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Periodic Report

PROJECT PERIODIC REPORT

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1. Publishable summary

Summary description of project context and objectives

Research on ELF MF as a potential risk factor for childhood leukemia has been performed since the late 1970s, and more than 30 epidemiological studies have been published since then. However, even though ELF MF have officially been classified as potentially carcinogenic, after 40 years of research, studies in animals and cells have provided only weak support for a causal relationship between sub-millitesla-level ELF-MF and malignant diseases. Biophysical studies also do not support such a relationship, and 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 settle this controversy by contributing to a sound assessment of the risks of childhood leukemia associated with exposure to ELF-MF. 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 on four processes in particular: 1) the epigenetic dynamics associated with hematopoietic cell lineage commitment and differentiation; epigenetic signatures will be monitored genome-wide, and mechanisms underlying eventual “misprogramming” will be 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 leukaemia in advanced genetically-modified animal models. In a final phase, results of this and other projects will be combined, and a risk assessment will be performed by adapting and applying the procedures outlined by the International Agency for Research on Cancer 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 Switzerland.

Description of work performed and main results

In work package 1 (WP1; Literature Database), a complete cross-disciplinary database covering all the scientific and engineering aspects relevant for the development of the ARIMMORA project has been created (http://arimmora-fp7.eu/refbase/). This database is available for all project partners and is particularly relevant for the preparation of WP9 (Risk Assessment).

WP2 (Exposure Matrix for Children Including Power Lines, Transformers & Near-Field Sources) aims to improve knowledge of exposure to ELF-MFs in children from prenatal life to adolescence (0-16 years old) by personal exposure assessments, determination of the contributions of near-field sources in comparison to power line fields, and translation of the incident fields into field quantities in tissues and organs. Exposure models that enable prediction of tissue exposures should be developed. In the first period, the performance and calibration of the applied exposimeters for personal exposure measurements were validated, and a study protocol was defined. Up until month 18, 49 and 70 children were measured during the spring/summer season, and 72 and 72 during the winter season, in Italy and Switzerland, respectively. Preliminary analysis shows little overall difference between the three study groups (built-in transformer, high voltage power line, control) regarding the geometric means of the measurements. A linear mixed model clustering for children of the same family and the geometric mean as target variable revealed no significant differences according to group, country, age, or urban setting for the summer season. This preliminary finding needs further investigation with the full data set. Preliminary comparison of spot- and personal measurements indicates that spot measurements tend to be more extreme on both ends of the measurement scale, which is important for risk assessment. Specifically, personal measurements of children under high exposure at home are lower than spot measurements, whereas personal measurements of children under low exposure at home tend to be higher. However, this preliminary

finding needs further investigation with the full data set.
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 distribution of fields from sources such as induction stoves, compact fluorescent bulbs, and train cars has been collected. For the development of exposure models, solvers and post-processors based on existing literature and on the Dosimetry Intercomparison Laboratories have been validated. The models, postures, and types of sources to be analyzed for the determination of the exposure as function of age and environment were selected, and the dosimetric evaluation of uniform exposure of fetuses and young children is ongoing. Numerical models of power lines and simulations have been completed to evaluate the exposure of child models. Transformer configurations are ongoing. Different fetal orientations for 3-month gestation are available, and a 3-year-old child model, which will be posable, has been developed for this project. An analysis of uniform exposure of pregnant woman models has been completed.

The task of WP3 (Exposure systems, dosimetry & quality control) is 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 partner laboratories. A novel high-performance in vivo exposure system (sXvELF) was developed, manufactured, validated, and installed at the animal facility at CBM in Madrid. In addition, field and environmental monitoring systems were installed at the University of Veterinary Medicine in Hannover. Importantly, numerical dosimetry that relates the induced fields and currents in rats and mice to those in children was performed.

WP4 (Development of Instrumentation for Exposure Assessment and Surveillance) has the objective to close gaps regarding 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. Measurement equipment that determines the magnitude and spatial gradient of the incident magnetic fields simultaneously has been designed. Validation and calibration of the instrumentation and transformation coefficient functions is in progress. Numerical post-processing tools for the evaluation of quantities requested by the standards agencies were developed, and the transformation coefficient functions (TCF) for the most important exposure scenarios reflecting the entire user population as a function of usage and distance have been determined.

In WP5 (ELF-MF perception, signaling, and impact on epigenetic stability), the hypothesis that ELF-EMF may induce MAPK signaling and downstream cellular processes is tested. A series of cell lines for the induction of ERK1/2 phosphorylation were screened. Statistically significant induction of phosphorylated ERK1/2 could be observed in non-transformed cell lines whereas cancer-derived cells did response less or not at all. The observed activation was generally quite weak and takes longer to become evident compared to induction by canonical mitogens. However, the extended screening for cell lines that respond to ELF-EMF by ERK1/2 activation yielded a good basis for further investigations of cause and consequence.
As a prerequisite for the genome-wide analysis of the epigenetic landscape of leukaemic 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 indication for substantial epigenetic alteration by the ELF-EMF was found. However, a subtle difference of ChIP-seq read distribution of active (H3K4me2) chromatin between sham- and ELF-EMF-exposed cells was identified, which required further investigations as it might be of biological or technical nature.

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, 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 in all treatment groups. Although the effect was moderate, it was significant. Whether the observed reduction of CTL has a functional effect cannot finally be concluded from the studies.
2) A new 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 mice of this background are currently 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'/5', for 20 hours per day. Analysis of the bone marrow of unexposed mice showed that, at 6 months of age, specific alterations of B-cell development could already be detected in the form of an increase of the BM pro/pre-B-cells and immature B-cells. However, like in humans, the mouse model does not develop a too-rapid leukemic process. Moreover, the appearance of this fusion protein in the mouse model does not commit the premalignant target cell to evolve into a malignant disease, as a significant proportion of the model animals developed do not show 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 generated mouse model is an ideal model for in vivo modeling of a possible ELF MF effect on B-ALL.
3) The ELF-MF effects on the hematopoietic system of the 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.

WP7 (Biophysical modelling) combines all tasks related to biophysical modelling 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 of 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 second task “micro dosimetry” will be performed in the second period. The third task concerns the investigation of potential mechanisms by modelling the biophysical interaction based on the results of the micro-dosimetry and applying appropriate modelling tools and supporting data from WP5 and WP6. As our literature review has shown that the effective exposure parameters, the potential test systems and target molecules have not been identified yet, we have performed two literature reviews: 1) on the reported positive effects of low-level EMF and 2) on a potential interaction mechanism. Based on these two reviews, we concluded that the radical pair mechanism is the most promising and will be our focus at the beginning of the second period. In addition, we will also investigate the disruption of the mitogenic spindles and possible biological quantum effects.

Based on the achievements of the project, WP8 (Dissemination to standards) deals with the dissemination of the results to the relevant standard committees. Until now, representative of the consortium became member of the corresponding working groups.

The objective of WP9 (Risk assessment) is to carry out risk assessment by performing an evaluation of the carcinogenicity of extremely low-frequency magnetic fields (ELF-EMF) based on studies conducted within this project and setting them into the context of recent studies conducted outside the consortium, adapting and applying procedures as outlined by the International Agency for

Research on Cancer for their Monograph Program on the evaluation of carcinogenic risks to humans. As the work package is based on the results of the other work packages, the main workload of the work package will be addressed at the end of the project.

WP10 (Management) is dedicated to the management of the ARIMMORA project. A project office to assist and facilitate the work of the coordinator, perform the day-to-day management of the ARIMMORA project, and to act as the intermediary between the consortium bodies and European Commission was installed. Special emphasis was placed on dissemination, which included creation of the attractive project website, 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. It provided a forum for extensive discussions that is available neither at scientific meetings nor at the official standard/assessment group meetings, namely to focus on those experiments that are not in line with current understanding of EMF interaction with biological organisms. Studies of the ARIMMORA project were presented and extensively discussed there together with world-renowned experts in leukeamia. The workshop provided highly valuable input on all work packages, in particular WP5, WP6, and WP7. More information on ARIMMORA can be found on the installed website: www.arimmora-fp7.eu.

Expected final results and potential impacts

If a causal relationship is established between magnetic fields at the microtesla level and cancer, and the underlying mechanisms can be identified, ARIMMORA will strongly contribute to our understanding of the biological effects triggered by ELF-MF. It will further support the hypothesis that exposures well below current protection levels have adverse health effects and possibly contribute to increased childhood leukaemia susceptibility. In that case, precautionary measures to reduce leukaemia risks could be devised based on quantitative risk models of ELF-MF exposure. Identifying mechanisms could also give further insight into the aetiology and cellular progression of childhood leukaemia in general, opening the door to its prevention and treatment.