

Project Progress Summary

Section 1: PROJECT IDENTIFICATION		NOT CONFIDENTIAL
Title of the project: Risk Evaluation of Potential Environmental Hazards from Low Energy Electromagnetic Field (EMF) Exposure Using Sensitive in vitro Methods		
Acronym of the project: REFLEX		
Type of contract: Shared Cost RTD		Total project cost (in euro) 3.149.621 €
Contract number QLK4-CT-1999-01574	Duration (in months) 52 Months	EU contribution (in euro) 2.059.450 €
Commencement date 1 February 2000		Period covered by the progress report 1 February 2000 – 31 May 2004
<u>PROJECT COORDINATOR</u>		
Name Prof. Dr. Franz Adlkofer	Title Executive Director	Address Pettenkoferstr. 33 D-80336 München
Telephone +49 89 5309880	Telefax +49 89 53098829	E-mail address prof.adlkofer@verum-foundation.de
Key words (5 maximum - Please include specific keywords that best describe the project.). Electromagnetism, Bioeffects, Risk to Health		
World wide web address (the project's www address) ---		
List of participants Provide all partners' details including their legal status in the contract i.e., contractor, assistant contractor (to which contractor?)		
<ol style="list-style-type: none"> 1. Prof. Dr. Franz Adlkofer, VERUM - Stiftung für Verhalten und Umwelt, Pettenkoferstrasse 33, D80336 München, Germany, Tel: +49 89 5309880 / Fax: +49 89 53098829 / E-mail: prof.adlkofer@verum-foundation.de (contractor) 2. Prof. Dr. Rudolf Tauber, Institut für Klinische Chemie, Universitätsklinikum Benjamin Franklin, Hindenburgdamm 30, D-12200 Berlin, Germany, Tel: +49 30 84452555 / Fax: +49 30 84554152 / E-mail: Tauber@ukbf.fu-berlin.de (contractor) 3. Prof. Dr. Hugo W. Rüdiger, Abteilung für Arbeitsmedizin, Universitätsklinik für Innere Medizin IV, Währinger Gürtel 18-20, A-1090 Wien, Austria, Tel: +43 1 404004701 / Fax: +43 1 4088011 / E-mail: hugo.ruediger@akh-wien.ac.at (contractor) 4. Dr. Anna M. Wobus, Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstrasse 3, D-06446 Gatersleben, Germany, Tel: +49 39482 5256 / Fax: +49 39482 5481 / E-mail: wobusam@ipk-gatersleben.de (contractor) 5. Dr. Angeles Trillo, Insalud, Ramon y Cajal Hospital, Carretera Colmenar km.9, E-28034 Madrid, Spain, Tel: +34 91 3368699 / Fax: +34 91 3368171 / E-mail: angeles.trillo@hrc.es (contractor) 6. Prof. Dr. Dariusz Leszczynski, Radiobiology, STUK - Radiation and Nuclear Safety Authority, Laipatie 4, FIN-0881 Helsinki, Finland, Tel: +358 9 75988694 / Fax: +358 9 75988556 / E-mail: dariusz.leszczynski@stuk.fi (contractor) 7. Prof. Dr. Hans-Albert Kolb, Institut für Biophysik, Universität Hannover, Herrenhäuser Strasse 2, D-30419 Hannover, Germany, Tel: +49 511 7622612 / Fax: +49 511 7623830 / E-mail: kolb@mbx.biophysik.uni-hannover.de (contractor) 8. Dr. Isabelle Lagroye, Laboratoire PIOM, ENSCPB, 16 Av. Pey Berland, F-33607 Pessac Cedex, France, Tel: +33 (0)5 40002821 / Fax: +33-(0)5 40006631 / E-mail: i.lagroye@enscpb.fr (contractor) 9. Prof. Dr. Ferdinando Bersani, Università degli Studi di Bologna, Viale Berti Pichat 6/2, I-40127 Bologna, Italy Tel: +39 (0)51 2095122 / Fax: +39 (0)51 2095050 / E-mail: bersani@gpaxbof.df.unibo.it (contractor) 10. Prof. Dr. Niels Kuster, Institut für Integrierte Systeme, ETH Zentrum, Gloriastrasse 37/39, CH-8092 Zürich, Switzerland Tel: +41 1 632 2737 / Fax: +41 1 632 1057 / E-mail: niels.kuster@ifh.ee.ethz.ch (contractor) 11. Prof. Dr. Francesco Clementi, Cattedra di Farmacologia, Università degli Studi di Milano, Via Vanvitelli 32, I-20129 Milano, Italy Tel: +39 (0)2 50316962 / Fax: +39 (0)2 7490574 / E-mail: f.clementi@in.cnr.it (contractor) 12. Dr. Christian Maercker, Ressourcenzentrum für Genomforschung GmbH (RZPD), TP3 EG, Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany; Tel: +49 6221 424741 / Fax: +49 6221 424704 / E-mail: c.maercker@dkfz.de 		

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 were to be employed in the REFLEX project to investigate cellular and sub-cellular responses of living cells exposed to EMF *in vitro*.

Results and Milestones: The strengths of REFLEX are based firstly on the adoption of a common technological platform for ELF-EMF and RF-EMF exposures that allow 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 REFLEX project showed that ELF-EMF had genotoxic effects on primary cell cultures of human fibroblasts and on other cell lines. These experiments were obtained in two laboratories, but have yet to be repeated in a third 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 REFLEX laboratories: 1) ELF-EMF at a flux density of about 2 mT upregulated the expression of early genes, such as p21, c-jun and egr-1, in p53-deficient mouse embryonic stem cells, but not in healthy wildtype 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. With respect to radiofrequency electromagnetic fields (RF-EMF), data showed that RF-EMF produced genotoxic effects in fibroblasts, granulosa cells and HL60 cells. Cells responded to RF-EMF exposure between SAR level 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 downregulated the expression of neuronal genes in neuronal precursor cells and upregulated the expression of early genes in p53-deficient embryonic stem cells, but not in wildtype 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 and Beneficiaries: 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 will be used for risk evaluation by WHO, IARC and ICNIRP.

Future Actions: The REFLEX project has created novel results. From a scientific point of view, it has to be stated very clearly that the REFLEX data do not prove a causal link between EMF exposure and any adverse health effects. The genotoxic and phenotypic effects, which have been reported within REFLEX, clearly require further studies. These studies should include extensive external replications of the key observations reported, initially using the same technological platform. A further objective should be the extension of REFLEX investigations to appropriate animal models (e.g. genetically modified mice) and human volunteer studies.