike intervals predominated under irradiation if its intensity was below 0.4 mW/cm^2. Shifts of the opposite direction prevailed under irradiation if its intensity was beyond 0.5 mW/cm^2.
CONCLUSION: Thus, it has been established that 1-min microwave exposures produce shifts in pulse flows of populations of cortical neurons. Changes of interspike intervals occur. Possibility of different effects of microwave exposures exists. Dependence of neuronal rearrangements upon intensity of irradiation is nonlinear.
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HUMAN – ELF

P-129

EFFECTS OF 50 HZ EMF ON THE HUMAN MELATONIN PROFILE. G.R. Warman*, H. Tripp*, J. English* and J. Arendt*. Centre for Chronobiology, School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey GU2 7XH, United Kingdom.

INTRODUCTION: AC magnetic fields have been proposed to have a weak zeitgeber (entraining) effect (2), and alter melatonin secretion (1, 3) in humans. The effects of magnetic fields on the human melatonin profile have, however, proved notoriously difficult to replicate.
OBJECTIVES: The present controlled study aimed to examine the effects of high level circularly polarised 50 Hz magnetic fields (MF) on the human melatonin profile.
METHODS: 2 hour pulses of 200-300 μT 50 Hz MF were administered between circadian time (CT) 7 and CT 16 in a double-blind sham controlled design. Pre-treatment baseline nights on each (sham and treatment) leg served to assess circadian phase, and posture and diet were strictly controlled. Plasma melatonin was analysed from blood samples drawn every 30-60 min between 17:00 and 10:00 each night.
RESULTS & DISCUSSION: No statistically significant changes in average melatonin onset time (mid-range crossing) were observed following MF exposure. Statistically significant changes in melatonin onset variability were observed following MF exposure compared to sham controls (paired t-test, $p=0.023$, $n=14$). Interestingly, preliminary data also suggest that the CT of MF may influence the magnitude and direction of the response observed. This observation could account for the lack of consistent responses observed in previous studies.
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This work was supported by the National Grid Company plc.

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AN UNUSUAL CASE OF HIGH EXPOSURE TO MAGNETIC FIELDS IN A DAYCARE CENTER.

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OBJECTIVES: The Quebec Public Health Department investigated the case of a daycare center where a computer interference had been noticed when an outdoor pavement heating device was in operation. This 31 kW heating device (800 A) is used to melt snow and ice on the pavement (25 m X 1.5 m).

METHODS: A survey with spot measurements was carried out indoors and outdoors using an Emdex Lite from Enertech Consultants Ltd (broadband measurements in the 40 – 1000 Hz frequency range) and an EFA-200, Narda Safety Test Solutions from Wandel & Goltermann (broadband measurements in the 5 – 32 000 Hz frequency range). Measures were taken when the device was on and off, at 1.0 meter above the floor, in the center and in four corners of the three main rooms. In addition, four measures were taken in the nursery at the head of infant beds. Six outdoor measures were also taken at 0.5 and 1.0 meter above the heated pavement. When measures with the Emdex Lite were found to be above 70 μT, measures were done using the EFA-200.

SUMMARY OF THE RESULTS: The arithmetic mean (AM) of indoor measures when the device was off was 0.06 μT (range: 0.02 – 0.44 μT). When the heating device was on, the AM of indoor measures was 6.48 μT (range: 0.46-19.8 μT). At the head of the infant beds, located near the wall adjacent to the heated pavement, the AM of the measures was 15.8 μT (range: 9.45-20.5 μT). AMs of outdoor measures when the device was off were 0.44 and 0.39 μT at 0.5 and 1.0 m respectively above the pavement. When the system was on, the AMs were 111.7 μT (range 72 – 140 μT) and 53.2 μT (range 36 – 72 μT) respectively.

CONCLUSION: A common device used for heating outdoor pavement can be a significant source of outdoor and indoor exposure to magnetic fields for toddlers and adults. In this daycare center, the mean indoor exposure was multiplied by 100 when the device was on. The ICNIRP guideline for the general public exposure (83 μT at 60 Hz) and the ACGIH recommendation for people with cardiac pacemaker (100 μT at 50/60 Hz) were exceeded regularly at 0.5 meter above the pavement.

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PULSED MAGNETIC FIELD INDUCED ANALGESIA: A STUDY OF ELECTRIC CURRENT AND HOT/COLD STIMULUS INDUCED PAIN IN NORMAL SUBJECTS AND CHRONIC PAIN PATIENTS.

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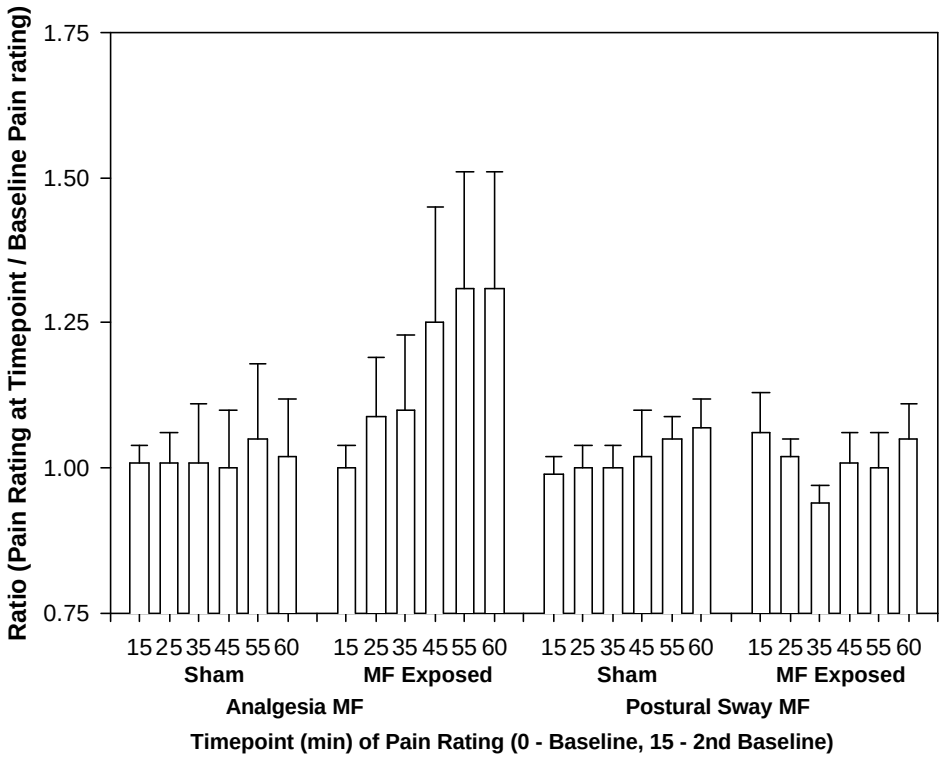
INTRODUCTION: The observation that non-specific earth-strength extremely low frequency (ELF) magnetic fields (MF) can attenuate opioid-induced analgesia has been, with the exception of orientation and navigation effects, perhaps the most reliable and reproducible MF effect yet reported.

OBJECTIVES: This study will examine the effectiveness of a specific pulsed magnetic field to induce analgesia in acute pain conditions, such as electric current induced pain, and hot/cold stimulus induced pain. Further, the subject pool will include normal subjects and chronic pain patients.

METHODS: This will be a double blind study using a heat/cold pain stimulus. Ethics approval has been granted by the Review Board for Health Sciences Research involving Human Subjects, University of Western Ontario. Based on previous study data (see fig) and assuming a desired minimum statistical power

of 0.80 we estimate approximately 12 subjects per group per experiment will be required to achieve an Eta^2 (estimated effect size) of 0.40 [http://www.statsol.com/tools/stattools/sampmeantool.html]. Our previous studies utilised an electric shock-induced painful stimulus. The electric shock-induced pain stimulation method has numerous drawbacks including gender differences, high variance, and low ecological validity [Hebert et *al* 1999]. Hot/cold pain is an alternate method to electrical pain stimuli in that, it is an ethologically relevant human pain, produces far less gender difference and less variability. [Lautenbacher & Rollman 1993]. Subject groups will consist of normal healthy volunteers and chronic pain patients. Using normal controls and pain patients with different pain etiologies allows for a better understanding of pain treatment efficacy and specificity. Subjects will complete visual analogue scales [Scrimshaw & Maher 2001] before during and after the exposure conditions of either sham exposure (no pulsed magnetic field) or pulsed magnetic field exposure. The exposures will be carried out initially within a uniform MF volume created by a three-axis orthogonal Helmholtz coil array [Thomas *et al* 2001]. Subjects will be seated in a comfortable chair mounted within the coil array, and will be asked to rate the level of induced discomfort/pain using a standardised protocol [McDermid *et al* 1996]. Preliminary data (see Fig) has indicated that a specific pulsed magnetic field exposure can induce hypoalgesia (pain relief) in human subjects. Each subject will be exposed to a single exposure condition per session, with subjects returning for the remaining exposure condition within a one-week period. Initial exposure conditions will be randomly assigned but balanced across the study to remove selection bias.

CONCLUSION: It is expected that given the previous ELF MF research on nociceptive processing in animal models [Thomas *et al* 1997], and the human EEG-SEP studies [see BEMS 2002 abstract, Cook *et al*], our results will demonstrate that a brief exposure to a pulsed ELF MF will reduce both the sensory and affective qualities of the pain perception.



Ratio of electrical current (mA time point / mA baseline) applied to the web of the left thumb required to produce a subjective 'moderate' pain rating in male and female university students (N=12) while exposed to either a sham or pulsed magnetic field. A ratio greater than 1 could be interpreted as an induction of analgesia (or hypoalgesia), and less than 1, hyperalgesia. Individual subjects were randomly assigned to either an analgesia-inducing MF exposure group (N=6), or postural sway altering MF group (N=6). Sham and MF exposure trials were held at least 1 week apart, and both experimenter and subjects were kept blind

to the exposure conditions until the completion of the study (double-blinded). Electric current recordings at each time point (15-60 min) were divided by the averaged baseline readings taken at time point 0 (not shown) and 15. Time point 15 was a second control time point, with exposures (sham or MF) starting at time point 25. The results show a significant interaction between MF type (analgesia vs. postural sway) and exposure condition (sham vs. MF exposed) [$F_{1,9}$=8.9, P = 0.03, Eta^2=0.64]. Note: this is preliminary (unpublished, R. Cooper *et al*, 2001) data and should not be construed as conclusive evidence of the MF induction of hypo- or hyperalgesia. Error bars represent the S.E.M.

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P-132

CORTISOL LEVELS IN URINE FROM WORKERS WITH OCCUPATIONAL EXPOSURE TO STATIC AND 50 HZ MAGNETIC FIELDS. B. Haugsdal, T. Tynes. Norwegian Radiation Protection Authority, N-1345 Østerås, Norway.

OBJECTIVE: In the present study we investigated the stress parameter cortisol in urine samples from two groups of night shift workers exposed to either static or 50 Hz magnetic fields. The study was initiated because of our previous finding of a positive correlation between the melatonin metabolite 6-sulfatoxymelatonin (aMT6s) in urine and exposure to both static and 50 Hz magnetic fields. As melatonin is known to be a powerful antioxidant, an increased production could be interpreted as the organisms compensatory response to increased stress.

MATERIALS and METHODS: Two groups of healthy men, all working in short shift cycles, took part in this study. During work hours, all participants were equipped with a magnetic field monitor, HI-3550 (Holaday Industries, Inc.), integrating either static or 50 Hz fields respectively. In the first group (Plant A) the participants (n=9, mean age 47.0 +/- 7.9 years) were exposed to static magnetic fields under two conditions. During one period, while working in the pot room, individual mean levels of exposure were in the range of 2.3 - 4.9 mT. During another period, while working in the control room, the corresponding levels were 0.58 - 2.0 mT. In the second group (Plant B), the study subjects (n=13, mean age 37.9 +/- 10.5 years) were exposed to 50 Hz magnetic fields with individual mean levels of exposure in the range of 3.1 - 78.8 µT. At both plants urine was sampled at three consecutive nights at work (23:00-07:00 hr). The urine volume from each interval was measured and recorded, and a pair of 15 ml samples were transferred to coded bottles and frozen for later analysis. Levels of cortisol in sample parallels from our previous melatonin study were determined by radioimmunoassay (RIA). Statistical analysis was performed by the mixed procedure, SAS version 6.12 (SAS institute, Cary, N.C).

RESULTS: Levels of cortisol in samples from night work periods were not correlate with magnetic field exposure, neither at plant A nor at plant B. When samples from the three consecutive nights were compared, we were unable to demonstrate a phase change in the excretion of cortisol.

DISCUSSION: Cortisol has important functions in many metabolic processes as well as anti-inflammatory effects, and its concentration in plasma and urine is a sensitive indicator of stress. In our study, the level of magnetic field exposure at plant B is correlated to temperature, but neither the heat stress nor the magnetic field exposure was observed to influence the level of cortisol in urine sampled from night work periods.

This work was supported by grants from the Confederation of Norwegian Business and Industry, Norsk Hydro and Borregaard Industries Ltd.

P-133 Student

SIGNIFICANT INCREASE OF LEUKOCYTES, NK AND INTERLEUKINE 2 IN HUMANS AFTER THE END OF A 0.4 µT-12 ??T SUBCHRONIC EXPOSURE. F. Szabazon[1*], L. Bonhomme-Faivre[2*], S. Deoux[3**], R. Santini[4]. [1]Association Guersanté, 32 rue Guersant 75017 Paris, France. [2]Department of Pharmacy, Hôpital Paul-Brousse 14, avenue Paul-Vaillant-Couturier 94802 Villejuif, France [3]Society Medieco, BP 278 Andorra la Vella, Andorra. [4]Institute of Applied Sciences, 20 avenue Albert-Einstein 69621 Villeurbane, France.

OBJECTIVES: We have previously observed in a human population exposed to environmental low frequency (50Hz) electromagnetic field produced by transformers a significant fall in total and CD4 lymphocyte counts with leukopenia and neutropenia (1). We report here variations of immunological parameters and serum IL2 values in 7 women (36.8 ± 9.6 years) during and after exposure to the electromagnetic field of a transformer station in another site. The exposed subjects have been working during three months 8 h/24h in premises located above behind transformers. IL2 is a lymphokine produced by activated T lymphocytes and exerts also an overall effects on the cells that participe in the immune response ie B,NK,LAK cells.

METHODS: The average daily exposure of the magnetic field strength on the site varied from 0,4 µT to 12 µT. The electric field at 50 Hz was 2 V/M to 10 V/M in the exposed room (EFM 130 electric field measurement stockbridge MA 01266 USA). Total peripheral blood lymphocytes and CD4, CD3,CD8 and NK cells were counted with coulter epic Profile. Serum concentration of IL2 was measured with an enzyme linked immunosorbent assay (Immunotech). All data were expressed as a mean ± standard deviation. Analysis of differences between the exposed period and 4 to 6 months after the end of exposure was done using the Student's t test apparied .

RESULTS: Lymphocytes, CD8 and NK values were lower than the Reference of the laboratory, during the exposure. Four to 6 months after the end of exposure leukocyte counts had statistically increased as well those of NK cells and IL 2 values. Lymphocyte, CD3, CD4, CD8 and polynuclear neutrophil counts increased from 3.8 to 27 %.

	During exposure (1)	4 to 6 months after exposure (2)	Ratio (1)/(2) %
Leukocytes	4753 ± 910	5509 ± 903*	+ 16%
NK	75 ± 33	258 ± 221*	+ 244 %
R CD4/CD8	3.2 ± 1.02	2.6 ± 0.96*	- 18 %
Total Lymphocytes	1327 ± 228	1652 ± 409	+ 24.5 %
CD8	287 ± 97	366 ± 137	+ 27%
CD4	817 ± 179	848 ± 125	+ 3.8 %
CD3	1122 ± 275	1258 ± 250	+ 12 %
Poly Neutro.	2897 ± 849	3115 ± 702	+ 7.5 %
IL2 (pg/ml)	12.4 ± 3.9	19.7 ± 4.5*	+ 58.8 %

(_____)mean values lower than normal.

Hematological and immunological parameters during the exposure and 4 to 6 months after the end of exposure to 0.4-12 µT (values ± SD).

Student's t test apparied *p<0.05 compared to period exposure

CONCLUSION: Total lymphocytes,NK and CD8 blood counts of seven women exposed to environmental ELF in rooms located aside or above electrical transformers during three months were lower than normal but a significant increase of leukocytes, Natural Killer and IL2 values was observed 4 to 6 months after the end of exposure indicating a deleterious effect of the exposure and confirming our previous observation

about potential immunological disorders due to transformer station effect in humans (1) and that after removal from exposure, hematological values can return to normal ones (2).
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P-134 Student

NORMAL SUBJECTS EXPOSED TO SPECIFIC PULSED 200µT MAGNETIC FIELDS: EFFECTS UPON THE ELECTROENCEPHALOGRAM. C.M. Cook, R.E. Thornhill, A.W. Thomas and F.S. Prato. Lawson Health Research Institute, Nuclear Medicine and Mr, St. Joseph's Health Centre and University of Western Ontario, 268 Grosvenor St., London, Ontario, CANADA, N6A 4V2.

BACKGROUND: Previous studies in our laboratory have determined the efficacy of using a specific pulsed (200 µT) extremely low frequency magnetic field (ELF MF) to affect nociceptive responses in snails and mice ('analgesia' MF) [1;2]. We have also determined that human standing balance can also be altered by using another specific pulsed MF ('postural sway' MF) [2]. In a recent pilot study, we found that normal human subjects responded differentially to the two different pulsed ELF MFs with subjects differing in the pain perception (see BEMS 2002 abstract, Rollman *et al)*. This study will assess the effect of these specific pulsed MFs quantitatively using electroencephalography (EEG). In a recent review paper [3], we have addressed the recent studies examining ELF MF effects upon human neuro- and psychophysiology. A number of previous studies of ELF MF effects upon the resting EEG have found changes within specific frequency bands, but due to the paucity of experiments, specific conclusions (and hypotheses) are difficult to make. Kecent studies suggest that the use of wavelet analyses may be better suited to the analysis of the EEG to allow a degree of time-frequency representation. To date, no EEG studies have utilized this method of analyzes to determine the effect of ELF MF exposure.
OBJECTIVE: This work in progress is assessing how two specific pulsed MFs affect the resting EEG. Our hypothesis is that a brief exposure to a specific pulsed ELF MF will likely affect those brain regions that are typically activated during the resting state, such as the prefrontal and parietal cortices.
METHODOLOGY: Normal subjects (n=20, gender matched) will be randomly assigned to receive either the 'analgesia MF' or the 'postural sway MF' over two different sessions. Subjects will be placed within 3 orthogonal, nested square Helmholtz coils [4] with the uniform magnetic field volume centred at the head level. In each session, subjects will be exposed to both an ambient sham and a specific pulsed ELF MF (<500µT, 0-3 kHz) in a 30 minute exposure periods. EEG will be recorded using a commercial electrode cap *(Quik-Cap,* Neuroscan labs, Sterling, VA) from 12 scalp locations (F3, F4, Fz, C3, C4, Cz, Pz, O2, O3, Oz, T3, T4) during both sham and MF exposure conditions. Real time data acquisition will be performed using a Neurodata Acquisition System (Model 12 Model 15, Astro-Med, Inc., West Warwick, RI) and processed using Scan 4.2 (Neuroscan labs, Sterling, VA). The EEG will be wavelet transformed using a continuous wavelet transformation (CWT) in Matlab to assess the time-frequoncy components.
References.
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P-135

CIRCULARY POLARIZED 50 HZ MAGNETIC FLUX DENSITIES OF 96 µT CANNOT INFLUENCE MICROCIRCULATION OF THE SKIN IN HEALTHY HUMAN VOLUNTEERS AND IN PERSONS SUFFERING FROM ELECTROMAGNETIC HYPERSENSITIVITY. J. Reißenweber[1], F. Wenzel*[2], E. David[1] J. Grote*[2], A. Wojtysiak*[1], M. Pfotenhauer*[1].
[1]Electropathological Research Center, Witten/Herdecke University, D-58453 Witten, Germany.
[2]Department of Physiology I, University of Bonn, D-53115 Bonn, Germany.

INTRODUCTION AND OBJECTIVE: Electromagnetic hypersensitivity (E. H.) is a growing problem in modern industrial societies. Until now no objective screening test for self-reported E. H. could be established. As crawling sensations are frequently mentioned by E. H. patients we wondered whether alterations in cutaneous microcirculation possibly linked to exposure in magnetic fields might be involved in the development of crawling sensations and further health problems. The major objective of the present preliminary study was to answer the question if cutaneous microcirculation may be influenced by weak alternating magnetic fields in the magnitude of everyday life in healthy and E. H. persons.
METHODS: During experiments all participants were lying in a bed placed within three magnetic coils of 180 centimeter diameter each. Circularly polarized 50 Hz magnetic flux densities and total body exposure were chosen because they are known to show a maximum biological activity (Kato et al. 1994). As measurement of microcirculation of the thumb is a well-established method we decided to use an infrared laser Doppler blood flow instrument for our diagnostic purposes. Glass optical fibers of the laser Doppler Flowmeter did not interfere with applied magnetic flux densities. Seven nonsmoking healthy volunteers (3 females, 4 males, mean age = 25) participated in the study. Additionally, 3 nonsmoking persons typically concerned by E. H. (3 males, mean age = 37,3) could be examined: Before starting the experiments all participants gave their informed consent. Following acclimatisation to the environment for 30 min at an air temperature of 25 °C and a humidity of the air of 70% each participant laid on a bed standing in a triangle of magnetic coils. The finger-print tissue of the thumb was chosen for experiments because it is rather homogenous. The laser Doppler flow (LDF) was continuously recorded for 50 minutes even under hyperemia and ischemia conditions. Heart rate was determined as well as peripheral arterial oxygen saturation was measured by pulse oximetry. Reactive hyperemia was performed by arterial congestion and hyperventilation was carried out by 10 deep breaths.
RESULTS AND DISCUSSION: In the present preliminary experiments we observed no vasodilatative effect of field exposure on cutaneous microcirculation neither in healthy volunteers nor in E. H. persons both under reactive hyperemia and hyperventilation. A significant rise of LDF during reactive hyperemia could be observed independently from field exposure. Conversely hyperventilation caused a decrease in microcirculation and LDF again independently from field exposure. Those phenomena are already known from general physiology. In the present experiments the increase of microcirculation provoked by reactive hyperemia may be interpreted as a species of rebound phenomenon after arterial congestion and cannot be explained by field influence because it similarly occurs in fields and without fields. Consequently, this experiment provides evidence that the experimental design used and the results obtained herewith are reliable. However, what is new in our investigations is the result that magnetic flux densities of nearly 96 µT are not able to significantly change blood flow. Thus, reactive hyperemia and hyperventilation provoke significant differences in cutaneous microcirculation in comparison to normal microcirculatory situations – independently from the status field on/field off. However, LDF measurements in weak magnetic flux densities do not seem to be helpful in this respect. The special focus of future LDF measurements should be on testing if for instance during exposure to stronger magnetic fields of various frequencies laser Doppler flowmetry could be helpful to detect persons concerned by E. H.
CONCLUSIONS: In result LDF measurements in healthy volunteers did not provide significant differences between status field on/off. However, it was possible to provoke significant alterations of LDF by both reactive hyperemia and hyperventilation independently from field status. Even in E. H. persons LDF

measurements did not result in significant differences between status field on/off. Thus, according to our preliminary results circularly polarized 50 Hz magnetic flux densities of 96 μT do not influence cutaneous microcirculation of the thumb in healthy human volunteers and in persons suffering from self-reported E. H.
Reference.
Kato M, Honma K, Shigemitsu, T, Shiga, Y: Circularly polarized 50 Hz magnetic field exposure reduces pineal gland and blood melatonin concentrations of Long-Evans rats. Neuroscience Letters 166 (1994) 59-62.
We are indebted to Mr. Manfred Rulhoff, electrical engineer, for skillful technical advice.

HUMAN – RF

P-136

PRELIMINARY STUDY ON SYMPTOMS EXPERIENCED BY PEOPLE LIVING IN VICINITY OF CELLULAR PHONE BASE STATIONS. R. Santini[1], P. Le Ruz*, J.M. Danze*, P. Santini*, M. Seigne*. [1]National Institute of Applied Sciences (INSA)- Bâtiment Louis Pasteur- 69621 Villeurbanne (France).

INTRODUCTION: Biological effects of electromagnetic fields (microwaves pulsed in extremely low frequency) emitted by cellular phone are relatively well know (1, 2). But, in our knowledge, there is no study about symptoms experienced by people living in the vicinity of cellular phone base stations (BS).
METHODS: Here are presented results of a survey study using questionnaires returned from 530 subjects (270 men and 260 women) living in France in vicinity of BS. Those subjects were enrolled by the way of information's given by press, radio, Web sites, … about the existence of a study on people living near BS. Subjects were volunteer to participate to the study. The questionnaire was fill up by subjects, without the presence of a person in charge of the study, and was returned (generally by mail) to a responsible of the study. From responses obtained it appears that 19.6 % of subjects were situated at < 10 meters (m) from BS, 26.2 % between 10 and 50 m, 13.8 % between 50 and 100 m, 9.6 % between 100 and 200 m, 10.1 % between 200 and 300 m, 20.7 % (referent group) were at > 300 m or not exposed to BS. Frequencies of complaints for 18 different Non Specific Health Symptoms (NSHS), reported in part in “radiofrequency sickness” (3), were studied by CHI-SQUARE test with Yates correction, in relation with distance from BS (comparison with the referent group) and sex. A $p < 0.05$ was considered as a significant difference. Subjects responses about symptoms were 0 = “never”, 1 = “sometimes”, 2 = “often” and 3 = “very often”.
RESULTS: Table shows results for 16 NSHS for responses “often” and “very often”. Some complaints are expressed significantly only in the near vicinity (< 10 m) of BS: nausea, loss of appetite, visual perturbations, moving difficulties. Significant difference is observed as far as 100 m from BS for irritability, depression, loss of memory, dizziness, 200 m for headache, sleep disturbance, discomfort, cutaneous problems. Beyond 200 m from BS, only tiredness is significantly more often reported as compared to the referent group. We have also observed (not in table) a libido decrease significantly more often reported for distances: < 10 m, 10/50 m and 50/100 m from BS. For women it was not observed significant difference for premature menopause in relation with distance from BS. Women significantly complained more often than men of nausea (< 10 m from BS), headache (10/50 m, 50/100 m, 100/200 m and 200/300 m from BS). Men significantly complained more often than women of libido decrease at 50/100 m from BS. Discussion: Our results show that some symptoms are not significantly expressed beyond 10 m from BS (distance where microwave intensity is high) and that some symptoms are still significantly expressed far from BS (distance where microwave intensity is normally much lower). It can be hypothesized that human sensibility to electromagnetic fields emitted by BS is not the same for all NSHS (specifics receptors implication?) and appears to be in relation for example, with sex. From those results and in relation with radioprotection,

minimal distance of people from BS should not be < 300 m specially in cases of day nursery, schools, hospitals, geriatrics centers, …

References.

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(3) (3) A.G. Johnson Liakouris. Radiofrequency (RF) sickness in the Lilienfeld study: An effect of modulated microwaves? Arch. Environm. Health. 1998. 53: 236-238.

Symptoms	Distances of subjects from base stations in meters (m)											
	< 10 m		10 - 50 m		50 - 100 m		100 - 200 m		200 - 300 m		> 300 m …	
	2	3	2	3	2	3	2	3	2	3	2	3
Tiredness	76 *	72*	63.5*	50.9*	60.6	56.6*	64.2	41.1	66.6*	43.7	40.7	27.2
Irritability	32.8	23.2*	41.7*	25.7*	47.2*	44.1*	25.8	4.1	25	9	18	3.3
Headache	51*	47.8*	40*	26.1*	40.6*	36.7*	60.7*	31.2*	19.3	0	15.6	1.8
Nausea	14.5*	6.9	8.4	3	5.7	3.8	2.4	4.6	0	2.3	2.1	1.1
Loss of appetite	20.4*	8.3	8	5.5	5	5	6.9	0	4.2	0	3.3	3.3
Sleep disturbances	41.3*	57.1*	41.4*	57.5*	46.9*	58.5*	45.8*	50 *	33.3	35.5	13.8	21.1
Depression	16.9	26.8*	21.6	19.7*	11.6	24 *	16.2	3.1	13.6	2.5	10.3	3.7
Discomfort	28 *	45.4*	25.2*	18.9	30.6*	12.8	15.7*	0	9.7	5.1	2.4	8.1
Concentration difficulties	39.3	28.8*	37.5	16.6	34.2	26.4*	25	12.5	43.3	5.5	26.7	7.1
Loss of memory	27.8	25.4*	29.4	26.6*	37.1*	29*	25	15.6	17.2	11.1	17.9	5.8
Cutaneous problems	18.1*	17.1*	6.6	10.8	11.1*	11.1	13.9*	7.5	8.7	0	1.2	4.6
Visual perturbations	14.5	24.3*	23	13.5	22	7.1	2.5	4.9	15	2.8	13.6	4.1
Hearing difficulties	33.3*	17.4	17.7*	12	8.3	15.5	7.7	7.7	11.6	9.5	5.6	8.7
Dizziness	10	12.5*	17.3*	7.5 *	9.6	9.6 *	12.2	2.7	7.7	5.2	6.2	0
Moving difficulties	5.6	7.7 *	8.2	1.7	3	3	0	0	2	0	2.9	1
Cardio-vascular problems	10.1*	13*	15.3*	9.	12.3*	7.4	8.7	0	8.5	6.5	1	3

Table: Percentages of complaints for 16 Non Specific Health Symptoms experienced by 530 people (men + women) living near cellular phone base stations in relation to their distances from base station. Responses: 2 = “often”, 3 = “very often”. Significant of results: * = $p < 0.05$ as compared to referent group (> 300 m or not exposed to base stations).

P-137

SUBJECTIVE SYMPTOMS AMONG MOBILE PHONE USERS - A CONSEQUENCE OF ABSORPTION OF RADIOFREQUENCY FIELDS? J. Wilén, M. Sandström and K. Hansson Mild. National Institute for Working Life, Umeå, Sweden.

We have previously, in an epidemiological study [Oftedal et al, 2000; Sandström et al, 2001] studied the prevalence of subjective symptoms among mobile phone users were we found as an interesting side-finding

that the prevalence of many of the subjective symptoms increased with increasing calling time and number of calls per day. This was valid even in an adjusted analysis where confounding factors such as psychosocial workload, gender and occupation were taken into consideration.
When using the mobile phone, the head of the user will absorb a certain amount of radiofrequency energy. Therefore it is of interest to go one step further and study whether the prevalence of subjective symptoms increases with increasing rate of absorption and the total absorbed energy per call and per day. In this extrapolative study, we have selected people from the epidemiological study who used any of the four most common GSM devices. We make use of the information about prevalence of symptoms, calling time per day and number of calls per day from the epidemiological study and combine it with measurements of Specific Absorption Rate (SAR).
Three volumes in the head have been defined (above the ear, on the ear and below the ear) and the maximum SAR averaged over a cube of one-gram tissue (SAR_{1g}) have been measured in each volume. Two new exposure parameters Specific Absorption per Day (SAD) and Specific Absorption per Call (SAC) have been introduced and obtained as combinations of the measured SAR_{1g}, calling time per day and number of calls per day.
The results indicates that high SAR values might be an important factor for the prevalence of symptoms, especially in combination with long calling times per day. A more extensive discussion about the results will be presented at the conference.
Reference.
Oftedal G, Wilén J, Sandström M and Hansson Mild K 2000: Symptoms experienced in connection with mobile phone use. *Occup. Med.-Oxf.* 50:237-245.
Sandström M, Wilén J, Oftedal G and Hansson Mild K 2001: Mobile phone use and subjective symptoms. Comparison of symptoms experienced by users of analogue and digital mobile phones. *Occup. Med.-Oxf.* 51:25-35.

P-138

BRAIN CANCER INCIDENCE TRENDS IN THE US BY AGE AND BRAIN LOBE 1973-1998. L. Erdreich[1], E. Lau[2]*, J. Cahill[2]*. [1]New York, New York 12170, USA. [2]Menlo Park, California 94025, USA.

OBJECTIVE: Surveillance of disease trends is a tool assessing the plausibility of links between environmental changes and disease and for identifying issues for further investigation. The objective of this project was to calculate annual incidence rates over time for gliomas, the most common brain cancer, to identify changes, and to assess whether any such changes were correlated with use of cellular telephones. Most analyses of brain cancer trends do not consider the anatomical site within the brain where the disease occured. We examined trends for gliomas in the temporal lobe in order to focus on the location of the brain that has the highest exposure to radiofrequency energy from cell phone use.
METHODS: The surveillance period included the years prior to the introduction of cell phones through the period of increasingly widespread use. The study included 32,967 malignant glioma cases compiled from nine population-based cancer registries sponsored by the National Cancer Institutes. Identification of malignant gliomas was based on the histology code assigned using the *International Classification of Diseases for Oncology* (ICD-O) 2nd Edition. The ICD-O system also includes codes for the primary tumor site by brain lobe. Glioma incidence was assessed by year of diagnosis in the total adult population aged 15 and over, in males only, and in three different age groups. These analyses were repeated for gliomas that occurred in the temporal lobe. Incidence rates were age-adjusted using the 2000 US census population as the standard. Trends in glioma incidence rates were examined separately for 1979-1985, and for 1986-1998, using Poisson regression analysis. The former period precedes cellular phones, while latter period includes the period of rapidly expanding cell phone use.
RESULTS: The standardized incidence rates in adults 15-44 and 45-64 years have not shown an increase during the period after 1985, which coincides with the increasingly widespread use of cellular telephones.

The incidence in the population aged 65 and over increased with time through the period prior to 1985, although the incidence rates since 1990 appeared to have leveled-off. The increase in this age group has been attributed to the increasing use of magnetic resonance imaging devices to detect tumors in the elderly. For all the categories examined, the slope parameters expressed as a percentage of change in the incidence rate were actually lower in the 1986-1998 period. For temporal lobe gliomas among adult males, the average rate of increase was 1.77% from 1973-1985 and -0.06% for 1986-1998.
CONCLUSIONS: This surveillance provided no evidence for an increase in malignant gliomas in adults, in males, or in gliomas in the temporal lobe that would support the hypothesis that cell phone use increases the risk of glioma. While conclusions about tumor promotion or causality cannot be based on these data alone, they contribute to the overall evidence about risks of cell phone use. To determine if cell phone use were correlated with initiation, rather than promotion, these incidence trends would need to be followed into the future to allow for the long induction period of these cancers.
This research was supported by Nokia, Inc. and Exponent.

P-139 Student

EFFECTS OF ELECTROMAGNETIC FIELDS WITH TWO DIFFERENT SAR DISTRIBUTIONS ON THE HUMAN SLEEP EEG AND HEART RATE. J. Schuderer*[1], R. Huber*[2], T. Graf*[2], K. Jütz*[2], A.A. Borbély*[2], N. Kuster[1], P. Achermann*[2]. [1]Foundation for Research on Information Technologies in Society (IT'IS), Zurich, Switzerland. [2]Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland.

OBJECTIVE: Recently, we reported that both EMF exposure during sleep [Borbély et al., 1999] and exposure during waking prior to sleep [Huber et al., 2000] resulted in comparable effects on the sleep EEG. Exposure setup and protocols differed in the two studies. The objective of the present abstract is to extend the analysis of our two previous studies and to provide a detailed dosimetry, including functional regions of the brain to enable a broader discussion and interpretation of the EEG findings.
METHODS: In the first study 24 subjects were exposed during sleep to an intermittent EMF (15 min on/off; 900 MHz; modulated with 2, 8, 217, 1736 Hz; duty cycle 7/8, spatial peak specific absorption rate 1 W/kg). In the second study 16 subjects were exposed for 30 min to an EMF (same modulation) during the waking period preceding sleep. Either the left or right hemisphere or neither was exposed. Both studies were carried out with healthy young male subjects (age 20-25 years) in a double-blind cross-over placebo controlled design. EEG (9 derivations) and ECG recordings were performed during the entire sleep episodes. Effects on EEG topography were assessed by comparing the different derivations.
DOSIMETRY: The dosimetry was conducted with numerical and experimental tools, whereby the simulations were performed with the simulation platform SEMCAD. A detailed and accurate head model was used. It is based on 121 magnetic resonance images (MRI) of a healthy female volunteer's head that was discretised with a maximum resolution of $1mm^3$. 23 different head tissues were discriminated. Mean SAR, standard deviation and 1g-averaged spatial peak SAR for the different tissues as well as for specific head areas (left side, right side, total head) were extracted. Uncertainties due to changes in head position with respect to the antenna as well as uncertainties due to head size or due to the presence of the electrodes were carefully assessed. The numerical results were verified with the near-field scanner DASY3 using the latest free field and dosimetric probes.
RESULTS: In both experiments spectral power in the 9-14 Hz frequency range of the EEG in non-REM (NREM) sleep was initially increased compared to sham exposure. No topographical differences of the EMF effect were observed in the two studies. Unilateral EMF exposure during waking induced no hemispheric asymmetry of EEG power during NREM sleep. Weak effects on cardiac activity were observed. Exposure during sleep affected heart rate variability during sleep, whereas exposure prior to sleep reduced the heart rate during waking and stage 1. The simulated SAR distribution revealed a similar exposure of left and right cortex and thalamus when EMF exposure was bilateral (during sleep). Unilateral

exposure (prior to sleep) resulted in an asymmetrical exposure of the cortex. The SAR ratio left/right was larger than 6 for all tissues investigated except for the thalamus. Left and right thalamus were exposed to a similar degree.
CONCLUSIONS. The results indicate that EMF emitted by mobile phones affect brain physiology. EMF exposure did not result in topographical differences, and no asymmetrical EEG effect was present after unilateral EMF exposure. Two explanations may be considered: 1) The attenuated field reaching the non-exposed hemisphere is sufficient to affect the EEG. 2) Subcortical regions (e.g., thalamus) may contain the most sensitive structures to EMF, and their bilateral cortical projection may explain the absence of a hemispheric asymmetry. The simulated SAR values of subcortical brain regions support this notion.
References.
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The studies were supported by the Swiss National Science Foundation, grant 3100-053005.97, the Human Frontiers Science Program grants RG-81/96 and RG-0131/2000, Swisscom, the Swiss Federal Office of Public Health (experiment 2), and the Foundation for Sustainable Mobile Communications (simulation of SAR distribution).

P-140

INFLUENCE OF ELECTROMAGNETIC FIELDS EMITTED BY HANDHELD MOBILE RADIO OF THE TETRA SYSTEM ON COGNITIVE PERFORMANCE AND WELL-BEING OF HUMANS. S. Eggert, K. Hentschel[*], I. Ruppe[*], H. Neuschulz[*], G. Kaul[*], S. Goltz[*], N. Kersten[*]. Federal Institute for Occupational Safety and Health, Noeldnerstr. 40-42, D- 10317 Berlin, Germany.

INTRODUCTION: Since the start of the digital operated mobile communication networks an arising interest of the scientific community as well as of the general public in possible health risk caused by the EM-radiation of such systems has to be stated. In particular, if using hand-held TETRA-transceivers (mobile radio) at work, close-to-head exposure and long lasting exposure (compared with normal mobile phones) are of special interest.
OBJECTIVES: There are two important differences between the public GSM-system and the non-public TETRA-system: The carrier frequency (380 – 400 MHz) and the frame repetition frequency (17.65 Hz) of TETRA are significant lower than those of GSM.
At the same level of radiated RF power, this leads to a deeper penetration into the head of the user and to a higher probability of an impact on the function of the brain.
The aim of the study is to investigate, whether the radiation of handheld TETRA – transceivers influences the cognitive performance and well-being of humans.
METHODS AND MATERIAL: A sample of 30 male volunteers were investigated in a double blind trial. The subjects were exposed alternating to the radiation of one of two generic antennas, fitted on both sides of the head in “intended use position”. The antennas were fed via cable by a rf-source consisting of a signal generator and an amplifier.
Resulting SAR has been measured by use the DASY-Equipment (KUSTER). SAR-Basic restriction for occupational exposure were not exceeded.
To determine the cognitive performance of the subjects, the Wiener Testsystem and the number-show test have been used. During a resting situation the influence on psychological basic activity was examined using Autokinetic Light Test.

The experiment has been finished in 2001. Evaluation of acquired data is in progress. It is performed like in a former series of comparable tests using GSM-exposure, where a very subtle, positive influence on the concentration performance and a better information processing were found.
PRELIMINARY RESULTS: The preliminary results *) of the investigation can be summarised as follows:
The subjects were not able to detect the presence of the EM-field
No influence on well-being could be found
*) Final results will be available at the BEMS meeting

PEER REVIEWED LATE ABSTRACTS

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ANALYSIS OF THE CELL DIVISION CYCLE IN SACCHAROMYCES CEREVISIAE UNDER STRONG STATIC MAGNETIC FIELDS AFTER UV IRRADIATION. M. Ikehata[1*], Y. Takashima[2*], J. Miyakoshi[3] and T. Koana[1,2*]. [1]Railway Technical Research Institute, Kokubunji, Tokyo 185-8540, Japan. [2]Tokyo Institute of Tecnology, Kanagawa 226-8502, Japan. [3]Kyoto University, Kyoto, Kyoto 606-8501, Japan.

OBJECTIVE: The aim of this study is to examine the possible biological effects of exposure to strong static magnetic fields on the cell division cycle of the budding yeast *Saccharomyces cerevisiae* with or without previous stress treatments by UVB irradiation.
METHODS: *Saccharomyces cerevisiae* XD83 *(Mata/Mat alpha, lys1-1/lys1-1, arg4-4/arg4-17, RAD*), W303-1a *(Mata, ade-2, RAD)*, X12-6B *(Mata, ade-2, rad1)*, X59-10A *(Mata, ade-2, rad9)*, JG-18 *(Mata, ade-2, rad18)* were obtained from American Type Culture Collection. For magnetic field exposure, we used a superconducting magnet (JS-500, Toshiba Co., Japan) which is able to generate up to 5 T homogenous static magnetic field surrounding the center of the bore (diameter; 20cm). Mid-log phase cells were harvested and washed with 0.1M phosphate buffer. Cells were re-suspended with 24 ml of YPC media and 12 ml poured into two L-tubes. One tube was incubated within the homogeneous region of a static magnetic field while the other was incubated in a conventional incubator. Temperature was maintained at 30 ± 0.5 ºC in both cultures. To study the effect of magnetic field on stress response, a similar experiment was performed except that the cells were irradiated with UVB immediately before re-suspending the cells in 24ml of YPD media. During UVB irradiation, the cells were suspended in ice-cold phosphate buffer and irradiated by UV light (CSL-4C, Cosmo Bio Co., Japan) with a 245nm band filter. UVB density was 0.1 $Jm^{-2}s^{-1}$. During the exposure period, 300 µl of aliquot of each sample was fixed with ethanol every 60 min (up to 6 h). At least 10,000 cells of each time point were analysed by FACS Calibur and Cell Quest ™ (Becton Dickinson, USA) for volume, complexity and DNA content of each cell after staining with propidium iodide.
RESULTS: The profile of DNA content distribution (G1 phase: app. 25%, S phase: app. 20%, G2/M Phase: app. 55%) did not change up to 6 h in the control group. When cells were exposed to a 5 Tesla static magnetic field, the DNA content distribution was not affected through the experimental period. In the UV irradiation study, distribution of DNA content changed drastically and cells in the S phase appeard to accumulate after 4 h from UV irradiation (1-18 J/m^3 depending on gentypes) except the case of *rad9* stain (DNA repair sheckpoint protein deficient). No significant difference in the profile of DNA content distribution was observed at each time point between cells that were either exposed or not exposed to static magnetic field emmediately after UV irradiation. These results suggest that exposure to static magnetic field did not affect either cell division cycle or cellular response to DNA damage by UV irradiation.

CONCLUSION: Experimental results suggest that exposure up to 5 T static magnetic field does not cause any alteration of cell cycle and cell cycle arrest is due to DNA damage cause by UV irradiation in *S. cerevisiae*.

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