VERUM
FOUNDATION FOR BEHAVIOUR AND ENVIRONMENT

VERUM Foundation
Josephspitalstrasse 15/IV
80331 München
Phone: +49 (0)89 207040-142
verum@verum-foundation.de
http://www.verum-foundation.de

REFLEX

Risk Evaluation of Potential Environmental Hazards from Low Energy Electromagnetic Field Exposure Using Sensitive *in vitro* Methods

Contract number: QLK4-CT-1999-01574

Duration: 1 February 2000 – 31 May 2004

Total project costs: 3,149,621 EUR | **EU contribution:** 2,059,450 EUR

Contractors: VERUM - Stiftung für Verhalten und Umwelt, München, Germany | Freie Universität Berlin, Germany | Medizinische Universität Wien, Austria | Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany | INSALUD - Instituto Nacional de la Salud , Madrid, Spain | STUK - Radiation and Nuclear Safety Authority, Helsinki, Finland | Leibniz Universität Hannover, Germany | Universita degli Studi di Bologna, Italy | ENSCPB - Ecole Nationale Supérieure de Chimie et Physique de Bordeaux, France | IT'IS - Foundation for Research on Information Technologies in Society, Zürich, Switzerland | Universita degli Studi di Milano, Italy | RZPD - Ressourcenzentrum für Genomforschung GmbH, Heidelberg, Germany

OBJECTIVES: Exposure to electromagnetic fields (EMF) in relation to health is a controversial topic throughout the industrial world. So far epidemiological and animal studies have generated conflicting data and, thus, uncertainty regarding possible adverse health effects. This situation has triggered controversies in communities especially in Europe with its high density of population and industry and the omnipresence of EMF in infrastructures and consumer products. These controversies are affecting the siting of facilities, leading people to relocate, schools to close or power lines to be re-sited, all at great expense. The causality between EMF exposure and disease can never be regarded as proven without knowledge and understanding of the basic mechanisms possibly triggered by EMF. To search for those basic mechanisms powerful technologies developed in toxicology and molecular biology have been employed in the REFLEX project to investigate cellular and sub-cellular responses of living cells exposed to EMF *in vitro*.

RESEARCH AND RESULTS: The strengths of REFLEX was firstly based on the adoption of a common technological platform for extremely low-frequency EMF (ELF-EMF) and radiofrequency EMF (RF-EMF) exposures that allowed the replication of positive findings between the collaborating partners. Secondly, on the adoption of the post-genomic technologies (DNA micro-arrays and proteomics) that enables very large numbers of potential cellular effects to be examined simultaneously without prejudice as to mechanisms. The data obtained in the course of the project showed that ELF-EMF had genotoxic effects on primary cell cultures of human fibroblasts and on other cell lines. These results were obtained in two laboratories and confirmed in two additional laboratories outside the REFLEX project, while no such effects could be observed in a further laboratory. ELF-EMF generated DNA strand breaks at a significant level at a flux density as low as 35 µT. There was a strong positive correlation between both the intensity and duration of exposure to ELF-EMF and the increase in single and double strand DNA breaks and micronuclei frequencies. Surprisingly, this genotoxic effect was only observed when cells were exposed to intermittent ELF-EMF but not to continuous exposure. Responsiveness of fibroblast to ELF-EMF increased with the age of the donor and in the presence of specific genetic repair defects. The effect also differed among the other types of cells examined. In particular, lymphocytes from adult donors were not responsive. Chromosomal aberrations were also observed after ELF-EMF exposure of human fibroblasts. The following observations were made in different laboratories: (1) ELF-EMF at a flux density of about 2 mT up-regulated the expression of early genes such as p21, c-jun and egr-1 in p53-deficient mouse embryonic stem cells, but not in healthy wild-type cells; (2) ELF-EMF (0.1 mT) increased the proliferation rate of neuroblastoma cells; and (3) ELF-EMF (0.8 mT) enhanced the differentiation of mouse stem cells into cardiomyocytes. However, no clear-cut and unequivocal effects of ELF-EMF on DNA synthesis, cell cycle, cell differentiation, cell proliferation, and apoptosis were found.

Data showed that RF-EMF produced genotoxic effects in fibroblasts, granulosa cells and HL60 cells. Cells responded to RF-EMF exposure between a specific absorption rate (SAR) of 0.3 and 2 W/kg with a significant increase in single and double strand DNA breaks and in micronuclei frequency. Chromosomal aberrations in fibroblasts were observed after RF-EMF exposure. RF-EMF at a SAR of 1.5 W/kg down-regulated the expression of neuronal genes in neuronal precursor cells and up-regulated the expression of early genes in p53-deficient embryonic stem cells but not in wild-type cells. Proteomic analyses on human endothelial cell lines showed that exposure to RF-EMF changed the expression and phosphorylation of numerous, largely unidentified proteins. Among these proteins is the heat-shock protein hsp27, a marker for cellular stress responses. There was no evidence that RF-EMF affected processes such as cell proliferation, apoptosis or immune cell functionality.
For both ELF-EMF and RF-EMF, the results of the whole genome cDNA micro-array and proteomic analyses indicated that EMF may activate several groups of genes that play a role in cell division, cell proliferation, and cell differentiation. At present the biological relevance of these findings can not be assessed.

BENEFITS: The REFLEX data have made a substantial addition to the data base relating to genotoxic and phenotypic effects of both ELF-EMF and RF-EMF on *in vitro* cellular systems. The data neither preclude nor confirm a health risk due to EMF exposure nor was the project designed for this purpose. Its value lies in providing new data that will enable mechanisms of EMF effects to be studied more effectively than in the past. Furthermore, the REFLEX data provide new information that should be used for risk evaluation by WHO, IARC, and ICNIRP.

PUBLICATIONS (team leaders in bold)**:**

2000

Platero C, Verbiest K, Úbeda A, **Trillo MA**, Gonsalvez J, Bartolomé J (2000) Platform opened for the processing and management of bio-medical images. XXI Jornadas of Automática:1-7.

2002

Ivancsits S, Diem E, **Rüdiger HW**, Jahn O (2002) Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts. Mutat Res 519(1-2):1-13.

Leszczynski D, Joenväärä S, Reivinen R, Kuokka R (2002) Non-thermal activation of hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: Molecular mechanism for cancer- and blood-brain barrier-related effects. Differentiation 70(2-3):120-9.

Platero C, **Trillo MA**, Úbeda A (2002) Processing of biochemical images for the study of the influence of GSM electromagnetic radiation on neural stem cells. XXIII Jornada of Automática:1-7.

2003

Ivancsits S, Diem E, Jahn O, **Rüdiger HW** (2003) Intermittent extremely low frequency electromagnetic fields cause DNA damage in a dose dependent way. Int Arch Occup Env Health 76(6):431-6.

Ivancsits S, Diem E, Jahn O, **Rüdiger HW** (2003) Age-related effects on induction of DNA strand breaks by intermittent exposure to electromagnetic fields. Mech Age Dev 124(7):847-50.

Schuderer J, **Kuster N** (2003) Effect of the meniscus at the solid/liquid interface on the SAR distribution in petri dishes and flasks. Bioelectromagnetics 24(2):103-8.

2004

Capri M, Mesirca P, Remondini D, Carosella S, Pasi S, Castellani G, Franceschi C, **Bersani F** (2004) 50 Hz sinusoidal magnetic fields do not affect human lymphocyte activation and proliferation in vitro. Phys Biol 1(3-4):211-9.

Capri M, Scarcella E, Bianchi E, Fumelli C, Mesircas P, Agostini C, Remondini D, Schuderer J, Kuster N, Franceschi C, **Bersani F** (2004) 1800 MHz radiofrequency (mobile phones, different Global System for Mobile communication modulations) does not affect apoptosis and heat shock protein 70 level in peripheral blood mononuclear cells from young and old donors. Int J Radiat Biol 80(6):389 -97.

Czyz J, Guan K, Zeng Q, Nikolova T, Meister A, Schönborn F, Schuderer J, **Kuster N**, **Wobus AM** (2004) High frequency electromagnetic fields affect gene expression levels in tumor suppressor p53-deficient embryonic stem cells. Bioelectromagnetics 25(4):296-307.

Czyz J, Nikolova T, Schuderer J, **Kuster N**, **Wobus AM** (2004) Non-thermal effects of power-line magnetic fields (50 Hz) on gene expression levels of embryonic stem cells – the role of tumour suppressor p53. Mutat Res 557(1):63-74.

Leszczynski D, Nylund R, Joenväärä S, Reivinen J (2004) Applicability of discovery science-approach to determine biological effects of mobile phone radiation. Proteomics 4(2):426-31.

Nylund R, **Leszczynski D** (2004) Proteomics analysis of human endothelial cell line EA.hy926 after exposure to GSM 900 radiation. Proteomics 4(5):1359-65.

Pilger A, Ivancsits S, Diem E, Steffens M, Kolb HA, **Rüdiger HW** (2004) No effects of intermittent 50 Hz EMF on cytoplasmic free calcium and on the mitochondrial membrane potential in human diploid fibroblasts. Radiat Envir Biophys 43(3):203-7.

Schuderer J, Schmid T, Urban G, **Kuster N** (2004) Novel high resolution temperature probe for RF dosimetry. Physics Med Biol 49(6):N83-92.

Schuderer J, Samaras T, Oesch W, Spät D, **Kuster N** (2004) High peak SAR exposure unit with tight exposure and environmental control for in vitro experiments at 1800 MHz. IEEE Transactions on Microwave Theory and Techniques 52(8):2057-66.

Schuderer J, Spät D, Samaras T, Oesch W, **Kuster N** (2004) In vitro exposure systems for RF exposures at 900 MHz. IEEE Transactions on Microwave Theory and Techniques 52(8):2067-75.

Schuderer J, Oesch W, Felber N, **Kuster N** (2004) In vitro exposure apparatus for ELF magnetic fields. Bioelectromagnetics 25(8):582-91.

Ventura C, Maioli M, Asara Y, Santoni D, Mesirca P, Remondini D, **Bersani F** (2004) Turning on stem cell cardiogenesis with extremely low frequency magnetic fields. The FASEB J 19(1):155-7.

2005

Diem E, Jahn O, **Rüdiger HW** (2005) Non-thermal DNA breakage by mobile phone radiation in human fibroblasts and transformed GFSH-R17 (rat granulosa) cells in vitro. Mutat Res 583(2):178-83.

Ivancsits S, Pilger A, Diem E, Jahn O, **Rüdiger HW** (2005) Cell type specific genotoxic effects of intermittent extremely low frequency electromagnetic fields. Mutat Res 583(2):184-8.

Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jotchev G, Schuderer J, **Kuster N**, **Wobus AM** (2005) Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. FASEB J 19(12):1686-8.

Winker R, Ivancsits S, Pilger A, **Adlkofer F**, **Rüdiger HW** (2005) Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low frequency electromagnetic fields. Mutat Res 585(1-2):43-9.

2006

Antonini RA, Benfante R, Gotti C, Moretti M, **Kuster N**, Schuderer J, **Clementi F**, Fornasari D (2006) Extremely low-frequency electromagnetic field (ELF-EMF) does not affect the expression of alpha3, alpha5 and alpha7 nicotinic receptor subunit genes in SH-SY5Y neuroblastoma cell line. Toxicol Lett 164(3):268-77.

Nylund R, **Leszczynski D** (2006) Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome- and proteome-dependent. Proteomics 6(17):4769-80.

Remondini D, Nylund R, Reivinen J, Poulletier de Gannes F, Veyret B, **Lagroye I**, Haro E, **Trillo AM**, Capri M, Schlatterer K, Gminski R, Fitzner R, **Tauber R**, Schuderer J, **Kuster N**, **Leszczynski D**, **Bersani F**, **Maercker C** (2006) Gene expression changes in human cells after exposure to mobile phone microwaves. Proteomics 6(17):4745-54.

2008

Benfante R, Antonini RA, **Kuster N**, Schuderer J, **Maercker C**, **Adlkofer F**, **Clementi F**, Fornasari D (2008) The expression of PHOX2A, PHOX2B and of their target gene dopamine-beta-hydroxylase (Dbeta H) is not modified by exposure to extremely-low-frequency electromagnetic field (ELF-EMF) in a human neuronal model. Toxicol In Vitro 22(6):1489-95.