Project Progress Summary

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) 43 Months	**EU contribution** (in euro) 2.059.450 €

Commencement date 1 February 2000	**Period covered by the progress report** 1 February 2000 – 31 January 2003

PROJECT COORDINATOR

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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?)

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Objectives: Exposure to electromagnetic fields (EMF) is a controversial topic throughout the industrial world. Despite the fact that possible effects of EMF on processes controlling key cell functions, have not been investigated adequately to date, it has become a matter of concern that the rapidly increasing exposure to EMF may cause, in addition to functional disorders, cancer and neurodegenerative diseases. This fear 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. So far epidemiological and animal studies have generated conflicting data and, thus, uncertainty regarding possible adverse health effects. Clearly, mere continuation or replication of this kind of research, without the introduction of innovative concepts, will prolong the uncertainty as to whether EMF does, or does not, represent a health risk. 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 state-of-the-art methods recently developed in toxicology and molecular biology are being employed in the REFLEX project to investigate cellular and sub-cellular responses of living cells exposed to EMF in vitro.

Results and Milestones: 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 and other cell lines 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 35 µT and there is a strong correlation between the increase in single and double DNA strand breaks and the increase in micronuclei frequencies. The genotoxic response to ELF-EMF is different from cell type to cell type and between the same cell types from different individuals. ELF-EMF activates the DNA repair in human fibroblasts. The DNA repair is, however, not error-free as demonstrated by chromosomal aberrations following ELF-EMF exposure. There is also evidence that ELF-EMF alters the expression of genes in various cell systems. This was found in differentiating embryonic stem cells of mice, if the stem cells were deficient of the p53 gene. These data again suggest that it may be the genetic background whether or not cells respond to ELF-EMF. Since the flux density had to be as high as 2.3 mT before a significant difference between exposed and sham-exposed stem cells could be observed, it is not clear yet, how to assess the biological relevance of the findings. No clear and unequivocal differences in DNA synthesis, cell cycle, cell differentiation, cell proliferation and apoptosis between exposed and sham-exposed cells of various origin were observed after ELF-EMF exposure. More data in this area of research are needed before definite conclusions can be drawn.
With respect to radiofrequency electromagnetic fields (RF-EMF), it is also proven, that RF radiation produces genotoxic effects in living cells. HL60 cells, a human promyelocytic cell line, respond to RF-EMF exposure at a SAR below 2 W/kg, the presently valid safety limit, with a significant increase of single and double DNA strand breaks and micronuclei frequencies. A comparable increase in DNA strand breaks was found with human fibroblasts and granulosa cells of rats after exposure at similar SAR values. In addition, RF-EMF at a SAR of 1.5 W/kg upregulates the expression of early genes, such as p21, c-jun and c-myc, in p53-deficient embryonic stem cells, but not in healthy wildtype cells. After lowering the SAR, no effect on the expression levels of these genes was observed anymore. Again, whether or not embryonic stem cells respond to RF-EMF is dependent on their genetic background and, of course, also on the magnetic field strength applied. Additional evidence that RF-EMF may alter the gene and protein expression is obtained in a study on a human endothelial cell line. The data show that the exposure to RF-EMF changes the protein expression of numerous, yet largely unidentified proteins. Among these proteins are the heat shock proteins hsp27 which may - besides mostly positive effects - increase the permeability of the blood/brain barrier and inhibit the programmed cell death (apoptosis) and, thus, contribute at least theoretically to the development of brain tumours. 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. No effect of RF-EMF on human peripheral blood mononuclear cells representing the immune system was observed. While genotoxic effects and effects on gene and protein expression caused by RF-EMF have been convincingly demonstrated, there is no evidence so far that RF-EMF may also affect the vital processes of cell proliferation and apoptosis.

Benefits and Beneficiaries: The REFLEX project has a number of social, technological and economic implications. First of all, the data obtained so far clearly demonstrate that it is not appropriate anymore to claim that adverse health effects emanating from EMF can be excluded with great certainty. Our data do at present neither preclude nor confirm a health risk due to EMF, but they offer a sound basis for further research that is urgently needed. Despite of this limitation, one benefit of the REFLEX project is already obvious: methods that can be used to determine threshold values for EMF exposure are at present successfully developed. It seems in fact to be possible to establish so-called "no-effect-levels" which could protect exposed people from adverse health effects. Furthermore, there is a fair chance of finding defined cellular/molecular markers for the introduction in molecular epidemiological studies in order to increase their reliability. Epidemiological studies have to be considerably improved before their results can be accepted as final proof of whether

or not EMF is a disease-causing environmental factor of significance. As long as uncertainty exists, the REFLEX data may promote the recommendation of behaviour rules for the population and for industry expressed in clear and compelling guidelines which could influence both consumer habits and consumer products. The definition of threshold levels could be used for the establishment of new standards for the manufacturers of EMF-generating products. Since it is totally unrealistic to even consider excluding EMF-generating products from society, the option remaining for industry is product improvement. The possibility of defining health risks from EMF exposure also has consequences for industrial processes, in particular through the normative impact on safety provisions. It might lead to new and modified process technologies and con-sequently to changes in the industry concerned. But the establishment of new standards also offers chances for the most advanced industries to realise competitive advantages. With regard to human health and/or quality of life the reduction of exposure following the delineation of threshold levels, the development of reliable methods for long-term risk assessment and the elimination of un-certainty all have benefits. In addition, EMF may also have specific beneficial effects, applicable for instance in the treatment of certain diseases, such as psoriasis, and many others. By clarifying the mechanisms underlying such effects REFLEX could again contribute to the promotion of human health. In the case of zero and/or beneficial effects of EMF, the implications for society and industry are equally clear: security of long-term socio-economic planning at the private, community and industrial levels, reassurance of society, with positive changes to consumer behaviour, and economic advantage. By achieving its immediate objective of defining the biological effects of EMF, REFLEX will make a major contribution to the promotion of human health and the quality of life within the European Union.

Future Actions: After three years of research, it is obvious that the REFLEX project is creating an immense amount of new results, some of which no one could foresee at the start of the project. As quite often in science, the problems existing will not be diminished by this progress. Nor will controversies in the public discussion of possible hazardous effects of EMF come to an end; on the contrary, public debate is more likely to become even more intense in the future. From the scientific point of view, it has to be stated very clearly that the REFLEX data do not prove a link between EMF exposure and any health risk as claimed by quite a few. But the effects on the genome of living cells, which have been demonstrated in in vitro studies, may render such an assumption more plausible. The new data force us to explore the possible consequences with more intellectual and material engagement than before. In the remaining time, research on genotoxic effects of RF-EMF will be intensified. To investigate the genotoxic effects of RF-EMF in more detail the same approach as with ELF-EMF will be used. It is especially important to find out whether RF-EMF is able to activate DNA repair mechanisms and to produce chromosomal aberrations the same way ELF-EMF does. Furthermore, a gene expression profiling analysis will be carried out for several cell systems to see how to make the best use of this technology in any follow-up project. A sub-array containing genes potentially influenced through EMF exposure will be available at the end of the research period. Quite obviously, however, we will not be able to answer the numerous open questions arising from our findings until the end of the research period; but the available time can be used to create a firm basis for a new research project, which should be built upon the powerful new methodologies of genomics and proteomics. Since many cellular responses after EMF exposure may be related to protein modification, an important step will be the development of high-throughput proteomic assay tools for the functional proteome analysis after EMF exposure.