Summaries and assessments of selected studies

In the period from end of January to mid of May 2017, 106 new publications have been identified, and 12 of these were discussed in depth by BERENIS. Based on the selection criteria, six of these publications were selected as the most relevant ones. Their summaries and assessments are provided below.

1) Experimental animal and cell studies

Global analyses of proteins and epigenetic marks of cells exposed to electromagnetic fields (Kuzniar et al. *2017, Manser* et al. *2017)*

Unlike most cell studies that test a specific hypothesis in regard to the effect of electromagnetic fields (EMF) on a molecular mechanism, Kuzniar *et al.* (2017) and Manser *et al.* (2017) used an approach without specific hypotheses. Due to recent technological advances in quantitative analyses of biological molecules, it was possible to globally compare the amount of proteins and the presence of epigenetic[1] marks in exposed and non-exposed cells. This wealth of information on the overall condition of the cells facilitates drawing conclusions about potentially affected cell functions by means of complex bioinformatics methods. The authors make their data publicly available, thereby providing an interesting resource for further EMF-related research.

The *in vitro* study by Kuzniar *et al.* (2017) compared a remarkably high fraction of the entire set of proteins (proteome) with semi-quantitative mass spectrometry. Using human fibroblasts, human osteosarcomas and mouse embryonic stem cells, they were systematically exposing them to an extremely low frequency magnetic field (ELF-MF) (50 Hz, 2 mT, 5/10 min on/off) for 15 hours, and to radiofrequency UMTS or Wi-Fi signals (2.1 GHz, 45 V/m; 5.8 GHz, 9.5 V/m) for 24 hours. These datasets were then compared to the respective controls using several statistical approaches. Exposure to the different EMF signals did not result in any indication for a significant and consistent impact on the proteome or specific cellular mechanisms. The authors note that the intrinsic experimental variability is generally larger than the effect of the exposure. Thus, for example, indications for a slight fluctuation of a DNA repair protein could not be confirmed by classic analytical methods. Furthermore, a database-based network analysis was conducted accounting for all potentially influenced proteins under all exposure conditions. This analysis indicates a potential influence on the regulation of histone modification, which plays a central role for the epigenetic status of cells.

The cell study of Manser *et al.* (2017) conducted in Switzerland investigated the impact of ELF-MF (50 Hz, 1 mT, 5/10 min on/off) on the stability and modification of epigenetic information. The analysis focused on biomarkers for the activity status of genes, specifically, the methylation status of histone H3, and the methylation of the DNA. These epigenetic marks change during cell differentiation,

[1] „Epigenetic“ means inheritable information which is not directly deposited in the sequence of base pairs (genetic coding). The epigenetic characteristics of a cell determine the gene activity, thus which genes are active and which ones are not read. Epigenetics therefore enable characteristic gene activity patterns depending on the type and function of the cells, based on identical genetic information.

defining the development status and cell type. Typically, they are strongly altered in cancer cells that have lost their cell identity. The authors investigated the effect of the ELF-MF on the epigenetic profile of leukaemia and blood stem cells that developed into granulocytes. Similar to the study of Kuzniar *et al.* (2017), no statistically significant and consistent effects of the exposure were found, while control conditions with known impact on the epigenetic landscape (developmental status and addition of trichostatin A) led to substantial changes. Furthermore, there was only little evidence that exposure had an effect on functionality and proliferation of the cells. Although the authors did not find any controlled alteration of the epigenetic profiles, they made an interesting observation. The variability of the epigenetic modifications was depending on the exposure condition, though not equally in all gene elements, and pronounced differently depending on the type of chromatin modification. For example, important regulatory gene elements were less affected than regions outside of genes. This could be interpreted in a way that the exposure influences the background noise of the epigenetic profile of cells. Whether this can lead to pathologic situations in the long term or have an impact on development processes remains to be clarified in further studies. However, this impact could indeed be a possible functional explanation for findings of recent studies describing EMF effects on cell differentiation processes.

Impact of radiofrequency electromagnetic fields on mitochondria (Sun et al*. 2017)*

Mitochondria are organelles that play a central role for the energy supply of cells. They occur in varying numbers in all cell types and bear their own circular DNA, coding the genetic information for part of the proteins that are required for fulfilling their important cellular function. The interesting part of the study by Sun *et al.* (2017) is that they focused on the investigation of mitochondrial changes, which have hardly been analysed so far. The authors exposed human leukaemia cells to a radiofrequency (RF) EMF for 5 days (900 MHz, 120 $\mu W/cm^2$, 4 hours/day) or radioactive γ-radiation (positive control), and investigated a variety of parameters relevant for mitochondrial function after 30 minutes, 4 hours and 24 hours. Comparable to the impact of radioactive exposure, a temporary increase of free radicals (ROS) was observed upon RF-EMF exposure. This increase (up to 50%) was most pronounced after 30 minutes, still measurable after 4 hours but disappeared after 24 hours. Simultaneously, oxidative damage of mitochondrial DNA increased with similar dynamics. In addition to mitochondrial DNA damage, the authors observed a reduction of the protein amount and gene expression of factors important for the control of the mitochondrial copy number and gene activity. This led to a significant reduction of the quantity of mitochondrial DNA and the availability of ATP as a carrier of energy 30 minutes after exposure. However, these differences were not detectable anymore after 24 hours. The findings of this study are generally compatible with results of other studies related to oxidative stress. Yet, further investigations and independent confirmation of these results will be needed in order to assess their validity and relevance. At present, it is unclear whether such temporary changes due to EMF could lead to mitochondrial diseases in the long term, or could influence their etiopathology.

2) Epidemiological studies

Maternal cell phone use during pregnancy and child behavioural problems (Birks et al. *2017)*

The study of Birks *et al.* (2017) addressed the question whether mobile phone use of women during pregnancy leads to behavioural problems of the children. The authors based their analysis on cohort data from five countries (Denmark, The Netherlands, Norway, Spain and Korea), with a total of more than 80'000 participating mother-child pairs. Both maternal cell phone use and child behavioural

problems were assessed through questionnaires filled in by the mothers. With regard to mobile phone use, data were collected prospectively in three of the cohorts, thus before the children were born. For the remaining two cohorts, data on mobile phone use were collected retrospectively at the same time as assessing potential behavioural problems at the age of five to seven. Children whose mothers used their mobile phone often during pregnancy (more than 4-6 times or more than one hour per day) had a 22% higher risk of having hyperactivity or inattention problems. No increased risk was found with regard to general behavioural or emotional problems. The analysis took account of several potential confounders, such as civil status, education, history of psychopathology, smoking and alcohol consumption during pregnancy, body mass index and height. The results were robust in a multitude of sensitivity analyses, and independent of whether mobile phone use was assessed prospectively or retrospectively.

The large database is a strength of the study, but there are also some weaknesses. The questions related to mobile phone use, behavioural problems and co-factors varied in the different countries, and no assessment was made about the actual strength and the timing of the children's RF-EMF exposure during pregnancy. Although the association as such has been established rather consistently, it remains unclear whether mobile phone use of the mothers during pregnancy was actually the cause of the observed behavioural problems of the children. Other factors related to maternal cell phone use could have played a role as well. It is quite likely that mothers who frequently used a mobile phone during pregnancy have continued doing so in the following years, which potentially also had an effect on their educational style.

Perceived and modelled exposure by mobile phone base stations, nonspecific symptoms and sleep disturbances (Martens et al. *2017)*

The study of Martens *et al.* (2017) modelled the exposure by mobile phone base stations at the home addresses of participants of the AMIGO cohort study in the Netherlands. In addition, perceived exposure to mobile phone base stations, radio and TV (0 = not at all; 6 = very much), nonspecific symptoms, and sleep disturbances were assessed by questionnaire. Exposure was modelled for two points in time (2011/2012 and 2013). Individuals with an exposure >0.14 V/m were considered to be exposed (10% of study sample). The subjective appraisal of exposure as well as the assessment of nonspecific symptoms and sleep disturbances took place at three different points in time in 2011/2012, 2013 and 2014. Cross-sectional analyses of the 2011/2012 data with 14,829 study participants aged between 31 and 65 years, and longitudinal analyses after one (n=2,228) and two years (n=1,740) did not show any correlations between modelled RF-EMF exposure and occurrence of nonspecific symptoms and sleep disturbances. However, the authors found an association between the participants' own perception of exposure and the occurrence of health problems. The observed correlation might indicate a nocebo phenomenon among participants who were aware of the presence of mobile phone base stations in their vicinity, and assessed their own exposure as relatively high. The authors' interpretation of the overall findings is that exposure by mobile phone base stations at the residential location does not lead to substantial adverse effects on health.

This is one of the few longitudinal studies on the topic. The number of study participants is relatively high, and exposure was modelled by using a validated approach. The standardised questionnaire allows good comparability of the data. Nevertheless, a weakness of the study is that perceived exposure included radio and TV, which was not the case regarding modelled exposure. This may have contributed to the lack of correlation between these two exposure measures. Furthermore, the differences in exposure levels are small, and RF-EMF exposure by own use of communication devices is not considered. The latter is usually considerably higher than RF-EMF of fixed site transmitters and

the authors state that they cannot exclude the possibility that total RF-EMF exposure is associated with symptoms.

3) Theoretical study related to mechanisms

A physical mechanism of magnetoreception (Binhi & Prato 2017, including comments of Barnes & Greenebaum 2017 and Prato & Binhi 2017)

In recent years, effects of weak ELF-MF have been observed in various biological systems, partly showing unusual dose-response relationships with regard to intensity and frequency. From a physical point of view, these findings are debatable because according to the classic principles of thermodynamics, the extent of energy induced by low magnetic field exposure is small compared to thermal noise in the physiological environment. According to this, no biological reaction is to be expected. The study of Binhi & Prato (2017) addresses precisely this issue. They present a theory for the primary step of a magnetic field (frequency range < 100 Hz and intensity < 100 µT) coupling with biological structures. The model that they present is generally applicable, and based on only three common physical principles and one assumption. The three principles are:

- "Sensors" for the external magnetic field must be molecules/ions that possess a magnetic moment themselves.
- In the geomagnetic field, the magnetic moment of these molecules performs a precession (analogue to a gyroscope). The presence of an additional alternating magnetic field or the shielding of the geomagnetic field disturbs this precession.
- Additionally, the thermal movement of the molecules in the surroundings disturbs the precession, but does not disrupt it.

The assumption is that the precession of the magnetic moment is coupled with a biochemical reaction, and causes a reaction cascade. Disturbances of the precession thus cause changes of the biochemical cascade. The type of the coupling is kept open. Under the abovementioned premises, the authors show that biological effects of weak ELF-MFs are theoretically possible despite thermal noise, also in the absence of the geomagnetic field. The model predicts complex dose-response relationships and further observables which can be tested in biological experiments.

The model is elegant, as it relies on only few physical principles, and basically can be applied with regard to any kind of coupling to a chemical reaction. Nevertheless, the type of this coupling remains open. If the model predictions can be confirmed in experiments, this would be an essential step towards a mechanistic understanding of low dose ELF-MF effects.

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Contact

Dr Stefan Dongus
BERENIS Secretariat
Swiss Tropical and Public Health Institute
Department of Epidemiology and Public Health
Environmental Exposures and Health Unit
Socinstr. 57, P.O. Box, CH-4002 Basel, Switzerland
Tel: +41 61 284 8111
Email: stefan.dongus@swisstph.ch

Additional information:

BERENIS - Swiss expert group on electromagnetic fields and non-ionising radiation

List of abbreviations (pdf)