	EUROPEAN COMMISSION RESEARCH AND INNOVATION DG	Periodic Report

Project No: 282891

Project Acronym: ARIMMORA

Project Full Name: Advanced Research on Interaction Mechanisms of electroMagnetic exposures with Organisms for Risk Assessment

Periodic Report 2

Period covered: from 01/04/2013 **to** 30/06/2014

Start date of project: 01/10/2011

Project coordinator name:
Prof. Niels Kuster

Version: 1

Date of preparation: 20/06/2014

Date of submission (SESAM): 12/09/2014

Project coordinator organisation name:
Foundation for Research on Information Technologies in Society

Periodic Report

PROJECT PERIODIC REPORT

Grant Agreement number:	282891
Project acronym:	ARIMMORA
Project title:	Advanced Research on Interaction Mechanisms of electroMagnetic exposures with Organisms for Risk Assessment
Funding Scheme:	FP7-CP-FP
Date of latest version of Annex I against which the assessment will be made:	29/07/2011
Period number:	2nd
Period covered - start date:	01/04/2013
Period covered - end date:	30/06/2014
Name of the scientific representative of the project's coordinator and organisation:	Prof. Niels Kuster Foundation for Research on Information Technologies in Society
Tel:	+41442459696
Fax:	+41442459699
E-mail:	nk@itis.ethz.ch
Project website address:	arimmora-fp7.eu

1. Publishable summary

Summary description of project context and objectives

Research on exposure to extremely low frequency magnetic fields (ELF-MF) as a potential risk factor for childhood leukemia has been conducted since the late 1970s, and more than 30 epidemiological studies have been published since then. However, even though the International Agency for Research on Cancer (IARC) classified ELF MF as possibly carcinogenic in 2001, animal and cellular studies have provided only weak support for a causal relationship between sub-millitesla-level ELF-MFs and malignant diseases. As biophysical studies also do not support such a relationship, it remains difficult to rule out chance and experimental bias as possible explanations for the detected association between childhood leukemia and exposure to ELF MF.
The ARIMMORA (Advanced Research on Interaction Mechanisms of electroMagnetic exposures with Organisms for Risk Assessment) project aims to resolve this controversy by conducting a sound risk assessment on the association between childhood leukemia and ELF-MF exposure. This task requires a better understanding of the levels and temporal patterns of ELF-MF exposures of children in their daily lives and normal activities; extensive biological investigations to detect possible interaction mechanisms at the cellular and sub-cellular levels; and advanced biophysical simulations with efficient numerical solvers to support the experiments. The impact of ELF-MF exposure is investigated for four processes in particular: 1) the epigenetic dynamics associated with hematopoietic cell lineage commitment and differentiation; epigenetic signatures are monitored genome-wide, and mechanisms underlying eventual “misprogramming” are addressed in gene promoter models; 2) the alteration of signalling processes in cells; 3) the induction of possible cytotoxic effects on CD8-positive T-cells; and 4) the genesis and evolution of childhood leukemia in advanced genetically-modified animal models. In a final phase, the results of ARIMMORA and other projects will be combined, and a risk assessment will be performed by adapting and applying the procedures outlined by the IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.
The ARIMMORA consortium consists of ten world-renowned research groups – from Switzerland, Germany, Spain, Israel, France, and Italy – with expertise ranging from engineering to biology. This three-year project is coordinated by Prof. Niels Kuster, Director of the IT’IS Foundation for Research on Information Technologies in Society in Zurich, Switzerland.

Description of work performed and main results

In work package 1 (Literature Database), a complete cross-disciplinary database covering all the scientific and engineering aspects relevant to the development of the ARIMMORA project was created (http://arimmora-fp7.eu/refbase/) and has been continually updated during the course of the project. This database is available to all project partners, and it is particularly relevant for the preparation of WP9 (Risk Assessment).
WP2 (Exposure Matrix for Children Including Power Lines, Transformers & Near-Field Sources) aimed to broaden the knowledge base on ELF-MF exposure in children from prenatal life to adolescence (0 – 16 years old) by conducting personal exposure assessments, by determining the contributions of near-field sources in comparison to power line fields, and by translating the incident fields into field quantities in tissues and organs. Exposure models that enable prediction of tissue exposures were developed. Personal exposure measurements (of children in the range of 5-12 years old) were performed in Italy and Switzerland during both cold and warm seasons. Bedroom measurements were also conducted before or after the personal measurements.
The near-field sources to which children and pregnant women may be exposed were selected and categorized by exposure type based on a thorough literature review. The spatial and temporal field distributions from sources were collected, and measurements were performed on a range of representative sources.
The models, postures, and types of sources to be analyzed for the determination of the exposure as a function of age and environment have been selected, and the dosimetric evaluations of uniform exposures of fetuses and young children are ongoing.
The exposures due to underground power lines and transformer stations have also been analyzed; however, shortcomings in the assessments of the shielding effectiveness were detected, and additional studies were consequently initiated.
Based on the above criteria, generic exposure models for assessing the exposures with less overestimation than current state-of-the–art evaluations have been developed. As soon as these

models are fully validated, they will be disseminated to the relevant standardization groups in the course of work package 8.
The task of work package 3 (Exposure systems, dosimetry & quality control) was to provide well-characterized exposure equipment for the biological experiments of WP5 and WP6. Two in vitro systems sXcELF were adapted to the needs of ARIMMORA and installed in the partners' laboratories. A novel high-performance in vivo exposure system (sXvELF) was developed, manufactured, validated, and installed at the CBM animal facility in Madrid, Spain. In addition, field and environmental monitoring systems were installed at the University of Veterinary Medicine in Hannover, Germany. Importantly, numerical dosimetry to relate the induced fields and currents in rats and mice to those in children was performed.
The objective of work package 4 (Development of Instrumentation for Exposure Assessment and Surveillance) was to close the gaps regarding the sound assessment of ELF-EMF exposure, measurement equipment, and extensions of numerical tools. The instrumentation requirements were evaluated by conducting measurements and simulations in various exposure scenarios. The second prototype of a novel measurement equipment was developed to determine the magnitude and spatial gradient of the incident magnetic fields simultaneously. Numerical postprocessing tools for the evaluation of quantities requested by the standards agencies were developed, and the preliminary transformation coefficient functions (TCF) for the most important exposure scenarios reflecting the entire user population as a function of usage and distance were determined. The validation and calibration of the instrumentation and transformation coefficient functions are in progress.
In work package 5 (ELF-MF perception, signaling, and impact on epigenetic stability), the hypothesis that ELF-MFs may induce MAPK signaling and downstream cellular processes was tested. A series of cell lines for the induction of ERK1/2 phosphorylation were screened. Statistically significant activation of ERK1/2 phosphorylation was observed in non-transformed cell lines, whereas cancer-derived cells responded less or not at all. Dose response experiments indicated that the signal reached the maximum at ~7 µT, and remained high at 1 mT. Exposure to weaker ELF-EMF intensities (10, 1, 0.5 and 0.15 µT) revealed that ERK1/2 phosphorylation was already detected in some cell lines at 0.15 µT and became elevated thereafter, while the phosphorylation in other cells was hardly detected at low exposures. The observed activation was generally quite weak and takes longer to become evident compared to induction by canonical mitogens. Although dysregulation of the ERK1/2 cascade is well linked to cancer, it is clear that its activation is not the only change required for the transformation process. Therefore, it is too early to speculate whether the observed low activation of the ERK1/2 cascade is related to the possible induction of leukemia or other diseases induced by ELF-EMF.
As a prerequisite for the genome-wide analysis of the epigenetic landscape of leukemic cells intermittently exposed to a 50 Hz ELF-EMF for about 3.5 cell cycles, the experimental conditions were successfully established.
So far, neither a disturbance of cell growth or viability nor a substantial epigenetic alteration by the ELF-MFs has been detected. Thus, no target regions responding to ELF-MF exposure could be identified and validated as a potential biomarker for exposure.
The overall objective of WP6 (Mechanisms and animal models) is to gain mechanistic insights on the impact of ELF-MF exposure in animal models by applying three different strategies.
1) The effect of ELF-MF exposure especially on changes in the immune system in CD-1 mice was investigated. The mice were exposed (20 h/d, 7 d/wk) to continuous linear polarized sine-shaped 50 Hz electromagnetic fields of 10 µT, 1 mT, and 10 mT, starting with pregnant dams and continuing to F1 offspring up to 90 days of age. At different time points, samples of spleen and peripheral blood were taken to evaluate potential changes in the number and activation status of T-cell, B-cell, and monocyte/macrophage compartments ex vivo by means of flow cytometry. On day 28, a reduced number of CD8+ cytotoxic T-lymphocytes (CTL) was seen in peripheral blood at all exposure levels compared to sham treatment. No alterations of this cell compartment were observed in spleen of all treatment groups. The effect was moderate but significant. Whether the observed reduction of CTL has a functional effect cannot be definitively concluded from these studies. None of the tested doses of the ELF-MF signal demonstrated a significant induction of micronuclei in the peripheral blood erythrocyte fraction. The absence of an effect speaks against a profound direct DNA-damaging potential of ELF-MF exposure.
Finally, gene expression profiling indicates that ELF-MF at high dose may pose a certain risk at an individual level, and this risk may depend on the complex interplay among environmental, genetic, and epigenetic factors. Future studies on the risk assessment of ELF-MF exposures should aim to define the genomic/epigenomic landscapes that predispose individuals to potential adverse effects of

ELF-MF.

2) A new transgenic mouse model of childhood B acute lymphoblastic leukemia (B-ALL) has been generated in which the human B-ALL-associated first genetic lesion, TEL-AML1, is expressed in the stem/progenitors compartment of the hematopoietic system. Breeding pairs of these transgenic mice are being exposed to a 50 Hz magnetic field of 1.5 mT with both fundamental and harmonic content, with and an on/off cycle of 10 min/5 min, for 20 hours per day. Analysis of the bone marrow of unexposed mice showed that, at 6 months of age, specific alterations in B-cell development could already be detected in the form of an increase in BM pro/pre-B-cells and immature B-cells. However, like in humans, the leukemic process in the mouse model does not develop very rapidly. Moreover, the appearance of this fusion protein in the mouse model does not commit the premalignant target cells to develop malignant disease, as a significant proportion of the model animals developed show no alterations, similar to observations in children who harbor TEL-AML1 fusion gene but never develop B-ALL, indicating that secondary cooperative changes in the mouse genome seem to be necessary for disease expression. Thus, the transgenic mouse model generated is ideal for in vivo modeling of possible ELF MF effects in B-ALL.
3) The ELF-MF effects on the hematopoietic system of two inbred rat strains, Lewis and Fischer 344 (F344), which are genetically related to the same background strain but differ in their sensitivities to stress, carcinogens, and ELF-MF exposure, were compared to identify relevant alterations in blood, spleen, and bone marrow. The present findings indicate that ELF-MF exposure has a functional impact on rat haematopoietic cells by affecting the proliferative capacity of lymphocytes from spleen and bone marrow ex vivo. The observed effects revealed sex and strain differences, and the data suggest that B- and T-cells as well as their interactions might be affected by ELF-MF exposure. The functionality of bone marrow cells from female rats, in particular F344, was predominantly decreased after exposure to 100 µT, indicating a lower responsiveness of lymphocytes and probably immunosuppressive effects in these rats. In contrast, increased functionality was observed, e.g., in spleen cells from male Lewis rats after ELF-MF exposure, revealing enhanced lymphocyte sensitivity. The evaluation of differential blood counts showed alterations in exposed F344 in terms of increased proportions of neutrophil granulocytes, decreased lymphocytes, and an increased ratio of neutrophils to lymphocytes, which is an indicator for inflammation and certain diseases, including cancer.
Furthermore, investigations of apoptosis were performed by determining two markers of apoptosis, Annexin V and caspases. In addition to directly addressing apoptosis, a screening assay was performed by simultaneously determining 90 different rat cytokines in the cell culture medium to examine differences in cellular signalling after in vivo ELF-MF exposure associated with cell death, immune cell suppression, and activation.
Work package 7 (Biophysical modeling) combines all tasks related to biophysical modeling with the objective to determine interaction sites and mechanisms. The first task was to determine the induced magnetic and electric fields at a macroscopic level for the setups and various cell dishes for field directions developed in WP3, i.e., averaged fields induced in the cell medium of the in vitro studies (WP5) and in specific tissues examined in the in vivo experiments (WP6), that can be correlated to those determined in WP2 for children. Mappings between child age or weight and the experimental rats and mice were developed such that the induced fields over the course of the experiments could be related to childhood exposure. Organ specific dosimetry for mice, rats, and children was performed. The comprehensive literature review of microdosimetry confirmed that it is unlikely that interactions occur via the induced electric fields but rather via direct interaction with the magnetic fields. This was also supported by the third task on potential mechanisms. Based on our two literature reviews on 1) the reported positive effects of low-level EMF and 2) a potential interaction mechanism, we concluded that the radical pair mechanism is the most plausible model. This was followed by a more focused literature review to collect specific evidence and the most effective exposure conditions to confirm the radical repair mechanism for the cells employed within ARIMMORA. Correspondingly, the in vitro exposure systems have been updated to conduct the most sensitive experiments for verifying or falsifying the derived hypotheses. The corresponding additional experiments have been initiated and will be conducted in the coming months.
Based on the project achievements, WP8 (Dissemination to standards) deals with the dissemination of the results to the relevant standard committees. A representative of the consortium became a member of the corresponding standardization working groups.
The objective of WP9 (Risk assessment) is to carry out a risk assessment by evaluating the carcinogenicity of ELF-MFs based on studies conducted within this project and to compare the

results with those from recent studies conducted outside the consortium, and by adapting and applying procedures as outlined by the IARC Monographs Program on the evaluation of carcinogenic risks to humans.
Furthermore, literature is continually reviewed and evaluated regarding its possible impact on the execution of ARIMMORA, especially in reference to the epidemiologic and mechanistic data on the aetiology of childhood leukemia, and, more specifically, on EMF-related findings. Within ARIMMORA, this has been investigated by retrieving data from completed childhood leukemia studies on how established or other suspected factors influencing the risk of leukemia may affect relapse or survival after leukemia. In addition, the epidemiological evidence leading to the classification of ELF-MFs as possibly carcinogenic based on studies of childhood leukemia has been extensively reviewed, in particular to identify any exposure parameters that might be identified as relevant to a possible mechanism. Briefly summarized, it appears that the average exposure over the whole day – measured either by median or geometric mean –shows the most consistent relationship compared to, for instance, fluctuations, peaks, or specific time windows. Also, the association appears to be independent of the field source, i.e., the effects are similar for low voltage sources and very high voltage sources that produce the same magnetic field intensity.

WP10 (Management) is dedicated to the management of the ARIMMORA project. Special emphasis has been placed on dissemination, which includes the creation and maintenance of the attractive project website, the distribution of informative leaflets, and foremost, the unique workshop organized on Monte Verita 'EMF Health Risk Research - Lessons Learned and Recommendations for the Future – 7 Years Later.' The workshop brought together leading researchers in the field of EMF and health as well as government health protection experts and standardization committees from around the world to analyze and synthesize newly available research results. Studies of the ARIMMORA project were presented and extensively discussed with world-renowned experts in leukemia. The workshop provided highly valuable input on all work packages, especially WP5, WP6, and WP7. More detailed information about ARIMMORA can be found on the project website: www.arimmora-fp7.eu.

Expected final results and potential impacts

ARIMMORA will strongly contribute to our understanding of the biological effects triggered by magnetic fields at the microtesla level and the underlying mechanisms and thus help to evaluate the causality between ELF-MFs and cancer. It will clarify the hypothesis that exposures well below current protection levels have adverse health effects and possibly contribute to increased childhood leukemia susceptibility. In that case, precautionary measures to reduce leukemia risks could be devised based on quantitative risk models of ELF-MF exposure. Identifying the underlying mechanisms can also provide further insight into the aetiology and cellular progression of childhood leukemia in general, opening the door to prevention and treatment.